W1190

Systemic *E. coli* or *H. pylori* Lipopolysaccharide Induces a Transcriptional Up-Regulation of nNOS in the Dorsal Vagal Complex

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Systemic administration of low doses of *E. coli* LPS modulates gastric motor function through NO synthesis in the brainstem (Quintana *et al.* 2001). Presence of nNOS in the dorsal vagal complex (DVC) and a physiological role for NO in this area modulating gastric motility have been reported (Krowicki *et al.* 1997). However, less is known about nNOS expression in the DVC under pathophysiological circumstances. AIM: To analyze nNOS expression and distribution in the DVC of rats pre-teated with *E. coli* or *H. pylori* LPS at doses that modulate gastric motor function. METHODS: *Gastric motility determination:* An intraluminal latex balloon was inserted into the stomach of anaesthetized fasted rats. *H. pylori* LPS (5, 40 or 100 μ/kg, i.v.) or saline (1ml/kg, i.v.) were administered 30 min before 2-deoxy-D-glucose (2DG, 200 mg/kg, i.v.) and intragastric pressure (IgP) was monitored for 60 min. *nNOS expression and distribution analysis:* conscious rats received *E. coli* LPS (40 μg/kg i.p.), *H. pylori* LPS (100 μg/kg i.p.) or saline (1 ml/kg i.p.) and were sacrificed 120 min later. a) nNOS mRNA was quantified by real time RT-PCR (LightCycler) in a section (2.5 mm) of the brainstem containing the DVC. b) Serial coronal sections (50 μm) along the rostro-caudal axis of the brainstem were processed for nNOS immunohistochemistry. RESULTS: *H. pylori* LPS prevents in a dose-dependent manner (5,40 and 100 μg/kg) the increase in IgP induced by 2DG administration (40±18, 84±23* or 119±22* % of inhibition, respectively). *E. coli* or *H. pylori* LPS significantly increased nNOS mRNA expression in the brainstem (160±7* % or 140±13* % respectively compared to vehicle-treated rats). nNOS immunoreactivity was present along the DVC nuclei with a distribution region dependent. The highest density of positive cells was found at the rostral level although some nNOS-stained cells were found at caudal and intermediate level. *E. coli* or *H. pylori* LPS treatment for 120 min significantly increased nNOS-immunopositive cells (270±34* or 265±19*, respectively) compared to vehicle-treated rats (184±13) at the most rostral level of the DVC (-13.44 to -13.15 mm from Bregma). In all cases n≥3. (*)P<0.05, Student's t-test. CONCLUSION: A transcriptional up-regulation of nNOS in rostral DVC is induced by peripheral administration of low doses of either *E. coli* or *H. pylori* LPS which suggest a role for NO synthesis in this specific area in the control of gastric motor function under endotoxemia.

W1191

Effects of Hyperglycemia on Responses to Norepinephrine (NE) in Rat Dorsal Motor Nucleus of the Vagus Neurons (DMV)

Kirsteen Browning, Richard Gillis, R. Alberto Travagli

Diabetes results in gastric dysfunction and ultimately in gastroparesis. Catecholamines play an important role in the brainstem circuits involved in the esophageal distention-induced gastric relaxation. Experimental evidence suggests that monoamine levels are disrupted in the brainstem of diabetic rats. The aim of the present study was to determine whether the sensitivity of brainstem gastric-projecting neurons to NE is altered during hyperglycemia. DMV neurons innervating the stomach were identified by application of the retrograde tracer DiI to discrete gastric regions. Acute hyperglycemia was induced via streptozotocin injection (65mg/kg, i.v.) 5-7 days prior to experimentation. Rats with blood glucose levels ≥300mg/dl for at least 5-7 days were used. Whole cell patch clamp recordings were made from gastric-projecting DMV neurons in thin brainstem slices. The glucose concentration in the recording solution was adjusted to the glycemia levels at time of the sacrifice. All electrophysiological recordings were performed at room temperature. Following hyperglycemia, differences were apparent in the basic biophysical properties of DMV neurons. Neurons innervating the antrum-pylorus, for example, had a small membrane input resistance (389±37MΩ compared to 719±89MΩ in control, p≤0.05) and a shorter duration action potential (3.6±0.3ms compared to 5.1±0.4ms in control, p≤0.05). Differences were also apparent in the responses of DMV neurons to application of NE (0.1-300μM); in hyperglycemic rats more DMV neurons responded with an inhibition (see table below). In addition, in control rats, NE inhibited glutamatergic but not GABAergic synaptic transmission to gastric-projecting DMV neurons. Following hyperglycemia, however, NE was also able to inhibit GABAergic synaptic transmission in a concentration-dependent manner. Our data suggest that following acute hyperglycemia, gastric-projecting DMV neurons are more likely to exhibit α2 mediated responses to NE. Supported by NIH grant DK 57105.

Postsynaptic Responses of DMV neurons to NE

		Inward Current	Outward Current	No Response
Fundus	Control	46%	0%	53%
	Hyperglycemic	13%	40%*	47%
Corpus	Control	42%	10%	48%
	Hyperglycemic	33%	17%	40%
Antrum/Pylorus	Control	26%	21%	53%
	Hyperglycemic	8%	75%*	17%*
Total Gastric	Control	37%	12%	41%
	Hyperglycemic	18%*	44%*	38%

* p≤0.05 compared with control

W1192

Colonic Nociception via Nucleus Submedius is Modulated by Pontine Centers in the Rat

Eva Barkova, Geoffrey K. Turnbull, John W. Downie

The thalamic nucleus submedius (Sm) receives input from colon nociceptors via the spinothalamic tract in the rat. A pontine center, Barrington's nucleus, is now believed to play a role in colon as well as in bladder function. We hypothesized that the responses of Sm neurons to noxious colon stimuli may be modulated by Barrington's nucleus (perhaps via the locus coeruleus). To date, there are no published data on the possiblity. Experiments were carried out in 22 urethane-anesthetized male rats. Blood pressure was monitored through a carotid artery catheter and the activity of rectus abdominus muscle (EMG) was assessed via silver wire electrodes. Noxious stimuli were applied to the toes using previously standardized clips and to the colon by inflation of a 5 cm balloon to 80 mmHg for 30 sec using a barostat. The brain was exposed to allow recording from the nucleus submedius of the thalamus with a monopolar tungsten electrode. A glass pipette was inserted into Barrington's nucleus for injection of 5 mM $CoCl_2$, a temporary neural blocker. After each experiment, the site of $CoCl_2$ injection was confirmed by the presence of 1% FluoroGold which was incorporated into the $CoCl_2$ solution to permit this localization. Results: We recorded 51 units in Sm that were excited by contralateral noxious toe pinch, 4 were inhibited. Colon distension to 80 mmHg produced EMG activation and caused a vasodepressor response. Noxious colon distension also excited 27 units in Sm and inhibited 13 others. Injection of $CoCl_2$ into the Barrington's nucleus/locus coeruleus region ipsilateral to the Sm electrode blocked the response to colon distension in 10 of 12 Sm units tested. Blockade of the Sm unit response was not associated with block of EMG or blood pressure responses. Conclusions: These data point to previously unrecognized modulation of colon nociception in the Sm by Barrington's nucleus and /or locus coeruleus. The results also suggest either that Sm does not mediate the viscerosomatic and cardiovascular components of colon nociception or that the unblocked side is sufficient to sustain these responses.

W1193

Acute Physical and Psychological Stress and Its Influence on Autonomic Outflow to the Gut in Irritable Bowel Syndrome

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Background : Stress is likely to influence symptoms in the irritable bowel syndrome (IBS). We studied the effect of acute physical and psychological stressors on symptoms, visceral sensitivity and gut-specific autonomic tone in healthy volunteers and patients with IBS. Methods : 24 patients (20 women) with constipation-predominant IBS (Rome II criteria) and 12 healthy volunteers (8 women) underwent either a physical (cold-water hand immersion) or a psychological (dichotomous listening to music) stressor on two separate visits. Assessments included: perception of stress (visual analogue scale); psychological state (Hospital Anxiety and Depression); systemic autonomic tone (heart rate and blood pressure); gut-specific autonomic tone (laser Doppler flowmetry of rectal mucosal blood flow (RMBF)); and visceral sensitivity (anal and rectal electrosensitivity). Results : The IBS group reported higher levels of perceived stress at baseline ($p<0.01$). RMBF fell during physical stress by 29.6± 2.8% and 28.7± 3.9% (p=NS), and during psychological stress by 24.4± 2.1% and 23.5± 4.3% (p=NS) in IBS and control groups respectively. Rectal perception thresholds decreased during physical stress [23.2± 6% vs 0.63% (IBS vs control group, $p<0.05$)], as did rectal pain thresholds [27.0± 4% vs 1.3± 5% (IBS vs control group, $p<0.001$)]. During psychological stress, rectal perception thresholds decreased [19.4± 6% vs 8± 6% (IBS vs control group, $p<0.01$)], as did rectal pain thresholds [28.4± 4% vs 3.4± 3.8% (IBS vs control group, $p<0.001$)]. There was no correlation between changes in sensitivity and autonomic tone. Conclusions: Acute physical and psychological stress alters gut-specific autonomic tone in both normal volunteers and IBS patients, and is associated with an increase in visceral sensitivity in IBS only. Heightened sensitivity with similar autonomic tone suggests a mechanism for visceral sensation independent of autonomic nerves. The stress response was similar in both health and IBS groups, arguing against the notion of an imbalance of autonomic nerves in Irritable Bowel Syndrome.

W1194

Pancreatic Polypeptide (PP) Targets Selective Populations of Pancreatic-Projecting Brainstem Vagal Motoneurons

Kirsteen Browning, F. Holly Coleman, R. Alberto Travagli

We have shown recently that discrete subpopulations of vagal motoneurons project selectively to the pancreas. Studies conducted by other groups have reported that these pancreatic-projecting vagal motoneurons have cells with distinct morphological characteristics. Our recent studies have suggested that a discrete subpopulation of vagal motoneurons regulates pancreatic exocrine secretion and that these neurons can be identified using pancreatic polypeptide (PP), which inhibits pancreatic exocrine secretion selectively. This study was designed to investigate the actions of PP on identified pancreatic-projecting neurons of the DMV. Whole cell recordings were made from identified pancreatic-projecting neurons of the dorsal motor nucleus of the vagus. In control rats, PP (100nM) induced an outward current (44±15pA) in 10/74 neurons. PP-responsive neurons were found to have a higher input resistance (551±47MOhm compared to 447±22MOhm) and a smaller action potential afterhyperpolarization amplitude (16±1.7mV compared to 19±0.9mV). Perfusion with PP also inhibited the amplitude of evoked excitatory postsynaptic currents (EPSCs) in a concentration-dependent manner (27±4.5% inhibition to 100nM PP) in 15/21 neurons. Furthermore, in 6/10 neurons PP(100nM) inhibited the amplitude of the inhibitory postsynaptic currents (IPSCs) by 23±3.9%. Alteration of the paired pulse ratio suggested a presynaptic site of action on both EPSCs and IPSCs. Interestingly, the majority of the PP-responding neurons have a soma shape that can be defined as multipolar, while the majority of the non responding neurons have a soma shape that can be defined as bipolar. Our data indicate that the electrophysiological and morphological properties of vagal motoneurons that respond to PP differ from the properties of non-responsive neurons. These data suggest that vagal motoneurons controlling pancreatic exocrine functions comprise a distinct subpopulation. Supported by NSF 0235741.

W1195

Peripheral Urocortin 2 Suppresses Water Avoidance Stress and CRF-Induced Colonic Motor Stimulation in Rats

Million Mulugeta, Paul R. Saunders, Yvette Tache

Background: Water avoidance stress (WAS) or corticotroin releasing factor (CRF)-stimulate colonic motor function in rodents through activation of both central and peripheral CRF_1 receptors (Gastroenteroly 2000,119:1569, Brain Res 2001,893:29). CRF_1 receptors mediate stress or CRF-induced ACTH response whereas CRF_2 curtails the response (Nat.Genet 1998,19:162-;Peptides 2001,22:733-). Both CRF_1 and CRF_2 are expressed in colonic myenteric nervous system. Aim: Determine the role of peripheral CRF_2 in stress and CRF-induced colonic motor response by using selective CRF2 receptor agonists and antagonists in rats. Methods: Non fasted male SD rats were injected ip with either saline (0.3 ml) or a selective CRF2 agonist, urocortin 2 (ucn 2, 10, 20 μg/kg). Five minutes later, rats were placed in their home cage or submitted to a 60 min WAS. Colonic motor response as fecal pellet output/h was monitored. In a separate experiment fasted rats were pre-treated with either saline or Ucn 2 (10 μg/kg). Five minutes later rats were briefly anesthetized (isoflorane) and a 5 mm plastic bead was placed 4 cm past the anus. At the same time, CRF (3 μg/kg, 0.1 ml) was injected iv (tail vein). Rats were placed in their home cage and bead expulsion time scored (1-6, correspond to expulsion times of ≤15, ≤30, ≤60, ≤120, and ≤240 min, scores of 7-9 correspond to the un-expelled bead position in the colorectum. Groups of rats were pre-treated (10 min) with vehicle (0.3 ml sc) a selective CRF_2 antagonist, astressin2-B (100 μg/kg) to assess the WAS and CRF (3 μg/kg)-induced colonic motor response. Results: WAS increased the number of fecal pellet output to 3.5 ± 0.3/h compared with 0.2 ± 0.1 in control. Ucn 2 (10 μg/kg) blunted the WAS-induced response (0.8 ± 0.2 pellet/h) vs vehicles (3.5 ± 0.3/h) and astressin2-B blocked the Ucn 2 inhibitory effect. In saline injected rats, the bead expulsion time score was 5 ± 1. CRF (3 μg/kg, ip) significantly reduced the expulsion time to a score of 3 ± 1. The CRF-induced acceleration of transit was normalized with Ucn 2 (6 ± 1). Ucn 2 alone had no effect on colonic bead transit (5 ± 1 for saline vs 7 ± 1 for Ucn 2). Blockade of the CRF_2 receptor with astressin2-B inhibited the effect of Ucn2 against the CRF-induced colonic transit acceleration (6 ± 1 vs 3 ± 1). Conclusion: Systemic injection of the selective CRF_2 agonist Ucn 2, antagonizes both CRF and WA stress-induced colonic stimulation in rats. Activation of peripheral CRF_2 may mediate a stress coping mechanism in the colonic response to stress. Supported by DK 57238 (YT).

W1196

Abnormal Ghrelin and Pancreatic Polypeptide Responses in Gastroparesis

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Ghrelin has been suggested to function as an appetite-stimulating signal from the gut to the brain acting through a vagal afferent pathway. Pancreatic polypeptide (PP) is released from the pancreas after a meal, an effect related to the cephalic phase of digestion and presence of food in the stomach. The increase in PP in response to sham feeding is mediated through a vagal efferent pathway. Systemic ghrelin also rises in part to cephalic phase of vagal efferent stimulation. Vagal nerve dysfunction has been suggested to play a role in diabetic gastroparesis (GP), but has not been well investigated in idiopathic gastroparesis. Alterations in ghrelin and its effects on appetite and energy regulation could play a role in GP. AIM: 1) To investigate the presence of vagal nerve dysfunction in patients with idiopathic or diabetic GP. 2) To test the hypothesis that alterations in ghrelin concentrations occur in GP. METHODS: 13 normal subjects (34 ± 3 (SEM) yrs; BMI - 26 ± 2; 6M/7 F), 11 patients with idiopathic GP (38 ± 4 yrs; BMI - 26 ± 2; 3M/8F) and 10 patients with diabetic GP (48 ± 3 yrs; BMI - 31 ± 3; 0M/10F) underwent sham feeding, consisting of seeing, smelling, tasting, but not swallowing, a bacon and cheese toasted sandwich. Serial blood samples were obtained during fasting and every 5-min during 30 min of sham feeding. RIA was used to measure plasma ghrelin and PP. RESULT: See table. Sham feeding was characterized by a significant increase in PP and ghrelin in normal controls and patients with idiopathic GP. The change in PP and ghrelin levels in diabetic GP was significantly less than in normal subjects. SUMMARY & CONCLUSIONS: Systemic ghrelin concentrations increased significantly with sham feeding in normal subjects but not in diabetic GP. In contrast, normal ghrelin responses were present in idiopathic GP. Vagal nerve dysfunction, as evidenced by an impaired increase of PP with sham feeding, is present in diabetic GP but not idiopathic GP. Thus, increases in ghrelin which occurs in part through a vagal efferent pathway is impaired in diabetic GP and may play a role in the altered satiety seen in these patients.

Group	Fasting PP Level (pg/ml)	Increase in PP with Sham Feeding (pg/ml)	Fasting Ghrelin Level (pg/ml)	Increase in Ghrelin with Sham Feeding (pg/ml)
Normal Subjects	371 ± 118	294 ± 102 †	1359 ± 186	216 ± 29 †
Idiopathic GP	147 ± 60	413 ± 233	1467 ± 145	277 ± 104 †
Diabetic GP	117 ± 40	35 ± 11 • †	1173 ± 117	45 ± 37 •

Results expressed as mean ± SEM. Symbols: † = p < 0.05 vs. no change from fasting; • = p < 0.05 vs. normal subjects.

W1197

Altered Reflex Control of Gastric Motility in Mice with Experimentally-Induced Colitis

Michael Monroe, Nate Wallace, Craig Schneider, Pamela Hornby, Jeffrey Palmer

Vagal activation rapidly inhibits macrophage TNF release via cholinergic receptors, suggesting that the vagus nerve is a protective physiological mechanism for attenuating inflammatory responses (Nature 421:384, 2003). The vagus could also modulate upper gut motility in response to colonic inflammation via vago-vagal motor reflexes. To test this hypothesis, we assessed colonic distention-evoked gastric relaxation in control and TNBS-treated mice. Methods: Female C57BL6 mice were either untreated or received intracolonic TNBS (1.0-2.0 mg in 30% ethanol) to induce colonic inflammation. At 5-7 days they were urethane anesthetized and two non-compliant balloons were inserted into the proximal stomach and distal colon. Rigid cylinder piston-type miniaturized barostats (Distender Series IIR, G&J Electronics, Toronto, Canada) were used to record gastric volume and apply colonic distention under isobaric conditions. Data were displayed using PowerLab Chart software (ADInstruments, Colorado Springs, CO). A ramp low-pressure (0-30 mmHg) distention protocol did not significantly change gastric volume in untreated mice (maximal change 5.37 ± 4.96 μl) but significantly increased gastric volume in TNBS treated mice (maximal change 60.63 ± 25.26 μl). In TNBS-treated mice, pretreatment with 25 mg/kg ip L-NAME, a non-selective NOS inhibitor, prevented the increase in gastric volume (maximal change 18.75 ± 11.25 μl). Cervical vagotomy completely abolished changes in gastric volume in response to colonic distention in mice with colitis. Morphine sulfate pentahydrate, a potent anti-nociceptive m-opioid receptor agonist, did not attenuate the evoked change in gastric volume (maximal change: vehicle 108 ± 20 μl versus 1,10 and 30 mg/kg morphine 140 ± 18 μl, 94 ± 12 μl, and 60 ± 15 μl, respectively). These data demonstrate that inflammation in the distal colon alters reflex relaxation of the stomach via vagally-mediated, non-nociceptive pathways that utilize release of NO to evoke gastric relaxation. Decreased thresholds for vago-vagal reflexes that result in increased gastric volume may contribute to symptoms associated with IBD.

W1198

Involvement of Peripheral Adrenergic Receptors in the Intestinal Circulatory and Metabolic Responses Due to Stimulation of Central H3 Receptors

Rafal Obuchowicz, Michal Pawlik, Ryszard Sendur, Tomasz Brzozowski, Wieslaw Pawlik

Histamine is an biogenic amine that when released fulfills important role as neuromodulator via simulation of H3 subtype receptors endowed in the neural endings of both adrenergic, cholinergic and peptidergic system. The aim of the present study was to explore the role of the interaction of central H3 receptors with adrenergic neurons in the control of mesenteric vasculature and oxygen uptake in the intestine of the rat. Wistar rats were used. Five experimental groups were set. Mesenteric blood flow (MBF), microcirculatory blood flow (LDBF) and arterial pressure (AP) were measured. Intestinal oxygen uptake (VO2) was calculated from arterio-venous oxygen difference (AVO2) and MBF. Vascular conductance (C) was calculated as equation of MBF and AP. Intracerebroventricular (i.c.v.) administration of H3 receptor agonist (R-alpha-methyl histamine (RAMH) - 10 umol/kg i.c.v.) evoked significant increase of AP by 28 %, MBF was decreased by 12 %, C was decreased by 21 %, VO2 was decreased by 19 %. Central administration of H3 receptor agonist (RAMH) - 10 umol/kg i.c.v.) after application of H3 receptor blocker (Clobenpropit - 0,4 umol − kg i.v) caused no major changes in intestinal blood flow and oxygen uptake. I.C.V. administration of H3 receptor agonist (RAMH - 10 umol/kg i.c.v.) after pretreatment with 6-hydroxydopamine (6-OHDA - 50 mg/kg i.p.), evoked no significant changes in blood flow and oxygen uptake. I.C.V. administration of H3 receptor agonist (RAMH) - 10 umol/kg i.c.v.) after application of alpha2 receptor blocker guanabenz (20 ug/kg i.v.) evoked marked decrease of AP 27 %, significant increase of MBF, C and VO2: 33 %, 41 % and 23 % respectively. Central administration of H3 receptor agonist (RAMH) - 10 umol/kg i.c.v.) in the rats pretreated with beta2 receptors blocker (nadolol 2 mg/kg i.v.) evoked significant increase of AP by 36 %, significant decrease of MBF, C and VO2 by 26 %, 28 % and 20 % respectively. We conclude that exogenously stimulated central H3 receptor subtype exhibit profound stimulatory influence on centrally located sympathetic neurons. Observed activation of adrenergic system affects intestinal micro and macrocirculation and metabolic activity via stimulation of peripheral alpha2 and beta2 adrenergic receptors.

W1199

Leptin and CCK Interact Synergistically to Induce Gastric Relaxation: Mediation by Vagal Low-Affinity CCK-A and Leptin Receptors

Yuanxu Lu, Shi-Yi Zhou, Jen Yu Wei, Chung Owyang

Leptin is key in long-term control of feeding and energy balance. Recent studies show that leptin and CCK interact synergistically via vagal pathways to induce short-term inhibition of food intake. Though peripheral leptin does not affect gastric emptying, leptin may affect gastric motility via a synergistic interaction with CCK. To test this hypothesis, we recorded the intragastric pressure response to leptin in anesthetized rats using a balloon attached to a catheter in the gastric body. Leptin (0.05-2μg/kg, I.V.) did not affect gastric motility, whereas CCK (0.5μg/kg) inhibited intragastric pressure (-1.2 ± 0.2 cm H20). When gastric motility returned to baseline 15 min after CCK administration, leptin was infused and was observed to dose-dependently decrease gastric pressure (threshold 0.1 μg/kg, ED_{50} 1μg). This CCK-8 synergistic action lasted up to 45 min after its administration. Pretreatment with substance P did not affect the motility action of leptin, suggesting a stimulus-specific CCK and leptin interaction. Hexamethonium pretreatment abolished leptin-evoked gastric relaxation. Bilateral truncal vagotomy and perivagal capsaicin reduced the CCK relaxant effect by 60% and completely abolished the motility action of leptin after CCK treatment. To identify the CCK receptor affinity state we performed in vivo studies using CCK-OPE, a high-affinity CCK receptor agonist and low-affinity antagonist. CCK-OPE (10μ/kg) did not affect intragastric pressure, nor did CCK-OPE pretreatment enhance the motility action of leptin. CCK-OPE abolished the effect of CCK and leptin interaction on intragastric pressure, suggesting CCK acts on low- but not high-affinity CCK-A receptors to enhance leptin action on gastric motility. To characterize vagal efferent mediation of leptin action, we showed that L-NAME and a VIP antagonist reduced leptin's relaxant effect by 65 ± 7% and 45 ± 8%, respectively. L-NAME and VIP antagonist combined completely abolished the motility action of leptin after CCK treatment. We conclude that CCK pretreatment potentiates leptin's ability to evoke gastric relaxation. This synergistic interaction is mediated by vagal low-affinity CCK-A receptors using vagal afferent pathway, which in turn stimulates NO and VIP release from the gastric myenteric plexus to induce gastric relaxation. Contrary to previous reports, leptin likely mediates gastric relaxation postprandially when the plasma CCK level is elevated.

W1200

Role of Brain-Gut Axis in Healing of Gastric Ulcers

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Background and Aim: Previous studies demonstrated the pivotal role of capsaicin-sensitive peptidergic sensory neurons and vagal nerves in the maintenance of gastric mucosal integrity. The aim of the present study was 1) to examine the effect of the functional ablation of sensory neurons with neurotoxic dose of capsaicin and surgical vagotomy on the course of healing of gastric ulcer in rat, and 2) to compare the ulcer healing action of leptin in rats with or without inactivation of sensory neurons in rats. Material and Methods: Gastric ulcers were induced by acetic acid method in Wistar rats. Three series of experiments (A, B and C) were performed. In the first series (A), the course of ulcer healing was compared in rats with intact and capsaicin-inactivated sensory neurons. The ulcer area and gastric mucosal blood flow were determined by planimetry and H2 gas clearance method, respectively. In addition, gastric mucosal gene expression of proinflammatory cytokines (TNFα, IL1β), cyclooxygenases (COX-1, COX-2) and transforming growth factor alpha (TGFα) were determined by RT-PCR. For assessment of angiogenesis, the expression of platelet endothelial adhesion molecule-1 (PECAM-1) was analyzed by Western blot. In the series B, the ulcer healing was determined at day 8 and 16 after ulcer induction and accompanying changes in GBF in rats with or without vagotomy were recorded. In the series C exogenous leptin (10 μg/kg i.p.) was given to rats with intact or deactivated sensory neurons for 7 days after ulcer induction. Results: Ablation of sensory neurons delayed significantly ulcer healing, increased the gastric mucosal gene expression of proinflammatory cytokines (IL1β, TNFα), decreased the mRNA expression for TGFα and inhibited PECAM-1 expression in the ulcerated mucosa. The ablation of sensory neurons had no effect on the mRNA expression of COX-1 and COX-2. Vagotomy significantly delayed ulcer healing and led to a decrease in GBF at ulcer margin. Treatment with exogenous leptin accelerated ulcer healing and increased gene mucosal expression of iNOS mRNA and this effect was attenuated after the deactivation of sensory neurons. Conclusions: Vagal and sensory neurons contribute to the ulcer healing process in the stomach mainly due to the increase of gastric mucosal blood flow, stimulation of mucosal growth factors expression and release of nitric oxide due to the activation of iNOS. The ulcer healing action of some gastroprotective factors such as leptin is attenuated in rats with inactivated sensory neuron

W1201

Vago-Vagal Reflex Responses to iv Ghrelin and CCK in Rats: A Combined Ultrasonometry-Dual Nerve Recording Study

David W. Adelson, Hovsep Kosoyan, Eric Chun, Mulugeta Million, Yvette Tache

Date et al. have established the role of vagal afferent neurons in iv. ghrelin-induced increased food intake, and shown reciprocal effects of ghrelin and CCK on mass vagal afferent discharge (Gastroenterol. 123:1120-8, 2002). AIM: Elucidate specific patterns of vago-vagal response to iv. ghrelin and CCK-8 and compare subdiaphragmatic gastric vagal efferent (SGVE) and afferent unit (SGVA) activity with motility changes. METHODS: Antral (ANT), pyloric (PYL), and duodenal (DUO) motility was monitored via ultrasonometry crystals (Sonometrics Inc.) affixed to the serosa. Using a quadripolar recording electrode, multi-unit impulse activity was recorded from finely divided distally-cut (SVGE) or proximally-cut (SVGA) bundles of the SGV nerve, or from each simultaneously, in urethane-anesthetized male rats. Neuronal and motility responses to iv. ghrelin (10 μg/kg), CCK-8 (3 μg/kg), and vehicle were recorded. Motility data were acquired using SonoLab software and output to a Spike2 acquisition system (CED, Ltd); electrophysiological data were acquired using Spike2. Multi-unit neuronal activity was sorted to yield single- or distinct multi-unit records. Responses were quantified as % change in mean discharge for 20 min post-injection (or 5 min for SVGA after CCK) normalized to mean discharge 5 min pre-injection. RESULTS: In 5 rats, ANT, PYL, and DUO motility was recorded along with impulse activity from 5 SVGE bundles and 3 SVGA bundles. In 2 preparations, SVGE and SVGA bundles were recorded simultaneously. Ghrelin increased amplitude of pacemaker-driven activity in ANT and PYL, and caused large irregularly-timed coordinated phasic motion changes in ANT, PYL and DUO beginning 1-3 min post-ghrelin injection. In 4/4 single SVGA units inhibited by ghrelin (-32 ± 6.6%), CCK caused excitation of +120±37%. In 4/4 SVGA units excited by ghrelin (28±6.2%), CCK caused excitation of +68±34%. One SVGA unit was inhibited by both ghrelin and by CCK. CCK caused inhibition of SVGE discharge (-26±6.3%) in 9 distinct SVGE multi-units that were excited by ghrelin (48±22%). In 3 SVGE single units, however, CCK caused marked excitation (90±45%) while ghrelin caused mild inhibition (-30%±16%). CONCLUSIONS: Vago-vagal reflex responses to iv ghrelin or CCK occur in a common subpopulation of SVGA and SVGE fibers, with opposite directions of response for each peptide, respectively. In addition to this commonly activated pool of neurons, specific SVGE are potently excited by CCK-8 but not by ghrelin.

W1202

Different Pseudoaffective Cardio-autonomic and Colonic Response to Colorectal Distension in Flinders Sensitive Line and Flinders Resistant Line Rats

Takeshi Kamiya, Lu Wang, Yufang Wang, Yukang Mao, John Bienenstock, Gervais Tougas

Background: In patients with irritable bowel syndrome (IBS), autonomic function testing reveals vagal dysfunction and increased sympathetic tone in constipation predominant IBS, whereas diarrhea-prone patients have increased parasympathetic activity. Furthermore, autonomic function may alter visceral perception. Flinders Sensitive Line (FSL) and Resistant Line (FRL) rats are selectively bred on the basis of a high (FSL) or a low (FRL) responsiveness to cholinergic stimuli. The autonomic response to colorectal distension (CRD) has not been examined in FSL and FRL. Aims: 1) investigate the differences in cardio-autonomic responses to CRD between FSL and FRL. 2) examine the relationship of intracolonic pressure and volume. Methods: Under anesthesia, a balloon was placed in the distal colon and inflated to 30, 50 and 80mmHg. Heart rate was measured through ECG. Results: CRD induced a pressure-dependent bradycardia in FSL, while heart rate increased during CRD in FRL (*p<0.05, FSL vs. FRL). Intracolonic volume in FSL rats is greater than that in FRL rats at any given pressure (*p<0.05, FSL vs. FRL). Conclusions: While CRD induces an increased sympathetic response in FRL rats, in FSL the response to CRD is mainly vagal. Colonic compliance to CRD is greater in FSL rats in keeping with increased vagal input during distension while increased sympathetic activity during CRD in FRL rats results in a lesser compliant colon. FSL and FRL rats have different cardiac and colonic reflex responses to CRD and these differences are based on different autonomic reflex responses.

Table: Maximum Heart Rate Response to CRD And Intracolonic Pressure-Volume Relationship (mean±SEM, n=5-7)

CRD Pressure (mmHg)	HR During CRD (% of Resting HR)		Intracolonic Volume (ml)	
	FSL	FRL	FSL	FRL
30	98.1±1.1	101.3±0.6	2.4±0.1	1.8±0.2
50	93.9±1.6*	104.7±1.6	3.3±0.1*	2.3±0.3
80	89.5±2.0*	109.0±1.0	4.2±0.1*	3.2±0.3

W1203

Altered Colonic Barrier and Mitochondrial Function to CRH in Chronically-Stressed Rats

Maria Vicario, Javier Santos, Mar Guilarte, Carmen Alonso, Maria Antolin, Esteban Saperas, Juan R. Malagelada

Chronic stress induces marked alterations on epithelial barrier function along with morphological changes in mitochondria of rat colon. These abnormalities might help to understand irritable bowel syndrome (IBS) relapse by life stress. However, the contribution of superimposed stressors to IBS relapse remains largely unknown. We have investigated colonic epithelial and mitochondrial responses to superimposed stressors on a rat model of chronic stress that resembles IBS pathophysiology. WKY rats were crowded (8 rats/cage) or sham-crowded (2 rats/cage) for 15 days and sacrificed 1h or 1 week later. Six hours before sacrifice rats were injected ip. with CRH (5 μg) or saline. Distal colon was mounted in Ussing chambers to measure short-circuit current (Isc), an indicator of ion transport. Isc responses to serosal addition of carbachol (10^{-5}M) and forskolin (10^{-5}M) were also assessed. Transmural colonic segments were processed for western blot analysis of cytochrome-c-oxidase subunit I (COX-I), an indicator of mitochondrial function, and succinate dehydrogenase (SDH), an indicator of mitochondrial mass. Results: COX-I expression in distal colon was enhanced by CRH administration in sham-stressed and chronically-stressed rats. However, CRH-induced COX-I expression in stressed rats 1h was clearly superior to sham and stressed rats 1 week. SDH expression was not modified by CRH administration in either sham or stressed rats. Colonic Isc-increase to CRH was markedly reduced in stressed 1h compared to sham-stressed rats (Sham:87 ± 17 μA/cm²; stressed 1h: 43 ± 11; stressed 1 week: 72 ± 14; p<0.01). Isc-responses to forskolin and carbachol were also significantly reduced in stressed 1h compared to sham-stressed rats. However, these epithelial responses were mostly recovered by 1 week after stress cessation (Table). Conclusions: Despite mitochondrial ability to respond to new stressors seems preserved in the rat colon of chronically-stressed rats, epithelial barrier function is, although transiently, severely compromised. Altered response to incoming acute stimuli in a sustained chronic stress situation may help to understand the pathophysiology of IBS.

Colonic Isc responses (Δ vs basal, μA/cm²)

	Sham	CS 1h	CS 1week
Carbachol	68±20	33±15*	59±18
Forskolin	77±21	36±16*	64±17

CS, crowding stress; mean±SD; n=6 rats/group, 2-4 tissues/rat; * p<0.05 vs sham

W1204

CNS Administered OFQ/N Affects Colonic Propulsion in OFQ/N Ligand Knockout Mice but not in ORL-1 Receptor Knockout Mice

Robert Kraichely, Eric Gaumnitz, Paul Bass, Miles Epstein

Background: Orphanin FQ/Nociceptin (OFQ/N), an endogenous opioid-like peptide, has been previously demonstrated to inhibit colonic transit in a murine model through both central and peripheral sites. Its mechanism of action is incompletely understood. We investigated the activity of CNS administered OFQ/N on OFQ/N ligand knockout mice and in ORL-1 (OFQ receptor) knockout mice. Methods: We examined the latency for expulsion of a 3mm glass bead from the distal colon following intracerebroventricular injection of OFQ/N or vehicle (saline). We also examined contraction of isolated segments of colon in isolated tissue bath following administration of OFQ/N. Results: We observed a four to five-fold increase in bead expulsion latency with 5 nmol i.c.v. injection of OFQ/N in wild type mice compared to the same strain of mice injected with saline. In knockout mice homozygous for absence of the ORL-1 receptor receiving 5 nmol OFQ/N i.c.v., no increase in latency was noted compared to saline injection in wild type mice. OFQ ligand knockout mice displayed a trend towards increased latency compared to wild type mice following 5 nmol OFQ/N i.c.v. suggesting super-sensitivity. Additionally, isolated tissue bath with colon from ORL-1 knockout mice demonstrated no stimulation to a variety of doses of OFQ/N whereas wild type colon displayed dose-dependent stimulation. Conclusion: These data affirm the ORL-1 receptor as necessary for the observed OFQ/N effect on colonic propulsion and also support the OFQ/ORL-1 pathway as an independent modulator of colonic motility. Supported by NIH grant DK 57018.

W1205

Mast Cell Activation and Afferent Sensitivity in *Nippostrongylus Brasiliensis* (NB) Infected Rats

Kirk Hillsley, Bingxian Wang, Christine Booth, Ronald H. Stead

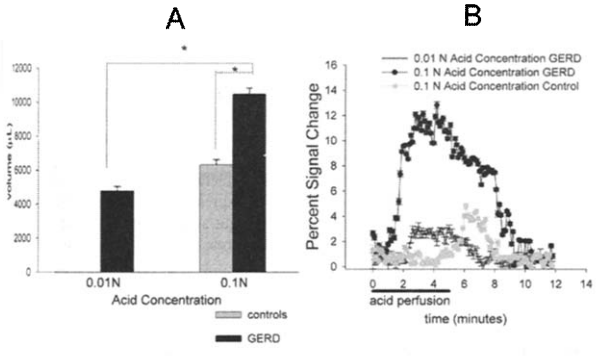
Background: Intestinal mucosal mast cells (IMMC) are intimately associated with mucosal nerves (including vagal axons) in rat jejunum; and sensitized mast cells can activate extrinsic afferents. The mucosal innervation undergoes extensive remodeling after Nb infection, raising the possibility that the afferent response to mast cell activation might change in the post-inflammatory state. Therefore, we employed 2-methyl-5HT (2m5HT; a selective activator of vagal afferents) and Nb antigen to investigate any changes in afferent sensitivity following Nb infection. Methods: Sprague-Dawley rats (sham and Nb infected 5 & 10 weeks (W) previously) were anaesthetized (sodium pentobarbital, 75 mg/kg). Extracellular recordings were made from mesenteric afferents using conventional techniques. 2m5HT (100μg/kg i.v.) was administered as a test of vagal innervation. Nb antigen (200μg/kg i.v.) was administered to determine if degranulation of mast cells activated mesenteric afferents. Jejunal sections from each animal were stained for IMMC using Alcian blue. Data are expressed as mean ± SEM. Results: Alcian blue staining demonstrated a 3 fold increase in the number of IMMC in infected vs sham animals at 10W ($p=0.0001$, $n=10$), confirming infection with Nb. 2m5HT evoked a brief intense activation of afferents in both sham and infected afferents. There was no significant difference in the magnitude of the peak 2m5HT-evoked response between the two populations at 5W (sham = 2.3 ± 0.4 fold increase, infected = 3.1 ± 0.7 fold increase, $p=0.42$, $n=6$) or 10W (sham = 4.1 ± 0.6 fold increase, infected = 5.6 ± 0.7 fold increase, $p=0.19$, $n=9$). Administration of Nb antigen to sham infected rats had no discernible effect on mesenteric afferent discharge. However, administration of Nb antigen caused a dramatic increase in nerve firing ($n=4/4$) in Nb infected rats. The Nb response was initiated following a latency of 46.7 ± 3.3 secs. The response peaked after 156.7 ± 23.3 secs with a mean increase of 28.7 ± 0.9 $imps^{-1}$ over baseline. The average duration of the afferent response to Nb antigen was >15 mins. Conclusions: Vagal afferent sensitivity to 2m5HT is not compromised after Nb infection. IMMC are increased and Nb antigen potently activates mesenteric afferents in infected animals, but not in sham animals. Additional studies are required to confirm that IMMC activation stimulates vagal afferents. This work was partially supported by a grant from Johnson & Johnson PRD, Belgium.

W1206

Correlation Between Acid Concentration and fMRI Activity During Heartburn in GERD Patients

Mark Kern, Candy Hofmann, Tanya Rittmann, Reza Shaker

Although cortical regions activated during heartburn have been defined previously, the influence of acid pH levels on cortical activity has not been fully studied. AIM: To determine and compare cortical fMRI activity associated with esophageal infusion of two concentration of acid (0.01N and 0.1N HCl) in GERD patients (GP) and healthy controls (HC). METHODS: Eight GP (ages: 21-50 yrs, 4 M) and 10 HC (ages: 19-50 yrs, 4 M) were studied during two fMRI scanning sequences (TR = 6000 msec, 250 repetitions) in which there were alternating 5-min intervals of acid and saline infusion (1 ml/min). RESULTS: While both 0.1 and 0.01 N HCl in GP induced changes in cortical fMRI activity with heartburn, only 0.1 N HCl resulted in changes in cortical activity in HC with no heartburn. Regions of cortical activity were similar between groups and involved the sensory/motor, insular, cingulate, prefrontal and parietal/occipital regions. Total fMRI activity volume was significantly different between HC and GP during 0.1N HCl infusion as well as between 0.1N and 0.01N infusion within the GERD group (Fig. A). In GP, the maximum percent fMRI signal increase during 0.1 N was significantly greater than that of 0.01 N HCl infusion. (Fig. B) fMRI signal activation latency in GP for 0.1N HCl infusion (56 ± 5s) was significantly shorter than that of HC (258 ± 6s) ($p<0.001$). Activation latency for 0.01N in GP (144 ± 12s) was significantly longer than that of 0.1N infusion (56 ± 6s) ($p<0.001$). CONCLUSIONS: In GERD patients: 1) Cortical fMRI signal change and activity volume in response to esophageal acid exposure is inversely related to infusate pH. 2) Latency period for fMRI signal activation associated with heartburn development is directly related to infusate pH.



W1207

Biofeedback-Assisted Relaxation Decreases Rectal Tone and Increases Pain Thresholds to Distention

Antonia Perello, Monica Perona, Emma Barthe, Cristina Puigdellivol, Agustin Balboa, Fermin Mearin

Visceral hypersensitivity is a key factor in the pathophysiology of gastrointestinal functional disorders. Patients with irritable bowel syndrome present lower pain thresholds during rectal distention. In these patients the modulation of visceral perception represents one of the main therapeutic goals. Aim: To assess the effect of one session of biofeedback-assisted relaxation in rectal tone, compliance and perception thresholds. Methods: Using an electronic barostat, rectal tone, compliance and perception thresholds were measured before and during the assisted systemic relaxation in twelve healthy volunteers (8 women). The relaxation level was continously evaluated through heart rate and electrodermal register. Results: All participants achieved progressive systemic relaxation demonstrated by the heart rate and electrodermal conductance decreases. During relaxation perception thresholds of urgency, discomfort and maximum toleration were significantly increased; rectal tone decreased whereas rectal compliance did not changed (Table). Conclusion: This is the first study to demonstrate that biofeedback-assisted relaxation is capable of decreasing rectal tone and visceral sensitivity in healthy volunteers. This technique should be evaluated in patients with irritable bowel syndrome.

	Pre-relaxation	During-relaxation	p
Heart rate (bpm)	79.2 ± 17.6	61.0 ± 6.6	< 0.005
Electrodermal (mho)	7.3 ± 3.3	3.1 ± 2.2	< 0.005
Compliance (ml/mmHg)	7.5 ± 2.7	7.8 ± 2.6	ns
Tone (ml)	60.0 ± 12.5	78.7 ± 19.3	0.02
1st perception (mmHg)	9.3 ± 4.9	13.0 ± 3.0	0.05
Sensation of urgency (mmHg)	23.3 ± 7.9	29.2 ± 7.7	0.02
Discomfort (mmHg)	25.0 ± 7.2	30.3 ± 7.3	0.03
Intolerable sensation (mmHg)	29.5 ± 6.3	35.6 ± 7.3	0.02

Mean±SD

W1208

Central Neuronal Activation and Mesenteric Afferent Sensitivity During Postoperative Ileus in the Mouse Jejunum

Mario H. Mueller, Dimitrios Kapitoglou, Joerg Glatzle, David Grundy, Martin Kreis

Introduction: Small bowel manipulation leads to a profound ileus and and an increase in spinal Fos expression suggesting prolonged activation of spinal afferent pathways. The extent to which vagal afferents may also be influenced has not been investigated. We aimed to examine intestinal afferent sensitivity and brainstem Fos expression in mice during postoperative ileus. Methods: C57BL/6 mice underwent laparotomy followed by small bowel manipulation to induce an ileus or left untouched as a sham-treatment group. The animals were killed 24h later. The brainstem was removed for Fos IR. 3 cm segments of jejunum were placed in Krebs buffer. Extracellular multiunit mesenteric afferent nerve discharge was recorded at baseline during distension and following serosal application of bradykinin (BK 1∝M). Whole nerve afferent discharge was quantified as peak increase above baseline. Fos IR was quantified in the nucleus of the solitary tract (nTS). Leucocyte infiltration into the intestinal muscularis was used an index of post-operative inflammation. Data are mean ± SEM and were compared by unpaired Students×t-test. Results: The number of leucocytes infiltrating the muscularis was elevated during ileus (39 ± 9vs1.8 ± 1/mm2 in sham controls; $P=0.008$). This was associated with an increase in the number of Fos-positive neurons in the nTS following surgery compared to sham controls (Bregma:-7.70mm, 30 ± 9vs6 ± 2; $P=0.01$, -7.32mm, 107 ± 26vs6 ± 2, $P=0.016$, $N=4$). Spontaneous discharge was higher in ileus animals compared to sham segments (17 ± 1 vs. 12 ± 2 imp/s, $P=0.02$, $N=6$), but not in the peak response to ramp distension (D at 60mmHg = 85 ± 6vs70 ± 4 imp/s, $P=0.07$). The response profile was different in ileus with a significantly increased response at low distending pressures (2-20 mmHg) compared to controls (18 ± 2imp/s vs 9 ± 2, $P<0.05$). The level of firing after BK adminstration was not significantly different during ileus (58 ± 8imp/s vs39 ± 9imp/s, $N=8$, n.s.). Conclusion: Small bowel manipulation leads to a markely increase in brainstem Fos expression indicating that vagal afferent pathways are activated during postoperative ileus. Mechanical manipulation leads to an augmented afferent sensitivity to low distending pressures. The increased sensitivity to low threshold distension might be due to local inflammatory processes, but is not reflected by changes in afferent sensitivity due to chemical stimulation suggesting there may be compensatory changes to limit inflammation-induced hypersensitivity.

W1209

Exploring the Temporal Characteristics of Excitability in the Human Brain Gut Axis Using Transcranial Magnetic Stimulation

Peter Paine, Shaheen Hamdy, Anthony Hobson, Satish Mistry, Elizabeth Gardener, Qasim Aziz

Background: Transcranial magnetic stimulation (TMS) allows the study of brain-gut pathways to the human pharynx and esophagus and has been used to explore mechanisms of swallowing recovery from dysphagia after stroke (1). TMS may also provide a neurophysiological method for predicting swallowing outcome in cerebral injury. However TMS measurement in the brain-gut axis poses unique challenges that needs more rigorous evaluation if it is to have clinical utility. The aim of this study was to determine the temporal reproducibility of TMS in evoking corticofugal electromyographic (EMG) responses in the human esophagus. Methods: EMG was recorded via an intraluminal catheter from the proximal striated esophageal muscle in response to TMS in 8 healthy subjects. For 6 different stimulus intensities (SI) (range 5% below motor threshold (MT) to 20% above), 20 consecutive TMS stimuli were delivered over a single scalp point at 5-second intervals across 3 time points, 40 minutes apart. The amplitudes for each EMG response were measured and the means

sequentially calculated for each SI and then log transformed. The repeatability coefficients (RC) for the 3 time points were then calculated for each SI for the 20 means and presented as an exponential ratio. Results: TMS was well tolerated and esophageal EMG responses were easily recorded from all subjects. At 5% below MT an optimal RC of 2 was achieved after 15 stimuli. A higher number of stimuli did not yield further improvement in repeatability. For all other SI this level of optimisation was achieved at between 5 and 10 stimuli. The largest reduction in RC was achieved over the first few stimuli for all SI. Conclusion: An optimal RC of 2 can be achieved for esophageal TMS if 15 stimuli are applied at 5% below MT and between 5 and 10 for all other SI above this. As the RC is an exponential ratio this implies that any change in magnitude over time elicited of less than double falls within the physiological limits of variability. Thus, given these parameters, TMS can be used reliably in future studies of patients with dysphagia after brain injury. Reference: (1) Hamdy S et al. Recovery of swallowing after dysphagic stroke relates to functional re-organisation in the intact motor cortex. Gastroenterology. 1998. 115 (5):1104-12

W1210

Dynamic Imaging of Cortical Activity During Temporally Distinct Stages of Esophageal Pain Processing

Anthony R. Hobson, Paul Furlong, Qasim Aziz

Background: Different components of the pain experience often occur in spatially coincident brain regions, but in different temporal time windows. Unlike metabolic brain imaging techniques, Magnetoencephalography (MEG) allows real-time imaging of the brain's neural activity. The objective of this study was to use MEG to dissociate the neural correlates of the temporally distinct stages of esophageal pain processing. Methods: 8 subjects (2 female, mean age 34yrs) were studied with 151-channel whole cortex MEG recorded in seven frequency bands (1-40Hz). 25 epochs lasting 20 seconds were recorded and each epoch consisted 4 x 5 second periods. Period 1 = rest, period 2 = anticipation, period 3 = Painful esophageal distensions (4 at 1Hz) and period 4 = post pain. Spatial analysis utilized SPM99 and temporal analysis utilized virtual electrode placement into regions of interest and time-frequency wavelet analysis. Results: Anticipation phase: Predominant activation was seen in the mid / posterior cingulate and supplementary motor / motor regions (1-15Hz). In addition, unilateral activation was seen in the operculainsular cortex (30-40Hz) for several seconds prior to the onset of the stimulus. Pain phase: Predominant activity was bilateral sensorimotor and operculainsular cortices (5-30Hz) and wavelet analysis revealed that this activity persisted for several seconds into the post pain period before diminishing. Subtraction of the pain and anticipation periods demonstrated pain-related activity in the perigenual and mid-anterior cingulate, which again persisted for several seconds following cessation of the stimulus. Conclusions: This data reveals that many of the spatially discrete brain regions within the visceral-cortical pain matrix are synchronously active during the different temporal stages of pain processing. However, subtle differences in the strength and timing of neuronal activity can be delineated with MEG. This reveals that regions of the cingulate cortex involved in cognitive processing are more active during the anticipation phase of the study than during pain itself. Additionally, cingulate regions that process the emotional aspects of pain are more active during the pain and post pain periods. Detailed study of this complex interaction throughout the cortex will be pivotal in unravelling the functional relevance of these structures in the generation and modulation of visceral pain processing.

W1211

Modulation of Endogenous Pain Inhibition Circuits During Visceral Stimulation

Steven M. Berman, Bruce Naliboff, Brandall Suyenobu, Lin Chang, Wilbert Gordon III, Jean Stains, Emeran A. Mayer

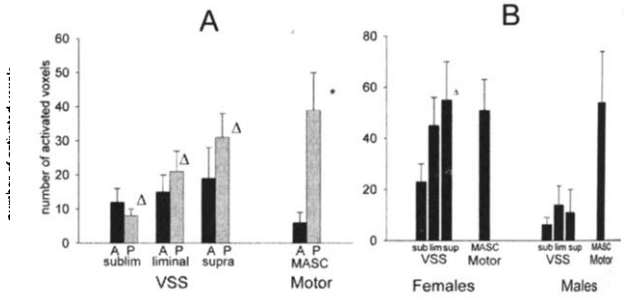
BACKGROUND: Brainstem periaqueductal grey (PAG), a region mediating integrated autonomic responses and pain inhibition, is organized into functionally distinct columns that receive ascending nociceptive input and descending excitatory and inhibitory influences from medial prefrontal/cingulate cortices and amygdala. Male IBS patients show reduced PAG activation during colorectal distension, suggesting inadequate pain inhibition and/or altered autonomic response. AIMS: Use functional MRI to evaluate activations and deactivations in the PAG and amygdala of IBS patients (IBS) and healthy control subjects (Ctrl) during sham and delivered rectal distension. METHODS: Study 1: 13 Ctrls (7 male) received 90 trials containing 15 sec of sham (5 mmHg), low (25 mm Hg), or moderate rectal distension (45 mm Hg). Data were normalized to a standardized brain image space and Statistical Parametric Mapping software assessed BOLD peak changes in amygdala and PAG regions of interest (ROIs). Study 2: 7 new Ctrls and 5 IBS-C (all female) were assessed, with a structural MRI acquired to allow analyses in native space. RESULTS: Study 1: Rectal pressure modeled as a linear increase from rest to low to moderate distention elicited activation in 58% of amygdala and 85% of PAG ROIs. Deactivation was seen in 69% of amygdala and 42% of PAG ROIs. Greater pressure increased the proportion of both activations and deactivations. Study 2 findings were similar to study 1. Average activation peaks in both right and left amygdala were in the basolateral (BL) nucleus, which channels sensory inputs to the basomedial nucleus (BM). Both deactivation peaks were in the BM, which receives input from BL and sends a major output to PAG. There were no group differences for amygdala responses or PAG activation. However, moderate pressure produced PAG deactivation in 90% of IBS vs 21% of Ctrl ROIs. CONCLUSIONS: 1) Rectal pressure reliably increased both activations and deactivations in amygdala and PAG subregions of Ctrls. 2) Similar PAG activations between IBS and Ctrls, together with the more prominent PAG deactivations seen in IBS are consistent with normal spinal nociceptive input to PAG (mediating activation) in combination with greater descending inhibitory input in IBS. 3) In view of similar activations and deactivations in amygdala subregions between the groups, the difference in descending input may originate from medial prefrontal/anterior cingulate cortices. Supported by NIH grants DK 48351 and P50 DK64539 (EAM), NR 04881 (BN), AR 46122 (LC), and Novartis

W1212

A Closer Look at Insular Cortex Activity During Hindgut Related Sensory/Motor Function

Arthi Sanjeevi, Adeyemi Lawal, Mark Kern, Candy Hofmann, Reza Shaker

Regional differential activation within the insular cortex in response to gut motor and sensory function has not been systematically studied. AIM: To map the gender specific, insular activities associated with rectal distension (viscerosensory stimuli, VSS) and volitional maximum anal sphincter contraction (MASC). METHODS: We studied 14 healthy volunteers (ages 18-35; 8 F). Insular activity was recorded during MASC and during barostat controlled rectal distension by a paradigm driven fMRI technique using fine slices (voxel size 2.5 x 2.5 x 3mm) to facilitate detailed mapping of insular functional anatomy. Measurements were made at perception threshold, 10 mmHg below (x2) and 10 mmHg above the perception threshold (non-painful). fMRI data was analyzed using autocorrelation techniques. RESULTS: Both anterior and posterior insula are recruited during VSS but only the posterior insular recruitment increased with increasing stimulus intensity ($P=0.028$). During MASC both males and females exhibited a larger volume of activity in the posterior compared to the anterior insula ($P=0.012$) (Fig.A). VSS in females was associated with a significantly larger volume of insular activity compared to males in the liminal and the supraliminal range ($P<0.05$). In females but not in males this recruitment increased with higher stimulus intensity ($P=0.009$) (Fig.B). CONCLUSIONS: In healthy individuals: 1) during hindgut related motor and sensory functions, posterior insula exhibits more robust activity than anterior insula. 2) Unlike anterior insula, recruitment of posterior insula is stimulus-intensity dependent. 3) Insular recruitment during intestinal visceral sensation is gender dependent.



W1213

Systemic Glucagon-like Peptide-2 Signals Satiety and Differentially Affects the Central and Enteric Nervous System

Laurie E. Wallace, Keith A. Sharkey, Mark G. Swain, David L. Sigalet

Glucagon like peptide (GLP-2) is an intestinal trophic factor. Central administration into the lateral ventricle of the brain results in increased Fos activity in the dorsomedial hypothalamic nucleus (DMH) and an inhibition of feeding behavior. However, physiologically GLP-2 is primarily produced from the distal intestine, and acts systemically. Aim: To examine the effects of systemic GLP-2 on the enteric nervous system (ENS), central nervous system (CNS) and related feeding activity. Methods: Fasted rats received an i.p. injection of GLP-2 (0.2 mg/kg) or saline (control) and were sacrificed 1 hour later. The presence of Fos immunoreactivity was assessed in the ENS of the small bowel and also in hypothalamus. In separate experiments, rats received either a GLP-2 or saline i.p. injection at the same dose and were placed in an activity chamber to determine if there was an effect on feed seeking behaviour, a centrally mediated effect. Results: Systemic GLP-2 increased ENS activity throughout the small bowel, as detected by Fos immunoreactivity; ileal myenteric plexus: GLP-2, $37.0+3.4$ cells/ganglia vs. control, $23.7+2.1$, $p<0.05$. Similar results were seen in the jejunal myenteric and jejunal and ileal submucosal plexuses. In contrast, CNS Fos labelling was reduced in the hypothalamic areas related to feeding: Paraventricular nucleus: GLP-2, $62.6+6.9$ cells vs. control, $85.2+3.2$, $p<0.05$ with similar results in the dorsal medial hypothalmus; no difference was seen in the arcuate nucleus. In the feeding study, intake was reduced at 2 hours (GLP-2, $2.04+0.53$ grams of food eaten vs. control $4.82+0.68$, $p<0.05$) as well as ambulatory activity with GLP-2 treatment (GLP-2, $978.3+233.8$ movements vs. control $2594.8+329.4$, $p<0.05$). By 16 hours, intake was not affected by GLP-2 (GLP-2, $19.98+0.69$ grams of food eaten vs. control $25.15+2.33$, $p>0.05$) yet activity continued to be reduced (GLP-2, $4237.0+1034.8$ movements vs. control $12652.0+2178.5$, $p<0.05$). Conclusion: Taken together, decreased Fos expression in the brain, reduced food intake and activity and stimulated Fos activity in the gut suggest that systemic GLP-2 has a differential affects on the CNS and ENS and plays a role in signalling satiety.

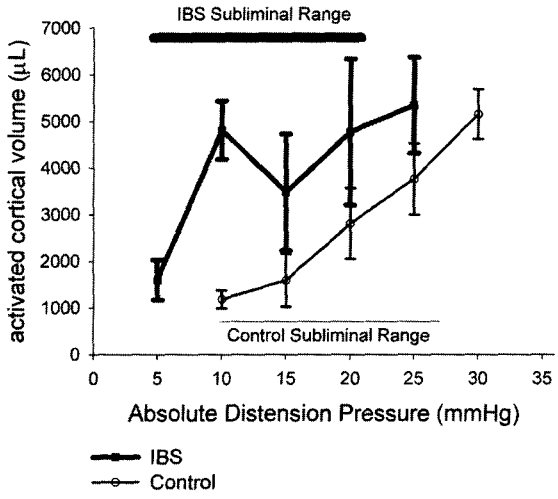
W1214

Characterization of the Subliminal Visceral Sensory Domain in IBS Patients

Mark Kern, Harjot Sidhu, Candy Hofmann, Reza Shaker

Functional magnetic resonance imaging (fMRI) cortical activity due to subliminal visceral stimulation has been shown in healthy subjects to extend over a range of rectal distension (RD) pressures, however, the definition and cortical response for the range of subliminal RD pressures in patients with IBS has not been examined. AIMS: 1) Define the range of subliminal RD pressures that elicit fMRI activity in a group of IBS patients. 2) Compare the magnitude and cortical topography of subliminal RD pressure range in IBS patients to controls. METHODS: We studied 9 IBS patients (Rome II criteria) (age: 20-42, 9 female) and 8 healthy control subjects (age: 21-37, 4 female). fMRI activity was recorded (x 2) over

the whole brain during barostat-controlled RD starting at 5mmHg and at incrementally increasing pressures (5mmHg increments). Pressure was increased to the perception threshold with an additional scan at 10 mmHg above the perception threshold. For each pressure, the brain was scanned for 120 s of alternating distention and rest (15s each). RESULTS: The lowest subliminal pressure that elicited a detectable fMRI signal 10 ± 1 mmHg (range 5-20mmHg) in the IBS patients was significantly lower ($p<0.05$) compared to controls (14 ± 2 mmHg, range 10-30 mmHg). (Fig) The average perception threshold pressure in IBS (21 ± 2 mmHg) was also significantly lower than that of controls (29 ± 3 mmHg, $p=0.039$). The regions of fMRI cortical activity were similar between the two groups involving the sensory/motor, parietal/occipital, cingulate gyrus, prefrontal and insular regions. CONCLUSIONS: The range of subliminal visceral sensory domain relative to perception threshold in IBS patient is similar to that of controls; however, it extends to significantly lower pressures.

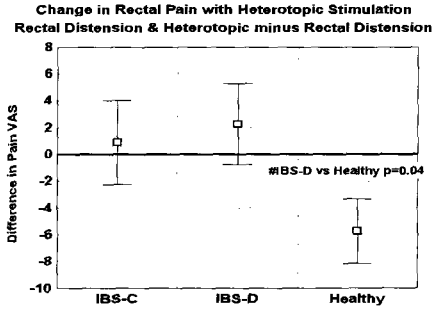


W1215

Abnormal Supraspinal Descending Modulation of Visceral Pain in IBS Patients with Constipation and Diarrhea

Clive H. Wilder-Smith, Daniel Schindler

Abnormal visceral sensation is observed in many patients with Irritable Bowel Syndrome (IBS). Amongst other abnormalities, changes in sensory thresholds and discrimination have been shown in IBS compared to healthy controls. In a previous functional MRI study focussing on supraspinal descending modulation of visceral pain distinct and widely divergent activation patterns were demonstrated for IBS patients with diarrhea (IBS-D), with constipation (IBS-C) and healthy controls (HC). The aim of this study was to further examine descending modulation of nociception in individual IBS subgroups using a well-validated heterotopic stimulation test. 20 HC, 20 IBS-D and 20 IBS-C (Rome 2, pain >30mm VAS during run-in), all females, mean age 41yr, were included. Individual pain thresholds were determined with a barostat and rectal bag using an ascending methods of limits (AML) distension protocol (5mmHg steps, 30s inflations/deflations, barostat cutoff 60mmHg). Subsequently, 120-second distensions at pain threshold pressure plus 10% were performed alone and during ice water immersion of the left foot (heterotopic stimulation of supraspinal descending inhibitory pathways; Willer et al., Bouhassira et al.). Rectal pain intensity was scored by VAS during rectal stimulations without and with heterotopic stimulation. Mean changes in rectal pain during heterotopic ice water stimulation are shown in the figure. The difference between IBS-D, but not IBS-C, and HC was significant ($p=0.04$ and $p=0.07$, resp.). The mean (95% CI) titrated rectal distension pressures used for stimulation were 43 mmHg (38-48) in IBS-D, 49 mmHg (43-55) in IBS-C and 52 mmHg (48-57) in HC (IBS-D lower than HC $p=0.03$). Activation of supraspinal descending modulation using a validated paradigm reduced rectal pain in healthy controls, but not in IBS-D or IBS-C. The demonstrated aberrant descending modulation of visceral pain may be an important mechanism in IBS. The center for modulation is extensively influenced by emotional, attentional and cognitive pathways and regulates central sensory input.



W1216

Effect of Acute Stress on Visceral Sensitivity After Previous Chronic Stressful Experience

Sylvie Bradesi, Ines Schwetz, James McRoberts, Gordon Ohning, Peter G. McLean, Wendy J. Winchester, Gareth A. Hicks, Emeran A. Mayer

We have previously shown that repeated exposure to water avoidance stress in rats leads to a sustained (30 days) enhancement of nociceptive response to colorectal distension (CRD) (Bradesi et al, 2003). Generalized stress sensitization after repeated exposure to the same stressor can also enhance the response to a subsequent heterotypic stressor (Stam et al, 1996). Aims: To evaluate whether an acute homotypic or heterotypic stressor can produce visceral hyperalgesia following a period of sustained chronic stress-induced visceral hyperalgesia. Method: Male Wistar rats were implanted with electrodes in the abdominal muscles, for electromyographic measurement in response to colorectal distension (CRD) (10, 20, 40, 60 mmHg; 20 s duration; 4 min inter-stimulus interval). Baseline visceromotor response (VMR) to CRD was recorded on day 0, then rats were subjected to 1hr water avoidance (WA) stress daily, for 10 consecutive days (day 1 to 10). VMR to CRD was then assessed on day 30 to verify the development of stress-induced visceral hyperalgesia (SIVH). 2 groups of rats were used. Between day 50 and 70 (after normalization of colonic sensitivity to CRD), rats were submitted once, either to 1h WA (homotypic) stress or 1h partial restraint (heterotypic) stress (PRS). Response to CRD was measured immediately after the acute WA or PRS and again 5 days later. Results: In rats previously submitted to repeated WA stress, the VMR to CRD measured on day 30 was enhanced by 71 ± 27 % compared with baseline (day 0). The increase was significant for the pressure of distension 40 and 60 mmHg ($p<0.05$). Between day 50 and 70, the VMR to CRD returned to baseline. Neither 1 hr WA nor 1hr PRS, applied at this time, restored the visceral hyperalgesia previously observed. In both groups, the VMR to CRD measured immediately after the acute stressors or 5 days later was similar to baseline. Conclusion: Chronic water avoidance stress leads to a long lasting but reversible visceral hyperalgesia. Exposure to a homotypic or heterotypic after normalizaton of the visceral nociceptive response failed to produce acute stress-induced visceral hyperalgesia and was unable to reactivate the sustained visceral sensitivity. Supported by GlaxoSmithKline (Neurology & GI Centre of Excellence for Drug Discovery), Harlow, UK and NIDDK grant 1 P50 DK 64539

W1217

Cerebral Response Patterns to Perception Threshold Adapted (PTA) Rectal Stimulation Reveal Alterations in the Emotional Processing of Visceral Sensory Input in Patients with Irritable Bowel Syndrome (IBS) Even at the Level of Subconscious Stimuli

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Purpose: One major factor in the genesis of symptoms in IBS is visceral hypersensitivity. The underlying mechanisms of irregular visceral perception are not yet clearly understood. Previous brain imaging studies indicated alterations in the central processing of mechanical visceral stimuli. However, it is still unknown whether these observations are due to altered peripheral (e.g., sensitisation at the level of sensory neurons or the spinal cord) or central (e.g., abnormal emotional processing of regular visceral sensations) mechanisms. The aim of this study is to further investigate perceptive and emotional processing of sensory input from the viscera, and to distinct their alterations in IBS by analysing cerebral response patterns to PTA rectal stimulation at levels of aware and subconscious intensities in IBS patients and controls, using functional magnetic resonance imaging (fMRI) of the brain. Methods: In 8 IBS patients and 8 controls the individual perception threshold (IPT) at rectal balloon distension (RBD) was determined beforehand. For fMRI measurements we used a block design of threshold adapted subliminal (IPT -10 mmHg) and supraliminal (IPT + 10 mmHg) stimuli alternating with off-periods. A gradient echo T2*-weighted sequence was used for the functional scans (16 slices). Statistical maps were constructed using the general linear model. Results: Both groups, though higher signal intensities were indicated in controls, showed significant ($p<0.001$) activations at supraliminal stimulation by RBD in the secondary somatosensory, the insular and the prefrontal cortex, and in the periaqueductal grey. However, activation of the cingulate cortex as part of the limbic system revealed distinct differences between groups: Only controls showed significant activation in the midcingulate cortex at supraliminal and in the anterior cingulate at subliminal stimulation. Conversely, patients responded to supraliminal RBD with a significant anterior cingulate deactivation. Conclusion: This fMRI-study indicates that in IBS cerebral discriminative perception patterns of PTA visceral stimuli correspond to controls. However, distinct differences in activation patterns of the cingulate cortex in IBS suggest alterations in the emotional processing of visceral sensory input even at the level of subconscious stimuli.

W1218

Effects of TNF on Neurons of the Rat Dorsal Motor Nucleus of the Vagus (DMV)

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Elevated circulating levels of the cytokine tumor necrosis factor-α (TNF) are associated with the immune response to antigenic challenge, chronic inflammatory disorders, radiation sickness, and carcinogenesis. TNF causes a dramatic, vagally mediated gastroinhibition. Our previous neurophysiological studies show that the TNF-induced gastroinhibition could be due to both withdrawal of vagal cholinergic excitation and activation of vagal NANC pathways. The aim of the present study was to investigate the effects of TNF on the membrane of identified gastric-projecting vagal motoneurones.

Whole cell patch clamp recordings were made from gastric-projecting DMV neurons in thin brainstem slices. Neurons were perfused with TNF (1-100pM) for 2 minutes. Slices were

pretreated with antagonists ($GABA_A$, bicuculline; α_1 adrenoceptor, prazosin; ionotropic glutamate, kynurenic acid; synaptic transmission, TTX) for 5-10 minutes before re-application of TNF in the presence of the antagonist.
Perfusion with 100pM TNF excited 46% of the neurons tested (278 ± 24% increase in firing rate, 8.5 ± 4mV depolarization, 45 ± 14pA inward current; N = 11), inhibited 37% of the DMV neurons (61 ± 19% decrease in firing rate, 5.6 ± 1.3mV hyperpolarization, 30 ± 2.2pA outward current; N = 9) and had no effect on the remaining 4 neurons. Both the inward and outward TNF-induced currents had a reversal potential close to -100mV, suggesting an action of TNF on potassium channels of the DMV membrane. The percentage of DMV neurones excited by TNF was not altered by pretreatment with kynurenic acid or TTX (12 of 25, i.e. 50%). The percentage of neurons inhibited by TNF, however, was reduced following TTX pretreatment (2 of 15, i.e.13%).
Our data suggest that the excitatory effects of TNF are due to both a direct effect on the DMV neuron as well as the release of an excitatory neurotransmitter other than an excitatory amino acid (EAA). The inhibitory effects of TNF might be due to both a direct effect on the DMV cell as well as to the release of an inhibitory neurotransmitter. Furthermore, it is unlikely that the inhibitory neurotransmitter is GABA but, rather, is more likely to be catecholaminergic.
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W1219

Effect of Central Corticotropin-Releasing Factor on Hepatic Circulation in Rats: A Role of CRF_2 Receptor in the Brain

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Backgrounds: Central neuropeptides play roles in physiologic and pathophysiologic regulations through autonomic nervous systems. Corticotropin-releasing factor (CRF) is distributed in the central nervous system and acts as a neurotransmitter to regulate gastric functions through vagal-muscarinic pathways. We have recently demonstrated that central CRF aggravates experimental acute liver injury in rats. In the present study, the central effect of CRF on hepatic circulation was investigated. Methods: Hepatic blood flow was determined by laser Doppler flowmetry in urethane-anesthetized rats. Portal pressure was simultaneously monitored. CRF (0.1-4 nmol), urocortine II (a specific CRF_2 rceptor agonist; 2.5-100 pmol), or saline vehicle was injected intracisternally, and changes in hepatic blood flow and portal pressure were observed for 120 min. We examined the effects of various pretreatments with atropine, 6-hydroxydopamine, hepatic plexus denervation, or hepatic branch vagotomy, respectively. Results: Intracisternal injection of CRF (0.2-4 nmol) caused a dose-dependent decrease in hepatic blood flow with a maximum response occurring 60 min postinjection, and the inhibition lasted up to 105 min. Portal pressure was dose-dependently elevated by intracisternal CRF (0.2-4 nmol) concurrently with the decrease in hepatic blood flow. These changes of hepatic circulation by intracisternal CRF were abolished by 6-hydroxydopamine and hepatic plexus denervation, but not by atropine or hepatic vagotomy. Urocortin II injected intracisternally decreased hepatic blood flow and elevated portal pressure at doses within the picomolar range (5-50 pmol). Conclusion: These data suggest that CRF acts in the brain to decrease hepatic blood flow and elevate portal pressure through central CRF_2 receptor and sympathetic-noradrenergic pathways.

W1220

A Selective CRF_2 Receptor Agonist, Urocortin 2, Blunts Colorectal Distention-Induced Activation of Rat Inferior Splanchnic Afferents *in Vitro*

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Background: Activation of peripheral CRF_2 receptor by Ucn 2 blocks repeated colorectal distention (CRD)-induced visceral hyperalgesia in conscious rats (Gastroenterology, 124:A-46, 2003). The mechanism and site of action of peripheral CRF_2 mediated visceral analgesia is not known. Aim: Determine whether CRF_2 receptor modulates CRD-induced stimulation of inferior splanchnic afferent activity (ISA) in an *in-vitro* preparation and detect CRF_2 receptor expression in the lumbo-sacral spinal dorsal root ganglia (DRG) in rats. Methods: Experiments were performed in rat *in-vitro* isolated colorectal-inferior splanchnic nerve preparation as previously described (Gastro.120:A328, 2000). Single unit activities were recorded from distal cut end of inferior splanchnic nerve strands. CRD was delivered by a mini latex balloon (diameter ~9mm when inflated with 0.4ml air). The response of ISAs to CRD (0.4 ml air for 20s) was studied. Vehicle (0.1ml saline), human Ucn 2 (1, 2 and 3 μg), the selective CRF_2 antatonist, astressin2-B (20 μg) and bradykinin (1 μg) were injected interarterially (ia). The ISA responses were quantified as (Q) representing the 100-s of net change of spike activity during and post treatment. In a separate group of rats, spinal L6-S1 DRGs were collected and CRF_2 expression assessed by RT-PCR. Results: Eight units were analyzed from 4 experiments. The response magnitude Q for sequential i.a injections of vehicle 0.1ml, Ucn 2 (1, 2 and 3 μg), astressin2-B (20 μg), Ucn 2 (2 μg) and bradykinin (1 μg) in the absence of CRD were 1.11 ± 0.13, 1.34 ± 0.23, 1.32 ± 0.13, 1.49 ± 0.14, 1.27 ± 0.07, 1.28 ± 0.07 and 6.36 ± 1.03, respectively. Only bradykinin's effect was significant. However, Ucn 2 (1, 2 and 3 μg) lowered, dose dependently, CRD-induced ISAs response to 62 ± 14, 54 ± 14 and 44 ± 10 ($P<0.05$) spikes/20-s respectively from 78 ± 15 in vehicle. The inhibitory effect of Ucn 2 at 3 μg/kg was prevented by astressin2-B. $CRF_2\beta$ gene expression was detected in the L6-S1 DRGs. Conclusion: Ucn 2 blunts CRD-induced firing of ISAs through activation of peripheral CRF receptors, presumably $CRF_2\beta$ receptors, which may contribute to the visceral analgesic effects of Ucn 2. Supported by NIH grants DK 3061 and P50 DK64539 (YT).

W1221

CRF but Not Corticosterone Is Involved in the Increased Gut Paracellular Permeability of Adult Rats Previously Submitted to Neonatal Maternal Deprivation

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Background/aims: Neonatal maternal deprivation has been shown to increase gut paracellular permeability and to alter the hypothalamo-pituitary-adrenal (HPA) axis function in adult rats. Links between these two physiological alterations have not been reported. The aim of the present study was to determine whether CRF and/or glucocorticoids were involved in the postponed increase in gut permeabilty observed in adult rats that were neonatally submitted to maternal deprivation. Methods: Male Wistar rat pups were separated from their dam 3h per day, from postnatal days 2 to 14, or left undisturbed. At 12 weeks of age, gut paracellular permeability was assessed by oral administration of ^{51}Cr-EDTA (1μCi/rat), and expressed as the percentage of radioactivity collected in the urine for 24 hours (mean ± SEM for each group). Rats were treated 12h before, just before (1h), and 12h after ^{51}Cr-EDTA gavage with either saline, the glucocorticoid receptor antagonist, mifepristone (4mg/kg, SC), or the non-selective CRF1/CRF2 receptor antagonist, α-helical CRF(9-41) (250μk/kg, IP). Plasma corticosterone concentrations were determined by HPLC in two other groups of rats. Results: Maternal deprivation increased plasma corticosterone levels from 89.5 ± 29.5 to 138.1 ± 16.7 ng/ml ($p<0.05$) and gut paracellular permeability from 2.7 ± 0.2 to 4.8 ± 0.5 % ($P<0.05$). In deprived rats, α-helical CRF(9-41) treatment suppressed the increase in gut paracellular permeability (2.1 ± 0.2 vs. 4.8 ± 0.5 %; $p<0.05$), while mifepristone had no effect (4.1 ± 0.2 vs. 4.8 ± 0.5 %; $p>0.05$). In undisturbed rats, mifepristone (2.7 ± 0.2 vs. 2.2 ± 0.3 %; $p>0.05$) and a-helical CRF(9-41) (2.7 ± 0.2 vs. 2.4 ± 0.2 %; $p>0.05$) had no effect on gut paracellular permeability. Conclusion: These results suggest that CRF is involved in the increase of gut paracellular permeability observed in adult rats that were previously submitted to neonatal stress. This effect appears not linked to the activation of the HPA axis and/or the subsequent increase in plasma corticosterone levels.

W1222

Effect of Neonatal Maternal Separation Stress (NMSS) on Pain Threshold and Neurochemical Responses of Colonic Mucosa and Spinal Cord to Nocioceptive Colonic Balloon Distension in Adult Rats

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Background: Childhood stress has been implicated as a contributing factor of irritable bowel syndrome (IBS). It has also been reported that neonatal maternal separation stress (NMSS) induces visceral hyperalgesia in rats. The effect of psychological stress on neurochemical change of brain-gut axis is unclear. Objective: To study the effect of NMSS on neurochemical response of colonic mucosa and spinal cord to nocioceptive stimuli in adult rats. Method: Male neonatal SD rats were randomly divided into 2 treatment groups on postnatal day 2-21: (1) Exposure to a 3-hour daily maternal separation (MS) or (2) Non-handling (NH) control group. When reaching adulthood (Day 60), these animals were subjected to stimulation by colonic balloon distension (CBD) at 6 cm from anus for assessment of abdominal withdrawal reflex (AWR). (Al-Chaer et al. Gastroenterol 2000;119:1276). Pain threshold was defined as balloon pressure that provoked a nocioceptive (grade 2) AWR response to graded CBD. Distal colon and lumbrosacral spinal cord (L6-S1) were harvested for expression of serotonin (5HT+) cells in colonic mucosa and Fos-like immunoreactive nuclei in the dorsal horn by immunohistochemistry before and after CBD, respectively. Results (Table): Before CBD, there was no difference in number of 5HT+ colonic cells and Fos-like immunoreactive nuclei at dorsal horn between MS and NH groups. After CBD, both 5HT+ colonic cells and Fos-like immunoreactive nuclei were significantly higher in MS as compared to NH group. Pain threshold was significantly lower in MS. Conclusions: NMSS results in changes in responsiveness of visceral nocioceptive neural pathway with exaggerated neurochemical responses and visceral hyperalgesia in adulthood. This observation supports the notion that childhood adversity may contribute to visceral hyperalgesia and development of IBS.

Comparison of 5HT+ cells and Fos-like nuclei between MS and NH groups before and after CBD

	5HT (no./10^6μm² mucosal area)		Fos (no./bilateral dorsal horn)		
	Pre-CBD	Post-CBD	Pre-CBD	Post-CBD	Pain threshold (mmHg)
MS (N=8)	288.1(26.8)	386.8(38.0)	16.8(8.6)	81.0(28.7)	20.0(8.4)
NH (N=8)	286.9 (87.4)	291.0(62.4)	15.7(9.9)	42.3(16.4)	26.9(8.3)
P value	0.97	<0.001	1.0	0.04	0.045

W1223

Phosphatidylinositide 3-Kinase Regulates Hormone-Induced Calcium Signals in Pancreatic Acinar Cells by Inhibition of Endoplasmatic Reticulum Ca^{2+}-ATPase

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Background & Aims: Calcium is a key mediator of hormone-induced digestive enzyme secretion in pancreatic acinar cells. Abnormal Ca^{2+} responses contribute to pathologic effects of high-dose cholecystokinin-8 (CCK) on exocrine pancreas. Phosphatidylinositide 3-kinase (PI3K) was demonstrated to regulate Ca^{2+} transport in some cell types. Furthermore, we recently showed that the gamma isoform of PI3K regulates the pathologic Ca^{2+} response and the Ca^{2+} sensitive trypsinogen activation in acinar cells. The present study sought to determine the mechanisms of PI3K involvement in CCK- and carbachol-induced Ca^{2+} responses in pancreatic acinar cells. Methods: $[Ca^{2+}]i$ was measured in Fura-2-AM-loaded rat pancreatic acinar cells either in suspension or in individual cells. In permeabilized cells

calcium changes were monitored with Fura-2 acid. Formation of inositol 1,4,5-trisphosphate (IP3) was measured by radioimmunoassay. Results: PI3K inhibitors, LY294002 and wortmannin, inhibited the magnitude of calcium mobilization (by 30%) and the Ca^{2+} influx (up to 50%) induced by both physiologic (1-100 pM) and supraphysiologic (1-100 nM) concentrations of CCK. Ca^{2+} mobilization induced by 0.1 mM carbachol was decreased by 25%. In individual cells PI3K inhibitors decreased by 34% the width of the $[Ca^{2+}]i$ peak induced by 100 nM CCK. Also, oscillations induced by 50 pM CCK subsided much more rapidly in the presence of LY294002. The inhibition of PI3K neither decreased the CCK-induced IP3 production nor affected the IP3-induced Ca^{2+} mobilization in permeabilized cells. PI3K inhibitors did not affect Ca^{2+} mobilization induced by thapsigargin, a specific inhibitor of endoplasmic reticulum Ca^{2+}-ATPase (SERCA). Moreover, blockade of SERCA abolished the inhibitory effects of LY294002 and Wortmannin on the hormone-induced Ca^{2+} mobilization and Ca^{2+} influx. This suggests that PI3K mediates the effects on hormone-induced calcium signals via inhibition of SERCA. Conclusion: The results indicate that in pancreatic acinar cells PI3K regulates Ca^{2+} mobilization and Ca^{2+} influx induced by CCK and carbachol by inhibiting SERCA. The inhibitory effect of PI3K on SERCA may be important for pathologic Ca^{2+} responses in exocrine pancreas.

W1224

A Mitotic PKA-Mediated Pathway Induces the Association of HP1γ to Chromosomal Stability Regulators in Pancreatic Cancer Cells

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The cAMP-dependent protein kinase A (PKA) signaling pathway has been associated with the maintenance of chromosomal stability, such as proper chromosomal segregation. Interestingly, not only has chromosomal instability been considered a hallmark of pancreatic cancer, but aberrant PKA signaling has also been detected in these tumors. In particular, inhibition of the PKA pathway has been shown to induce growth arrest and apoptosis in pancreatic cancer cell lines. Our laboratory has been interested in the relationship between chromatin dynamics and chromosomal instability in the development of pancreatic cancer. Recently, we have focused our studies on characterizing the function of the human homologs of HP1, a drosophila chromatin protein that plays a key role in chromosomal stability, transcriptional repression, and embryonic development. We have observed that pancreatic cancer cells express three HP1 proteins, HP1α, HP1β, HP1γ with an enrichment of the latter isoform. Therefore, we have investigated whether PKA-signaling is involved in selective posttranslational modification of individual HP1 isoforms. Indeed, we find that PKA exclusively phosphorylates the pancreas-enriched isoform, HP1γ, at residue serine 93 in pancreatic tumor cell lines. Immunofluorescence experiments demonstrate a selective association of phosphorylated HP1γ with mitotic chromosomes, supporting a role in the regulation of chromosomal function. In conjunction, western blot analyses of cells arrested at various stages of the cell cycle reveal that levels of PKA-phosphorylated HP1γ are highest during mitosis, while the total amount of HP1γ remains constant throughout the cell cycle. In vitro and in vivo studies determine that this PKA-phosphorylated form of HP1γ specifically interacts with Ku70, a protein that induces the enzymatic activity and DNA recruitment of Werner (WRN) protein. The Ku70-WRN complex functions in recombination, DNA repair, and chromosomal stability. Together, these results reveal the existence of a novel mechanism by which the function of Ku70 and WRN may be regulated by a metaphase-induced chromosomal form of HP1γ, suggesting a role for this protein complex in the maintenance of chromosomal stability. RU is supported by the Mayo Cancer Center, the Lustgarten Foundation and NIH Grants DK52913 and DK5662.

W1225

Stimulation of ATP Release from Dog Pancreatic Duct Epithelial Cells (PDEC) by Bile Acids

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Background: ATP may be released from an epithelial cell to act on the same cell (autocrine effect) or on neighboring cells (paracrine effect). We previously reported that ATP interacts with $P2Y_{11}$ and $P2Y_2$ receptors on dog PDEC to induce both cAMP and calcium signaling pathways and activate CFTR and calcium-activated K^+ and Cl^- channels (Am. J. Physiol. 275:G104-113 & 280: G795-804). We also observed that bile acids, such as taurodeoxycholic acid (TDCA) and taurochenodeoxycholic acid (TCDCA), at a concentration of 1 mM, modulated the secretory pathways of PDEC monolayers without evidence of cellular toxicity, as assessed by LDH release or transepithelial resistance (TER) (Am. J. Physiol. 283: G1042-1050). We now report that TDCA and TCDCA also stimulate ATP release from these PDEC. **Methods:** Polarized PDEC, derived from the main pancreatic duct of a dog, were cultured on Transwell inserts (Costar), using medium conditioned with gallbladder myofibroblasts. ATP release from confluent PDEC monolayers (TER: 0.8-2 $k\Omega.cm^2$) was assessed through ATP concentrations, measured using luciferin/luciferase bioluminescence, in either the luminal compartment, facing the apical membrane, or serosal compartment, facing the laterobasal membrane. **Results:** Baseline ATP concentration was higher in the luminal (vs. serosal) compartment (luminal: 54.1 +/- 11 ALU [mean +/- SEM, n = 18]; serosal: 3.21 +/- 1.1 ALU [n = 12]). Because bile acids interfered with the luciferin/luciferase reaction, only contralateral effects were assessed. Luminal addition of TDCA stimulated serosal ATP release in a concentration-dependent manner, with 300 μM, 1 mM, and 2 mM TDCA eliciting, respectively, ATP concentration increases of 9 +/- 6.4 -fold, 29 +/- 8 -fold (*), and 69 +/- 24 -fold (*) from baseline, 2 min following addition of TDCA (mean +/- SEM, n = 4, (*): $p < 0.05$ by one-tailed t-test). In contrast, serosal addition of up to 2 mM TDCA did not stimulate luminal ATP release. A similar response was observed with luminal TCDCA, but not tauroursodeoxycholate. **Conclusion:** In polarized PDEC, higher basal ATP release occurs across the apical membrane vs. laterobasal membrane. Luminal treatment with TDCA and TCDCA (300 μM - 2 mM) stimulated a fast serosal ATP release, but not vice-versa. This contralateral ATP release may signal a response to the potential noxious effect of certain bile acids; it is also likely mediated through intracellular signaling mechanisms, currently under investigation. *Supported by NIH RO1-DK55885 and VA Merit Review.*

W1226

Pancreatic Intraepithelial Neoplasia Displays Features of Gastrointestinal Metaplasia

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Pancreatic cancer (PC), the fourth leading cause of cancer-related death in western societies, is thought to develop through a series of non-invasive duct lesions termed Pancreatic Intraepithelial Neoplasia (PanIN). To further explore the underlying molecular changes associated with PanIN, we assessed global gene expression between microdissected samples of PanIN lesions and normal ductal epithelium. Amplified RNA (aRNA) was reverse transcribed to generate labeled cDNA, which was competitively hybridized to cDNA microarrays representing 15,000 transcripts. Fifty-five genes were identified to be differentially expressed between PanIN-1/2 lesions and normal ducts by a factor of >3 (35 upregulated and 20 downregulated in PanIN). A cluster of non-pancreatic gastrointestinal epithelial markers including pepsinogen C, MUC6, KLF4 and TFF1 were also upregulated in PanINs. These markers were validated using a combination of real time RT-PCR, in-situ hybridization and immunohistochemistry in human samples of microdissected normal epithelium, PanIN and pancreatic cancer cell lines. Semiquantitative RT-PCR for other markers of non-pancreatic gastrointestinal epithelium not represented on the microarrays showed additional upregulation of Gastrin, Hox A5, GATA4, GATA5, GATA6, Villin 1, Villin 2, Forkhead 6 and Hlx B9. These data demonstrate upregulation of a significant number of gastrointestinal markers in early PanIN lesions, and suggest that the development of early PanIN may occur through a process that involves gastrointestinal differentiation.

W1227

Association of PAR-2 SNPS with Familial and Sporadic Pancreatitis

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Background: Proteinase-activated receptors (PARs) represent a subset of the transmembrane G-protein-coupled receptor family. PARs have a unique mechanism of activation through proteolysis of their receptor by serine proteases. PAR-2 is expressed in the pancreatic duct cells and is activated by tryptase and trypsin. Activation of PAR-2 evokes intracellular calcium signaling and bicarbonate secretion in duct cells. This may provide an important protective mechanism against trypsin-associated pancreatitis. Aim: To determine whether genetic polymorphisms in PAR-2 gene are associated with chronic pancreatitis. Methods: 51 familial pancreatitis probands, who tested negative for cationic trypsinogen mutations, 104 sporadic chronic pancreatitis patients were studied plus 48 healthy controls. Genetic analysis was performed on DNA isolated from peripheral blood by polymerase chain reaction and subsequent direct DNA sequencing. Results: PAR-2 has 2 exons that encode the amino terminal exodomain and the transmembrane domain respectively. Three previously unreported single nucleotide polymorphisms (SNPs) were identified in Exon 1. SNP-1 was found in the 5 untranslated region at position -45 (-45C/T), which could potentially affect the polymerase processing. SNP-2 (4C/A) was synonymous. SNP-3 (63C/T) resulted in a serine to phenylalanine substitution. Statistical comparisons using Armitage trend tests revealed a significantly higher proportion of the T/C heterozygote at position -45 in healthy controls when compared with sporadic and familial pancreatitis subjects (54% vs. 31% vs. 32%; $p=0.004$). The familial pancreatitis subjects also had significantly higher prevalence of the T/T genotype than did control subjects (54% vs. 31%; $p=0.023$). SNP-2 and SNP-3 were uncommon without statistically significant differences between patients and controls. A synonymous SNP (621 C/T, I207I) was identified in Exon 2. Conclusions: 3 novel SNPs were identified in Exon 1 of PAR-2 gene. The T/C heterozygote at -45 is statistically more common in the control subjects. The high risk genotype in familial pancreatitis is T/T.

SNP at position -45

Genotypes	Control (n=48)	Sporadic (n=104)	Familial (n=51)
T/T	15 (31%)	52 (50%)	27 (54%)
T/C	26 (54%)	32 (31%)	16 (32%)
C/C	7 (15%)	20 (19%)	7 (14%)

W1228

Protease Activated Receptor-4 (PAR-4) Polymorphism in Familial and Sporadic Chronic Pancreatitis

Nevin Oruc, Georgios I. Papachristou, Janette Lamb, George Charlton, Adam Slivka, David C. Whitcomb

Protease activated receptors (PARs) are a family of G—protein coupled receptors that are activated by serine proteases through proteolysis and autostimulation by a tethered ligand. PAR4, a newly described family member, is highly expressed in pancreas and is activated by trypsin, thrombin, and the neutrophil protease cathepsin G. PARs utilize intracellular calcium signaling and cause bicarbonate secretion in duct cells. We hypothesize that the PARs are an important sensor for intrapancreatic trypsin activation and trigger duct cell secretion to flush activated digestive enzymes out of the pancreas as part of a defensive mechanism. Mutations in the PARs might eliminate this sensor, allowing prematurely activated trypsin to remain within the pancreas and lead to pancreatitis. Aim: To screen genetic polymorphisms in PAR4 gene locus and to determine if any polymorphism is linked to chronic pancreatitis. Methods: 52 Familial pancreatitis (FP) probands, family members of FP cases, 96 sporadic chronic pancreatitis patients (CP) and 48 healthy controls (HC) were screened for polymorphisms in the PAR-4 gene. DNA isolated from blood cells was amplified and genetic analysis of PAR4 gene was performed by subsequent direct DNA sequencing in the Genomics and Proteomics Core Laboratories of the University of Pittsburgh. Results: PAR4 has three exons, the first coding for the amino terminal exodomain and the second for the transmembrane domain. Three common single nucleotide polymorphisms (SNPs) were identified in exon1, intron1 and exon2 at —104T/C, IVS109+22G/A and 358G/A resulting in an A120T substitution in the second intramembrane portion of the receptor.

The IVS109+22AA genotype was significantly more common in CP patients than controls ($p<0.01$). FP patients also had higher frequency of AA genotype in SNP-2 but the difference did not reach statistical significance. Seven additional uncommon SNPs were also found in PAR4 gene IVS109+121C/T, 504C/T, 729G/A, 929C/T, 966C/T, 976C/G, 1051G/T). Conclusions: Ten SNPs were identified in the PAR4 gene locus. The PAR4 IVS109+22 AA genotype appears to be associated with the development of chronic pancreatitis in a subset of patients.

Genotype frequencies of SNPs in PAR4 gene locus

	SNP1			SNP2			SNP3		
	CC	CT	TT	GG	GA	AA	GG	GA	AA
FP	18(35%)	20(38%)	14(27%)	34(65%)	13(25%)	5(10%)	32(61%)	16(31%)	4(8%)
CP	41(43%)	39(40%)	16(17%)	54(56%)	29(30%)	13(14%)	20(57%)	9(26%)	6(17%)
HC	14(29%)	23(47%)	11(24%)	31(65%)	16(33%)	1(2%)	29(60%)	16(33%)	3(7%)

W1229

VAV-2 Recruits SRC-Rho-Phosphoinositide 3-Kinase Pathways During Cholecystokinin-Stimulated Pancreatic Acinar Exocytosis

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Background: Several lines of evidence have been accumulated that the Src family and phosphoinositide 3-kinase (PI3K) are involved in the signal transduction pathways in various cell types. We previously demonstrated that Src and phosphoinositide 3-kinase participate in signal transduction and pancreatic exocytosis. RhoA also plays an important role in mediating stimulus-secretion coupling and interacts with Src in acinar cells. A retroviral oncogene Vav-2 is a member of guanine nucleotide exchange factors for the Rho family. However, the precise mechanism has not completely been ascertained. Aim: We attempted to evaluate the role of Vav-2 in Src /RhoA/phosphoinositide 3-kinase pathways during CCK-stimulated pancreatic acini. Methods: Isolated acini were obtained from male Sprague-Dawley rats. Immunoprecipitation (IP) and Western immunoblotting (WB) were performed and intact acini were incubated with or without cholecystokinin-octapeptide (CCK). Protein expressions of Vav-2, Src, RhoA, and PI3-K were analyzed by WB and their interactions were examined by IP. Amylase secretion was also measured. Results: In WB study, protein expressions of Vav-2, Src, RhoA, and PI3-K were located at 95kDa, 60kDa, 21kDa, and 85kDa respectively; CCK (10pM and 10nM) enhanced their expressions 2-4 fold over basal after 20 min cell stimulation. Furthermore, the Src inhibitor, herbimycin A (3mM), Rho inhibitor, pravastatin (100mM), and PI3-K inhibitor, wortmannin (3mM) attenuated the intensities of RhoA and PI3-K bands, following stimulation with CCK. Pretreatment of herbimycin A, pravastatin, and wortmannin also inhibited amylase secretion in CCK-treated pancreatic acini without altering basal secretion. IP study showed that Src was co-immunoprecipitated with Vav-2, RhoA, and PI3-K in response to CCK stimulation, indicating that Src forms immunocomplex with Vav-2, RhoA, and PI3-K. RhoA was also co-immunoprecipitated with Src and Vav-2 following CCK stimulation, indicating that RhoA forms immunocomplexes with Vav-2 and Src. Conclusion: We conclude that Vav-2 recruits Src/RhoA/PI3-K signaling pathways during CCK-stimulated pancreatic exocytosis.

W1230

Vanilloid Receptor-1 Expressed in Pancreatic Islet Beta Cells Modifies Insulin Secretion in Rats

Yasutada Akiba, Shinichi Kato, Ken-Ichi Katsube, Masahiko Nakamura, Koji Takeuchi, Hiromasa Ishii

Capsaicin-sensitive afferents including vanilloid receptor-1 (VR-1) and calcitonin gene-related peptide (CGRP) participate in the physiological regulation of pancreatic endocrine and exocrine. However, the effect of capsaicin on insulin secretion is controversy. We have found that VR-1 is present in islet cells as well as in neurons in rat pancreas and that chronic ethanol treatment increases VR-1 expression in rat pancreas. The present study was undertaken to investigate the expression of VR-1 in rat pancreas and beta cell line (RIN) and examine the effect of capsaicin on insulin secretion. Expression of VR-1 in pancreas and RIN was examined by immunofluorescence double-labeled with CGRP, western blot or RT-PCR. To investigate the direct effect of capsaicin on islet beta cells, RIN was treated with capsaicin (10^{-11} to 10^{-6} M) with or without a VR-1 inhibitor, capsazepine (10^{-7} M) or EDTA (10^{-7} M) and the secreted insulin in the medium was measured with ELISA. Effect of capsaicin on plasma insulin level in rats was also examined by the administration of capsaicin (10 mg/kg, s.c.). VR-1-positive nerve fibers were recognized around the pancreatic ducts, vessels and islets colocalized with CGRP. VR-1-positive endocrine cells were recognized in islets. VR-1 was expressed in pancreas shown by western blot and also detected in RIN by RT-PCR. Capsaicin dose dependently increased insulin secretion at the range from 10^{-11} to 10^{-9} M, but higher dose at the range from 10^{-8} to 10^{-6} M had less effect. The increased insulin secretion by capsaicin was inhibited with capsazepine or EDTA. In vivo administration of capsaicin increased plasma insulin level 1 hr after the treatment. We demonstrated that VR-1 is functionally expressed in rat islet beta cells and that VR-1 is activated with capsaicin and acts as a calcium channel to release insulin. Modulation of insulin secretion with VR-1 may be related with the abnormal sensitivity of serum glucose in chronic pancreatitis and chronic ethanol treatment. This study also may account for the influences of capsaicin on the food intake and energy consumption as well as the pathophysiological regulation of pancreatic endocrine.

W1231

The Protein Tyrosine Phosphatase SHP2 in Pancreatic Acini: Activation by Growth Factors and Their Cellular Mechanisms of Activation

Jose A. Tapia, Robert T. Jensen

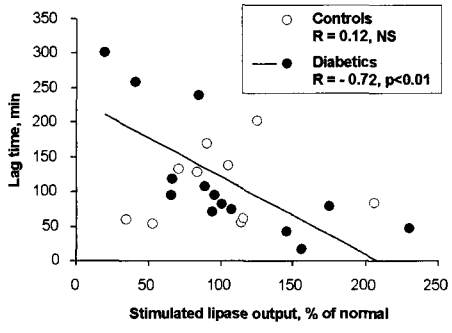
BKG: Tyrosine kinases (PTK) and phosphatases (PTP) play a central role in signalling cascades. Whereas PTK has been well studied in pancreas, little attention has been paid to PTP. The SH2 domain-containing PTP 2 (SHP2) is involved in the signaling of numerous stimuli, and with activation undergoes tyrosine phosphorylation (TYR-P). SHP2 plays a role in cell growth, differentiation, and death. A recent study suggests SHP2 is present in pancreas and may be stimulated by some growth factors (GF's). **AIM:** To determine whether SHP2 is present in rat pancreatic acini and study its regulation and activation. **METHODS:** Phosphotyrosine immunoprecipitates and lysates were analysed by Western blot (WB) or subjected to a phosphatase assay. Immunohistochemistry (IH) and RT-PCR were used to confirm the presence of SHP2 in acini. **RESULTS:** WB and IH showed SHP2 in acini mainly in the cytosol (i.e. 97%). SHP2 mRNA expression in acini was shown by RT-PCR. Some GF's (HGF, PDGF, EGF), but not others (bFGF, Insulin, VEGF, IGF), increased SHP2 TYR-P. TYR-P was maximal with HGF (1 nM), reaching 24+/-2-fold within 5 min., whereas PDGF caused a 10+/-4-fold and EGF a 1.4 fold-increase. CCK, carbachol, bombesin, secretin and VIP did not stimulate SHP2 TYR-P. Depletion of intracellular Ca^{2+} by thapsigargin, or adding GF109203X, a PKC inhibitor, or both, had no effect on HGF-/PDGF-stimulated TYR-P of SHP2. The Src kinases inhibitor, PP2, completely inhibited the SHP2 TYR-P by PDGF, however only slight inhibition (<5%) was seen with HGF. The inactive analogue, PP3, had no effect. SU6656, a more selective Src inhibitor, slightly inhibited (<5%) the SHP2 TYR-P stimulated by HGF and PDGF. PI3-K inhibitors (LY 294002, Wortmannin) had no effect on stimulated SHP2 TYR-P, while inhibiting HGF-/PDGF-stimulated Akt activation. Inhibitors of MEK or p38MAPK did not modify the HGF-/PDGF-stimulated SHP2 TYR-P. **CONCLUSIONS:** These data show SHP2 is present in rat pancreatic acini, mainly in cytosol, and some GF's, such as HGF and PDGF, cause its TYR-P and activation, however activation of GPCR's, such as CCK, does not activate SHP2. SHP2 stimulation is independent of PKC, PI3-K or MAPK activation, and Ca^{2+} changes, but is minimally modulated by Src kinases. Because studies show the importance of HGF in pancreatic growth, differentiation and response to injury, and the role of SHP2 in these processes in other cells, our study suggests its activation will also play a major role in HGF's actions in the pancreas. *JAT was supported by MECD.*

W1232

Gastric Emptying Disturbances Correlate with Pancreatic Exocrine Dysfunction in Patients with Long-Standing Diabetes Mellitus

Jutta Keller, Sebastian Brueckel, Axel Gresens, Christine Jahr, Christiane Fibbe, Peter Layer

Introduction: Gastric emptying disturbances in diabetes mellitus are caused by autonomic neuropathy. Apart from gastroparesis in the majority of affected patients and accelerated emptying in a subgroup, we and others have shown mild to moderate pancreatic exocrine insufficiency in diabetic patients, but pathomechanisms are unclear. Because intact pancreatic innervation is a prerequisite for enzyme response even to both endogenous and exogenous stimulation, it is conceivable that autonomic neuropathy contributes to diabetic exocrine dysfunction. Hypothesis: Decreased pancreatic exocrine secretion correlates with delayed gastric emptying in long-standing diabetes mellitus, suggesting a common neural mechanism. Methods: 10 healthy subjects and 14 patients with diabetes mellitus (10 type I, 4 type II, duration 15.3±4.0 yrs, HbA1C 8.4±0.3 %, BMI 25.6±1.0 kg/m2, mean±SE) received a standardized secretin-cerulein test (SCT) and a 13C-octanoic acid breath test (OABT) on two study days. Results: Outputs of all pancreatic enzymes in response to maximal exogenous stimulation were significantly decreased in diabetics compared with controls (mean±SE, U/min: trypsin: 310±36 vs 445±39, $p=0.016$, lipase 3137±456 vs 4632±600, $p=0.046$, amylase 394±42 vs 700±54, $p=0.00009$). Moreover, cumulative 13C-excretion in response to the OABT was significantly lower than in healthy controls (18.9±2.1 vs 25.8±2.1 % of dose over 4 h, $p=0.028$). Only in diabetics, exogenously stimulated enzyme output was negatively correlated with half time and lag time of gastric emptying (R-0.72, $p<0.01$, Fig.). Conclusions: In patients with diabetes mellitus, the degrees of pancreatic exocrine insufficiency and gastric emptying disturbances are tightly correlated, suggesting that autonomic neuropathy is involved as a common pathomechanism.



W1233

Effects of cAMP on Carbachol Stimulated Zymogen Activation, Secretion, and Cell Injury

Anamika M. Chaudhuri, Thomas R. Kolodecik, Fred S. Gorelick

Background: A hallmark of pancreatitis is premature activation and retention of pancreatic zymogens in the pancreatic acinar cell. We have shown that increasing intracellular cAMP enhances cerulein induced zymogen activation in the rat pancreatic acinar cell, but the effects of cAMP on acini treated with cholinergic stimulation are unknown. We hypothesized that agents which enhance acinar cell secretion might reduce injury. Aim: To examine the effects of increased intracellular cAMP on carbachol induced zymogen activation, secretion, and cell injury. Methods: Isolated acini were treated with physiologic and supraphysiologic doses of carbachol. Cellular cAMP levels were increased with either 100 nM secretin or the cell permeable cAMP analogue, 100 uM 8-Br-cAMP. Samples were analyzed using chymotrypsin, trypsin, amylase and LDH assays to measure zymogen activation, secretion, and cell injury, respectively. To detect secretion of activated enzymes into the media, cell and media samples were probed by immunoblot using a chymotrypsin antibody. Results: Secretin and 8-bromo-cAMP enhanced amylase secretion in response to physiologic carbachol from 17% to 25% and 32% respectively. Additionally, 8-Br-cAMP, but not secretin, enhanced secretion in response to supraphysiologic carbachol from 12% to 20%. Compared to carbachol alone, secretin caused a 1.3 - 1.5 fold increase in zymogen activation. However, a larger increase (2.5 - 5 fold) was observed with 8-bromo-cAMP. Secretin and 8-Br-cAMP both ameliorated physiologic carbachol induced cell injury, reducing LDH release nearly to control levels. 8-bromo-cAMP, but not secretin, reduced supraphysiologic carbachol induced LDH release from 7% to 4.5%. Immunoblot analysis revealed that adding 8-Br-cAMP to cells treated with supraphysiologic carbachol (unlike other treatments) caused a decrease in chymotrypsin found in the cells and an increase of chymotrypsin in the media. Conclusion: cAMP-stimulated pathways may reduce cellular injury by causing the secretion of active enzymes from the pancreatic acinar cell.

W1234

Mathematical Modeling of Bicarbonate Conductance-Disrupting CFTR Mutations in Pancreatic Duct Cells Predict Inhibition of Pancreatic Fluid Secretion

David C. Whitcomb, G. B. Ermentrout

Background. Human pancreatic duct cells secrete high concentrations of bicarbonate through a CFTR-dependent mechanism. CFTR is permeable to both chloride and bicarbonate, and the conductance of these anions are independently regulated. Recently, a number of mutations in the NBD1 domain of CFTR have been described that limit bicarbonate conductance through CFTR but not chloride. Aim. To use a physiologically-based, mathematical model of pancreatic duct cell secretion using experimentally derived parameters to model the effects of CFTR mutations altering bicarbonate and/or chloride secretion on pancreatic fluid secretion. Methods. A new mathematical model was developed simulating a duct cell within a proximal pancreatic duct and included a sodium-2-bicarbonate cotransporter (NBC) and sodium-potassium pump (NaK pump) on a chloride-impermeable basolateral membrane, CFTR on the luminal membrane with a baseline 0.2 to 1 bicarbonate to chloride permeability ratio. The relative permeability of chloride and bicarbonate were independently altered. The model was integrated over time using XPPAUT and the effect of pancreatic fluid secretion was calculated. Results. This model predicts robust bicarbonate secretion with opening of the CFTR, generates and maintains pancreatic fluid secretion with bicarbonate concentrations greater than 140 mM and returns to basal levels with CFTR closure. Secretion also stopped with inhibition of the NaK pump. Limiting CFTR permeability to bicarbonate, as seen in some CFTR mutations, markedly inhibited pancreatic bicarbonate and fluid secretion whereas limiting chloride had minimal effects on overall fluid secretion. Conclusions. A simple CFTR-dependent duct cell model can explain active, high volume, high concentration bicarbonate secretion in pancreatic juice that reproduces the experimental findings. CFTR mutations that predominantly effect bicarbonate permeability are predicted to markedly inhibit pancreatic fluid secretion. Epithelial cells in other organs that utilize CFTR for chloride secretion would not be effected by these mutations. Thus, some CFTR mutations may specifically target the pancreas.

W1235

A Novel Approach for Real-time Time Measurement of Pancreas Chloride Output *in vivo*: The Differential Effect of Cholecystokinin (CCK) and Secretin

Tamer Coskun, Joseph R. Reeve Jr., Gary M. Green, Marshall H. Montrose III

Chloride ion is an important component of pancreatic fluid and bicarbonate secretion. AIM: 1) To develop an in vivo technique for quantitatively and simultaneously measuring pancreatic chloride output 2) To investigate the effect of CCK or secretin on pancreas chloride output. METHODS: Fasted Sprague-Dawley male rats were anesthetized by Inactin. After trachea and jugular vein cannulations, 3 cannulas were placed to the common bile-pancreatic duct. The first cannula shunts the bile from liver. The second cannula is used to perfuse the common-bile duct with chloride-free solution containing chloride-sensitive fluorophore MQAE (145 mM Na Gluconate + 5 mM HEPES + 20 μM MQAE at pH 7.4). The third cannula is placed to the duodenal junction of the common bile duct to collect the pancreatic effluent. Duodenum is perfused with previously collected bile (50:50 Bile:Saline + 2 mg/ml trypsin). Pancreatic chloride output was determined by 1) 433 nm emission ratio measurements of MQAE fluorescence with the calibration for known chloride concentrations 2) chemical determination of chloride in pancreatic effluent with a chloridometer. Chloride measurements were collected online every 10 seconds. Total CO_2 content of effluent was detected by blood-gas analyzer. The chloride content of collected bile was measured by chloridometer. Secretin or CCK was administered iv at 500 pmol/kg/h. RESULTS: Basal bile chloride and tCO_2 contents (mM) were 91.18 ± 0.91 and 34.18 ± 0.65, respectively. Neither CCK or secretin modified bile chloride or tCO_2 concentration. CCK caused a significant increase in pancreatic effluent chloride and tCO_2 concentration (Δ increase: 3.33 ± 0.84 mM and 1.08 ± 0.29 mM, respectively) which returned to basal values in 90 min. However, secretin caused a sustained increase in pancreatic effluent chloride and tCO_2 concentration during the stimulation period (Δ increase: 4.74 ± 1.21 mM and 4.23 ± 1.05 mM, respectively). After pancreatic chloride output reached a plateau via secretin stimulation, co-infusion of CCK did not cause an additional increase in pancreatic chloride (from 7.39 ± 1.41 mM to 9.63 ± 1.67 mM) or tCO_2 (from 3.68 ± 0.67 mM to 3.85 ± 0.77 mM) output. CONCLUSIONS: 1) We developed a very sensitive, on line technique for measurement of chloride in vivo. 2) CCK and secretin show distinct stimulatory patterns on pancreas chloride secretion. 3) CCK or secretin acts on the same cell type in pancreas to stimulate chloride secretion, probably acinar cell.

W1236

Luminal Leptin Stimulates Pancreatic Enzyme Secretion in Rats

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Background : Leptin, secreted from adipocytes regulates food intake however this peptide could be also releaved from the stomack into the gastrointestinal tract. In spite of the presence of leptin receptors in the pancreas the role of leptin in the regulation of pancreatic exocrine function hasn't been elucidated yet. Aim : To determine the secretory effects of leptin given intraduodenally (i.d.) under basal conditions and following CCK-A receptor blokade. Rats with capsaicin deactivated afferent nerves (AN) have been used in the part of the study. Materials and methods: The secretory studies were carried out on anesthetized Wistar rats, weighing 300-350 g. The animals were surgically equipped with silicone catheters, one of them was inserted into pancreato-biliary duct, the other one- into duodenum. The experiment started 2 hours after surgery. Following i.d.. administration of leptin at doses of 0.01; 0.1; 1.0 or 10.0 μg/kg, the samples of pancreatic juice were collected in 15 minutes aliquots. To deactivate AN capsaicin was given to the rats at total dose of 100 mg/kg, 10 days before the test. Tarazepide, CCK-A receptor antagonist at dose 2,5 mg/kg was given i.p. to the rats, 15 min prior to the administration of leptin. The volume of pancreatic juice, and protein and amylase contents of each sample was measured. Results: Leptin given i.d. significantly and dose-dependently increased pancreatic protein and amylase secretions. Above effects were totally abolished by capsaicin deactivation of AN or pretreatment of the rats with tarazepide. Conclusion : Luminal leptin could be involved in the physiological stimulation of pancreatic enzyme secretion. Above pancreatic secretory effects of leptin depends on CCK release via duodeno-pancreatic reflexes.

W1237

Zymogen Activation in a Reconstituted Pancreatic Acinar System

Edwin C. Thrower, Fred S. Gorelick

Premature activation of zymogens within the pancreatic acinar cell is a pivotal early step in initiation of acute pancreatitis, but the mechanisms underlying this phenomenon are largely unknown. Cytosolic events, particularly an increase in intracellular Ca2+, regulate the activation of zymogens within intracellular compartments. To examine the mechanisms that mediate zymogen activation we have developed an in vitro assay using pancreatic acinar cell fractions. In initial studies, rat pancreas was homogenized, subjected to centrifugation to form a 100,000 x g membrane fraction, and cytosol. To detect active enzymes, membrane fractions were loaded with a membrane-permeable fluorogenic chymotrypsin substrate. Cytosol or buffer was added and chymotrypsin activity was assayed for 15 min. Finally, chymotrypsin activity was assayed in the presence and absence of either 5 mM ATP or 1 mM free Ca2+. We observed slight ATP-dependent chymotrypsinogen activation in the membrane fractions alone. However, the addition of cytosol resulted in ATP-dependent activation that was 3-fold greater than membranes without cytosol. To confirm the ATP dependence of zymogen activation, the non-hydrolyzable ATP analogue, AMP-PNP (5mM) was used in the place of ATP and did not increase chymotrypsin activity. The ATP stimulation was related to the concentration, a maximal effect being observed between 1 and 5 mM ATP. To further examine the organelles responsible for this activation, zymogen granule and microsome-enriched fractions were studied. Both fractions exhibited ATP and cytosol-dependent chymotrypsinogen activation. When these data were normalized to amylase content, chymotrypsin activity was up to 10-fold higher in microsomes, compared to zymogen granules. Finally, effects of Ca2+ on chymotrypsinogen activation were assayed. When 1 mM Ca2+ was included in the assay buffer, ATP-dependent chymotrypsin activation was enhanced by up to 30 % for zymogen granules and microsomes. Inclusion of 1 mM EGTA in the assay buffer reduced activation to that seen in the absence of Ca2+ and ATP. Based on this reconstituted pancreatic acinar system, zymogen activation is dependent on ATP and cytosol and can be enhanced by Ca2+. Further, zymogen granules and other cellular organelles appear to have the potential to support zymogen activation. This assay provides a novel technique to directly examine the mechanisms of zymogen activation.

W1238

Inhibition of c-Jun N-terminal Kinase Attenuated the Activation of Rat Pancreatic Stellate Cells

Kazuhiro Kikuta, Atsushi Masamune, Masahiro Satoh, Noriaki Suzuki, Tooru Shimosegawa

Background: In response to pancreatic injury or inflammation, pancreatic stellate cells (PSCs) are transformed ("activated") from their quiescent phenotype into highly proliferative myofibroblast-like cells which express the cytoskeletal protein α-smooth muscle actin, and produce type I collagen and other extracellular matrix components. Activated PSCs have been implicated to play important roles in pancreatic fibrosis and inflammation. The molecular mechanisms responsible for PSC activation remain to be elucidated, but the activation of signaling is likely to play a role. c-Jun N-terminal kinase (JNK) is a member of mitogen-activated kinase family, and activated in response to stresses and cytokines. The role of JNK in the activation of PSCs remains unknown. Aim: To clarify the role of JNK pathway in the

activation of PSCs by using SP600125, a novel inhibitor of JNK. Methods: PSCs were isolated from rat pancreas tissue and used in their culture-activated, myofibroblast-like phenotype unless otherwise stated. Activation of JNK was determined by Western blotting using anti-phosphospecific JNK and c-Jun antibodies. Activation of the transcription factors was examined by electrophoretic mobility shift assay. The effects of a selective JNK inhibitor, SP600125, on the monocyte chemoattractant protein (MCP)-1, collagen production, and proliferation were examined. The effect of SP600125 on the serum-induced activation of freshly isolated PSCs in culture on plastic was also examined. Results: Interleukin-1b activated both JNK1 and JNK2, whereas platelet-derived growth factor-BB activated only JNK1. SP600125 inhibited IL-1β-induced JNK activity and activator protein-1 activation, but did not affect the activation of extracellular-regulated kinase, p38 mitogen-activated protein kinase, and nuclear factor κB. SP600125 inhibited platelet-derived growth factor-induced proliferation of PSCs, and interleukin-1β-induced monocyte chemoattractant protein-1 expression. SP600125 inhibited serum-induced collagen production. In addition, SP600125 attenuated spontaneous activation of quiescent PSCs in culture on plastic. Conclusions: Inhibition of JNK pathway modulated profibrogenic and proinflammatory actions in PSCs, implying a potential application of JNK pathway inhibitors for the treatment of pancreatic fibrosis and inflammation.κ

W1239

Protease-activated Receptor-2 in Rat Pancreatic Stellate Cells

Atsushi Masamune, Kazuhiro Kikuta, Noriaki Suzuki, Masahiro Satoh, Tooru Shimosegawa

Background: In response to pancreatic injury or inflammation, pancreatic stellate cells (PSCs) are transformed ("activated") from their quiescent phenotype into highly proliferative myofibroblast-like cells which express the cytoskeletal protein α-smooth muscle actin, and produce type I collagen and other extracellular matrix components. Activated PSCs have been implicated to play important roles in pancreatic fibrosis and inflammation. Trypsin and tryptase, which are agonists for protease-activated receptor-2 (PAR-2), are involved in the pathogenesis of pancreatitis. Aim: To clarify the expression of PAR-2 and its role in PSCs. Methods: PSCs were isolated from rat pancreas tissue after perfusion with collagenase P. The expression of PAR-2 was examined by Western blotting and immunostaining. Trypsin, PAR-2 activating peptide (SLIGRL-NH_2, corresponding to the rat PAR-2 tethered ligand), and tryptase were tested for their ability to affect proliferation, monocyte chemoattractant protein-1, and collagen production in culture-activated PSCs. Activation of transcription factors was examined by electrophoretic mobility shift assay. Activation of mitogen-activated protein (MAP) kinases was assessed by Western blotting using anti-phosphospecific MAP kinase antibodies. The effect of PAR-2 agonists on the activation of freshly isolated PSCs in culture was also examined. Results: PAR-2 expression was observed in culture-activated PSCs whereas the expression was little in freshly isolated PSCs. PAR-2 agonists activated activator protein-1 and MAP kinases (extracellular-signal regulated kinase, c-Jun N-terminal kinase, p38 MAP kinase), but not nuclear factor κB. PAR-2 agonists induced proliferation of PSCs, through the activation of extracellular-regulated kinase. PAR-2 agonists increased type I collagen production, but not monocyte chemoattractant protein-1. PAR-2 agonists did not initiate the spontaneous activation of freshly isolated PSCs in culture on plastic. Conclusions: Our results suggest a role of PAR-2 in the development of pancreatic fibrosis through the increased proliferation and collagen production.

W1240

Microarray Profiling of Pancreatic Gene Expression in Ethanol Fed Rats: Down-Regulation of Protective Genes May Underlie the Sensitizing Effect of Alcohol to Pancreatitis

Ilya Gukovsky, Constanze Kubisch, Aurelia Lugea, Hidekazu Tsukamoto, Stephen J. Pandol, Craig D. Logsdon

Background & Aims: Mechanisms of alcoholic pancreatitis remain elusive. Alcohol abuse is believed to sensitize the pancreas to the inflammatory, cell-injury and fibrosing responses of pancreatitis. Recently, we developed rat models demonstrating such sensitizing effect of ethanol feeding to responses of acute and chronic pancreatitis. Here, using microarray analysis, we studied changes in pancreatic gene expression induced by ethanol feeding. *Methods:* Rats were pair-fed for 8 weeks control or Lieber-DeCarli ethanol diet. Pancreatic RNA was isolated and subjected to microarray analysis with Affymetrix RAE230A Gene-Chips. We also measured pancreatic gene expression in a model of alcohol-mediated pancreatitis (1) in rats that received cyclosporin A for the last 2 weeks of feeding and were subjected to 1 episode of cerulein pancreatitis 7 days before the end of feeding. These animals were killed 3 h (to study acute responses) or 2, 4, and 7 days (for "chronic" responses) after cerulein treatment. *Results:* Between control and ethanol fed rats, we observed 593 probe-sets with $p<0.01$, where 159 are expected by chance. We further restricted the analysis to genes with $p<0.01$ and fold-change>2, yielding 105 probe-sets affected by ethanol feeding alone (with 28.7 expected by permutation testing). Importantly, the profile of these changes is drastically different from those observed during initiation of pancreatitis (2). In particular, we did not find changes in gene expression of cytokines, chemokines and other inflammatory molecules that are greatly induced in acute pancreatitis. On the other hand, we found down-regulation of a number of genes the products of which are thought to play a protective role in pancreatitis. For example, the expression of metallothioneines type 1 and 2 was decreased about 5-fold by ethanol feeding. These data suggest that ethanol impairs pancreatic stress defenses. *Conclusions:* A comprehensive analysis of pancreatic gene expression indicates that ethanol feeding does not by itself induce changes associated with pancreatitis. One mechanism by which ethanol may sensitize the pancreas to pancreatitis responses is by predisposing the pancreas to oxidative stress. 1. I Gukovsky, A Lugea, J Cheng, B French, N Riley, S French, H Tsukamoto S Pandol. *Alcoholism Clinical and Experimental Research* 26:140A, 2002. 2. B Ji, X Chen, D Misek, R Kuick, S Hanash, S Ernst, R Najarian, C Logsdon. *Physiol Genomics* 14:59-72, 2003.

W1241

Activation of JAK-STAT Pathway by Platelet-derived Growth Factor-BB in Rat Pancreatic Stellate Cells

Atsushi Masamune, Kazuhiro Kikuta, Masahiro Satoh, Tooru Shimosegawa

Background: In response to pancreatic injury or inflammation, pancreatic stellate cells (PSCs) are transformed ("activated") from their quiescent phenotype into highly proliferative myofibroblast-like cells which express the cytoskeletal protein α-smooth muscle actin, and produce type I collagen and other extracellular matrix components. Activated PSCs have been implicated to play important roles in pancreatic fibrosis and inflammation. Platelet-derived growth factor (PDGF)-BB is a potent mitogen for PSCs. We have shown that activation of extracellular-regulated kinase (ERK) is required, but not sufficient for the PDGF-induced proliferation, suggesting that other signaling pathways might be involved. Janus kinase-signal transducers and activators of transcription (JAK-STAT) pathway has been shown to be involved in PDGF-induced proliferation in NIH3T3 fibroblasts, human airway smooth muscle cells, and rat vascular smooth muscle cells. Aim: To clarify the role of JAK-STAT pathway in PDGF-induced proliferation in PSCs. Methods: PSCs were isolated from rat pancreas tissue after perfusion with collagenase P, and used in their culture-activated, myofibroblast-like phenotype. Expression of PDGF β-receptor in activated PSCs was examined by immunostaining and by Western blotting. STAT-specific binding activity was assessed by electrophoretic mobility shift assay. Activation of Src, JAK2, STAT3, and extracellular signal-regulated kinase (ERK) was determined by Western blotting using anti-phosphospecific antibodies. Cell proliferation was assessed by measuring the incorporation of 5-bromo-2'-deoxyuridine. Results: Culture-activated PSCs expressed PDGF β-receptor. PDGF induced STAT-specific binding activity, and activated Src, JAK2, STAT3, and ERK. PDGF-induced activation of ERK was inhibited by a selective Src inhibitor PP1, but not by a JAK2 inhibitor AG490. PDGF-induced proliferation was inhibited by a selective Src inhibitor PP1 and by a JAK2 inhibitor AG490. Conclusions: PDGF activated Src-JAK2-STAT3 pathway. Activation of ERK was dependent on Src, but independent of JAK2-STAT3 pathway. Activation of JAK-STAT pathway, in addition to ERK, may play a role in the proliferation of PSCs.

W1242

Angiotensin II Is an Autocrine Stimulator of DNA Synthesis in Rat Pancreatic Stellate Cells

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Background and Aim: The present study was conducted to examine the effect of angiotensin II on proliferation of rat pancreatic stellate cells (PSCs) and to elucidate its intracellular signaling pathway. Methods: PSCs were prepared from rat pancreas using collagenase digestion and centrifugation with Nycodentz gradient. PSC proliferation was investigated with [^{3}H]-thymidine incorporation assay. Presence of angiotensin II receptors in PSCs was investigated with western blotting and immunocytochemistry. Presence and secretion of angiotensin II were examined with immunocytochemistry and ELISA, respectively. Activation of EGF receptor and extracellular signal regulated kinase (ERK) was examined with western blotting using anti-phospho-EGF receptor and anti-phospho-ERK antibodies, respectively. Results: Angiotensin II type 1 and type 2 receptors were expressed in PSCs. Angiotensin II was present in and secreted from PSCs. Angiotensin II enhanced DNA synthesis in PSCs in a dose-dependent manner, which was blocked by angiotensin II type1 receptor antagonist losartan. Angiotensin II transactivated EGF receptor, and activated ERK. Both EGF receptor kinase inhibitor AG1478 and MEK1 inhibitor PD98059 blocked angiotensin II enhancement of DNA synthesis and ERK activation in PSCs. Conclusion: Angiotensin II is an autocrine stimulator of PSC proliferation. EGF receptor transactivation-ERK activation pathway is involved in its intracellular signal transduction.

W1243

Pancreatic Stellate Cells Inhibit Spontaneous Lymphocyte Apoptosis by IL-15

Gisela Sparmann, Peter Brock, Robert Jaster, Stefan Liebe, Joerg Emmrich

Background and aims: There is growing evidence that pancreatic stellate cells (PSC) produce cytokines and take part in the regulation of inflammatory processes in the pancreas. IL-15 stimulates lymphocyte proliferation and inhibits apoptosis of various cell populations. The aim of this study was to investigate whether PSCs produce IL-15 and thereby can affect the proliferation and apoptosis of lymphocytes. Methods: Primary PSCs were isolated from rat pancreas using density gradient centrifugation. The mRNA expression of IL-15 was demonstrated by RT-PCR, the IL-15 protein was analysed by immunoblotting. Lymphocytes were obtained from rat mesenterial lymphnodes (LnLy) and co-cultured with *in vitro* activated PSCs. Proliferation was monitored using ^{3}H-thymidine incorporation. Apoptosis could be quantified by the binding of annexin V-FITC with flow cytometry (FACScan). Results: It has been shown that PSCs express two splice variants of IL-15. The protein could be detected in cell lysates but not in the cell culture supernatant. The co-cultivation of lymphocytes with PSCs inhibited significantly the spontaneous apoptosis of LnLys. This effect was reduced by an anti-IL-15 antibody and by co-cultivation in the transwell system preventing cell - cell contact between PSCs and LnLys. Recombinant IL-15 decreased the apoptosis rate of LnLys. There was a slight stimulation of lymphocyte proliferation by both co-cultivation with PSCs and the addition of IL-15. PSC - LnLy co-cultures induced *vice versa* the proliferation of PSCs. Conclusions: The results suggest that the inhibition of spontaneous lymphocyte apoptosis in co-cultures with PSCs was at least partially mediated by cell-bound IL-15. The reduction of lymphocyte apoptosis by activated PSCs and the stimulation of PSCs by LnLys on the other hand might be crucial pathogenic mechanisms resulting in the persistence of the inflammatory process and the development of fibrosis during chronic pancreatitis. Supported by BMBF grant 01ZZ0108

W1244

The Role of Long Acting Octreotide in Treating Patients with Advanced Hepatocellular Cancer (HCC). A Randomized Placebo-Controlled Trial

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The overexpression of somatostatin receptors (SSTR) in the liver of some patients with HCC is well documented. The aim of the study was to estimate if the administration of octreotide long acting formulation (LAR) improves survival and quality of life in patients with advanced HCC. 127 cirrhotic patients stages A-B due to HBV-HCV and advanced HCC were enrolled. Scintigraphy with 111Indium labeled octreotide was performed for determination of SSTR. The patients with intense uptake of radionuclear compound were randomized to receive placebo or octreotide as follows: octreotide 0.5 mg every 8 hours for 6 weeks. At the end of week 4 and every 4 weeks octreotide LAR. Patients' follow-up worked out monthly, including estimation of quality of life (QLQ-C30), AFP levels bimonthly and tumor size every 3 months. Patients with negative SSTR detection were followed in the same manner (control group). Scintigraphy demonstrated SSTR in 61 patients. Thirty were randomized to receive placebo and 31 octreotide (7 were excluded due to diarrhoea). A significantly higher median survival time ($p<0,001$) and a 74% lower hazard of death were observed in the octreotide group. The overall survival in weeks was 45 (28-72) for the octreotide, 27 (19-34) for the placebo and 27 (21-33) for the control group. During the first year of follow-up, a 21%, 39% and 43% decrease in the QLQ-C30 score was observed in each group respectively. The proposed therapeutic approach showed to improve the survival and quality of life in SSTR positive HCC patients.

Results

				Etiology			Morphology		
	M	F	Age	HBV	HCV	HBV/HCV	Massive	Multi nodular	Diffuse
Octreotide group	17	7	69±7	12	11	1	17	5	2
Placebo group	22	8	70±6	13	14	3	20	6	4
Control group	36	30	69±6	30	32	4	45	13	8

W1245

Radio-frequency Ablation for Patients with Hepatocellular Carcinoma: Comparison with Percutaneous Ethanol Injection

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PURPOSE: To compare the therapeutic effect of radio-frequency ablation (RFA) with that of percutaneous ethanol injection (PEI) for the treatment of hepatocellular carcinoma (HCC) in patients with cirrhosis or chronic hepatitis. METHODS: One hundred and thirty-three patients were enrolled from the Department of Gastroenterology and Hepatology, Okayama University Medical School between January 1995 and August 2003. Seventy (mean age, 68y.o.) and 63 (mean age, 65y.o.) patients received RFA and PEI, respectively. RFA was performed by using a 17-gauge internally cooled-tip electrode attached to a 480-kHz monopolar RF generator (Radionics Inc., Burlington, MA). The electrode and PEI needles were percutaneously inserted into a targeted HCC under real-time US guidance by using a 3.5-MHz convex probe (Prosound II SSD-6500; Aloka, Tokyo, Japan). PEI was performed twice a week using 21-gauge needle four to eight sessions for each tumors. The therapeutic effectiveness was assessed with spiral CT performed 1 week after treatment. In cases of residual viable lesion, additional treatment with RFA or PEI was scheduled. All survival probabilities were estimated by means of the Kaplan-Meier method. Differences between the RFA and PEI groups were analyzed by means of the Wilcoxon test. RESULTS: Mean tumor size was 20mm and 18mm ($p=0.03$) and the mean numbers of tumors were 1.5 and 1.6 ($p=0.22$) in RFA and PEI group, respectively. One- and 2-year local-recurrence rates were 0% and 10% in the RFA group and 19% and 25% in the PEI group, respectively ($p=0.017$). One- and 2-year rates of occurrence of new tumors were 23% and 59% in the RFA group and 30% and 53% in the PEI group, respectively ($p=0.78$). One- and 2-year overall recurrence rates were 23% and 64% in the RFA group and 38% and 59% in the PEI group, respectively ($p=0.45$). One- and 2-year survival rates were 96% and 92% in the RFA group and 98% and 92% in the PEI group, respectively ($p=0.84$). CONCLUSION: RFA is superior to PEI with respect to local recurrence of HCC.

W1246

Combined Tamoxifen and Retinoids Affect Intratumoral Apoptosis Rate in a Rat HCC Model

Marion Ganslmayer, Matthias Ocker, Gabi Kraemer, Eckhart Hahn, Detlef Schuppan, Christoph Herold

Aim: Our in vitro data on hepatoma (HCC) cells showed that a combination of Tamoxifen and 9cis retinoic acid (CRA) induced apoptosis in an overadditive manner. Therefore we tested the combination therapy in vivo. Methods: Morris hepatoma cells MH777A were cultured in vitro (10th -15th passage) and implanted into the liver of adult (250-300g) immuncompetent Buffalo rats (n/group = 12). Between the seventh and the 21th postoperative day the rats received TAM (3mg/kg BW i.p.) and CRA (30mg/kg BW i.p.) alone or in combination. After euthanasia tumor growth of treated and untreated rats was compared. Anti-tumoral efficacy was evaluated using a score including tumor growth and metastazation. Apoptosis was assessed on paraformaldehyd fixed sections using the TUNEL method. For confirmation a immunhistochemical staining for bax was enclosed Results: Untreated rats developed well vasculated tumors with central necrosis reaching a diameter of 45 17 mm after 3 weeks. Metastasis into lung, kidneys, spleen and peritoneum occurred timedependently. After 5-6 weeks untreated rats died or had to be killed due to ascites and severely reduced condition. Treatment with TAM and CRA reduced tumor growth and metastasis only moderately. The combination enhanced the anti- tumoral effects clearly, inducing tumor reduction of 72 % and reduced metastasis of 50 %. Treatment increased apoptosis rates compared with untreated controls Conclusion: 1)) TAM and CRA as single agents show modest effects on tumor growth and apoptosis 3) Combined therapy showed overadditive effects. 4) Similarly apoptosis rate increased after combination therapy 5) A combination therapy may be a promising treatment for hepatocellular carcinoma.

	controls	TAM	CRA	TAM/CRA
tumor growth (%)	100	9041	8944	3820*
TUNEL pos. cells	105	167	2510*	3716*
BAx-pos. cells	110	149	208*	3914*

* significant vs controls ($p<0,05$)

W1247

The Study of Risk Factors of HCC and Recurrent HCC

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BACKGROUND
Hepatocellular carcinoma (HCC) frequently is liable to recur even after curative therapy because of intrahapatic metastasis or multicentric occurrence. The aim of this study is to clarify the risk factors for HCC recurrence (initial recurrence, secondary recurrence).
PATIENTS AND METHODS
We retrospectively evaluated 840 patients with HCC (mean age of 68 years; 567 men and 273 women) who had received a curative therapy of surgical resection or local ablation therapy for HCC were enrolled. Recurrence of HCC was determined by dynamic CT or MR imaging or ultrasonography every 2-3 months. To identify risk factors for the first and second recurrence of HCC after the curative therapy, 5 variables concerning of tumor and 11 clinical, biochemical, and virological variables were analyzed by both uni- and multivariate analysis. As for the first recurrence of HCC, the risk factors for the recurrence within one year, two years and after passing for two years and more after the curative therapy were analyzed, respectively. Also, to identify the risk factors for the second recurrence, the variables at both the initial and second treatment were analyzed.
RESULTS
The mean size of tumors was 23.2 mm (range 9-56 mm). Of 840 patients, 192 (23%) received surgical resection, and 643 (77%) received local abration therapy. The rate of cumulative recurrence in these patients at 1, 2, 3-year are 35, 58, 71% respectively. The multivariate analysis indicated that AST level (>40IU/L) (risk ratio(RR)=1.77, (95%CI; 1.17-2.77), $p=0.01$) and tumor number (≥2) (RR=1.4, (95%CI; 1.1-2.01), $p=0.01$) are predisposing factors for HCC recurrence within one year; tumor number (≥2) (RR=1.72, (95%CI; 1.18-2.5), $p=0.05$) and AST level (>80IU/L) (RR=1.51, (95%CI; 1.05-2.16), $p=0.03$) within two years; platelet count (<100000/μl) (RR=1.89, (95%CI; 1.23-2.90), $p=0.004$) after passing for two years and more. At 1,2,3-year after the curative treatment of recurred HCC were 51, 82, 86% respectively. The AST level at the initial treatment (>40IU/L) (RR=3.446, (95%CI; 1.19-9.96), $p=0.02$) was identified as an important predisposing factor for the 2nd HCC recurrence.
CONCLUSIONS
Tumor number and AST level were associated with HCC recurrence within one year or within two years, while platelet count, reflecting underlying liver, was associated with that after two years. In addition AST level was associated with the 2nd HCC recurrence. From the results, decreased AST levels, which reflect reduced activity stage, may contribute to the reduced frequency of HCC recurrence and may lead to higher survival rate.

W1248

The Histone-Deacetylase Inhibitor SAHA Potentiates Pro-Apoptotic Effects of 5-Fluorouracil and Irinotecan in Hepatoma Cells

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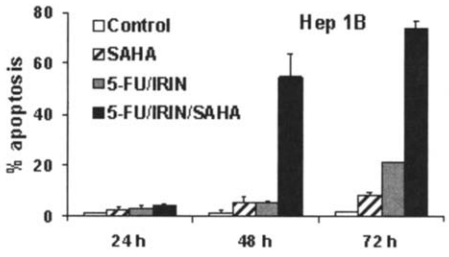
Aim: Treatment for advanced stages of hepatocellular carcinoma (HCC) remains unsatisfactory. While 5-fluorouracil (5-FU) and Irinotecan are first-line treatment options for other gastrointestinal tumors, the effect on HCCs is low. Previously, histone-deacetylase inhibitors (HDAC-I) like suberoylanilide hydroxamic acid (SAHA) showed anti-tumoral activities in a variety of human cancers in vitro and in vivo. Here, we investigated the effect of a combination of 5-FU, Irinotecan and SAHA on growth inhibition and apoptosis induction of HCC cell lines. Methods: HepG2, Hep1B and MH-7777A hepatoma cell lines and human foreskin fibroblasts as non-transformed controls were incubated with either 5-FU, Irinotecan and SAHA alone or on combination. Results: While single agents did not show any effects on growth of the investigated cell lines, combination of 5-FU and Irinotecan (both 10 mikroM) lead to a moderate increase in apoptosis and proliferation inhibition. Adding 1 mikroM SAHA increased apoptosis rates in hepatoma cell lines up to 92% after 72 h, while fibroblasts showed no response (5.5% apoptosis). Induction of apoptosis was paralleled by loss of the mitochondrial transmembrane potential, down-regulation of bcl-2 expression and activation of caspase 3 but not caspase 8. Conclusion: In summary, SAHA sensitizes HCC cell lines for treatment with an otherwise ineffective combination of 5-FU and Irinotecan and leads to mitochondrial apoptosis induction. The triple combination could lead to optimized treatment results in vivo and needs further evaluation.



W1249

Percutaneous Radiofrequency Ablation for Unresectable Liver Metastases of Colorectal Cancer

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Background; The liver is the most common site of metastases from colorectal cancer. At the present, surgery is supposed to be the only therapy that offers the possibility of cure for patients with hepatic metastatic diseases. However, only 25% of patients with colorectal liver metastases are candidates for liver resection, while the other patients are treated with chemotherapy or best supportive care. This study was conducted to clarify the efficacy and safety of percutaneous radiofrequency ablation for unresectable liver meatstases of colorectal cancer. Methods; From Feb. 2002 to Nov. 2003, twenty five patients with unresectable liver metastases from colorectal cancer were treated by percutaneous radiofrequency ablation. At the initial ablation, the mean number and size of tumor foci were 3.9+-2.5 (mean+-SD, range 1-12) and 39+-25.6 mm (mean+-SD, range; 18-138 mm). Patients were not considered for surgical metastatectomy due to extrahepatic metastases (15 patients), prior hepatic resection (5 patients), disease extent (more than 5 lesions, 6 patients), age (over 75 y.o., 6 patients), and/or comorbidity. Complications and survival after radiofrequency ablation were calculated. Survival rates were compared with those of the 15 patients who did not receive either surgical resection or radiofrequency ablation for metastatic liver tumor of colorectal cancer developing from 1999 to 2000. Results; No critical complications were observed in the patients treated by radiofrequency ablation. 12- and 18-month survival rates of the patients who received radiofrequency ablation for liver metastases were 83% and 67%, respectively, while those of the control were only 50% and 8%, respectively ($P=0.007$, log rank test). Conclusion; This study indicated that percutaneous radiofrequency ablation might safely prolong the prognosis of patients with unresectable liver metastases from colorectal cancer.

W1250

Survival and Recurrence Rates After Complete Radiofrequency Ablation for Hepatocellular Carcinoma; Value of Lens Culinaris Agglutinin-Reactive Alpha-fetoprotein (AFP-L3 Fraction)

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Background: Lens culinaris agglutinin-reactive alpha-fetoprotein (AFP-L3 fraction) is a newly developed tumor marker, highly specific for hepatocellular carcinoma (HCC). It is reported that the serum level of AFP-L3 fraction is correlated well with the biological malignant grade of HCC (J Gastroenterol Hepatol 2001; 16: 1378- 1383). The aim of this study is to evaluate the clinical value of the changes of AFP-L3 fraction after complete ablation of HCC by radiofrequency ablation (RFA) therapy, as compared with the changes of total AFP and des-gamma-carboxy prothrombin (DCP) levels. Materials and Methods: Among 396 patients with HCCs who underwent RFA, 197 patients with HCCs (initial treatment cases; $n=124$, recurrence cases; $n=73$) were evaluated as complete response with 5 mm-thick safety margin around the tumor. Three tumor markers (AFP, DCP, AFP-L3 fraction) were measured serially for an average of 650 days after RFA therapy in these patients, and its clinical significance was studied. All patients were classified into 3 groups. In group 1, the tumor marker was continuously positive before and after RFA therapy. In group 2, the tumor marker was positive before RFA therapy, but it changed to negative after RFA therapy. In group 3, the tumor marker was continuously negative before and after RFA therapy. We defined a 90 day evaluation period in which to observe changes in the tumor markers. Results: According to survival rates with both DCP and AFP-L3, the Kaplan-Meier curves showed significant differences. With regards to all tumor markers, significant differences were found between groups 1 and 3. However, only with AFP-L3 there was a significant difference between groups 1 and 2. With AFP and DCP there were no significant differences between groups 1 and 2. According to recurrence rates with only AFP-L3, the Kaplan-Meier curves showed a significant difference. With DCP, no significant difference was found between groups 1 and 3. Again, only with AFP-L3 there was a significant difference between groups 1 and 2. With AFP and DCP there were no significant differences between groups 1 and 2. The results of the multivariate analysis for both the survival and disease free rates showed that AFP-L3, stage and Child-Pugh had significant differences. Conclusion: AFP-L3 fraction level was indicated to be a better index than AFP or DCP in predicting survival and recurrence rates after complete RFA therapy.

W1251

Antitumor Effect of OK432-Contained Polyurethane Membrane As a Drug Delivery System on Adenocarcinoma in Mice

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Background/Aims: Recently we designed local and controlled release system of OK432 for the purpose of development of functional polyurethane membrane-covered metallic stent to improve long-term patency of stent that is applied to malignant bile duct obstruction. OK432 is low virulence strain, Su of Streptococcus pyogenes inactivated by penicillin G, and suggested to have anticancer effect as an immunopotentiator extensively used in Japan for adjuvant cancer therapy. One of its main mechanisms has been reported to induce apoptosis of cancer cells. We evaluate anticancer effect of controlled local delivery system of OK432 and its possible antitumor mechanism in mouse model of adenocarcinoma. Methods: We made an experimental model of cancer by injecting two different concentration ($8x10^4$, $1.5x10^6$ cell/100 μl) of cultured CT26 (a murine colon cancer cell line) into three six- to 10-week-old female BALB/c mice intradermally respectively. The OK432-containing or -noncontaining polyurethane membrane was inserted beneath preformed tumor mass at period of 5-7 day after cell injection. Then 18 day after cell injection, the tumor mass was harvested and measured its volume. We analyzed extent of the apoptosis of cancer cells in the extracted tumor tissues using TUNNEL staining. Results: In concentration of $8x10^4$ cell/100 μl, OK432 group (1,300 mm^3) was smaller than non-OK432 group (2,000 mm^3). In $1.5x10^6$ cell/100 μl, OK432 group (1,900 mm^3) lesser than non-OK432 group (3,500 mm^3). The tumor volume of $1.5x10^6$ cell concentration group was larger than of $8x10^4$ group. The TUNNEL staining showed a tendency to induce more apoptotic cells in OK432 group than in non-OK432 group, in both cell concentration groups. Conclusion: We documented the effectiveness of OK432 release system in inhibiting the tumor growth under animal model. This anticancer effect seemed to be by inducing the apoptosis of tumor cells mainly.

W1252

5-FU and IFN Alpha8 Combination Treatment Induce More Severe Apoptosis and Caspase Activities Than 5-FU and IFN Alpha2 Treatment in Hepatoma Cell Lines

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[Background] Interferon (IFN) is used for the treatment of patients with human carcinoma and viral hepatitis, and is now used for patients with hepatocellular carcinoma (HCC) in combination with 5FU with a favorite outcome. IFN alpha consists of more than 14 subtypes. IFN alpha 8 is the most effective subtype to induce apoptosis in several cancer cell lines. [Aim] To clarify the necessity for the IFN subtype selection in hepatocellular carcinoma treatment, we studied molecular mechanisms of the difference in apoptosis inducibility levels between IFN alpha subtypes (8 and 2) combined with 5-FU in hepatoma cell lines. [Methods] Five hepatoma cell lines (Hep3B,Huh7,HLE,PLC/PRL/5,HepG2) were tested for apoptosis inducibility by IFN alpha8, 2b and in combination with 5-FU. Western blotting was performed with antibodies specific to JAK/STAT, cyclin kinase inhibitor (p27Kip1,p21Cip1) molecules. ISRE activation was estimated by ISRE luciferase gene reporter assay. Caspase3,8 and 9 activities were analyzed by colorimetric protease activity assays using Ac-IETD-pNA as a caspase substrate. A cDNA microarray system that contains 718 cDNA of known IFN- and apoptosis-related genes (Japan Genome Solutions, Inc., Tokyo) was employed after 2 hrs and 24 hrs treatment with IFN alpha 8 and 2b on Hep3B. [Results] In Hep3B, apoptosis was induced more severely with IFN alpha8 than alpha2. Combination of 5-FU amplified this effect. Following experiments were analyzed with selected cell line Hep3B. The activation of JAK/STAT pathway, CDKI were same between the two IFN subtypes treatment. The cDNA microarray revealed that MAPKinase, EGF receptor and several chemokine receptors were differentially regulated between the two IFN subtypes at 2 hrs after treatment. The caspase 3,8 and 9 activities were higher with IFN alpha8 treatment than IFN alpha2 treatment when combined with 5-FU. [Conclusion] IFN alpha8 induced apoptosis more severely than IFN alpha2 even with combination of 5-FU. The vigorous activation of caspase may be associated with this effect.

W1253

Methylprednisolone Inhibits Low Flow Hypoxia-Induced Mitochondrial Dysfunction in Rat Liver

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The study investigated the mechanism by which methylprednisolone protects the liver from hypoxia-induced injury using male, fasted, pathogen-free Sprague-Dawley rats. Low flow hypoxia was produced by reducing afferent perfusate pressure from 10 to 2.5 cmH2O; isolated livers were portally perfused for 2 h. We measured mitochondrial membrane potential and hydrogen peroxide production by imaging rhodamine 123 and 2'-7' dichlorofluorescein fluorescence, respectively. Leakage of mitochondrial enzymes was also monitored by assaying mitochondrial aspartate aminotransferase activity in the outflow perfusate, and the radical scavenging effect of methylprednisolone was assessed by measuring luminol-dependent hydrogen peroxide chemiluminescence. Apoptosis in liver cells was determined using TdT-mediated dUTP-digoxigenin nick end-labeling. Rhodamine 123 fluorescence was significantly diminished in the hypoxic liver, especially in the region of the terminal hepatic venules, which is indicative of membrane depolarization in the mitochondria in those areas. Hypoxia-induced mitochondrial dysfunction was indicated by leakage of aspartate aminotransferase into the outflow perfusate, and increased 2'-7' dichlorofluorescein fluorescence indicated increased hydrogen peroxide levels, particularly in the midzone. Pretreatment with 30 mg/kg, 10 mg/kg or 3 mg/kg methylprednisolone inhibited the hypoxia-induced mitochondrial membrane depolarization and enzyme leakage, though hydrogen peroxide levels and apoptosis in sinusoidal endothelial cells were unaffected. In conclusion, methylprednisolone does not protect the liver from hypoxia-induced liver injury by suppressing hydrogen peroxide production. Instead, the beneficial effect of methylprednisolone appears to be related to its ability to protect against mitochondrial membrane depolarization under hypoxic conditions.

W1254

Pitavastatin Ameliorates Severe Hepatic Steatosis in Aromatase-deficient (Ar-/-) Mice

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BACKGROUND&AIMS: Tamoxifen is a potent antagonist of estrogen, and hepatic steatosis is a frequent complication in adjuvant tamoxifen for breast cancer. Impaired hepatic FA beta-oxidation in peroxisomes, microsomes, and mitochondria results in progression of massive hepatic steatosis in estrogen deficiency. This impairment, although latent, is potentially serious: About 3% of the general population in the United States is now suffering from nonalcoholic steatohepatitis associated with obesity and hyperlipidemia. Therefore, in the present study we tried to restore impaired hepatic FA beta-oxidation by administering a novel statin, pitavastatin, to aromatase-deficient (Ar-/-) mice defective in intrinsic estrogen synthesis. METHODS: Ar-/- mice aged 16 weeks were fed for 8 weeks with or without pitavastatin. The hematoxylin&eosin staining of the liver tissues and Northern blotting for the mRNA analysis from the liver were performed by the standard methods. The measurement of FA beta-oxidation activity and FA analysis were performed with freshly isolated liver. RESULTS: Light microscopy observation shows that numerous fat droplets regressed to few fat droplets in Ar-/- mice when it was treated with pitavastatin. Northern blot analysis of Ar-/- mice liver revealed a significant restoration of mRNA expression of essential enzymes involved in FA beta-oxidation such as very long fatty acyl-CoA synthetase in peroxisome, peroxisomal fatty acyl-CoA oxidase, and medium-chain acyl-CoA dehydrogenase. Severe hepatic steatosis observed in Ar-/- mice substantially regressed. Consistent findings were obtained in the in vitro assays of FA beta-oxidation activity. CONCLUSION: These findings demonstrate that pitavastatin is capable of restoring impaired FA beta-oxidation in vivo via the peroxisome proliferator-activated receptor-alpha-mediated signaling pathway and is potent enough to ameliorate severe hepatic steatosis in mice deficient in intrinsic estrogen.

W1255

Hepatic Hemangiomas: Possible Association with Female Sex Hormones

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Background: Hemangiomas are the most common benign tumors of the liver, often discovered incidentally. Although a relationship between estrogens and liver tumors has been reported, the association of hepatic hemangiomas with female sex hormones is not entirely clear. Aims: To prospectively evaluate the impact of female sex hormones on the natural history of liver hemangiomas. Methods: We followed 94 women with 181 hemangiomas diagnosed by ultrasound for a period of 1-17 years (mean 7.3 years). All patients underwent imaging follow-up every three months in the first year and annually after that. The location, number, size, and ultrasonographic pattern of the lesions were evaluated. Patients were evaluated by questionnaire for gynecologic and reproductive history. We also compared the change in number and size of hemangiomas in patients who received or did not receive exogenous hormonal treatment. Results: Age at first period was inversely associated with the size of hemangiomas ($r=0.181$, $p=0.015$) while age at menopause was positively correlated to number of hemangiomas detected at first ultrasound ($r=0.542$, $p<0.0001$). During follow-up no change in the ultrasonographic pattern or number of the hemangiomas was observed. An increase in the hemangioma size occured in 5/22 (22.7%) hormone therapy exposed patients compared to 8/72 (11.1%) controls. Three variables, ultrasonographic pattern, number of hemangiomas and hormone therapy, predicted whether or not a given hemangioma would increase in size. Hypoechoic pattern increased the risk of progression, while a hyperechoic pattern decreases that risk (OR 3.7, 95% CI 1.5-9.0, $p=0.003$), hemangioma number was inversely associated with the likelihood of progression (OR 0.27, 95% CI 0.11-0.68, $p=0.006$) and finally hormone therapy increased the risk of hemangioma enlargement (OR 3.02, 95% CI 0.99-9.12, $p=0.05$). Conclusions: Hepatic hemangiomas seem to be influenced by both endogenous and exogenous female sex hormones. Consequently, routine liver ultrasound follow-up in women with hepatic hemangiomas receiving hormone therapy appears appropriate.

W1256

A Paradigm Shift Towards Bloodless Liver Surgery: Radiofrequency Assisted Liver Resection Optimises Safety and Minimises Blood Loss and ICU Admission

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Liver resection remains major surgery, and is associated with high peri-operative morbidity (10-39%), significant mortality (0-8%), frequent need for intensive care unit (ICU) transfer, and a post-operative stay of 10-20 days. Excessive haemorrhage during hepatic resection remains a major complication, and is associated with a post-operative mortality as high as 17%. We have recently described a promising new technique of potentially bloodless liver resection, which involves applying radiofrequency (RF) energy during surgery. We report the results of its use in major liver resections for various primary and secondary liver tumours. In our centre for liver surgery, between January 2001 and April 2003, 85 consecutive resections were performed using this novel RF assisted technique. These results were compared with a previous series, from the period leading up to the end of 2000, of 80 liver resections from our group, in which the primary modalities used to limit liver blood loss were the standard, ubiquitous techniques known as PM (17.5% of cases) and TVE (76.3% of cases).

Using the new technique, median resection time was 60min (30-140), and the median blood loss was only 50mL (15-1500). The mean pre-operative and post-operative haemoglobin was 13.4 ± 1.6g/dL and 11.6 ± 1.5g/dL, respectively. Only 3 of the 85 patients were admitted to the ICU. Only two patients died in the post-operative period, and both these deaths were not related to the liver surgery. This yielded a post-operative mortality rate of 2.35%, which compares very favourably with the 7.5% observed in our previous series. Compared to the previous series of patients, in which currently established best surgical practice was observed, overall complications (47.5% vs 10.6%), need for blood transfusion (46.8% vs 8.2%), bile leak rate (8.8% vs 2.5%), ICU admission (83.8% vs 3.5%), and post-operative stay (18.9 days vs 9 days), were all significantly reduced. In both series, the liver tumours included metastases from colorectal cancer, primary hepatocellular carcinoma, cholangiocarcinoma, carcinoid tumours, gallbladder cancer, etc.

This novel technique of RF assisted liver resection appears to be safer than currently accepted best surgical practice, and is associated with minimal intra-operative blood loss, fewer biliary leaks and other complications, a reduced post-operative stay in hospital, and a drastically reduced need for ICU admission.

W1257

Acute Myopathy Associated with Liver Cirrhosis ; Hepatic Myopathy

Ok-Jae Lee, Tae-Hyo Kim

Background/Aim : Alcoholism and alcoholic liver diseases can be accompanied by myopathy which is called alcoholic myopathy. Many cirrhotic patients also have muscular symptoms and rhabdomyolysis. However, the myopathy associated with liver cirrhosis has not been established as a disease entity. We evaluated clinical characteristics of acute myopathy associated with liver cirrhosis. Methods : We reviewed retrospectively medical records of 5,440 patients with liver cirrhosis who had admitted to Gyeongsang National University Hospital from August 1997 to January 2003. Among them, 99 patients had developed acute myopathies and they were analyzed for clinical and laboratory parameters, and outcomes. Results : Child-Pugh classification at the time around onset of myopathy was A in 1 case, B in 33 (33.3%), and C in 63 (63.6%). Most frequent predisposing factor of myopathy was infection. The antibody for influenza was checked in idiopathic 18 cases with 50% positivity. Forty cases were complicated by acute renal failure, and 25 cases among them expired. 64 cases recovered, and the mortality rate was 31.2%. The mortality was highest in the cases with Child-Pugh class C compared to B or A. The major causes of death were hepatic failure. Conclusions: Acute myopathy can develop in liver cirrhosis and is one of the serious complications by various predisposing factors. It's frequency, severity and mortality depend on the underlying liver function, and are higher in advanced liver cirrhosis with decompensation. Influenza should be considered as an etiologic factor in the idiopathic cases. It is proposed that acute myopathy associated with liver cirrhosis could be called 'hepatic myopathy' and careful monitoring for hepatic myopathy should be necessary in the patients with advanced liver cirrhosis.

W1258

Utility of Liver Biopsy in Bone Marrow Transplant Patients

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Background and Aim: Hepatic dysfunction from veno-occlusive disease (VOD), graft-versus host disease (GvHD), drug toxicity, and infectious hepatitis are well known causes of morbidity and mortality in bone marrow transplant recipients. During the complex clinical management of these patients, review of liver histology is often contemplated. The purpose of our study was to assess the safety and utility of liver biopsy in this patient population. Methods: 1700 bone marrow transplants were performed at our institution from June1982 to December2002. Data from patients who underwent liver biopsy and consented were reviewed once they were identified through a computerized medical index system. A dedicated team of hepatopathologist reviewed the liver histology. The impact of the histologic diagnosis on subsequent patient management was obtained from the clinical records. Histologic diagnosis was used as a standard to assess the reliability of clinical diagnosis. Results: 61 patients, 39 males and 22 females, had a liver biopsy performed (27 transjugular, 29 percutaneous and 4 laparoscopic, 1 not specified). Histologic diagnosis and the impact of the results of liver biopsy on management are shown in the table. As a result of liver biopsy, management was changed in 37% of patients and included addition of medical therapy in 11 and stopping therapy in 5. Complications were observed in 16 (27%) patients, 8 associated with transjugular, 5 with percutaneous & 3 with laparoscopic biopsies. The difference in complications among these techniques was not statistically significant. Complications included 10 cases of pain or bleeding at the biopsy site, 4 subcapsular hemorrhages, 1 arrhythmia & 1 death. Conclusion: Liver biopsy, though infrequently obtained during the assessment of liver dysfunction in bone marrow transplant population can serve as an important diagnostic tool with significant impact on the clinical management of these patients. Although the rate of complications observed was higher than other studies, majority of them were minor. Hence, in this group of patient population with diagnostic dilemmas, liver biopsy is a reasonable diagnostic modality, which aids significantly in guiding specific therapy.

Histologic diagnosis and impact of liver biopsy

Biopsy Diagnosis (N)	N (%) with change in management
VOD (7)	3 (43)
Drug toxicity (7)	4 (57)
GvHD (14)	7 (50)
GvHD & drug toxicity (3)	1 (33)
Viral hepatitis (3)	2 (67)
Cholestasis (6), Amyloidosis (4), Hemosiderosis (1), Normal (1), GvHD & infection (1)	0
Non-diagnostic (13)	5 (38)
Total (60)	22 (37)

W1259

Liver Cirrhosis in Sickle Cell Disease

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Case series describing liver histology in sickle cell disease (SCD) report only occasional instances of liver cirrhosis. We searched our Center for patient databases from 1983-2003 and found 14 cases of liver cirrhosis documented by histology. Nine of these were diagnosed by liver biopsy while the patients were still alive. The diagnosis in the remaining 5 patients was made at autopsy. The mean age of the cirrhosis patients was 39 + 10.5 (SD) years. There were 7 males and 7 females. Thirteen of the patients have Hb SS and one patient has Hb SC disease. Thirteen patients had a history of multiple transfusions and one had a history of alcohol abuse. One patient, who had no transfusions before diagnosis of liver cirrhois, is thought to have had non-HFE hemochromatosis. Only one patient had a positive serology for hepatitis B virus. Three of the 9 patients diagnosed after 1995 were seropositive for hepatitis C virus. Mean serum chemistries are shown in the table below. Histological evidence of heavy iron overload (by iron stain) was present in all but one of the liver specimens examined. This one specimen had only a moderate degree of iron deposition. The mean and median follow up for the 9 patients diagnosed during life was 1 year (+ 1.67 SD) and 0.4 years, respectively. Five of the 9 patients diagnosed at liver biopsy died with a median time of 0.8 years from diagnosis to death. Four of them died of complications of liver cirrhosis and one died of a stroke. Two patients were lost to follow up 0.2 and 3.3 years after biopsy diagnosis. Only 2 cirrhotic patients are still alive after 4.7 and 2.2 years from their diagnosis. Both are on iron chelation therapy. Of the 5 patients diagnosed at autopsy only one died of cirrhosis complications. The cause of death in two additional patients was cardiac hemosiderosis. Our data suggest that in most SCD patients, liver cirrhosis is associated primarily with iron overload. Only one of our patients had no significant iron overload; and also this patient did not have viral hepatitis or an alcohol abuse history. However, all 14 of these patients had serum ferritin levels greater than 1000 ng/ml. Since a substantial number of patients were diagnosed at autopsy, a more frequent use of liver biopsy is recommended for SCD patients, particularly those with high ferritin levels. Ferritin is a good screening test for iron overload in sickle cell patients and should be used to identify patients for liver biopsy to confirm iron overload and start appropriate therapy.

Parameter	Mean	SD
Ferritin (ng/ml)	2737	1175
Iron (ug/dl)	128	40
Transferrin (mg/dl)	186	33
Transerrin sat. (%)	61.7	21.9

W1260

Utility of the MELD Score for Predicting Outcomes Following Cholecystectomy in Patients with Cirrhosis

Linda Perkins, Mark Jeffries, Tushar Patel

Background. Patients with cirrhosis have an increased risk of cholelithiasis but also have an increased risk of postoperative morbidity and mortality. Current pre-operative assessment of surgical risk is imprecise. The MELD score has been developed to assess mortality in persons with advanced liver disease. Aims. To assess pre-operative predictors of surgical risk, and to determine the utility of the MELD score as a predictor of outcomes following cholecystectomy. Methods. A computerized database was used to identify all persons who underwent a cholecystectomy at our institution between 1996 and 2003. All persons with cirrhosis (n=33) were identified, and the diagnosis confirmed on clinical, biochemical or histological grounds. A control group (n=30) comprised of patients without cirrhosis. Pre-operative clinical and biochemical parameters were obtained from the electronic database, and the MELD score assessed. Ninety day mortality and morbidity was determined. The utility of the MELD score was assessed by analysis of the area under the curve (AUC) of a receiver operator characteristic curve. Global patient charges were assessed at 30 and 90 days after surgery and included surgical, physician, and laboratory charges. Results. There were two deaths, both in cirrhotic patients. The overall risk of morbidity or mortality was increased in cirrhotics compared to controls (39.4% vs.16.6%, p=0.04). Post-operative morbidity was significantly associated with pre-operative elevations of INR >1.2 (p<0.001), bilirubin >1.0 (p=0.01), platelets <150 (p=0.04), or creatinine >1.4 (p=0.04). An elevated INR was most predictive of post-operative morbidity with an Odds Ratio = 16.9 (p<0.001), and a sensitivity = 73%, specificity = 86%. The MELD score accurately predicted post-operative morbidity with an AUC of 0.903 (0.8-1.0). A pre-operative MELD score ≥ 8 had a sensitivity of 91% and specificity of 77% for predicting post-operative morbidity. Persons with a MELD score ≥ 8 had increased resource utilization, with significantly increased 30-day (p=0.02) and 90-day (p=0.04) global charges and increased usage of RBC (p=0.03) or FFP/cryo (p=0.01). Conclusions. Morbidity and mortality following cholecystectomy are increased in cirrhotic patients compared to a control group. Pre-operative biochemical parameters, INR, bilirubin, platelets, and creatinine can predict increased morbidity in cirrhotic patients. The MELD score is useful in predicting post-operative morbidity and resource utilization.

W1261

Dietary Intake and Gastroesophageal Reflux Disease: A Cross-Sectional Study in Volunteers

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Background: Although diet has been associated with GERD, the role of dietary components (total energy, macro, and micronutrients) is unknown. We examined associations of GERD symptoms with intakes of specific dietary components. Methods: We conducted a cross sectional study in a sample of employees (non-patients) at the Houston VAMC. Gastro Esophageal Reflux Questionnaire (GERQ) was used to identify the onset, frequency and severity of GERD symptoms. Dietary intake (usual frequency of consumption of various foods and portion sizes) over the preceding year was assessed using the Block 98 food frequency questionnaire. Upper endoscopy was offered to all participants and esophageal erosions recorded according to the LA classification. We compared the dietary intake (macronutients, micronutrients, food groups) of participants with or without GERD symptoms, or erosive esophagitis (EE). Stepwise multiple logistic regression analyses were used to examine associations between nutrients and GERD symptoms or esophageal erosions, adjusting for demographic characteristics, BMI, and total energy intake. Results: 371 of 915 respondents (41%) had complete and interpretable answers to both heartburn and regurgitation questions and met validity criteria for the Block 98 FFQ. The mean age was 43 years, 260 (70%) were women, and 103 (28%) reported at least weekly occurrence of heartburn or regurgitation. Of the 164 respondents on whom endoscopies were performed, EE were detected in 40 (24%). Compared to participants without GERD symptoms, daily intakes of total fat, saturated fat, cholesterol, percentage of energy from dietary fat, and average fat servings were significantly higher in participants with GERD symptoms; with a dose response relationship between GERD and saturated fat, and cholesterol. The effect of dietary fat became non-significant when adjusted for BMI. However, high saturated fat, cholesterol, or fat servings were associated with GERD symptoms only in participants with BMI>25kg/m2 (effect modification). Fiber intake remained inversely associated with the risk of GERD symptoms in adjusted full models. Participants with EE had significantly higher daily intakes of total fat and protein than those without it (p<0.05). Conclusions: In this cross-sectional study, high dietary fat intake was associated with increased risk of GERD symptoms and EE, while high fiber intake correlated with reduced risk of GERD symptoms. It is unclear if the effects for dietary fat are independent of obesity.

W1262

Obesity Correlate with Gastroesophageal Reflux Symptoms in US Veteran Population

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CONTEXT: Gastroesophageal reflux and obesity are both increasing in prevalence in United States. It is estimated that heartburn affects 25 million adults in the United States on a daily basis. The significant data documenting the relationship between obesity and gastroesophageal reflux symptoms has been relatively lacking from within the US, and the emerging literature is chiefly from outside the US. A difference between sexes concerning this relationship has been proposed. OBJECTIVE: To evaluate the relation between body mass index and gastroesophageal reflux symptoms and to determine the difference between sexes concerning this relationship. DESIGN: Retrospective, cross sectional, case control study, conducted at Overton Brooks VA Medical Center, at Shreveport, LA. Evaluated the medical records of patients from the period October 1998 through June 2003. PARTICIPANTS: A total of 39,090 subjects, 37,073 men and 2,012 women were selected. Mean age of subjects was 65.4 years and controls was 63.0 years. The patients were selected on the basis of International Classification of Diseases (ICD) 9 codes for reflux esophagitis (530.11) and / .or reflux disease (530.81); the controls were individuals who did not fit these criteria. MAIN OUTCOME MEASURE: The data were analyzed using multivariate logistic regression with reflux as the dependent variable and BMI, age, and race as covariates. The SPSS statistical package (SPSS, Inc., Chicago, IL) was used to analyze the data and generate descriptive statistics. RESULTS: There was a dose-response association between increasing body mass index (BMI) and reflux symptoms in both sexes (P for trend <.001). Compared with those with a BMI less than 25, the risk of reflux was minimally increased among severely obese (BMI >35) men (OR, 1.01; 95% CI, .92-1.10) and moderately increased among women (OR, 1.55; 95% CI, 1.11-2.16). Reduction in BMI was associated with decreased risk of reflux symptoms. CONCLUSIONS: There is a significant association between increasing body mass and symptoms of gastroesophageal reflux. The association is weaker in men than in women. Of further interest, race did not influence the association between obesity and gastroesophageal reflux symptoms in our veteran population.

W1263

Symptom Severity, Quality of Life (QoL), and Psychological Distress in Patients with Erosive Esophagitis (EE) vs. Nonerosive Esophageal Disorders (NEED): Are They Really Similar?

Borko Nojkov, Michael J. Shaw, Susan Adlis, William D. Chey

Aims: Previous studies using non-validated clinical end points and generic QoL instruments suggest that symptom severity and QoL are similar in patients with EE and NEED. The goal of our study was to test this notion using validated survey instruments. We also formally assessed the degree of psychological distress between EE and NEED. Methods: Pts with heartburn >2x/week referred for EGD were recruited. Pts who had taken a PPI within 2 weeks of EGD were excluded. Eligible patients completed validated questionnaires including: Digestive Health Symptom Index (DHSI) which assessed upper and lower GI symptoms; Reflux Disease Questionnaire (RDQ) which assessed the severity and frequency of GERD symptoms, SF-12 which provided a generic assessment of QoL, QOLRAD a GERD specific QoL tool, and the Brief Symptom Index (BSI) are a measure of psychological distress. At EGD, pts were categorized as EE or NEED using the LA classification. Results: 105 patients have been recruited and 73 have completed the study to date (24 EE, 49 NEED). Of patients with EE, 33% had LA A, 43% LA B, and 24% LA C/D esophagitis. NEED patients were more likely to be female than EE patients. Upper GI symptom scores by DHSI were similar between EE and NEED (Table). RDQ scores revealed no significant differences in the severity of GERD symptoms, heartburn or regurgitation between groups. Generic QoL by SF-12 as well as GERD specific QOL (QOLRAD) were also similar between groups. There was no difference in the degree of psychological distress between groups by BSI. Conclusions: Symptom severity, impairment in QoL, and psychological distress are similar between EE and NEED. These findings confirm the notion that NEED patients do not represent a less severe form of GERD.

Survey score	EE	NERD	P-Value
DHSI-UGI	39.6 (13.8)	39.7 (13.1)	0.974
RDQ-GERD	17.2 (9.4)	16.3 (9.2)	0.684
SF12 physical	47.1 (10.2)	44.8 (9.1)	0.354
SF12 mental	47.3 (12.3)	46.0 (12.1)	0.691
QOLRAD	3.56 (1.33)	3.87 (1.32)	0.357
BSI-global score	55.9 (13.0)	57.0 (7.2)	0.566

W1264

Psychological Profile and QOL in Patients with Functional Heartburn

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Background: Many patients referred to our GI Physiology Laboratory with functional heartburn/functional chest pain often report symptom occurrence after a stressful event. Like IBS, these patients may have altered perception of pain related to a prior stressor. Anxiety, depression and quality of life (QOL) have not been reported in this patient population. Aim: To determine anxiety, depression and QOL in patients with functional heartburn/functional chest pain as defined by the Rome II Criteria. Methods: 67 patients were screened after informed consent. Patients underwent esophageal manometry, 24 h pH metry, EGD, esophageal balloon distention and Bernstein test. Patients filled out the following questionnaires at the 1st visit: Beck Depression Index, STAIT Trait Anxiety Index and SF-36 QOL. Patients that failed entry criteria were compared to the study population. Symptomatic patients on a stable dose of antidepressants for at least 3 months were eligible. Questionnaires were scored and means were generated and compared via t-tests employing a 0.05 level of significance. Results: Questionnaires from 50 patients with functional heartburn/functional chest pain (18 M, 32 F; Age 19-67 years) were compared to those not eligible for the study or dropped (N=17; 6 M, 11 F; Age 20-60 years). Patients with functional heartburn/functional chest pain had a mean Beck Depression Index of 3.88. Mean STAIT Trait Anxiety Scores were 29.06 (Present Feelings) and 29.04 (General Feelings). SF-36 Scores were as follows: Physical Functioning=89.9, Limitations due to Physical Health=94.0, Limitations due to Emotions=89.3, Energy/Fatigue=65.0, Emotional Well-Being=82.2, Social Functioning=86.0, Pain=87.2, General Health=76.3. There were no significant differences in Depression Index, or STAIT Trait Anxiety Scores between patient groups. General Health (SF-36) was significantly decreased in the screen fail group compared to the study group (66.2 vs 76.3; $P<0.03$). Conclusions: 1) Although the Beck Depression Index, STAIT trait Anxiety Index and the SF-36 QOL did not show depression, anxiety or decreased QOL, respectively: 1) These instruments may not be sensitive enough to detect alterations in psychological profiles or QOL in patients with functional heartburn/chest pain, or 2) Subjects may have developed compensating behaviors, and/or 3) Depression, Anxiety and decreased QOL may not be significant issues in this patient group.

W1265

The Impact of Baseline Scores on a Relevant Change and a Minimal Important Difference (MID) in Patient-Reported Outcomes (PROS) in Reflux Disease?

Ingela Wiklund, Ola Junghard

Purpose: To explore the impact of baseline scores on the relevant change and the minimal difference between treatments in PROs with a 7-point scale.
Patient and Methods: Data from 1774 endoscopy -ve patients with mild to severe heartburn treated with esomeprazole were analyzed. The PROs included the Gastrointestinal Symptom Rating Scale Reflux dimension, and the Sleep and Eat/drink dimensions in the Quality of Life in Reflux and Dyspepsia (QOLRAD) questionnaire. The baseline PRO 7-graded scores were classified as none/minor, mild, moderate or severe. The Overall Treatment Effect (OTE) was used to mirror the clinical relevance of the change and to establish the MID.
Results: The magnitude of the PRO change after 4 weeks of treatment differed considerably depending on the baseline score. The mean change in the Reflux dimension for patients with severe Reflux symptoms at baseline (n=361) was 3.5, the mean change in the QOLRAD Sleep dimension in patients with severe Sleep disturbances (n=308) was 3.3, and the mean change in the QOLRAD Eat/drink dimension in patients with severe Eat/drink problems (n=399) was 3.3. The corresponding changes in patients with none or minor symptoms/problems at baseline were 0.7, 0.4, 0.6 for Reflux (n=316), Sleep disturbances (n=638) and Eat/drink problems (n=298), respectively. In patients with moderate symptoms or problems the change scores were 2.5, 2.4 and 2.3 for Reflux (n=541), Sleep (n=389) and Eat/drink (n=570) dimensions respectively. Irrespective of baseline severity of symptoms or problems, around 80% of the patients considered their symptoms to be at least "moderately better" according to OTE. The MID for those with severe Reflux symptoms or Sleep and Eat/drink problems is estimated to be 2-5 times the MID for patients with no or minor Reflux symptoms or Sleep and Eat/drink problems. For both QOLRAD dimensions, a MID was estimated to 0.2 for patients with none/minor baseline problems, to 0.4, 0.6 and 0.9 for those with mild, moderate and severe baseline problems, respectively. For the Reflux dimension the MID increased from 0.3 to 0.9 when the severity of baseline symptoms increased from minor to severe. Hence, a 0.5 score change on a 7 point scale may represent a change perceivable by patients, but a difference of 0.5 score points probably represents an unrealistically high target when comparing two treatments.
Conclusion: The baseline score affects the magnitude of the change and the MID and must be considered when interpreting data.

W1266

Interpreting the Clinical Relevance of Treatment Differences in Patient-Reported Outcomes (PROs) in Patients Treated for Reflux Disease

Ola Junghard, Ingela Wiklund

Purpose: To explore what can be considered to be a clinically relevant treatment difference in PROs with a 7-graded response format by relating it to differences in rates of symptom resolution.
Patients and Methods: PRO data from a clinical trial (n=997), comparing omeprazole 20 mg with omeprazole 10 mg and ranitidine 150 mg bid in patients with reflux disease, were used in this analysis. The PRO data were collected using the Gastrointestinal Symptom Rating Scale (GSRS) Reflux dimension, which consists of two 7-graded items, one for heartburn and one for acid regurgitation. The mean change from baseline to 4 weeks is compared with the proportion of patients being symptom-free (i.e., score=None on both items in the GSRS reflux dimension) after 4 weeks of treatment. A treatment difference of 10 %-points is often considered as clinically relevant and is used as an anchor.
Results: The mean Reflux dimension score change was 1.48 for omeprazole 20 mg, 1.32 for omeprazole 10 mg and 1.15 for ranitidine. The corresponding proportion of patients with complete symptom relief during the past 7 days was 42%, 34% and 26%, respectively. Thus the difference between omeprazole 20 mg and ranitidine 150 mg of 0.33 score points corresponds to a difference in the proportions of symptom-free patients of 16%. The difference between omeprazole 20 mg and omeprazole 10 mg of 0.16 score points corresponds to a difference in proportions of 8%. Hence, a treatment difference of 10 %-points corresponds to a difference in mean Reflux dimension score of 0.2. The same relationship, that a difference of 10 % points corresponds to a difference in mean score of 0.2, was found when evaluating the GSRS heartburn item separately.
Conclusion: The results suggest that a treatment difference of 0.2 score points defines a clinically relevant treatment difference, at least in studies where a large proportion of the patients become symptom-free. A difference of 0.5 score points, previously suggested as a standard, is unrealistic and much too large to define a clinically relevant treatment difference in studies with highly effective treatments.

W1267

Cost of Illness in a Cohort of Patients with Gastroesophageal Reflux Disease Over 2 Years

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Purpose: In patients with gastroesophageal reflux disease (GERD) we aimed to assess reflux specific medication use (both prescribed and OTC) and disease-related costs in relation to patients and disease characteristics during 2 years of routine care (RC).
Methods: ProGERD is an international cohort study of 6215 outpatients with GERD. Patients were endoscoped and received initial treatment with a proton pump inhibitor (PPI) for 2 to 8 weeks. During the following observational period, patients received RC at the discretion of their primary care physician. After 1 and 2 years of RC, medication, and other patient and disease variables were assessed by patient questionnaires (response 90% and 86%). Direct cost data (medication, physician visits and hospital admissions) were calculated by multiplying disease related medical resource units with cost factors by unit. Indirect cost data (productivity loss) were calculated by multiplying days off work due to GERD with the average cost factor per day (societal perspective).
Results: Total disease-related direct costs in the ProGERD cohort (mean age 54 ±14, 47% female) amounted to a mean of 361 Euros ± 911 per patient per year (Medication 71%, Hospital care 22%, Physician care 7%). Of the total cohort, 43% took regular GERD medication. For those regular users, the mean costs were 526 Euros ± 365 (SD) per patient per year. 88% of them took PPIs, 3% H_2-blockers, and 9% other antireflux drugs. Of all patients, 30% did not use GERD medications on a regular basis (68% PPIs and 4% H_2 and 28% other antireflux drugs), with mean costs of 117 Euros ± 114 per patient per year. Of all employed persons (N=2413), 7% reported days off work (median 14 days per year). Those indirect costs amounted to 53 Euros ± 298 per patient per year. Significantly higher GERD-related costs were observed in patients with erosive GERD (486 Euros) vs non-erosive (324 Euros), with increasing duration of GERD and with the presence of concomitant diseases. Costs did not differ with respect to age, gender, and education.
Conclusion: In RC, costs of medication for GERD varied considerably, and contributed markedly to the disease-related total costs. Medication costs per patient were similar to those in patients with statin therapy for hyperlipidemia[1]. Patients with erosive or long-standing GERD had the highest total costs per year.
Reference: [1] Schwabe U, Paffrath D: Arzneiverordnungsreport 2003: Springer Verlag, Berlin.

W1268

Effect of Esomeprazole on Sleep Quality in Patients with GERD: Patient-Reported Outcomes from a Randomized Controlled Trial Using the Pittsburgh Sleep Quality Index (PSQI) Questionnaire

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Purpose: This study evaluated the effects of esomeprazole (20 or 40 mg once daily) and placebo on sleep quality, as measured by the use of validated instrument, in GERD patients with both sleep disturbance and moderate to severe nighttime heartburn. Methods: Patients with GERD (a history of erosive esophagitis or episodes of heartburn or acid regurgiation for ≥3 months) and all of the following symptoms were eligible for randomization: history of sleep disturbance ≥1 month; moderate to severe nighttime heartburn (by patient recorded daily diary) and sleep disturbance (e.g. trouble falling asleep, frequent awakenings, overall poor sleep quality) on ≥3 of the last 7 nights of a run-in period. Patients with conditions (other than GERD) known to affect sleep were excluded. Sleep medication was allowed if usage was stable (≥3 months) and was expected to remain stable throughout the study.

Patients were randomized to esomeprazole 20 or 40 mg or matched placebo qAM for 4 weeks. The PSQI questionnaire was administered at baseline and at the end of week 4. PSQI assessed sleep over the previous month on 7 dimensions (subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction), each rated on a 4-point scale (0 to 3, with 3 indicating more profound effect), which are added together to give a global score, with higher scores representing worse sleep quality. Percentage of patients with sleep quality defined as good (PSQI score < 5) at baseline and week 4 and number needed to treat (1/Absolute Risk Reduction) were also calculated. Results: See table. Conclusion: In this randomized controlled trial, esomeprazole provided a highly significant improvement in sleep quality, as determined by a validated instrument, in patients with moderate to severe nighttime heartburn and GERD-related sleep disturbance.

PSQI Scores, % of Patients with Good Sleep and NNTs

	Placebo (n=214)	Esomeprazole 20 mg (n=214)	Esomeprazole 40 mg (n=204)
PSQI baseline	9.6	9.6	9.5
PSQI week 4	7.5	5.8	6.0
PSQI change	-2.0	-3.8*	-3.5*
% patients with PSQI <5 at baseline	13.6	16.8	19.6
% patients with PSQI <5 at week 4	37.0	57.5	51.9
NNT		4.7	6.3

Significance: p<0.0001 vs placebo

W1269

Is the Diagnosis of Barrett's Worse Than the Disease?
Joel H. Rubenstein, John M. Inadomi

Background: Diagnosing a potentially life-threatening disease may adversely affect patient quality of life (QOL) independent of biologic effects. It is unknown whether the mere diagnosis of Barrett esophagus (BE) adversely impacts patients' preferences (health-state utility) sufficiently to impair the cost-effectiveness of endoscopic surveillance for esophageal adenocarcinoma.
Objective: To calculate the threshold impact on health-state utility incurred by diagnosing BE that would allow surveillance to remain cost-effective.
Design: A Markov model was developed to examine strategies of no surveillance, and surveillance every 3 years for patients with BE and no dysplasia (every 12 and 3 months for low-grade and high-grade dysplasia, respectively). Cost-utility was incorporated to account for patient preferences for various health states.
Data Sources: Published literature and the Center for Medicare and Medicaid Services.
Target Population: 50 year-old Caucasian men with symptoms of gastroesophageal reflux disease (GERD).
Time Horizon: 50 years of age until 80 years of age or death.
Perspective: Third party payer.
Outcome Measures: Threshold decrement in utility incurred by diagnosing BE based on incremental cost-effectiveness ratios (ICER) of $50,000 and $100,000 per quality-adjusted life-year (QALY) gained, comparing surveillance every 3 years to no surveillance.
Results: For an ICER of $50,000 per QALY, the decrement in utility could be as great as 9%, meaning that surveillance is cost-effective as long as diagnosing BE does not impair QOL by more than 9%. For an ICER of $100,000, the decrement could be as great as 10.5%.
Conclusions: The decrement in utility caused by diagnosing BE may be substantial without compromising the cost-effectiveness of endoscopic surveillance. Given that previous studies have found little impairment of QOL in patients with BE, it is likely that surveillance remains cost-effective despite the impact of diagnosing BE on health-state utility.
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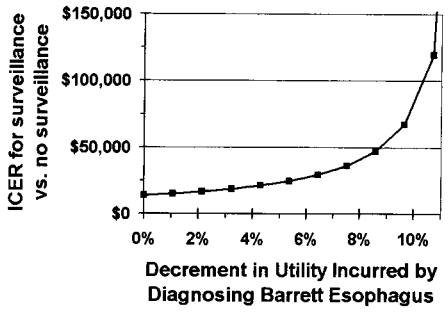


W1270

Helicobacter Pylori-Associated Ulcer Bleeding: Should We Test for Eradication after Treatment?
Heiko Pohl, Samuel R. G. Finlayson, Amnon Sonnenberg, Douglas J. Robertson

Background: Eradication of Helicobacter pylori (HP) after peptic ulcer hemorrhage substantially reduces the risk of recurrence. Because HP treatment is very successful it is unclear whether testing to confirm eradication is worthwhile. Objective: To examine whether patients with HP associated peptic ulcer hemorrhage should be tested for successful eradication after completion of antibiotic therapy. Method: A Markov cost-effectiveness model was developed that directly compared testing for eradication versus not testing for patients treated for HP after peptic ulcer hemorrhage. Probability estimates were based on published information regarding the likelihood of successful eradication of HP after treatment (85%), the sensitivity of testing to confirm eradication (90%) and the likelihood of recurrent bleeding complications in the presence (1%) and absence (10%) of successful eradication. Average costs were also derived from published information and included those associated with testing, retreatment as well as the downstream costs of recurrent bleeding complications. In a sensitivity analysis, the results were tested over a broad range of plausible probabilities and costs. Results: In the base case analysis strategy testing for HP eradication was both more effective and less costly (dominant strategy). Specifically, testing for HP eradication resulted in a benefit of 0.25 quality adjusted life years (QALYs) and cost $2572 less than the strategy of not confirming eradication. The finding of dominance of the test strategy was robust over a broad range of assumptions regarding the age of the cohort, the initial success of eradication, a variety of antibiotic treatment strategies, and the rate and costs of recurrent bleeding. Assuming an eradication rate of 95%, the test strategy becomes more expensive only if the cost of HP testing reaches $6213, however, even under these conditions it remains cost-effective. Conclusions: Patients with HP associated peptic ulcer bleeding should be tested to confirm eradiation of HP after completion of antibiotic treatment. Even if initial eradication rates are assumed to be high the strategy of confirmatory testing with treatment of those who failed initial therapy saves lives and decreases costs.

W1271

High Dose Oral Proton Pump Inhibition Decrease Re-Bleeding in High-Risk Patients with Acute Peptic Ulcer Bleeding: A Meta-Analysis
Marc Bardou, Youssef Toubouti, Myriam Martel, Elham Rahme, Alan Barkun

Background: Recent studies suggest oral high-dose proton pump inhibition (PPI) might be useful in the acute management of high-risk bleeding PUD. Aim: We conducted a meta-analysis to better characterize summary results of randomized controlled clinical trials on the efficacy of PPI following successful endoscopic treatment. Methods: We reviewed all randomized trials published between 01/90 and 04/03 assessing any PPI administration for the prevention of re-bleeding (RB), surgery (S), and mortality (M) in patients with acute PUD bleeding. We particularly assessed two PPI dosing schedules: high dose oral PPI (Oral-PPI, at least twice the usual daily dose) and high dose intravenous PPI (IV-PPI, 80 mg bolus followed by 6-8 mg/h constant infusion for 3 days). All comparisons were made between the groups initially randomized for in each study. A two-step meta-analysis included a linear mixed effect model clustering studies by control-type, in which the weighted rate differences (treatment minus control) were regressed against the year of publication, quality score of the study, mean age of all patients, and number of male patients. We adjusted for possible confounding (proportion of patients in shock, and year of publication). Secondly, a mean effect of the treatment category compared to both each control-type separately and all control-types combined were calculated. 95% confidence intervals for overall effect were determined taking into account within- and between-study variances Results: 18 studies were selected that included 1828 high-risk (Forrest Ia to IIb) patients (4 studies each for Oral-PPI and IV-PPI, with 474 and 680 patients, respectively). Compared to placebo, Oral-PPI significantly decreased RB (risk difference -11.0%; 95% confidence interval [-19.3%, -2.8%]) but not S (-2.2%; [-5.6%, 1.3%]) or M (-1.2%; [-3.0%, 0.5%]). As we previously reported, IV-PPI (usually post endoscopic therapy) significantly decreased RB compared to H2RA -20.2%, [-21.4%, -19.0%] and placebo (-15.6%; [-16.1%, -15.1%]). IV-PPI was also associated with a decrease in M compared to placebo (-2.1%, [-3.5%, -0.7%]). Conclusions: Oral high dose PPI's appear to decrease re-bleeding in patients with bleeding PUD and high-risk stigmata. No significant improvement in mortality was noted, probably in part due to its very low incidences in the trials. Additional studies are warranted to confirm the beneficial effects of oral high dose PPI, especially in Western patient populations.

W1272

The Influence of PPI Dose on Treatment Efficacy for Ulcer Bleeding Following Endoscopic Hemostatic Therapy (EHT): A Sub-Group Analysis from the Cochrane Collaboration (CC) Systematic Review
Grigoris I. Leontiadis, Linda McIntyre, Virender K. Sharma, Colin W. Howden

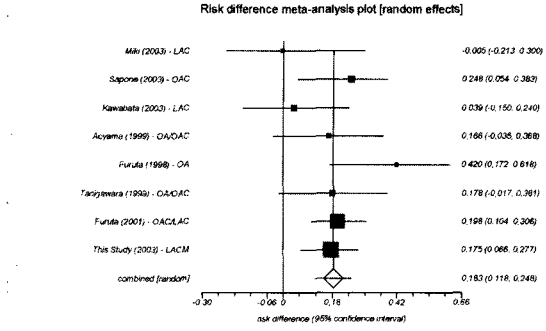
INTRODUCTION: In patients with ulcer bleeding, PPI treatment significantly reduces re-bleeding and surgical intervention rates but not mortality (CC review, *Am J Gastroenterol* 2003; 98: S49).
METHODS: In a pre-determined sub-group analysis, we examined the results of RCTs that had used pre-randomization EHT for patients with active bleeding or a non-bleeding visible vessel (NBVV), stratified by dose of PPI treatment - either high dose IV PPI (equivalent of omeprazole 80 mg bolus and 8 mg/hr infusion for 72 hr) or a lower dose. We measured statistical heterogeneity among RCTs and summarized data as odds ratio (OR) and 95% CI.
RESULTS: Of the 5 RCTs identified, 2 (340 patients) used high dose IV PPI, and 3 (339 patients) used a lower dose either IV or orally. There was no significant heterogeneity for mortality, re-bleeding or surgical intervention.
Among all 5 RCTs, pooled mortality rates were 2.7% for PPI and 5.2% for control (OR = 0.51; 95% CI 0.23, 1.12). Pooled re-bleeding rates were 11.6% for PPI and 25.7% for control (OR = 0.38; 95% CI 0.25, 0.57). Pooled surgical intervention rates were 4.5% for PPI and 7.3% for control (OR = 0.54; 95% CI 0.27, 1.08).
For the 2 RCTs that used high dose IV PPI, pooled mortality rates were 2.9% for PPI and 8.2% for control (OR = 0.36; 95% CI 0.13, 0.98). Pooled re-bleeding rates were 5.9% for PPI and 22.9% for control (OR = 0.21; 95% CI 0.10, 0.44). Pooled surgical intervention rates were 1.8% for PPI and 5.3% for control (pooled OR incalculable, due to zero event rate in 1 RCT).
For the 3 RCTs that used lower dose PPI, pooled mortality rates were 2.4% for PPI and 2.3% for control (OR = 1.01; 95% CI 0.26, 3.83). Pooled re-bleeding rates were 17.5% for PPI and 28.3% for control (OR = 0.54; 95% CI 0.32, 0.90). Pooled surgical intervention rates were 7.2% for PPI and 9.2% for control (OR = 0.68; 95% CI 0.30, 1.55).
CONCLUSIONS: When given after EHT, PPI treatment effectively reduces re-bleeding rates regardless of the dose used. The effects on re-bleeding and surgery are greater with high dose IV PPI, which also produced a marginal reduction in mortality.

W1273

CYP2C19 Polymorphism and Proton Pump Inhibitors (PPI) in Helicobacter pylori Eradication Therapy - A Metaanalysis

Gerhard G. Treiber, Matthias Schwab, Ulrich Klotz

Background: PPI are metabolised by the polymorphic CYP2C19. H.pylori eradication results according to different PPIs and dosages are inconsistent. Data from asian studies suggested that poor metabolisers of PPIs do better, data in caucasians are scarce. Objective/Methods: Meta-analysis of available literature data. Results: By mid of 2003, 12 studies met the inclusion criteria and reported differences in eradication outcome between wt/wt (wildtype) vs combined wt/mt and mt/mt (mutant) alleles. Significant (p = 0.0012) heterogeneity could be observed and was resolved (p = 0.23) by excluding 4 rabeprazole studies. 8 studies were based on racemic omeprazole or lansoprazole (see figure displayed). No bias was noted (Funnel diagrams). 1) All studies: DerSimonian-Laird pooled risk difference = 0.1361, approximate 95%CI = 0.0666 to 0.2055, p = 0.0001 2) Omeprazole/Lansoprazole studies (figure): DerSimonian-Laird pooled risk difference = 0.1828, approximate 95%CI = 0.1176 to 0.2480, p<0.0001 This absolute risk difference between wildtype and CYP2C19 mutant patients of around 20 % translates into a low NNT of 5 patients. Conclusions: Genotypic differences in CYP2C19 status affect H. pylori eradication rates in Caucasian patients as well as Asian patients if racemic omeprazole or lansoprazole is used (as both are mainly metabolised by CYP2C19). Testing for CYP2C19 genotypes appears to be clinically relevant - independently from antibiotic resistance - as about 20% lower eradication rates must be expected in wildtype patients, who represent two-third of Caucasian populations.



W1274

Does the Efficacy of PPI Treatment for Ulcer Bleeding Depend on the Nature of Pre-Randomization Endoscopic Stigmata? A *Post Hoc* Analysis from the Cochrane Collaboration (CC) Systematic Review

Grigoris I. Leontiadis, Linda McIntyre, Virender K. Sharma, Colin W. Howden

INTRODUCTION: In patients with bleeding peptic ulcer, PPI treatment significantly reduces re-bleeding and surgical intervention rates but not mortality (CC review, *Am J Gastroenterol* 2003; 98: S49).

METHODS: In a *post hoc* analysis, we examined the efficacy of PPI treatment in RCTs reporting outcomes for patients with active bleeding or a non-bleeding visible vessel (NBVV) separately from RCTs reporting outcomes for patients with adherent clot pre-randomization. Statistical heterogeneity was measured by the Mantel Haenszel method. Data were summarized as odds ratio (OR) and 95% CI.

RESULTS: 6 RCTs only included patients with active bleeding or NBVV; another 4 reported separate outcomes for such patients. Pooled summary results for these 10 RCTs (979 patients) are shown in the Table.

Two additional RCTs reported separate outcomes for 187 patients with an adherent clot at pre-randomization endoscopy. Since there was no mortality or surgical intervention in 1 RCT, heterogeneity testing was inapplicable and pooled OR incalculable for these outcomes. There was no heterogeneity between those 2 RCTs for re-bleeding (P = 0.57); pooled re-bleeding rates were 0% for PPI and 18.7% for control (OR = 0.04; 95% CI 0.01, 0.33).

CONCLUSIONS: PPI treatment reduced re-bleeding rates among patients with active bleeding, NBVV or adherent clot. There was a trend towards reduced mortality in patients with NBVV or active bleeding.

Pooled summary data for RCTs of PPI treatment in peptic ulcer bleeding in patients with active bleeding or a NBVV

Outcome	Pooled rates (%)		Heterogeneity	OR	95% CI
	PPI	Control			
Mortality	2.8	5.5	No (P = 0.79)	0.51	0.26, 1.01
Re-bleeding	13.5	29.6	No (P = 0.24)	0.36	0.25, 0.51
Surgical intervention	5.6	12.2	No (P = 0.69)	0.39	0.23, 0.65

W1275

Is There a Threshold PPI Dose That Decreases Non Lethal Outcomes in Patients with Non Variceal Upper GI Bleeding?

Alan Barkun, Myriam Martel, Youssef Toubouti, Elham Rahme, Joyce Strazzulla, Manon Petit

Background: Although high dose intravenous, and perhaps oral, proton pump inhibitors (PPI) are associated with improved outcomes in selected patients with non variceal upper gastrointestinal (NVUGIB), any optimal dosing schedule remains unknown. Aim: Using a registry composed of randomly selected patients with NVUGIB receiving a spectrum of disparate PPI dosing schedules, we attempted to seek a PPI threshold dose significantly associated with improved non lethal outcomes. Methods: The charts of randomly selected patients presenting at a single institution with a documented NVUGIB, defined by standardized criteria, over a 16-month period ending March 2003, were abstracted. Data included demographic, historical, physical examination, and initial laboratory information as well as the subsequent course in hospital (admission, endoscopic hemostasis, re-bleeding or continued bleeding (using a validated definition), surgery, discharge, or death), and the doses of pharmacological agents used, if any, as well as their precise timing with regards to non lethal outcomes, if present. A series of logistic regression equations were created using established risk models to determine any protective effect attributable to PPI's on non lethal outcomes and, more specifically, a possible dose-threshold. They included age, ASA score (1to 5 scale of illness severity), presence of high-risk endoscopic stigmata, performance of endoscopic therapy and various thresholds of total mean daily PPI doses. Results: Charts from 166 patients (mean age: 66.2 ± 17.5 yrs, 63.2% female) were reviewed. 21.4% of patients had an ASA risk score of 4 or 5, 34% had high-risk stigmata, and 30% underwent endoscopic hemostasis. Continued bleeding occurred in 12.7%, re-bleeding in 9.6%, and surgery in 9.6%. PPI's were administered in 74.6 % (oral PPI's in 66.7%, IV PPI's in 45.8%), with a mean total daily dose till discharge, death or a non lethal outcome of 55.6 ± 62.6 mg (24.8 ± 19.8 mg daily for oral, and 101.4 ± 82.7 mg daily for IV, respectively). Multivariate modeling demonstrated that both hemostatic therapy and PPI administration independently improved non lethal outcomes significantly. Doses ≥ 50-60 mg of PPI per day were associated with improved non lethal outcomes. No additional effect was noted with higher doses. Conclusion: This observational, retrospective analysis suggests that daily doses of PPI's over 50-60 mg may be required to improve non lethal outcomes, after adjustment for know confounders.

W1276

Maintenance of Improvement in Quality of Life Dimensions and Symptom Control Following Initial Treatment of Upper GI Symptoms with Esomeprazole Versus Placebo in Patients on Long-Term NSAID Therapy

Nicholas J. Talley, Christopher J. Hawkey, Neville Yeomans, James M. Scheiman, Joseph Sung, Roger Jones, Goran Langstrom, Jorgen Naesdal

Purpose: Two 6-month follow-up studies examined the efficacy of esomeprazole 20 and 40 mg in maintaining improvements in health related quality of life (HRQL) and symptom severity gained during previous studies testing 4 weeks' esomeprazole treatment.[1]

Methods: Patients who achieved upper GI symptom relief in the acute studies[1] were re-randomized into two identical, multi-center, placebo-controlled, double-blind studies. Patients received placebo, esomeprazole 20 mg or esomeprazole 40 mg qd orally for 6 months. HRQL and symptom severity were assessed at baseline and following 6 months' treatment (or at the last visit if patients discontinued treatment) using two validated instruments, the Quality of Life in Reflux and Dyspepsia (QOLRAD) questionnaire and the Gastrointestinal Symptom Rating Scale (GSRS). Both these instruments use 7-graded Likert scales to assess patients' responses.

Results: Esomeprazole 20 mg and 40 mg was more efficacious than placebo in maintaining improvements in HRQL and symptom severity during 6 months' continuous NSAID use (Table).

Conclusion: Following initial improvement on acute treatment, esomeprazole 20 and 40 mg maintained control of specific dimensions of HRQL and upper GI symptoms whereas patients on placebo tended to deteriorate over 6 months continuous NSAID exposure.

Reference: 1. Hawkey CJ, Yeomans ND, Jones R, et al. Gastroenterology 2003; 124(4 Suppl 1): A-107

Table: Mean change in QOLRAD and GSRS scores after 6 months' treatment following initial upper GI symptom relief. Higher GSRS scores indicate an increased symptom severity, higher QOLRAD scores indicate an enhanced quality of life.

Dimension	Study A			Study B		
	Placebo	Esomeprazole 20 mg	Esomeprazole 40 mg	Placebo	Esomeprazole 20 mg	Esomeprazole 40 mg
QOLRAD:	n=98	n=94	n=85	n=74	n=71	n=76
Emotional distress	-0.46	-0.17	-0.13*	-0.46	-0.21	-0.09
Sleep disturbance	-0.43	-0.13*	-0.14*	-0.53	-0.20	-0.10*
Food/drink problems	-0.55	-0.03**	-0.13*	-0.42	-0.19	-0.15
GSRS:	n=98	n=94	n=87	n=73	n=71	n=76
Reflux	0.52	0.10*	0.09*	0.47	0.01*	-0.17**
Abdominal pain	0.39	0.00	0.03*	0.37	0.10*	0.03*
Indigestion	0.16	-0.06	0.11	0.35	-0.01	-0.04*

ANCOVA: * p<0.05, **p<0.01 vs. placebo

W1277

Utilization of Antisecretory Medication in 100,000 Nova Scotia Seniors' Pharmacare Beneficiaries Between 1998 and 2002: A Cohort Database Analysis

Jordan T. Zacny, Ingrid Sketris, Chris Skedgel, George Kephart, Sander J. O. Veldhuyzen van Zanten

Objective: To study trends in the utilization of proton pump inhibitors (PPIs) and H2-receptor antagonists (H2RAs) in Seniors' Pharmacare (NU100,000) over a five year period. Methods: Data about use of PPI/H2RA prescriptions were analyzed from 1998 to 2002 to: 1) establish the period prevalence of PPI/H2RA use, 2) establish how many take PPIs/H2RAs continuously, intermittently and how many are prescribed a PPI either once daily (o.d.) or twice daily (b.i.d.) and 3) determine how many step up or step down therapy. For PPIs, a prescribed daily dose of ≥ twice the standard daily dose was considered high. Analysis included duration of use, dose of medications and switches to different doses or drugs. Indications and appropriateness of PPI/H2RA use was not assessed. Results: From 1998 to

2002, the total Seniors' Pharmacare population ranged from 101-115,000. During the study period, the percentage of beneficiaries using PPIs increased from 1.8 to 4.9% and from 13.9 to 15.4% for H2RAs. The average number of prescriptions per user increased from 4.4 to 6.0 for PPIs and 4.8 to 5.0 for H2RAs. The proportions of patients utilizing PPIs for 1-3, 4-6, 7-9 and 10-12 months of their first treatment year were 37, 17, 11 and 35%, respectively. For PPIs, 77% of prescriptions were for a standard dose (o.d.), 20% a high dose (b.i.d.) and 3% a low dose. Of those starting on a standard dose of an H2RA, 6% switched to a PPI. 25% of patients starting on a PPI began at a b.i.d. dose. Of the 75% starting on an o.d. dose of PPI, 5% switched to a b.i.d. dose. 84% of patients did not switch medication regimens. Conclusions: H2RA use was high and stable at 15% in this population. PPI use increased by 172% over the five year study period, from 1.8 to 4.9%. Over one-third of patients utilized PPIs for 10-12 months of their first treatment year. The majority of patients (84%) did not switch from the medication regimen they were started on. It will be important to prospectively assess the indications and appropriateness of PPI/H2RA use.

W1278

An Evidence-Based Analysis of Esomeprazole Therapy *Versus* Placebo for the Prevention of Gastric or Duodenal Ulcers in At-Risk Continuous NSAID Users

Neville Yeomans, James M. Scheiman, Christopher J. Hawkey, Nicholas J. Talley, Nimish Vakil, Jorgen Naesdal, Goran Langstrom

Purpose: This retrospective pooled analysis assessed the number needed to treat (NNT) and the absolute risk reduction (ARR) associated with esomeprazole 20 mg and 40 mg relative to placebo in preventing gastric ulcer (GU) and duodenal ulcer (DU) in at-risk patients.
Patients and methods: 1429 patients (*Helicobacter pylori*-negative, ≥18 years old) taking continuous COX-2-selective or non-selective NSAIDs were randomized into two placebo-controlled, parallel-group, multi-center studies of similar design. Patients were aged ≥60 years and/or had a history of GU/DU within the last 5 years. Patients received placebo or esomeprazole 20 or 40 mg orally once daily before breakfast for 6 months. The primary variable was the proportion of patients without GU/DU throughout 6 months of treatment (determined by endoscopy at 1, 3 and 6 months). ARR is the absolute reduction in GU/DU incidence for esomeprazole 20 or 40 mg relative to placebo. NNT is the inverse of ARR (1/ARR), and rounded up to the next whole number.
Results: See Table. The NNT was 9 and 8 for esomeprazole 20 and 40 mg, respectively. For patients taking non-selective NSAIDs or COX-2-selective NSAIDs, the NNT were similar (10 and 9, and 7 and 8, respectively, for esomeprazole 20 and 40 mg). Both doses of esomeprazole were well tolerated and numbers of adverse events were similar in all three treatment groups.
Conclusions: Esomeprazole is effective for reducing ulcer incidence relative to placebo in continuous users of NSAIDs, including COX-2-selective NSAIDs, at risk of ulcer development. For every 100 patients using continuous NSAIDs treated with esomeprazole 20 and 40 mg 12 treatment failures are prevented. Alternatively, for every 8 or 9 patients treated with esomeprazole, one ulcer per 6 months is avoided.

Table: Life table estimates (with 95% confidence intervals) of percentage of patients free from GU/DU throughout 6 months of continuous daily NSAID use, the number needed to treat (NNT) and the absolute risk reduction (ARR).

NSAID type		Placebo	Esomeprazole 20 mg	Esomeprazole 40 mg
All	n	452	459	467
	ARR (%)	-	11.8 (7.4-16.1)	12.4 (8.2-16.7)
	NNT (patients)	-	8.5 (6.2-13.4)	8.0 (6.0-12.2)
Non-selective	n	318	334	326
	ARR (%)	-	10.4 (5.0-15.7)	12.4 (7.3-17.5)
	NNT (patients)	-	9.7 (6.4-20.0)	8.1 (5.7-13.8)
COX-2-selective	n	134	125	141
	ARR (%)	-	15.7 (8.6-22.7)	12.4 (4.8-20.1)
	NNT (patients)	-	6.4 (4.4-11.6)	8.0 (5.0-21.0)

W1279

Methodologic Quality of Trials Affects Estimates of Treatment Efficacy in Functional (Non-Ulcer) Dyspepsia: A Meta-Analysis Based on Two Methodologic Criteria Scores

Neena S. Abraham, Paul Moayyedi, Sander J. O. van Zanten

Background & Aims: The evaluation of treatment options for functional (non-ulcer) dyspepsia (FD) remains problematic due to the varying methodologic quality of the existing literature. Our aims were to: (1) Evaluate efficacy of treatment for FD using objective quality criteria; (2) Determine the utility of the Rome II guidelines to assess methodological study quality, when compared to a gold standard quality criteria. Methods: A meta-analysis of all randomized placebo-controlled treatment trials of endoscopically investigated dyspepsia from 1979 to 2003 was performed using a standardised search and abstraction strategy. The Jadad score (high quality score 4 or 5 vs. low quality score 1-3) was used to assess methodologic quality. The treatment estimates using three key ROME II guidelines on study design (cut off of 0/3 or >0/3) were also compared to the Jadad score, to assess its validity in detecting methodologic quality. Results: Poor quality trials suggest benefit of prokinetic therapy in NUD (RR of remaining dyspeptic = 0.47; 95% CI = 0.39 to 0.56) not confirmed in high quality trials (RR of remaining dyspeptic = 1.0; 95% CI: 0.84 to 1.19). There is marked benefit of H2RA therapy in poor quality trials (RR of remaining dyspeptic = 0.68; 95% CI: 0.61 to 0.76) but marginal benefit in good quality trials (RR of remaining dyspeptic = 0.87; 95% CI: 0.79 to 0.97). Trial quality did not affect the small statistically significant benefit seen in trials of H. pylori eradication in NUD. Two high quality trials suggest a modest benefit with the use of PPIs in NUD, with no poor quality trials to provide a comparison. Similar results were obtained when the Rome criteria was used to score the methodologic quality of studies. Conclusions: The magnitude of benefit of prokinetic and H2RA therapies in NUD reported in previous meta-analyses has been overestimated. The quality of trials does have an impact on the estimates of treatment efficacy. The Rome II criteria for study methodology may be appropriate for judging NUD study quality when compared to a gold standard.

W1280

Medical Diagnoses in Functional Dyspepsia Patients

Olafur S. Palsson, William E. Whitehead, Victoria E. Barghout, Marsha J. Turner

Functional dyspepsia (FD) diagnosis is currently imprecise; at least 7 different International Classification of Disease (ICD-9CM) codes are commonly used. **Aims:** To examine whether additional ICD-9CM medical diagnoses, which might help identify FD patients, show excess association with FD in a large patient sample. **Methods:** All patients ≥20 years of age who were assigned any of 7 FD diagnoses (536.8, 789.06, 535.5, 537.89, 306.4, 536.9, and 537.9) in the University of North Carolina hospitals and clinics from 1999-2001 and had negative EGD findings (normal or only "gastroenteritis") were identified (n=712). Controls (n=1613) were randomly selected medical patients seen in the hospital system during the same period who did not receive any FD diagnoses. After similar disease entities had been grouped into diagnostic categories where appropriate, the prevalence of all diagnoses carried by at least 5% of EGD-confirmed FD patients were compared to controls. As FD patients averaged 4.6 times the prevalence of general diagnoses, only diagnoses with odds ratio (OR) above 6.2 (4.6 + 95% confidence interval) were considered to be significantly associated with FD diagnosis. **Results:** 41 non-FD diagnostic categories had a prevalence of more than 5% in FD patients, and 10 of these were significantly higher in FD patients vs controls: belching/bloating/flatulence, 5.6% vs. 0.2%, OR=30.2, p<.01; esophagitis/reflux/heartburn, 45.4% vs 4.0%, OR=11.4, p<.01; nausea ± vomiting, 22.9% vs 2.2%, OR=10.6, p<.01; hiatal hernia, 13.1 vs 1.4%, OR=9.6, p<.01; unspecified non-infectious gastroenteritis/colitis, 10.0% vs 1.1%, OR=9.5, p<.01; diarrhea, 13.8% vs 1.6%, OR=8.5, p<.05; constipation, 12.7% vs 1.7% OR=7.6, p<.05; IBS, 10.1% vs 1.4%, OR=7.1, p<.05; dysphagia, 8.2% vs 1.2%, OR=6.9, p<.05; and unspecified hypothyroidism, 5.1% vs 0%, p<.01 (no control cases; OR could not be calculated). **Conclusions:** Functional dyspepsia is highly associated with several other diagnostic categories that may be relevant to the condition: bloating and gas, reflux, nausea ± vomiting, hiatal hernia, non-specific gastroenteritis, and dysphagia. These could prove useful for identifying probable FD patients, but also highlight the challenge of separating FD from reflux. Consistent with previous studies, excess overlap of FD with bowel disorders was also observed. The association of FD and unspecified hypothyroidism (a diagnosis not given to any control patients) in our data is unexplained. [Supported by a grant from Novartis Pharmaceuticals and RO1 DK31369]

W1281

Systematic Review and Economic Evaluation of Helicobacter Pylori Eradication Treatment for Non- Ulcer Dyspepsia

Comfort Osonnaya, Kingsley Osonnaya, Ian R. Sanderson, Paul Swain

Objective- To evaluate efficacy and cost effectiveness of Helicobacter pylori eradication treatment in patients with non- ulcer dyspepsia infected with H pylori. Method- Seven electronic databases were searched for randomised controlled trials from January 1966 to October 2003. Experts in the field, pharmaceutical companies, and journals were contacted for information on any unpublished trails. Trials reports were reviewed according to predefined eligibility and equality criteria. Systematic review of randomised controlled trials comparing H pylori eradication with placebo or another drug treatment was carried out. Results were incorporated into a Markov model comparing health service costs and benefits of H pylori eradication with antacid treatment over one year. Main outcome measures- Relatives risk reduction for remaining dyspeptic symptoms (the same or worse) at 3 to 12 months. Cost per dyspepsia-free month estimated from Markov model based on estimated relative risk reduction. Results- Thirty-eight trials were included in the systematic review, 27 of which evaluated dyspepsia at 3 to 12 month in a total of 7453 patients. H pylori eradication treatment was significantly superior to placebo in treatment non-ulcer dyspepsia (relative risk reduction; 9%, 95% confidence interval; 4% to 14%), one case of dyspepsia being cured for every 15 people treated. H pylori eradication cost 58 UK pounds per dyspepsia-free month during first year of treatment. Conclusion- H pylori eradication may be a cost effective treatment for non-ulcer dyspepsia in infected patients but further evidence is needed for decision makers willingness to pay for relief of dyspepsia.

W1282

The Quality of Published Health Economic Analyses in Digestive Diseases: A Systematic Review and Quantitative Appraisal

Brennan M. R. Spiegel, Laura E. Targownik, Vincent DeRosa, Gareth S. Dulai, Ian M. Gralnek, Chiun-Fang Chiou

Background: Health economic (HE) analyses are increasingly common in the GI literature and are often cited to frame consensus guidelines. Whereas clinical trials are routinely subjected to critical appraisal, there has been no attempt to appraise the quality of GI HE analyses with a validated instrument. We therefore sought to: (1) systematically review and appraise the quality of GI HE analyses, and (2) identify predictors of study quality.
Methods: A systematic review was performed to identify GI HE analyses in GI and internal medicine (IM) journals since 1980. Using *a priori* criteria, three reviewers independently selected and assessed relevant manuscripts for quality using the QHES - a validated 16-item instrument designed to measure quality of HE analyses (score=0-100; >75=high quality). Reviewers were blinded to authors, institution, and funding source. Logistic regression was conducted to identify predictors of high quality, including: funding source, author characteristics, journal characteristics, journal impact factor (IF), presence of editorial, Web of Science citations/year, and reference to National Panel on Cost Effectiveness (NPCE) guidelines.

Results: There were 160 identified HE analyses published since 1980. The mean QHES score was 63 ± 18 and 29% of the studies met criteria for high quality. 77% failed to address potential biases in the model assumptions and 75% failed to disclose potential conflicts of interest. Five factors independently predicted high quality: (1) study disclosed potential conflicts of interest (OR for high quality = 4.0; 95%CI = 1.6, 9.7); (2) at least one author had advanced degree in health services or related field (OR = 3.5; 1.4, 8.8); (3) study cited NPCE guidelines (OR = 2.8; 1.6, 9.7); (4) study published in IM journal (OR = 2.5; 0.2, 7.2); and (5) study published in US journal (OR = 1.8; 0.7, 4.8).
Conclusion: Less than one-third of GI HE analyses meet criteria for high quality. The quality of analyses is limited by a prevalent failure to describe inherent biases and to disclose potential conflicts of interest. Traditional surrogate markers of quality (*e.g.* journal IF, presence of editorial, Web of Science citations) do not predict the quality of HE analyses. In contrast, quality is highly dependent upon author training and disclosure, journal characteristics, and adherence to national methodology guidelines. These data may be used to focus the attention of journal editors and reviewers in ensuring the future high quality of GI HE analyses.

W1283

Medication Taking Behavior in A Gastrointestinal Clinic: A Disconnect Between Patient Behavior and Physician Knowledge

Sunanda V. Kane, James Dang

Background: Many patients do not take their medications are prescribed; multiple reasons can account for this phenomenon. Aim: To quantify patient knowledge regarding medications and their level of communication with their physician regarding their concerns. Methods: An anonymous 5-item survey was distributed to patients waiting to be seen in a tertiary care gastroenterology clinic. Patients were asked to identify themselves as a New Patient (NP) or established patient (EP) and their underlying gastrointestinal condition as either Liver or Gastrointestinal. Five True/False questions asked about their understanding of the mechanism of their medications and their indication, satisfaction and whether their treating physician knew of all they were taking. Patients were not obliged to reveal participation in the survey to their treating physician. Results: Over 30 half-day clinic sessions, 250 patients agreed to participate (> 90% response rate). Fifty-two were NP and 198 were EP. The majority (82%) were GI patients. Fifteen percent admitted they do not know how their medications work, 22% dissatisfaction with their medications, citing side effects and cost as major reasons. New patients to the clinic were more likely to be dissatisfied with their medications than established patients (55% vs. 21%, $p < 0.05$). Twelve percent do not feel it was important to tell their physician all the medications they take. Conclusions: A significant number of patients are dissatisfied with their medications. Some patients do not understand how their medications work, which in addition to dissatisfaction, may lead to non-adherence.

W1284

Gastric Electrical Stimulation—A Ten Year Experience

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Introduction: Since the first human implantation in January 1992, Gastric Electrical Stimulation (GES) has undergone a number of clinical trials for the treatment of patients with gastroparesis and is now available for clinical use in both the US (as a Humanitarian Use Device) and in Europe. Patients: We evaluated the experience of 3 centers in the US implanting GES in terms of safety and efficacy. Mortality was compared with to a group of medical controls treated with traditional therapy and followed for up to 10 years (Diabetes 52: A191, 2003). Methods: All patients consented for GES implantation from 3 centers. Presenting diagnosis, type of implant, survival, most recent gastric emptying test, (compared to baseline symptoms) were documented. Results: The results of 3622 patient months (301.8 patient years) of therapy are listed in the table below. Of the patients consented, 133 had implants; 122 permanent and 11 temporary only. 8 patients have undergone explant, most commonly for infections, 11 patients underwent replacement of devices due to battery life or other technical problems. 13 patients died, most from their primary illnesses. Patient deaths were lower for both Idiopathic and diabetic patients with GES than controls. There were no deaths directly attributable to the devices. Conclusions: We conclude that Gastric Electrical Stimulation has shown itself to be both safe and effective over the last decade. Continued efforts to identify which patients will benefit most from GES, and under what conditions, are warranted. There appears to be an improvement in survival for some patients with GES, especially for patient with Diabetes Mellitus.

	Deaths DM	Deaths Non-DM	Control Deaths DM	Control Deaths Non-DM	TSS (0-50)	Vomiting (0-4)	GET 2 hr %	GET 4 hr %
Before	24	109	7	26	38.4	3.57	59.6	29.16
After	4/24 6%	9/109 12.1%	3/7 43%	2/26 13%	18.2	1.44	39.3	14.9

W1285

Outcome of Laparoscopic versus Laparotomic Cholecystectomy: A Population-Based Mortality Cohort Study

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Background Cholecystectomy (laparoscopic, LC or open, OC) is the preferred treatment in patients with symptomatic gallstones. Few controlled studies compare LC vs OC efficacy. Aim. To compare LC and OC by a population-based mortality cohort study. Methods Discharge records, Emilia Romagna Regional Health System (Italy), 1998-2002, including individual information on patient discharged from hospitals, coded according to ICDIX-CM. Inclusion criteria: OC and LC (51.22-23), cholelithiasis (574), age>/=18, and residence in the region. Exclusion criteria: diagnosis of tumours (140-239), pregnancy and newborns (MDC 14,15), trauma (800-999), surgical procedures associated with incidental cholelithiasis. Comorbidity: chronic diseases during index and previous (12 month preceding cholecystectomy) hospital admissions. Severity of cholelithiasis also included. Outcome: 30-day mortality. Logistic regression and multilevel models to adjust for confounding and to detect interaction by demographic characteristics, comorbidity, and severity of disease. Study population: 16526 LC and 7457 OC, in 83 hospitals. Results Procedure-specific mortality risk: 0.01 for OC and 0.0011 for LC. Crude OR = 0.09 (95% cl 0.05-0.15) for LC vs OC. Risk adjustment evaluated fitting logistic regression models to the observed data, selecting significant variables by stepwise method based on the Akaike Information Criterion. Final general risk adjustment model includes: gender, severe non ischemic heart disease, liver, pancreatic and renal chronic diseases, cholelithiasis severity. Age acts as an effect modifier. Risk adjusted ORs for LC vs OC were estimated separately for age strata. Adjusted OR for LC vs OC ranged from OR = 0.11 (95% cl 0.04-0.25) for age</=70 to OR= 0.28 (95% cl 0.12-0.66) for age over 70. Conclusion LC is associated with a lower mortality than OC. Adjusting for comorbidities and disease severity significantly reduced strong crude protective effect. LC advantage seems greater for age</=70. Results support the hypothesis that LC is more effective than OC; however, this outcome study cannot exclude that the observed lower mortality after LC is actually due to selective patient characteristics that cannot be detected by available hospital discharge forms.

W1286

Long Term Followup of Trigger Point Injection for Abdominal Wall Pain

Jose Nazareno, Terry Ponich, Jamie Gregor

Objective: Abdominal wall pain (AWP) is a common yet often overlooked source of abdominal pain. Trigger point injections (TPI) into the abdominal wall have been tried in the past. Few studies have looked at the longterm outcome from these injections. Methods: A retrospective chart review was performed on 130 consecutive patients who received TPI for AWP at the London Health Sciences Center, University of Western Ontario, London, Ontario, Canada. Patients were excluded if the chart was missing or information on the injection or the results was incomplete. Patients were included if they met previously validated criteria for AWP defined as: (1) fixed or localized pain AND (2) superficial or point tenderness (<2.5cm diameter) or a positive Carnett sign (increased pain with tensing abdomen). The diagnostic tests ordered to exclude AWP and the cost of investigating it were determined. Gender, location and radiation of the pain, GI symptoms, type of agent injected and presence of a surgical scar were examined to see if they were predictors of response. Results: 89 patients were included in the study, 79 of whom had long term followup available. The mean age was 42 years and 84% were women. The average length of followup was 24.9 months. The primary outcome shows that at followup, 34% of patients had complete relief, 43% had some relief, and only 22% had no relief. As well, an average of 4.33 diagnostic tests per patient were ordered simply to exclude other causes of abdominal pain. The cost of evaluating these patients to rule out a visceral cause of the pain was conservatively estimated at CD$764 per patient. Secondary analyses show that meeting the criteria for AWP (as outlined above), the absence of GI symptoms, and an upper abdominal location of pain were statistically significant predictors of a positive response to TPI. However, gender, radiation of the pain, the presence of a surgical scar, and the type of agent injected were not predictors of response to TPI. Conclusions: AWP is a prevalent yet often inadequately acknowledged and misdiagnosed condition. Our study demonstrates that trigger point injections, in patients who meet the criteria for AWP, are effective over the long term.

Efficacy of Trigger Point Injections for Abdominal Wall Pain

IMMEDIATE RELIEF	COUNT	PERCENT
complete	50	56.2%
some	29	32.6%
none	10	11.2%
LONGTERM RELIEF	**COUNT**	**PERCENT**
complete	27	34.2%
some	34	43.0%
none	18	22.8%

89 patients met the inclusion criteria. 79 had longterm followup. Average length of followup 24.9 months. Injection consisted of <15cc of local anesthetic with steroid.

W1287

How Do US Gastroenterologists Diagnose Irritable Bowel Syndrome?

Brian Mulhall, Brooks D. Cash, Philip Schoenfeld, William D. Chey

Irritable bowel syndrome (IBS) is a prevalent condition frequently diagnosed and managed by gastroenterologists (GE). In an effort to better describe current practices used to diagnose IBS and rule out organic gastrointestinal disorders, we distributed a survey to 120 gastroenterologists randomly selected from the ACG directory. This questionnaire requested demographic information and queried the use of diagnostic studies in the evaluation of patients with suspected IBS. Methods: Individuals were sent a questionnaire and asked to report the frequency of their use of various diagnostic studies for both diarrhea-predominant and constipation-predominant IBS: CBC, electrolytes, LAEs, TFTs, ESR, sprue antibody, stool O&P, culture, Giardia lamblia antigen testing. Additional testing with flexible sigmoidoscopy, colonoscopy, rectal biopsy, barium enema, lactose hydrogen breath testing, breath testing for small bowel overgrowth, FOBT and abdominal ultrasound were also assessed. GE were also asked about their use of the Rome and Manning criteria to diagnose IBS. Results: 41/120 (34%) completed the questionnaire. Of the respondents, 65% were in private practice, 17% were practicing at a university, and 2% were working within the VA system. Over 50% of respondents reported seeing between 11 and 30 patients per day, and almost 70% were working in a single or multi-specialty clinic. Though >90% of respondents were familiar with the Manning, Rome I, or Rome II criteria for IBS, 58% used clinical judgment alone as the primary means of making the diagnosis of IBS (20% used Rome II alone). 68% were familiar with the recently published AGA guidelines for the diagnosis of IBS. Of those who were familiar with the AGA guidelines, none followed the guidelines in a majority of

their IBS patients, and 3.7% followed the guidelines in patients with diarrhea predominant IBS (IBS-D). The most consistently used tests in clinical practice were FOBT, CBC, routine chemistries, and LAEs. Endoscopy was used in the majority of cases (25% sigmoidoscopy, 48% colonoscopy) and was more likely to be performed in those with IBS-D. Other studies were used less consistently. Tests for celiac sprue were conducted in 31% and for small bowel overgrowth in 4% for patients with suspected IBS-D. Conclusion: Though the majority of providers were familiar with at least one symptom based criterion, only a third used such criteria in clinical practice. Clinical practice behavior is inconsistent and does not adhere to published guidelines, based on a small sample of GE.

W1288

Constipation Is Often Undiagnosed or Misdiagnosed in Outpatient Clinics: Diagnosis of Constipation Is Associated with Better Outcomes

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Background: The proportion of patients with constipation in outpatient clinics who receive a diagnosis of constipation, and how such a diagnosis impacts outcomes, is not known. Aims: To examine the implications of clinical diagnosis and treatment outcomes for physician diagnosed and Rome II criteria defined constipation. Method: Patients (N = 1600; 76% female; mean age 53 years; 79% primary care) with clinical diagnoses of IBS, abdominal pain, constipation, or diarrhea seen in primary care and gastroenterology clinics at a large health maintenance organization (HMO) in the Northwestern U.S., completed mailed questionnaires following a clinic visit (59% response rate). Responders completed a 2nd survey 6 months later (76% response rate). Questionnaires included the Rome Modular Diagnostic Questionnaire, questions about prescribed medications, and rating scales for symptom improvement, satisfaction with medical care, and subjective overall health. Results: Of the 18% of respondents who had physician-diagnosed constipation, only 37.2% met ROME II constipation criteria, but 48.6% met ROME II IBS criteria. Of the 15% of respondents with self-reported constipation, the 54.4% who did not have physician-diagnosed constipation were less likely to receive a recommendation for over-the-counter or prescription laxative use (32% vs. 60%, p<.0001); at 6-month follow-up, these patients reported satisfactory relief of symptoms less frequently than those with physician-diagnosed constipation (49% vs. 66%, p = .01). Similarly, of the 22% who met Rome II constipation criteria, 74.4% did not have physician-diagnosed constipation and these patients received laxatives less commonly (22% vs. 64%, p<.0001) and reported satisfactory relief less often at follow-up (51% vs. 65%, p<.05). Satisfaction with medical care and self-ratings of overall health were equal among physician-diagnosed, undiagnosed Rome II, and undiagnosed self-reported constipation patients. Conclusions: Half of self-reported constipation and 3/4 of Rome II diagnosable constipation patients in this HMO sample did not have a physician diagnosis of constipation. These undiagnosed patients received far less medication for constipation and showed less improvement over a 6-month period. Half of patients with physician-diagnosed constipation had IBS according to Rome II criteria, and only about 1/3 met Rome II constipation criteria. [Supported by Novartis Pharmaceuticals and RO1 DK31369.]

W1289

Randomized Comparison of Audio Tape Recorded Enhanced Esophagogastric Cancer Consultations

Michael Stephens, Sarah Pellard, Carolyn Gent, Rhiannon Day-Thompson, Guy Blackshaw, Paul Edwards, Wyn Lewis

Background. Consultations to convey a diagnosis of esophagogastric cancer may be difficult and traumatic for patients and as many as 50% of patients are displeased with the information given to them by their doctors. Aims. The aim of this study was to assess the value of tape recording this consultation, to determine whether this might enhance patients' memory of key facts, and reduce anxiety prior to therapy. Methods. Fifty patients were allocated at random (stratified for sex) to have their consultations audio-taped or not. Twenty-six patients received taped consultations (median age 66 yr, 17m, 9 esophageal, 17 gastric cancers) and were compared with 24 control patients (69 yr, 19m, 11 esophageal, 13 gastric) who did not. All patients completed a hospital anxiety and depression (HAD) questionnaire and were re-interviewed one to two weeks later. Results. Twenty-three patients listened to their tapes (1 patient refused a tape, 2 elected not to listen to their tapes). Patients who had received taped consultations were less likely to forget key facts regarding their diagnosis and treatment options (0 of 26 patients) compared with patients who had not received a tape (8 of 24 patients, Chi2 = 10.3, DF 1, P = 0.001). HAD A scores were similar in both groups [tape 6 (2-10), no tape 6 (0-21)]. HAD D scores were lower in patients who had received a tape [2.5 (0-23)] compared with patients who had not [4 (0-10), P = 0.891]. Whether or not patients received a tape did not influence the surgical resection rate (tape 55% vs. no tape 50%, Chi2 0.105, DF 1, P = 1.0). Conclusion. All of the patients who listened to the tape found it helpful, and in broad terms, tape recorded interviews had a positive effect on the ability of patients and their families to participate in management decisions.

W1290

Validation of Noninvasive Disease Activity Indices for Ulcerative Colitis in U.S. Patients

Peter D. R. Higgins, John Mapili, Marc Schwartz, Sheryl Korsnes, Ellen M. Zimmermann

Background: None of the many disease activity indices for ulcerative colitis have ever been rigorously validated. The classic St. Mark's Index and the commonly used Ulcerative Colitis Disease Activity Index (UCDAI) require invasive endoscopy. The noninvasive Seo Index and Simple Clinical Colitis Index (SCCAI) have not been validated nor evaluated in U.S. patients. A validated index is critical to the performance and interpretation of clinical trials in ulcerative colitis, and a valid noninvasive index would lower costs and minimize subject discomfort. Aim: The noninvasive SCCAI and Seo Index were evaluated in U.S. patients by four standard validity criteria: criterion-convergent, criterion-predictive, content, and construct validity. Methods: Sixty-six consecutive patients were evaluated with the St. Mark's Index, UCDAI, SCCAI, Seo Index, and IBDQ. Criterion-convergent validity was evaluated by correlating the noninvasive indices with the St. Mark's Index. To evaluate criterion-predictive validity, the sensitivity, specificity, and c-statistic for prediction of patient-defined remission were calculated. Content validity was evaluated by performing factor analysis of the items in the four indices, to determine if the noninvasive indices include the significant domains. Construct validity was evaluated by testing for correlation of the non-invasive indices with the bowel symptom subscore of the IBDQ. Regression and factor analysis were performed to define the contribution of endoscopy to invasive indices.
Results: The SCCAI and Seo indices correlate well with the St. Mark's Index, with Spearman correlations of 0.87 and 0.69. Both the SCCAI and the Seo Index predict patient-defined remission well, with specificity, sensitivity, and c-statistic of (0.89, 0.77, 0.91) and (0.89, 0.81, 0.89). Factor analysis demonstrated that the SCCAI captured three of the four significant factors, while the Seo Index lacked two significant factors, but added a novel factor with laboratory tests. The SCCAI and the Seo Index both correlated well with the IBDQ bowel symptom subscore (0.85, 0.65). Endoscopy contributed minimal predictive value (change in r^2 = 0.002) to invasive indices, because endoscopy shared a common factor with hematochezia.
Conclusions: The noninvasive indices meet four criteria for validity in American patients. Both indices predict the clinical endpoint of patient-defined remission. These noninvasive indices can lower costs and subject discomfort in future clinical trials.

W1291

Perceived Role of Scientific Evidence in the Decision to Continue Use of Complementary Therapies by Canadians with Inflammatory Bowel Disease

Laura C. Vanderheyden, Marja J. Verhoef, Robert J. Hilsden

PURPOSE: To explore what factors affect whether patients with inflammatory bowel disease (IBD) who use complementary therapies (CT) for their disease would continue or discontinue use based on scientific evidence indicating that a therapy does not work. METHODS: This was a secondary analysis of data collected in a 2001 mail survey of which the primary objective was to examine CT use by people with IBD. The survey was mailed to 4,453 members of the Crohn's and Colitis Foundation of Canada (response rate 75.5%). The sample for this analysis consisted of 514 current users of CT for their IBD. A specific question on the survey asked, "If a scientific report came out saying that one of the therapies you currently use does not work, would you stop using that therapy?" RESULTS: Of the 514 CT users, 65% (n = 334) responded that they would continue CT use despite scientific evidence indicating that a therapy they use does not work; 35% (n = 180) would discontinue use. Demographic characteristics were not related to consideration of scientific evidence. As reported disease severity increased, respondents were more likely to discontinue CT use based on negative scientific evidence (p = 0.009). As the number of reported CTs used increased, participants were more likely to continue CT use despite negative scientific evidence (p = 0.015). Participants who reported a positive experience with CTs were more likely to continue use despite negative scientific evidence (p<0.03). Last, as participants' desired role in treatment decisions increased they were more likely to continue CT use despite negative scientific evidence (p = 0.037). CONCLUSIONS: Current users of CT place more emphasis on factors other than scientific evidence when deciding about CT use. A focused study to further explore these factors and the role of scientific evidence in patient treatment decisions is needed.

Perceived Effects of CAM on Symptoms by Users Who Would Continue and Discontinue Use Based on Scientific Evidence Indicating a Therapy Does Not Work

Symptom	Continue Use (% Reporting Improvement)	Discontinue Use (% Reporting Improvement)	95% CI for Difference (p-value)
Energy Level	76.0	63.1	4.3-21.6% (0.0026)
Nutritional Status	72.1	57.5	5.6-23.5% (0.0012)
Side Effects of Conventional Medicine	38.6	17.1	13.6-29.4% (<0.0001)
Sense of Well-Being	90.1	74.0	8.8-23.6% (<0.0001)
Stress Level	74.1	58.7	6.5-24.2% (0.0005)
Sense of Control Over Disease	77.6	68.6	0.6-17.3% (0.0299)
IBD Symptoms	83.4	58.7	16.3-33.2% (<0.0001)

W1292

Economic Burden of Acute Diverticular Hemorrhage in The U.S.: A Nationwide Estimate

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Background: Limited data exist on the epidemiology and economic burden of acute lower gastrointestinal hemorrhage associated with diverticular disease. Objective: To analyze a national database of in-hospital inpatient stays to study the epidemiology, outcomes and economic cost of patients admitted with diverticular hemorrhage. Methods: The National Inpatient Sample (NIS) databases of the Healthcare Cost and Utilization Project (HCUP) from year 1997 to 2001 was analyzed with ICD-9-CM diagnostic codes for acute diverticular hemorrhage. The NIS is a stratified sample of approximately 20 percent of US community hospitals, including roughly 7 million discharges from about 1000 hospitals. Results: The weighted national estimates of total number of discharges for diverticular hemorrhage in 2001 was 88,736 with 1.4% in-hospital deaths. Forty-three percent were men; 1.5% were in the age group of 18 to 44 years, 13.6% were between 45 and 64 years, 60.1% were between 65 and 84 years and 24.8% were 85 years or older. The mean length of hospital

stay was 4.9 days, and the mean hospital charges were $14,845 with an estimated national bill of $ 1,303,054,224. For majority of these patients, Medicafe was the primary payer; 15.3% had private insurance. The mean length of stays were 4.8 and 5.1 days for non-teaching and teaching hospitals, respectively; also Medicare patients had longer length of stay compared to patients with private carriers (5.0 days vs. 4.1 days). Patients in the northwest had maximum length of stay (5.5 days) and those in the west had the least (4.3 days). Although, the length of stay progressively decreased from 5.4 days in 1997 to 4.9 days in 2001, the mean hospital charge progressively increased from $12,096 in 1997 to $14,845 in 2001. Conclusion: Acute diverticular hemorrhage is associated with a significant and increasing national economic burden (in excess of 1.3 billion dollars in 2001). Most of theses patients are elderly and have Medicare as their primary payer. Differences exist in the length of hospital stays based on region, type of insurance and also, teaching status of admitting hospitals.

W1293

Diverticular Bleeding: Long Term Outcome and Risk Factors for Recurrence

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Purpose: Diverticular bleeding is the most common cause of acute lower gastrointestinal bleeding leading to hospitalization. Twenty five to forty percent of such patients will have recurrent bleeding within 4 years of their index bleed. Little is known about longer-term outcomes or risk factors, if any, predicting recurrent bleeding. The purpose of this study was to evaluate long-term outcome and identify potential risk factors for recurrence in patients admitted to the hospital for a first episode of diverticular bleeding Methods: A cohort of 146 patients admitted from 1976-1989 for acute lower gastrointestinal bleeding from diverticulosis was investigated using Kaiser Permanente databases. Individuals who had other sources of lower gastrointestinal bleeding or had previous hospitalizations for bleeding were excluded. Additional information about the index admission, readmissions, procedures and studies was gathered through chart review. A proportional hazards regression model was used to evaluate risk factors for subsequent admissions for bleeding. Results: The mean cohort follow up was 9.7 years. During that time, 65 patients (45 %) had 147 hospitalizations for rebleeding. Of the original cohort of 146 patients, twenty-three patients (16%) underwent colonic resections. Six patients (4%) died during readmission for bleeding. 31 patients, or forty-eight percent of patients who had a second admission for diverticular bleeding, had multiple rebleeding admissions. Patient age at initial hospitalization was the only baseline characteristic associated with an increased hazard of readmission (HR 1.03, p=0.03, CI 1.0-1.06). Gender, race, nadir hemoglobin, number of units transfused or use of NSAIDs, aspirin or coumadin do not appear to be associated with an increased hazard of rebleeding. Conclusions: 1. Patients admitted for diverticular bleeding have a 45% chance of having a readmission for rebleeding within 10 years of their index bleed and 48% percent of those will have one or more future admissions for bleeding. 2. Advanced age is weakly associated with an increased risk of rebleeding but gender, admit hemoglobin, nadir hemoglobin, number of units transfused, or use of aspirin, NSAIDs or coumadin do not appear to carry an increased risk of rebleeding.

W1294

Estrogen-Progesterone Therapy in Gastrointestinal Angiodysplasia: A Systematic Review of Controlled Clinical Trials

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BACKGROUND: Gastrointestinal angiodysplasia (GA) may cause recurrent bleeding requiring transfusion. Endoscopic or surgical therapy is associated with high re-bleeding rate. Combination therapy with estrogen and progesterone (EP) was shown to decrease GI bleeding in patients with GA due to Osler-Weber-Rendu (OWR) syndrome or von Willebrand's disease (vWD); these patients may be more likely to respond to treatment than those with idiopathic GA. The efficacy of EP in idiopathic GA is unclear as results from controlled clinical trials (CCT) are conflicting. Our aim was to critically appraise those CCTs of EP therapy in bleeding GA. RESULTS: Our literature search yielded 5 CCTs. Since 1 was an updated version of a previous trial, only the updated version was included. Of the 4 CCTs, 1 had a parallel group design, 1 was a crossover study, 1 a matched cohort study, and 1 a cohort study with the control group made up of those who discontinued EP or were on estrogen monotherapy; only the CCT with a parallel group design was placebo-controlled. Two CCTs were double-blinded and randomized. The CCTs used various EP drugs at different doses. Patients were followed throughout the treatment period that varied from 6 months to 3 years. The 4 CCTs included a total of 184 patients (including 14 in the crossover trial); 97 were treated with EP, 8 with estrogen, and 79 were not treated. Mean age of patients in the 4 CCTs ranged from 68 to 77 years old. Three CCTs included patients with GA confirmed by endoscopy; 1 included patients with recurrent GI bleeding of unknown etiology. Three CCTs listed blood transfusion criteria; 2 transfused when hemoglobin (Hgb) was < 10 g/dl; 1 when Hgb < 8.5 g/dl. EP was superior to control in 2 CCTs; one of which was a double-blinded, randomized (crossover) trial where 57% of patients included had either OWR or vWD; in the other, only those with idiopathic GA was included. Two CCTs did not show benefit of EP; these evaluated patients with idiopathic GA specifically. Only 1 CCT estimated a pre-specified sample size but failed to achieve it; this study did not show a difference in outcomes between the 2 groups. All CCTs reported adverse events (AE) from EP. Only 2 compared them with the control group; 1 showed higher AE in the EP group; 1 showed more cardiac deaths in the controls. CONCLUSIONS: Although 2 of 4 CCTs showed a benefit, studies were of low quality. Data from published CCTs do not justify EP treatment for idiopathic GA. AEs associated with EP further limit its use in clinical practice.

W1295

Predictors of Gastrointestinal Bleeding in Patients Receiving Thrombolytic Therapies for Acute ST-Elevation Myocardial Infarction

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Background: Treatment with thrombolytics in the setting of acute ST-elevation myocardial infarction (STEMI) is associated with a significant risk of gastrointestinal (GI) bleeding. However, predictors for GI bleeding in this population have not previously been well described. Methods: Data from 7 large randomized, controlled trials of thrombolytic therapies in patients with acute STEMI (GUSTO-I/IIb/III, ASSENT-2/3/3 Plus, HERO-2) were pooled to determine baseline factors predictive of GI bleeding. Patients in all 7 trials had acute STEMI and presented within 12 hours of symptom onset. Multivariable logistic regression was used to model the occurrence of moderate or severe GI bleeding using baseline factors. In addition, a 30-day survival model, adjusted for established predictors of mortality, was created to explore the impact of GI bleeding on mortality. Results: From these 7 trials, 10,711 patients were analyzed; 1482 experienced moderate or severe GI bleeding. Significant factors found to be independent predictors of GI bleeding are shown in the table. In addition, GI bleeding was found to be a significant predictor of mortality at 30 days (adjusted odds ratio 1.84, 95% confidence interval 1.41-2.39). Discussion: We revealed baseline factors predictive of significant GI bleeding, and also found that GI bleeding was an independent predictor of 30-day mortality. These analyses suggest the importance of early identification of patients at increased risk for significant GI bleeding following thrombolytic therapy. Further studies should prospectively evaluate treatment strategies in patients at increased risk of GI bleeding following thrombolytic therapy.

Predictors of GI Bleeding

Variable	Wald Chi-Square	Odds ratio	95% Confidence Interval
Age	229.1823	1.502	1.425-1.583
Weight≤70kg	41.4621	0.735	0.670-0.808
HR>60bpm	37.9053	1.101	1.068-1.135
Killip Class	20.4487	1.241	1.130-1.363
Weight>70kg	12.9820	0.896	0.844-0.951
Systolic BP≤135bpm	9.7048	0.928	0.885-0.973
Time from Symptom Onset to Treatment	9.0968	0.952	0.922-0.983
Baseline Hypertension	7.8095	1.175	1.049-1.316
Female Gender	7.7518	1.201	1.056-1.367
Diastolic BP	5.7441	0.940	0.893-0.989

Odds ratios reflect estimated odds of moderate/severe GI bleeding for a 10-unit increase in age, HR, BP, and weight; a one-hour increase in time to treatment; and a one-level increase in Killip class. The C-index for the model is 0.722.

W1296

Assessment of Patient Perception of Bowel Preparation Quality at Colonoscopy

Gavin Harewood, Curtis Wright, Todd Baron

Introduction: There is little published literature evaluating the accuracy of patients perceptions of the quality of their bowel preparation for colonoscopy. The aim of this study was to compare patients perceptions of the adequacy of their bowel preparation with the endoscopists rating at colonoscopy.

Methods: Outpatients undergoing elective colonoscopy completed surveys rating the quality of their colon preparation, type of bowel preparation agent used and proportion of preparation ingested. Both the patient and endoscopist were given the same scale to rate preparation quality: excellent (clear, water-like stool), good (semi-clear, liquid stool), fair (semi-solid stool), poor (solid stool). Patient responses regarding quality of bowel preparation were compared to endoscopists assessment of colonic preparation.

Results: In total, 474 patients were enrolled. Most patients (87.6%) claimed that they had ingested >90% of the preparation prior to the colonoscopy; 99.6% estimated their preparation to be excellent or good compared to 74.9% as judged by the endoscopist. Overall, patients perceptions of the quality of their bowel preparation were inaccurate when compared to the endoscopists rating (sensitivity, 75%; specificity, 34%; accuracy, 50%). Overall correlation with endoscopists rating was low, r = 0.08. Young patient age (<61 years (median age)) was an independent predictor of adequate bowel preparation (p=0.009) (see table). Neither proportion of preparation ingested nor type of preparation agent was predictive of preparation adequacy.

Conclusions: Patients are unreliable judges of the quality of their own bowel preparation, tending to overestimate the cleanliness of their colon. Conversely, patients concern that their preparation may be suboptimal is also inaccurate. These findings suggest that colonoscopy should not be canceled on the basis of a patients perception that the quality of their preparation is poor.

Adjusted odds ratios (and 95% confidence intervals) of variables predicting adequate quality bowel preparation

Variable	Odds ratio (95% C.I.)
Age (>61 yrs vs < 61 yrs (median age))	0.56 (0.36 - 0.86) *
Gender (male vs female)	1.21 (0.79 - 1.86)
Medication (narcotics vs none)	0.64 (0.29 - 1.55)
Prep agent (PEG vs sodium phosphate)	0.81 (0.52 - 1.27)
% prep taken (>90% vs <90%)	1.02 (0.51 - 1.92)

* p = 0.009

W1297

Conjoint Analysis (CA) of Patient Preferences for Colorectal Cancer (CRC) Screening Strategies

John K. Marshall, Deborah Marshall, Reed Johnson, Kathryn Phillips, Lehana Thabane, Gary Foster, Bernie O'Brien

BACKGROUND & AIM: The success of population screening for CRC depends on public uptake and adherence. Discrete choice experiments with CA measure preferences for services that differ in dimensions of process and outcome. We undertook a CA of CRC screening strategies.
METHODS: 6 key attributes of CRC screening strategies were chosen through structured focus groups in Canada and the U.S. CA surveys with 10 choice sets were prepared using a fractional factorial design to maximize D efficiency by maintaining level and utility balance and orthogonality. Surveys were mailed to a random sample of 1074 patients age 40-60 from an Ontario primary care network. β co-efficients from conditional logit regression estimated the marginal utilities of attribute levels. Those for out-of-pocket cost (OOP) were used to estimate marginal value of money and willingness to pay (WTP) for each level.
RESULTS: Of 547 surveys returned (51% male), 485 had complete responses. The relative importances of attributes were consistent with a priori hypotheses and respondents were willing to trade among attributes. Test sensitivity was given greatest weighting (measured by β coefficient range), followed by specificity. β coefficients for attribute levels and approximate WTP (in parentheses) vs. reference levels are summarized in the Table.
CONCLUSIONS: Consumers are willing to trade among attributes of CRC screening strategies, with test sensitivity perceived as most important. Better understanding of public preferences will enhance the design, promotion and uptake of CRC screening programs.
Funded by the Canadian Institutes of Health Research (CIHR)

β Coefficients and Estimated Willingness to Pay for Attibute Levels vs. Reference Level (Parentheses)

TEST	Scan	0.412 ($223)	Stool	0.026 ($74)	Scope	-0.143 ($34)	Enema	-0.243
SENS	90%	1.296 ($898)	70%	0.046 ($473)			40%	-1.342
SPEC	100%	0.553 ($355)	80%	-0.062 ($146)			50%	-0.491
PREP	None	0.388 ($218)	Diet	-0.137 ($39)			Purge	-0.251
PAIN	None	0.177 ($120)					Mild	-0.177

W1298

Does Published Data Support Recent Multi-Society Colon Polyp Surveillance Guidelines? A Systematic Review of the Evidence

Sameer Saini, Philip Schoenfeld

Background: Current multi-society guidelines (Gastroenterology 2003; 124: 544) stratify patients with a personal history of adenomas as low-risk (< 2 small adenomas at index colonoscopy) or high-risk (> 3 small adenomas or > 1 advanced adenoma at index colonoscopy) for advanced adenoma recurrence. These guidelines recommend five-year intervals between surveillance colonoscopies for low-risk patients and three-year intervals for high-risk patients.
Objective: To systematically review data about the prevalence of adenomas at three-year surveillance colonoscopy among low-risk patients and high-risk patients.
Methods: Computer searches of MEDLINE, PREMEDLINE, and EMBASE were performed to identify appropriate studies. Study-selection criteria were: (1) study design: prospective registry-based, prospective cohort, or prospective randomized controlled trial; (2) study population: patients with a personal history of adenomas; and, (3) intervention: completion of surveillance colonoscopy at an interval of ≥ 2 years. Independent, duplicate data extraction was performed by both authors. Data were extracted on: (1) prevalence of advanced and non-advanced adenomas at index and surveillance colonoscopies; (2) interval between colonoscopies; and, (3) risk factors associated with recurrent adenomas.
Results: Fifteen studies met study-selection criteria. No study stratified patients according to the low-risk and high-risk groups suggested by current surveillance guidelines. Studies used widely variable definitions of low-risk and high-risk groups and risk factors. The prevalence of recurrent advanced adenomas at surveillance colonoscopy was 1.4-8.2% in low-risk patients versus 3.2-26.1% in high-risk patients. Increasing number of adenomas and increasing size of adenomas at index colonoscopy were risk factors for advanced adenoma recurrence.
Conclusions: The prevalence of advanced adenomas at surveillance colonoscopy ranged widely and overlapped considerably between low-risk and high-risk groups. Prospective studies utilizing standardized definitions of low-risk and high-risk groups should be performed to validate the recent guidelines.

W1299

Barriers to Compliance with Screening Colonoscopy Among Hispanics and Non Hispanic Whites

Murtaza Parekh, Antonio Serna IV, Michael Urbano, Yasser Shaib, Tahir Qaseem

Purpose: Colorectal cancer (CRC) is the second leading cause of cancer related death in the US. In a previous study, it was found that Hispanics were less likely to present for a screening colonoscopy than Non-Hispanic Whites (NHW) at the University of New Mexico GI Endoscopy Center (p 0.0022). This study is designed to examine the barriers that may contribute to the difference in compliance with screening colonoscopy. Methods: One hundred and five patients scheduled for screening colonoscopy at the UNM GI Endoscopy Center who failed to undergo the procedure from October 2002 to December 2002 and May 2003 to August 2003 were contacted via telephone for a follow up questionnaire. This included reasons for non compliance as well as whether the procedure was explained to the patient. Ethnicity of the subjects was determined using the GUESS program which is 90-95% accurate in New Mexico. Fishers Exact Test was used to analyze the data and compare reasons for non-compliance among Hispanics and NHW. Results: There were 12 reasons patients gave for non-compliance which were placed in 5 groups: procedure specific (difficult prep, scared, didn't want procedure), logistics (at work, no ride), cost, unrelated circumstances (ill, out of town, death in family, not specified) or scheduling problems (forgot, scheduled too far in advance). There was no statistically significant difference among the groups when comparing ethnicity alone. However, when the additional variable of whether or not the procedure was explained was analyzed, there was a difference in barriers to compliance between Hispanics and NHW who were not explained the procedure ($p = .02$). For Hispanics, logistics was significant ($p = .05$), for NHW, procedure related issues were significant ($p = .01$). Conclusions: When comparing barriers to screening among Hispanics and NHW who were not explained the colonscopy procedure, there were statistically significant differences between the two ethnic groups in logistics and procedure specific barriers. Hispanics were more likely to cite logistics and NHW were more likely to cite procedure specific barriers. These specific problems, as well as education about the procedure, can be targeted for potential interventions to improve compliance.

W1300

Outcomes of Follow-up Colon Examination Among a Population-Based Cohort of Colorectal Cancer Patients

Stephen J. Rulyak, Margaret T. Mandelson, David A. Lieberman, Andrew D. Feld, J. T. Ylvisaker, Edward H. Wagner

Background: The benefits of follow-up colon examination among colorectal cancer survivors are uncertain, and the findings of surveillance colonoscopy are not well characterized. Methods: We studied all enrollees in Group Health Cooperative with local or regional colon or rectal cancer diagnosed between 1993 and 1999 who underwent surgical resection. Utilization of colon examination with colonoscopy or flexible sigmoidoscopy plus barium enema was determined from automated clinical records. Mortality was estimated using survival analysis, and findings of colon examinations were determined by review of pathology reports. Results: A total of 1292 cases of colorectal cancer were diagnosed during the study period, and 1002 patients met inclusion criteria. Five-year survival was 76.8% for patients who had at least one follow-up colon exam ($n = 652$) and 52.2% for those who did not ($n = 350$) [$p < 0.0001$]. Survival did not differ according to timing of follow-up (see table). After adjustment for age, gender, race, stage, and comorbidity, colon examination remained a predictor of survival [HR 0.58; 95% CI: 0.44-0.75]. A total of 936 colon examinations were conducted during follow-up (mean = 1.4 exams/patient). Exam findings included: carcinoma ($n = 10$; 1.1%), adenoma ($n = 206$; 22.0%), or hyperplastic polyps ($n = 159$; 17.0%). Advanced neoplasia (adenoma > 1 cm, villous adenoma, or carcinoma) was more common when initial follow-up occurred 36-60 months after diagnosis compared with exams performed within 18 months (see table) [$p = 0.02$]. Patients with adenomas prior to or at the time of diagnosis were more likely to have advanced neoplasia on follow-up compared to patients without a history of adenomas (12.1% versus 5.3%; $p = 0.002$). Patients with advanced neoplasia on initial follow-up frequently had advanced neoplasia on repeat exam (13/16 [81%]). Conclusions: Colorectal cancer patients who undergo follow-up colon examinations have improved survival, but the survival benefit can not be explained entirely by early detection of neoplasia. There does not appear to be additional benefit associated with earlier follow-up exams, but patients with a personal history of adenomas and those with advanced neoplasia at initial follow-up may warrant more intensive surveillance.

Outcome of Follow-up Colon Examinations

Initial Follow-up Interval	5-year survival	Advanced Neoplasia
<18 months (n=484)	78.0 %	6.9 %
18-35 months (n=110)	75.7 %	8.2 %
36-60 months (n=58)	77.3 %	15.5 %
No follow-up exam (n=350)	52.2 %	--

W1301

Diabetes Mellitus Is a Risk Factor for Colon Cancer: A Case Control Study

Vikas Khurana, Rambabu Chalasani, Tejinder Singh, Daniel L. Halberg, Charlton Fort

PURPOSE: To explore the association between diabetes mellitus and the risk of developing colon cancer in US veterans. BACKGROUND: The incidence of insulin resistance is increasing in the US where colon cancer remains the second leading cause of cancer death. The geographic patterns for colon cancer and diabetes are strikingly similar; both diseases were considered relatively rare before industrialization and their incidence usually increases in regions undergoing economic development. Mechanistically, insulin resistance has been associated with hyperinsulinemia, increased levels of growth factors including IGF-1, and alterations in NF-kappaB and peroxisome proliferator-activated receptor signaling, which may promote colon cancer through colonocyte stimulation. We further examined the association between diabetes and the incidence of colon cancer. METHODS: We conducted a retrospective, cross sectional, case control study, at Overton Brooks VA Medical Center, at Shreveport, LA. We evaluated the medical records of patients from the period October 1998 through June 2003. There were a total of 50715 patients in our database, of which 50697 were selected and 18 were excluded due to incomplete data. Mean age was 60.4 years and 91% were males. Multiple logistic regression analysis was done and the data was adjusted for obesity, smoking, use of aspirin and alcohol. RESULTS: Of the 50697 patients in the study, 8,974 (17.7%) had diabetes mellitus. Diabetic patients were 32% more likely to have colon cancer than patients without diabetes (Odds ratio 1.32; 95% CI 1.12 to 1.55). The data was controlled for aspirin use, obesity, smoking and alcohol use. Additionally, alcohol (odds ratio 1.33, 95% CI 1.11 to 1.61) and obesity (odds ratio 1.28, 95% CI 1.09 to 1.51) were identified as significant covariates in this model. DISCUSSION: Our data should be evaluated with caution, given the limitations of the population, the database and the fact that this is a case control study. Duration and degree of control of diabetes was not factored into the

analysis. Some factors known to increase the risk of colon cancer like family history and inflammatory bowel disease were not incorporated in the study. However the large size of the database was felt to limit the effects of these factors. CONCLUSION: Our data supports and adds to the growing evidence that diabetes is a risk factor for colon cancer in US veterans.

W1302

Natural History of Patients with Liver Cirrhosis in Sweden During the Last Decade

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Background: Liver cirrhosis is the most common liver disease in the western world. Data on the natural history of cirrhosis after the discovery of hepatitis C are limited. Previous studies from the 70s demonstrated very high mortality from gastrointestinal bleeding (Christensen et al. Gastroenterology 1981; 81: 944). Conflicting results exist in the literature whether prognosis of the patients with cirrhosis is dependent on the etiology. The aim of the study was to investigate the incidence, etiology and prognosis of liver cirrhosis in Sweden and to study whether the etiology influences the prognosis. Methods: All consecutive patients diagnosed with liver cirrhosis for the first time in Gothenburg Sweden (inhabitants 500.000) from January 1994 to June 2003 were included. Etiology of cirrhosis, complications, treatment, prognosis and cause of death were analyzed. The median follow-up (of those who survived 3 months) 36 months (range 3-96). Results: 778 patients were diagnosed (66% men) with a mean age of 60 ± 13 years, in patients with alcoholic liver disease (ALD) 59 ± 11 years and those with non-alcoholic liver disease (NALD) 63 ± 15 years ($p<0.05$). The mean incidence was 16 patients per 100.000 inhabitants per year (range 13-21). ALD was present in 413 (53%), Hepatitis C (HCV) in 75 (10%), ALD + HCV 73 (9%) and cryptogenic cirrhosis 116 (15%), PBC 31 (4%), NASH 15 (2%), Hepatitis B 16 (2%), PSC 8 (1%), others 31 (4%). Child-Pugh classes: 140 (18%) of the patients presented with class A, 257 (33%) B and 381 (49%) C. A total of 352 (45%) had esophageal varices and 451 (58 %) ascites at diagnosis. HCC developed in 62 (8%) and 51 (7%) of the patients underwent liver transplantation. 408 patients (54%) died during follow-up with 55% deaths among patients with ALD and 51 % with NALD ($p=$NS). 26 (3%) died within one week from the diagnosis of the cirrhosis and among those 15/778 (2%) died from their first variceal bleeding. 27 % of the patients died within 1 year from the diagnosis. The five-year survival was 50%. Causes of death: liver failure 34%, variceal bleeding 10%, GI-bleeding 4%, infections 10 %, HCC 13 %, other malignancies 7 %, non-liver related causes in 22%. Conclusions:The cause of liver cirrhosis was alcoholic liver disease in more than 50% of patients. Patients with ALD had similar prognosis as patients with other etiologies. Liver cirrhosis was associated with high mortality during the last decade but mortality from variceal bleeding has decreased and very few die from their first variceal bleeding.

W1303

Aggressive Local Ablation Therapy May Be Effective in Retaining Tumor Stage of Hepatocellular Carcinoma within Milan Criteria

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Backgrounds: The dropout rate from the waiting list for liver transplantation among transplant candidates with hepatocellular carcinoma (HCC) has been reported as 30-40% at 1 year, mostly due to tumor progression. It is challenging to suppress the tumor extent within Milan Criteria and to extend "safe" waiting time. Local ablation therapy may help in reducing the dropout rate. Aims: To calculate the probability of dropout due to tumor progression beyond Milan Criteria among cirrhotic patients with HCC in non-transplant setting, and to analyze the factors affecting the outcome. Methods: Retrospective cohort study was conducted using hospital database. A total of 458 patients were diagnosed with HCC at Department of Gastroenterology, Tokyo University Hospital between January 1997 and December 2001. Among 398 patients who underwent percutaneous local ablation therapy, 285 patients met Milan Criteria and were selected for analysis. Tumor progression beyond Milan Criteria was defined as "dropout". Death without tumor progression was also considered in calculating overall probability of dropout from the list. Cox proportional hazard model was used to evaluate the risk for dropout of the following factors; age, gender, hepatitis C, hepatitis B, child classification, history of alcohol consumption, ascites, serum albumin level, serum DCP, serum AFP, tumor size and number at the time of diagnosis. Results: After a median follow-up period of 16.7 (range 1-73) months, 200 of 285 patients (70%) were alive and retained within Milan Criteria, 26 (9%) died without tumor progression, and 59 (21%) dropped out. The tumor aspects at dropout were the increase in number in 28, size growth in 13, portal vein tumor thrombosis in 7, distant metastasis in 4, tumor seeding in 3, tumor rupture in 1, bile duct invasion in 1, and not specified in 2. The 1- and 2-year overall dropout rate were 8.5% and 18.3%, and the 1-, and 2- year dropout probability due to tumor progression were 5.6% and 12.3%. By Cox regression analysis, tumor size (>3cm, $p=.015$), high AFP level (>100ng/dl, $p=.036$), high DCP level (>100mAU/ml, $p<.0001$) and serum albumin (<3.5g/dl, $p=.026$) were found to be significant independent factors for dropout due to tumor progression. Conclusion: The 1 year dropout rate from waiting list was 8.5% in overall and 5.6% due to tumor progression, which was much lower than it had been reported. Local ablation may be helpful in reducing dropout rate, when this type of therapy is applicable.

W1304

Incidence of Gastroduodenal Ulcers in Patients with Arthritis and Previous Ulcer Bleeding After 6 Months of Celecoxib or Diclofenac Plus Omeprazole: A Randomized, Double-Blind Study

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Background & Aims The gastric safety of cyclooxygenase-2 inhibitors or the combination of a nonsteroidal anti-inflammatory drug with a proton pump inhibitor in high-risk patients is unclear. We sought to evaluate the incidence of gastroduodenal ulcers in patients with arthritis and a recent history of ulcer bleeding who received celecoxib or diclofenac plus omeprazole in a 6-month, prospective, double-blind, randomized gastrointestinal (GI) outcome study. Methods Patients with arthritis and a recent history of ulcer bleeding who were negative for *Helicobacter pylori* and had confirmed ulcer healing were randomly assigned to celecoxib 200 mg twice daily plus omeprazole placebo or diclofenac 75 mg twice daily plus omeprazole 20 mg once daily for 6 months. Endoscopy was performed in a treatment blinded fashion on patients who: (i) developed recurrent GI bleeding; and (ii) completed or exited the study without GI complications. The endpoint was ulcer with a diameter of at least 5 mm and a perceptible depth. Results 287 patients were enrolled (143 in the celecoxib group and 144 in the diclofenac plus omeprazole group). 24 patients developed recurrent GI bleeding; 16 of them were adjudicated to have ulcer bleeding (7 in the celecoxib group and 9 in the diclofenac plus omeprazole group). 2 patients defaulted follow-up and 2 died. Of 259 patients who did not develop GI complications, 222 underwent follow-up endoscopy (116 in the celecoxib group and 106 in the diclofenac plus omeprazole group. The 6-month cumulative incidence of ulcer was 19% (95% CI, 11.3 to 26.1) in the celecoxib group and 26% (95% CI, 17.1 to 34.1) in the diclofenac plus omeprazole group ($P=0.21$). The combined incidence of bleeding and endoscopic ulcers was 24% (95% CI, 16.3 to 31.8) in the celecoxib group and 31% (95% CI, 22.7 to 40.0) in the diclofenac plus omeprazole group ($P=0.21$). 65% of ulcers in the celecoxib group and 42% of ulcers in the diclofenac plus omeprazole group recurred at previous ulcer locations. Age above 75 (RR 5.8; 95% CI, 1.7 to 19.6), treatment-related severe dyspepsia (RR 5.5; 95% CI, 2.6 to 11.5) and coexisting medical conditions (RR 2.2; 95% CI, 1.2 to 4.0) were independent risk factors for recurrent ulcers. Conclusion The incidence of gastroduodenal ulcers was high among patients with arthritis and a recent history of ulcer bleeding who received celecoxib or diclofenac plus omeprazole.

W1305

Maintained Symptom Control with Esomeprazole Following Initial Treatment of Upper GI Symptoms of Patients on NSAIDs Including COX-2-Selective NSAIDs

Christopher J. Hawkey, Neville Yeomans, James M. Scheiman, Nicholas J. Talley, Joseph Sung, Roger Jones, Jorgen Naesdal, Goran Langstrom

Purpose: All NSAIDs, including COX-2-selective NSAIDs, cause upper GI symptoms which we have shown to be improved with esomeprazole[1]. We now report results of two studies showing maintained improvement over 6 months with esomeprazole.
Methods: Patients who achieved upper GI symptom relief (none/mild for last 7 days, maximum 2 days rated as mild) in the acute studies[1] were re-randomized into two identical, multicenter, placebo-controlled, double-blind studies. Patients received placebo, esomeprazole 20 mg or esomeprazole 40 mg once daily (qd) orally for 6 months. Upper GI symptoms (pain, discomfort or burning in the upper abdomen) were recorded on patient daily diary cards using a 7-grade scale from "none" to "very severe". Relapse was defined as moderate or severe symptoms (a score of 3-6 for ≥3 days in any 7 day period). The primary endpoint was the proportion of patients with relapse of upper GI symptoms (from diary cards) through 6 months of treatment.
Results: The pooled ITT population comprised 594 patients (34% were taking only COX-2-selective NSAIDs). Both doses of esomeprazole were significantly more effective than placebo in preventing the relapse of upper GI symptoms (Table). Compared with placebo, esomeprazole 20 mg and esomeprazole 40 mg also resulted in significantly ($p<0.05$) more patients with no heartburn (placebo: 62.4%; esomeprazole 20 mg: 75.5%; esomeprazole 40 mg: 81.3%) or acid regurgitation (placebo: 67.3%; esomeprazole 20 mg: 78.1%; esomeprazole 40 mg: 86.5%) at 6 months. The results are similar across patients taking non-selective and COX-2-selective NSAIDs.
Conclusion: Esomeprazole 20 mg and 40 mg qd are more effective than placebo in preventing relapse of upper GI symptoms in non-selective and COX-2-selective NSAID users over 6 months.
References:
1. Yeomans N, et al. Gastroenterology 2003; 124(4, Suppl 1): A 107

Table: Estimated cumulative proportion of patients with relapse of upper GI symptoms (diary card)

Pooled ITT population	Placebo (%) (95% CI)	Esomeprazole 20 mg (%) (95% CI)	Esomeprazole 40 mg (%) (95% CI)
Month 1	31.5 (25.1-37.9)	14.8 (9.8-19.9)	11.2 (6.7-15.7)
Month 3	35.9 (29.2-42.6)	20.9 (15.0-26.9)	20.3 (14.4-26.3)
Month 6	39.1 (32.2-46.0)	29.3 (22.3-36.2)	26.1 (19.4-32.9)
Log rank test, p-value vs. placebo	-	0.0059	0.0006

W1306

Gastrointestinal Prevention Strategies with Either Cox-2 Inhibitors or Generic and Non-Generic Proton Pum Inhibitors Plus NSAIDs Are Equally Cost-Effective in Patients with Risk Factors

Angel Lanas

Background: COX-2 selective inhibitors (coxibs) are GI safer than non-selective NSAIDs, but coxib penetration in European market is poor. The main barrier for the expansion is cost, since coxib are more expensive than the alternative PPI plus NSAIDs (generics are available in several countries). Aim: To evaluate the costs per ulcer bleeding event prevented with current strategies. Methods: Two strategies have been considered: a) prescription of standard doses of coxibs and b) prescription of generic NSAIDs plus either generic omeprazole or brand PPI. All published evidence has been analyzed (PubMed). No specific clinical scenarios have been considered if evidence was not available. Factors considered to determine costs were the number of patients needed to treat (NNT) to prevent a bleeding event, the cost of the drug (2002) and the period of time of treatment in NNT. Costs are given in Euros (E) and prices obtained from one European country where generic omeprazole is available. Results: Cost of hospitalizations of a bleeding event is not superior to 2661E. No strategy is cost-effective in patients with no risk factors (51127-71483E with generic PPI/NSAID; 95153-106515E with brand PPI/NSAIDs; 70463-123187E with coxibs). However, all strategies are cost-effective in the high-risk patient with previous ulcer bleeding (1360-1902E with generic PPI and NSAID; 2205-2835E with brand PPI and NSAIDs; 1701-2016E with coxibs; 1548-1756E with coxib plus PPI - a 50% reduction of complications needed). In the presence of other risk factors all strategies are cost-effective but to prevent one event is twice as expensive in the age range of 65-74 (10128-25641E with PPI and NSAID; 12318-14600E with coxibs) than in patients older than 75 (range:4051-5840E). No strategy shows clear superiority in terms of costs, unless the cheapest NSAID and PPI are always prescribed. If coxib therapy confirms a 50% reduction in the incidence of lower GI complications, this option may be more cost-effective than the others (2376-3261E with generic PPI plus NSAID; 1458-1728E with coxibs in the high-risk patient). Conclusions: The prescription of COX-2 inhibitors or either generic or brand PPI plus NSAIDs are only cost effective in patients with risk factors. Coxib therapy would be more cost-effective if it reduces by 50% the incidence of lower GI events. Generic PPI plus NSAIDs would be more cost-effective if the cheapest available drugs were prescribed.

W1307

Digestive Risk of NSAIDs Assessed by French GPs: Results of a National Survey in 1777 GPs

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Introduction: Gastrointestinal toxicity is the most common serious adverse event associated with the use of non-steroidal anti-inflammatory drugs (NSAIDs). Although several factors appear to be associated with an increase of the risk of NSAID-related complications, there are not definitive recommendations for adding gastroprotective agents (GPA) to a medical prescription of NSAIDs. The aim of this study was to analyze the factors that determine a NSAID-GPA co-prescription by GPs in clinical practice. Material and Methods: During a one week period, a quota-determined sample of 1777 French GPs included adult patients for whom an NSAID was prescribed. Patient characteristics and the rationale for co-prescription were obtained using a standardized questionnaire identifying previous and current digestive symptoms as well as the presence of one (or more) identified risk factor(s) (IRF) (age > 65, history of peptic ulcers or complications, concomitant use of aspirin, anticoagulants, corticosteroids). Analysis was performed by comparing the results observed in both groups of patients according to the presence or not of a co-prescription of a GPA (respectively NSAID-GPA+ vs NSAID-GPA- patients). Results: 20993 patients were included. The NSAID-GPA+ patients (n = 7072) were significantly older than the NSAID-GPA- patients (n = 13921) (respectively 55.7 versus 46.2 yrs, $p<0.01$). The prescription of a GPA was associated with at least one IRF in 41.2% of the cases. Among patients without IRF having received a GPA, 78.9% had a history of at least one of the following digestive disorders: chronic idiopathic dyspepsia, NSAIDs induced dyspepsia, gastroesophageal reflux disease. In all, 92.3% of NSAID-GPA+ co-prescriptions were associated with IRFs and/or a history of upper gastrointestinal (GI) symptoms. Finally 27.3% of the NSAID-GPA- patients had at least one IRF. Conclusion: In more than 9 patients out of 10 treated with NSAID, the co-prescription of a GPA is associated with IRF (41.2%) and/or a history of upper GI symptoms. However more than one-fourth of the patients treated with NSAIDs do not receive a GPA though they have at least one IRF. This study shows the necessity of establishing more precise recommendations for the prescription of a GPA in the course of NSAID treatments. Finally, additional studies should more precisely determine whether physicians take into account other factors for their prescription (predictable duration, quality of life of patients, compliance, associated pathologies, etc.).

W1308

Gastrointestinal Safety and Anti-Inflammatory Activity of HCT-1026, NO-Releasing Flurbiprofen, in Healthy Humans Volunteers

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Background. HCT-1026 is a nitric oxide (NO)-releasing derivative of flurbiprofen. Aims. To investigate the effect HCT-1026 on gastrointestinal mucosa and COX-1 and COX-2 activity in human healthy volunteers. Methods. We enrolled in a parallel-group, double-blind, placebo controlled study, 32 healthy subjects were randomly allocated to receive 7 days treatment with HCT-1026 (100 and 150 mg b.i.d), flurbiprofen (100 mg b.i.d.) or placebo. Upper endoscopies were performed before and at the end of treatment period and gastroduodenal lesions assessed using a pre-specified scoring system. Basal and post-treatment platelet aggregation in response to arachidonic acid (AA), TXB_2 plasma levels and ex vivo generation of PGE_2 and IL-6 from isolated granulocytes stimulated with endotoxin were assessed. Results. Flurbiprofen administration caused gastric and duodenal damage (total mean endoscopic score of 17.6 ± 2.0; $P<0.001$ versus placebo). Administration of HCT-1026 resulted in a significant attenuation of gastrointestinal damage: mean endoscopic score were 7.0 ± 1.9 and 8.0 ± 1.7 with 150 mg/bid (i.e. equimolar to 100 mg/bid flurbiprofen) and 100 mg bid HCT-1026, respectively. Flurbiprofen and HCT-1026, 150 and 100 mg bid, caused 96%, 95% and 96% inhibition of platelet aggregation induced by AA and >99% inhibition of in vivo TXB_2 formation and > 85% reduction of urinary 11-DH-TXB_2 excretion. All drugs inhibited ex vivo generation of PGE_2 as well as gastric PGE_2 and 6-K-$PGF1\alpha$. Finally, HCT-1026, 100 mg/bid but not flurbiprofen, inhibited endotoxin-induced IL-6 release from isolated granulocytes. Conclusions. HCT-1026 is a potent COX-1 and 2 inhibitor. In comparison with flurbiprofen HCT-1026 causes 80-100% reduction of gastric/duodenal ulcers. This data confirm that addition of an NO-releasing moiety to NSAIDs is an effective strategy to reduce gastrointestinal damage in humans.

W1309

Upper Gastrointestinal Bleeding and the Use of Non-steroidal Anti-inflammatory Drugs in Hemophiliacs

Shonda M. Asaad, Barbara Kroner, Sylvia Cohn, James Goedert, Elaine Eyster, Hemophilia Cohort Study Group/Multicenter

The use of non-steroidal anti-inflammatory drugs (NSAIDs) in persons with hemophilic arthropathy is controversial because of bleeding concerns, especially upper gastrointestinal (UGI) bleeding. The incidence of UGI bleeding in persons with hemophilia (PWH) is unknown. The purpose of this study was to determine the incidence of UGI bleeding and the relationship of UGI bleeding, obstruction or perforation to the use of conventional nonselective NSAIDs and cyclooxygenase selective inhibitors (COX-2) in PWH. UGI bleeding was defined as 1) hematemesis or detection of occult blood in the stools with endoscopically verified ulcer, or 2) melena, occult blood in the stools without hematochezia, or endoscopically verified gastritis, accompanied by a drop in hemoglobin of at least 2 grams or requiring red cell transfusion. The study population consisted of all PWH enrolled in the second Multicenter Hemophilia Cohort Study (MHCS II). Beginning May 1, 2002, data were collected prospectively on 1347 subjects from 51 hemophilia treatment centers, ages 13 to 89, mean 36.8, median 35 years. They were evaluated a mean of 7 months during the 12 month study period, totaling 810 person years (py) of follow up. 11 experienced UGI bleeding for an incidence of 1.4 per 100 py. None developed obstruction or perforation. 567 (42%) reported using some type of NSAID. 248 of these used NSAIDs within 2 weeks of the baseline visit, of whom 115 (46%) used only COX-2. Relative risk (RR) of bleeding was not increased in those on NSAIDs, but was 3-4 times higher in those with hemophilic arthropathy or impaired Karnofsky status, although the differences were not significant. RR of bleeding was increased 5.3 times (95% CI 1.3-20) in those with platelet counts below 130,000/cu mm. None with UGI bleeding had a clotting factor inhibitor or an abnormal prothrombin time. Bleeding RR was 2.2 (95% CI 0.5-11) with at least 4 alcoholic drinks per week. We conclude that the 1.4% annual incidence of clinically important UGI bleeding events in PWH is higher than the 0.1- 0.4% background rate reported in the general population not taking NSAIDs, but is similar to the 1-2% rate reported in non-hemophilic users of nonselective NSAIDs. After 810 py of follow up, the incidence of UGI bleeding is not significantly different in PWH taking NSAIDs compared to those who are not.

W1310

The Comparative Healing of Gastric Ulcers with Esomeprazole Versus Ranitidine in Patients Taking Either Continuous COX-2 Selective NSAIDs or Nonselective NSAIDs

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Purpose: Two studies with identical protocols compared the effects of esomeprazole or ranitidine on the healing of gastric ulcers associated with the use of nonsteroidal anti-inflammatory drugs (NSAIDs). Methods: Patients receiving either nonselective NSAIDs or cyclooxygenase-2 [COX-2] selective NSAIDs with confirmed gastric ulcers (≥5 mm in diameter but no ulcer >25 mm) and *H. pylori* negative were randomized into 1 of 2 double-blind, multicenter, parallel-group studies to receive esomeprazole 40 mg once daily, esomeprazole 20 mg once daily, or ranitidine 150 mg twice daily. The patient's existing NSAID therapy was continued during the treatment phase of the study. Patients underwent repeat endoscopy at week 4 and week 8. The primary endpoint of the studies was gastric ulcer healing status through week 8. Results: A total of 809 patients formed the combined intent-to-treat population: 262 (esomeprazole 40 mg once daily), 276 (esomeprazole 20 mg once daily), and 271 (ranitidine 150 mg twice daily). Overall 15% of patients were receiving COX-2 selective agents. Gastric ulcer healing rates for the 3 groups are shown in the Table. Both doses of esomeprazole and ranitidine were well tolerated and no safety concerns were raised. Conclusions: Esomeprazole is significantly more effective than ranitidine in healing gastric ulcers associated with the continuous use of NSAIDs, both all NSAIDs and nonselective NSAIDs only. Although the numbers are low, the results for patients taking COX-2 selective NSAIDs show a similar trend for increased efficacy of esomeprazole versus ranitidine.

Week 8 Gastric Ulcer Healing Rates for Different Acid Suppressive Regimens

Treatment	All NSAIDs % Healed (95% CI) (n=809)	COX-2 Selective NSAIDs % Healed (95% CI) (n=121)	Nonselective NSAIDs % Healed (95% CI) (n=686)
Esomeprazole 40 mg once daily	88.6* (84.7, 92.4)	94.6† (80.5, 99.1)	87.6* (83.2, 91.9)
Esomeprazole 20 mg once daily	86.6* (82.6, 90.6)	91.2‡ (75.2, 97.7)	86* (81.6, 90.3)
Ranitidine 150 mg twice daily	75.3 (70.1, 80.4)	80.0 (65.9, 89.5)	74.4 (68.7, 80.2)

*P<0.002 vs ranitidine 150 mg twice daily †P=0.051 vs ranitidine 150 mg twice daily ‡P=0.165 vs ranitidine 150 mg twice daily

W1311

Overall Control Of NSAID-Induced Dyspepsia with the Proton Pump Inhibitor (PPI) Pantoprazole: A Placebo Controlled Trial on the Onset of Symptom Relief

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Purpose: Treatment with NSAIDs causes dyspeptic symptoms in up to 40% of patients. While the proton pump inhibitor pantoprazole has shown to prevent NSAID lesions, we aimed to determine the efficacy of pantoprazole on the global relief and onset of symptom relief in NSAID-induced dyspepsia.
Methods: 747 patients with dyspeptic symptoms associated with NSAID treatment were recruited for this double-blind, placebo-controlled, multicenter trial. Patients were treated for four weeks and randomly assigned to receive either pantoprazole (20 mg o.d., N = 381 ITT) or placebo (o.d., N = 366 ITT). Only patients with no history of gastro-esophageal reflux or peptic ulcer disease, were eligible. Gastrointestinal symptoms were assessed utilizing a standardized questionnaire (interview) at entry and every 3 to 4 days, and the symptom load was calculated accordingly. Primary outcome parameter was the overall symptom load (cumulated symptom intensities) after 28 days. Comparisons were done using Wilcoxon rank sum test (two-sided, 5% level). In addition, we aimed to identify the earliest time point of a meaningful (significant) reduction of symptoms.
Results: Median symptom loads were significantly lower after 4 weeks in patients receiving pantoprazole than in the placebo group (28 vs 39, $p<0.0001$). Interestingly, already after 2 weeks the post hoc analysis yielded a significant difference in favour of pantoprazole (23 vs 27, $p<0.0015$) while differences after one week just failed significance (16 vs 17, $p<0.06$). The lower incidence of adverse events in patients treated with pantoprazole (20 vs 22 %) did not yield significance.
Conclusions: Compared to placebo, pantoprazole significantly decreased NSAID-associated dyspeptic symptoms. Effects occurred within the first week of treatment and the gain over placebo steadily increased up to 28 days of treatment.

W1312

Eradication of Helicobacter pylori Does Not Prevent Peptic Ulcers in Patients with Long-term Treatment with NSAIDs: A Randomized, Double Blind Placebo Controlled Trial

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Objective Both infection with *Helicobacter pylori* and use of NSAIDs are major causes of peptic ulcer disease (PUD). Eradication of *H. pylori* substantially decreases the rate of recurrence of PUD in patients not taking NSAIDs. However, studies on the benefit of eradication of *H. pylori* in NSAID users have had conflicting results. Therefore, we investigated whether *Helicobacter pylori* eradication in *H. pylori* infected patients receiving long-term treatment with NSAIDs reduces the incidence of PUD.
Methods For this randomised, double blind, placebo-controlled study, patients with a rheumatic disease were recruited from 8 outpatient clinics in the Netherlands. Inclusion criteria were long-term treatment with NSAIDs, age between 40 and 80. Patients who were positive for *H. pylori* on serological testing (ELISA) were eligible for the study. Patients were randomly assigned *H. pylori* eradication therapy with omeprazole 20 mg, amoxycillin 1000mg, and clarithromycin 500 mg twice daily, (OAC) or placebo for seven days. The primary endpoint was the presence of endoscopic gastric or duodenal ulcers (>5 mm) at the 3-month endoscopy. Secondary outcome measures were: complications of peptic ulcers, erosions, dyspepsia, quality of life, and adverse events. Endoscopy was performed 3 months after randomisation. Other outcomes were assessed during 12 months after randomization, with repeat endoscopy if clinically required.
Results Of 2761 patients screened, 1091 (40%) were positive for *H. pylori*. A total of 347 eligible patients (61% female, mean age 59 years, 87% Caucasian) consented to be included in the trial. About half of them were on chronic gastroprotective medication. At 3 months, endoscopic peptic ulcers were diagnosed in 6 (4%) and 8 (5%) patients in the eradication group and in the placebo group respectively ($p = 0.65$). During follow-up of 12 months, no symptomatic ulcers, ulcer bleedings, or ulcer perforation occurred in either group. No significant differences were found in the development of gastroduodenal erosions, dyspepsia, or quality of life.
Conclusion Eradication of *H. pylori* has no beneficial effect on peptic ulcer disease in patients with long-term treatment with NSAIDs.

Number of patients with peptic ulcers 3 months after randomization

Type of ulcer	Group		p value
	Eradication (n=155)	Placebo (n=160)	
Gastric	5 (3%)	6 (4%)	0.80
Duodenal	1 (1%)	2 (1%)	0.59

W1313

Prevalence of Troublesome Dyspeptic Symptoms, Symptomatic and Silent Ulcers in Patients Treated with NSAIDs: A Prospective Study in Primary Care

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Background: NSAIDs belong to the most frequently prescribed drugs in primary care. Side effects, especially of the gastrointestinal tract, are common. However, data gathered prospectively in routine clinical practice are scarce. The aim of the present study, therefore, was to evaluate the frequency of troublesome dyspeptic symptoms and ulcers in the upper gastrointestinal tract in primary care patients on NSAID treatment. Patients and Methods: Consecutive patients attending a private practice in Germany who required at least two weeks of NSAID treatment were asked to participate in this study. Patients below the age of 18, with troublesome dyspeptic symptoms at entry, a requirement for H2-blocker, PPI, misoprostol or sucralfate therapy, or a history of upper GI surgery were excluded, as were patients who refused endoscopy. A structured questionnaire and an upper GI endoscopy, including biopsy of the stomach, were undertaken in all patients. Results: 104 patients (median age 53 years, range 26 to 80 years; 91 women) entered the study during a period of 1 year. Four patients had to be excluded from the analysis because of a history of partial stomach resection (two) and late refusal of endoscopy (two). Moderate to severe (troublesome) dyspeptic symptoms occurred in 35 % of the patients, with epigastric pain being the most frequent complaint (20%). Seventeen patients showed ulcers at endoscopy (duodenal ulcer: n = 4; gastric ulcer: n = 11; duodenal and gastric ulcer: n = 1; cardia ulcer without evidence of reflux disease: n = 1). Patients with peptic ulcers complained more frequently of troublesome epigastric pain than patients without ulcer (35% vs 18%; p = ns), but 10 out of 17 ulcer patients had no (n = 7) or only mild dyspepsia. Risk factors for an ulcer were regular alcohol intake (OR 4.49, $p<0.02$), smoking (OR 5.11, $p<0.01$), and intake of the NSAID for less than 1 month (OR 4.95, $p<0.03$). Age, gender, *H.pylori status*, type of NSAID, and concomitant aspirin use were not significantly related to the occurrence of an ulcer. Conclusion: Average risk patients in primary care frequently develop troublesome dyspeptic symptoms during treatment with an NSAID. The prevalence of peptic ulcers during NSAID therapy was 16%, with many of the ulcers being clinically silent. Risk factors for ulcer development were alcohol consumption, smoking, and a short history of NSAID therapy

W1314

Erradication of Helicobacter Pylori Prevents the Appearance of Peptic Ulcer in Patients Treated with NSAID. A Meta Analysis

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Epidemiological studies show that the two main risk factors for the appearance of peptic ulcer are Helicobacter pylori (Hp) infection and the use of non-steroidal anti-inflammatory drugs (NSAID). However, it is not clear whether the eradication of Hp prevents the appearance of peptic ulcer in chronic NSAID users. Objective: By means of a meta-analysis, to evaluate whether Hp treatment prevents peptic ulcer in chronic NSAID users. Material and methods: A MEDLINE search was performed. Search strategy included the words: (NSAID OR non-steroidal anti-inflammatory drugs) AND (Helicobacter pylori) limited to randomized trials. Abstracts of the articles obtained and papers presented at EHPSG and AGA congresses from 1996 to 2003 were also examined. Inclusion criteria were: 1) randomized trials 2) that included positive Hp patients. 3) The patients were randomized in at least two branches of treatment; (Hp eradication + NSAID (E) vs no eradication + NSAID (NoE) or E vs NoE + proton pump inhibitors (PPI) (NoE + PPI). Main outcome was development of peptic ulcer during follow-up. Meta-analysis was conducted using conventional shareware (Review Manager 4.2). Peto Odds Ratio and 95% confidence intervals were calculated for each of the comparisons. Results:. Five studies were included, with a total of 945 patients. The rate of peptic ulcer in the NoE vs E groups was 74/481 (15.4%) vs 33/464 (7.1%) respectively in the intention to treat analysis. Peto Odds Ratio was 0.40 (IC95%;0.26-0.63). Per protocol analysis could only be carried out in 3 studies with a total of 302 patients. Results were similar: 33/152 (21.7%) developed an ulcer in the NoE group vs 12/150 (8%) in the E group. Peto OR: 0.33 (95%CI; 0.18-0.63). Two studies with a total of 385 patients analyzed E vs NoE + PPI. The results could only be evaluated by intention to treat analysis. The rate of peptic ulcer was 5/196 (2.6%) in the E group vs 0/189 (0%) in the NoE + PPI group. Peto OR 7.43 (1.27-43.64). Conclusion: In patients treated with NSAID and Helicobacter pylori infection, eradication reduces the risk of peptic ulcer. However, eradication alone is less effective than PPI treatment in the prevention of NSAID ulcers.

W1315

Endoscopic Characteristics and H. Pylori Infection in NSAIDs-Induced Gastric Ulcer

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Background and aim: In the recent years, H. pylori infection and nonsteroidal antiinflammatory drugs (NSAIDs) are deeply involved in the etiology of gastric ulcer. The incidence of gastric ulcer attributable to NSAIDs is expected to increase in the progress of aging society and when H. pylori eradication therapy is extensively performed in the future. Subjects and methods: Fifty patients (23 males, 27 females, mean age 66.5 years old) diagnosed to have NSAIDs-induced gastric ulcer in the past 2 years were used as the subjects of study. In addition, 100 patients with gastric ulcer that was induced by factors other than NSAIDs ("non-NSAIDs gastric ulcer") were provided as the control group. Their sex and age were matched with those in the NSAIDs-induced gastric ulcer group. The morphology of ulcer, number of lesions, size, onset site, incidence of bleeding ulcer, incidence of H. pylori infection were investigated in both groups. H. pylori was diagnosed by serum anti-H. pylori IgG antibody and Giemsa staining. Results: NSAIDs-induced gastric ulcer demonstrated significantly more multiple lesions (34/50; 68% vs 20/100; 20%, $p< 0.001$), higher incidence in the antrum (28/50; 56% vs 6/100; 6%, $p< 0.001$) and higher incidence of bleeding ulcer (17/50; 34% vs 4/100; 4%, $p< 0.001$) in comparison with non-NSAIDs gastric ulcer. There was no difference in the morphology and size of ulcer between the two groups. The rate of H. pylori infection in the NSAIDs-induced gastric ulcer group was significantly lower (24/50; 48% vs 96/100; 96%, $p< 0.001$) in comparison with non-NSAIDs gastric ulcer group. Conclusion: NSAIDs-induced gastric ulcer frequently occurs in the antrum with bleeding in comparison with non-NSAIDs gastric ulcer. The rate of H. pylori infection in the NSAIDs-induced gastric ulcer is significantly lower in comparison with non-NSAIDs gastric ulcer group.

W1316

U.S. Adults Often Use Over-the-Counter (OTC) Analgesics Inappropriately and Without Safety Concerns

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Background: While OTC analgesics, such as NSAIDs and acetaminophen, are commonly used, there is little information about how analgesics are used by US adults, specifically in regard to safety. Methods: This report combines results of two large surveys of adults in the United States. The first, done by Roper Starch in 1997, evaluated the perceptions of analgesic users on the effectiveness, safety, awareness of side effects, risk of complications, and belief that early warning signs would precede serious complications. The second much larger survey, done in 2003 by The National Consumer League (NCL), similarly assessed consumers' understanding of analgesic use. Results: (see table) Conclusions: OTC analgesics are often taken inappropriately and believed to be safe, as evidenced by 82% of users in 1997 and 41% of users in 2003 not being concerned about side effects. Almost 20% of OTC analgesic users considered themselves to be in less than good health. A third of users are likely to combine OTC analgesics with prescribed NSAIDs. Educational intervention for both patients and physicians is warranted to decrease GI adverse effects with OTC analgesics.

Characteristics of OTC Analgesic Use

Percent of Respondents	1997 Roper (n=258)	2003 NCL (n=3557)
Taking OTC analgesic daily	27%	15%
Taking more than recommended OTC dose	26%	44%
Ibuprofen-based analgesic	57%	50%
Naproxen-based analgesic	11%	15%
Aspirin analgesic	10%	18%
Acetaminophen analgesic	14%	45%
Current health is less than good	19%	14%
Concerned about side effects	18%	59%
Safe to combine OTC with Rx NSAID	-	33%

W1317

Survivin, an Anti-Apoptosis Protein, Is a Major Target for NSAIDs-Induced Gastric Mucosal Injury

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Background: NSAIDs cause gastrointestinal erosions and ulcers. Apoptosis is one of the mechanisms. Survivin, a member of the Inhibitors of Apoptosis Protein family, binds to microtubules, promotes cell cycle progression and inhibits apoptosis. It is expressed during fetal development, and is highly expressed in human cancers, including GI cancers. Recent studies demonstrate that it is expressed at high basal levels in normal human and rat gastric mucosa. Its potential role in gastric mucosal defense and injury is unknown. The aims of this study were to examine the roles of survivin in NSAIDs-induced gastric mucosal injury *in vivo*, and in maintaining integrity and survival of gastric epithelial cells *in vitro*. *Methods*: Rats were treated with 45mg/kg indomethacin (nonselective NSAID), 10mg/kg NS-398 and 10mg/kg celecoxib (COX-2 selective NSAIDs), and 40mg/kg SC-560 (COX-1 selective NSAID) for 3 and 8 hours. Gastric epithelial, RGM-1 cells with and without suppressed survivin expression (by siRNA) were treated with 0.5mM indomethacin for 6 and 24 hours. *Studies in vivo*: 1) The extent of macroscopic gastric mucosal erosions, 2) microscopic injury by quantitative histology, and 3) survivin expression in gastric mucosa by Western blotting and immunostaining. *Studies in vitro*: To determine the requirement for survivin in defense against injury, and survivin regulation in response to injury, we suppressed survivin expression in RGM-1 cells by siRNA, and examined its effect on 1) RGM-1 cell integrity, and 2) the extent of NSAIDs-induced RGM-1 injury by LDH release assay. *Results*: 1) Indomethacin treatment *in vivo* reduced survivin protein levels by ~50% compared to controls ($p = 0.017$), and this effect preceded the occurence of gastric erosions. 2) Selective COX-1 or COX-2 inhibitors did not alter survivin levels or cause severe mucosal injury. 3) Suppression of survivin expression increased RGM-1 cell injury by 230% ($p<0.001$), and enhanced NSAIDs-induced cellular injury by 250% ($p<0.001$) compared to controls. *Conclusions*: Our study demonstrates for the first time: 1) Indomethacin significantly reduces survivin levels in the gastric mucosa and this action precedes the occurence of gastric erosions, 2) Selective COX-1 or COX-2 NSAIDs do not reduce survivin levels or injure the gastric mucosa, and 3) survivin is a major molecular target for nonselective NSAIDs such as indomethacin.

W1318

Microsomal Prostaglandin E Synthase (mPGES) -1 Regulates Hepatocyte Growth Factor (HGF) Release in Gastric Fibroblasts Stimulated with Interluekin(IL)-1beta

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Background: Prostaglandins (PGs) are known to be important factors in maintaining gastric mucosal integrity. However, only limited information is available regarding the mechanism by endogenous PGs. Recent papers identified two PGES isozymes, that is cytosolic PGES and microsomal PGES-1, but the expression and roles of these enzymes are not clarified yet. We and others have shown that gastric fibroblasts play a key role in gastric mucosal repair through PGE2 and growth factors. We also demonstrated mPGES expression of gasric interstitial cells in ulcer tssues. Aims- To clarify the expression of cPGES and mPGES in gastric fibroblasts in vitro, and the effects of these enzymes on HGF release in these cells. Methods:Gastric fibroblasts were cultured in RPMI1640 and stimulated with and without IL-1beta in the presence or absence of selective COX-2 inhibitor: NS-398 or mPGES inhibitor:MK-886. The supernatant was harvested during 24 hour after the stimulation, and amount of HGF and prostaglandin (PG) E2 was measured by ELISA and EIA, respectively. Fibroblasts were also harvested to detect the expression of COX-1, COX-2, cPGES and mPGES-1 protein with Western blot analysis. Messenger RNA in the cell-lysates was also detected by real-time PCR with specific primers of COX-1, COX-2, cPGES and mPGES. PGES activity was measured by short incubation of the cell-lysates with PGH2, which is the substrate of PGES, under the presence or absence of MK886. Immunofluorescence staining of the fibroblats was also performed by specific antibodies against COX-1, COX-2, cPGES, and mPGES. Results:1. IL-1did not enhance the protein expression of COX-1 and cPGES, but that of COX-2 and mPGES, in cultured gastric fibroblasts during 24 hours. 2. Real-time PCR analysis also showed that IL-1 stimulated mRNA expression of COX-2 and mPGES in gastric fibroblasts in vitro. 3. PGES activity was increased in the cell-lysates of gastric fibroblasts stimulated with IL-1. 4. MK-886 significantly suppressed PGE2 and HGF release of gastric fibroblasts stimulated with IL-1, as well as NS-398. Additional PGE2 restored the inhibition of HGF release by MK-886. 6. COX-2 and mPGES immunoreactivity were co-localized in the same IL-1stimulated fibroblasts. Conclusion: These data suggest that mPGES plays an important role in HGF release in cultured gastric fibroblasts stimulated with IL-1, and might play an important role in regulating gastric integrity during gastric ulcer healing.

W1319

Lipoxin A_4 is Protective in Gastric Mucosal Ischemia-Reperfusion (I-R) Damage in Rats

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Background: Consecutive metabolism of arachidonic acid by aspirin-acetylated cyclooxygenase (COX)-2 and 5-lipoxygenase yields 15(R)-epi-lipoxin (LX)A_4. Alternatively, 15(S)-LXA_4 can be generated by combined action of 5- and 12- or 15-lipoxygenase. In the rat stomach, aspirin increased formation of 15(R)-epi-LXA_4 which counteracted aspirin-induced damage whereas inhibitors of COX-2 or the LXA_4 receptor antagonist Boc1 aggravated aspirin injury (Fiorucci et al., 2002). We examined whether LXA_4 generated in the absence of aspirin modulates gastric injury during I-R. Methods: In rats (4-6 rats/group), the celiac artery was clamped for 30 min. After 60 min reperfusion, gastric damage was assessed by calculation of a lesion index (LI). Gastric mucosal fragments were incubated for 10 min at 37°C to assess release of leukotriene (LT)C_4 (RIA) as marker of 5-lipoxygenase activity. Results: I-R alone induced minor damage (LI 4 ± 0.3). The LXA_4 receptor antagonist Boc1 (0.3-1 μg/kg, ip) or the 5-lipoxygenase inhibitor MK-886 (2.5-5 mg/kg, sc) dose-dependently increased I-R damage reaching a LI of 42 each at the highest dose used ($p<0.001$ vs controls). Oral dosing of indomethacin (3 mg/kg), the COX-2 inhibitors celecoxib (3 mg/kg) and rofecoxib (3 mg/kg), the COX-1 inhibitor SC-560 (7.5 mg/kg) or dexamethasone (2 mg/kg, sc) markedly (LI >33, $p<0.001$ each) aggravated I-R injury. The increase in damage induced by COX suppression was dose-dependently antagonized by LXA_4 (1.25-2.5 μg/kg, ip). Thus, at the dose of 2.5 μg/kg, LXA_4 diminished ($p<0.001$) injury to a LI <10 with all compounds studied. In contrast, LXA_4 (2.5 μg/kg) did not protect against injury induced by 70% ethanol (LI 36 ± 1.3 vs 37 ± 1.3 in controls). Furthermore, LXA_4 did not counteract the reversal of the protective effect of the mild irritant 20% ethanol (LI 11 ± 1) induced by indomethacin (2.5 mg/kg, LI 36 ± 2 vs 36 ± 3) or rofecoxib (0.2 mg/kg, LI 35 ± 2 vs 30 ± 1.1). MK-886 (5 mg/kg) reduced mucosal formation of LTC_4 by 59%. Conclusions: 1) The LXA_4 receptor antagonist Boc1 and the 5-lipoxygenase inhibitor MK-886 substantially aggravate I-R damage. 2) Treatment with exogenous LXA_4 reverses the damage-aggravating effects of COX inhibitors and dexamethasone in I-R. 3) LXA_4 does not protect against damage caused by 70% ethanol or the reversal of mild irritant-induced protection elicited by COX inhibitors indicating specificity of the LXA_4 effect. 4) The findings suggest that the gastric mucosal LXA_4 system is involved in the resistance of the gastric mucosa against I-R damage.

W1320

Selective Inhibition of COX-1, but Not COX-2, Produces Damage to the Gastric Mucosa of Portal Hypertensive Rats Without Affecting Normal Gastric Mucosa: Mechanistic Considerations

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BACKGROUND/AIMS: Inhibition of both cyclooxygenase isoforms (COX-1 and COX-2) is required to produce gastric mucosal damage and it has been suggested that this is due to a compensatory increase in COX-2 expression levels following selective COX-1 inhibition (*J. Physiol. Paris* 95: 21, 2001). Portal hypertensive (PHT) gastric mucosa has increased susceptibility to injury and impaired healing but the underlying mechanisms are not fully elucidated. This study was aimed to determine: 1) whether selective inhibition of COX-1 or COX-2 alone is sufficient to cause damage to PHT gastric mucosa; 2) how such damage compares to that caused by non-selective NSAIDs; 3) the possible mechanism(s) mediating damage caused by selective COX inhibition. *METHODS*: PHT was produced in rats by staged portal vein occlusion and splenic vein ligation. Sham operated (SO) rats served as controls. At 14 days, fasted rats received i.p. either vehicle; 40 mg/kg indomethacin (a non-selective COX inhibitor); 10, 20, 30, or 40 mg/kg SC-560 (a selective COX-1 inhibitor); or 10mg/kg NS-398 or celecoxib (selective COX-2 inhibitors) and gastric tissue was obtained 8 hrs later. *STUDIES*: 1) Extent of macroscopic injury. 2) COX-1 and COX-2 expression by Western blot and immunohistochemistry. 3) Portal vein pressure measurement. *RESULTS*: Indomethacin treatment caused gastric hemorrhagic lesions in both PHT and SO rats with damage being more severe in PHT vs. SO gastric mucosa (17.9 ± 4.7 vs. 5.4 ± 2.2 mm^2; $P<0.01$). Neither NS-398 nor celecoxib treatment caused gastric mucosal damage in either group. SC-560, at all doses tested, also did not produce any damage to SO gastric mucosa. In contrast, SC-560 treatment dose-dependently induced hemorrhagic lesions in PHT gastric mucosa (1.8 ± 1.0 — 2.7 ± 1.1 mm^2). COX-2 protein expression levels were significantly reduced by 49% ($P<0.05$) in gastric mucosa of PHT vs. SO rats treated with SC-560. *CONCLUSIONS*: 1) PHT gastric mucosa has increased susceptibility to damage caused by non-selective NSAIDs. 2) Selective inhibition of COX-1, but not COX-2, is sufficient to cause damage to PHT gastric mucosa. 3) Lack of compensatory increases in COX-2 expression levels may explain the injury produced to PHT gastric mucosa by selective COX-1 inhibition.

W1321

Gastrointestinal and Cardiovascular Safety of HCT-3012, NO-Naproxen, in Aspirin-Treated Arthritic Rats. Evidence That NO Compensates for COX-1 and COX-2 Inhibition

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Background. Administration of non steroidal anti-inflammatory drugs (NSAIDs) being or not selective inhibitor COX-2 to arthritic patients taking aspirin increases the risk of gastrointestinal (GI) bleeding. HCT-3012 is a NO-releasing derivative of naproxen with reduced GI and cardiovascukar (CV) toxicity. Whether HCT-3012 maintains GI safety in patients taking aspirin is unknown. Aims. To examine anti-inflammatory activity, GI and CV safety of HCT-3012 in comparison with naproxen and celecoxib in arthritic rats treated with aspirin. Methods. Arthritis was induced in Lewis rats by s.c. injection of Freund complete adjuvant (FCA). Rats were treated with naproxen (10 and 20 mg/Kg), HCT-3012 (14.5 or 29 mg/kg) and celecoxib (30 mg/kg) alone or in combination with aspirin (30 mg/Kg) from day 7 to 21. Animals were sacrificed on day 22 and gastric mucosal injury, prostaglandin E_2, plasma TXB_2,IL-6 and blood pressure assessed. Results. HCT-3012, naproxen and celecoxib, alone or in combination with aspirin effectively reduced hindpaw edema and number of tail nodules ($P<0.01$ vs arthritic, n=6/group). HCT-3012 alone or in association aspirin, but not naproxen or celecoxib, significantly reduced the plasma levels of IL-6 ($P<0.01$ vs arthritic). Naproxen administration caused more gastric damage than HCT-3012 and celecoxib ($P<0.001$). Administration of naproxen and celecoxib, but not HCT-3012, to rats treated with aspirin, exacerbates gastric injury caused by aspirin ($P<0.01$ vs naproxen and celecoxib alone). Gastric COX-2 expression was significantly increased in arthritic rats and its expression was further enhanced by naproxen and celecoxib in combination with aspirin, but not by HCT-3012. All treatments, but celecoxib alone, reduced the gastric PGE_2 and plasma TXB_2 ($P<0.01$ vs arthritic rats). Rats treated with naproxen alone or in combination with aspirin, showed significantly higher blood pressure than both control group and rats treated with HCT-3012 alone or in combination with aspirin ($P<0.05$ vs naproxen alone). Conclusions. In contrast to naproxen and celecoxib, HCT-3012 does not exacerbate GI damage caused by aspirin and might represent an alternative to selective and non-selective COX-2 inhibitors in the treatment of inflammatory conditions in aspirin-taking patients.

W1322

Induction of Claudin-4 and Other Tight Junction Related Proteins by NSAIDS in Cultured Gastric Cells

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Background & Aims: In addition to the anti-inflammatory action, non-steroidal anti-inflammatory drugs (NSAIDs) have other various positive and negative actions. Epidemiological studies have shown that prolonged use of aspirin or other NSAIDs reduces the risk of cancer and Alzheimer's disease. Furthermore, NSAIDs have gastrointestinal side effect, which becomes serious clinical issue. Since these actions of NSAIDs cannot be fully explained by the cyclooxygenase (COX)-inhibition by NSAIDs, COX-independent actions of NSAIDs are necessary to be identified. For this purpose, by use of DNA microarray analysis, we here searched genes whose expression is altered by NSAIDs in human gastric cancer cells (AGS). Methods: AGS cells were incubated with indomethacin for 24 hr and expression of genes (about 16, 000 genes) was analyzed by DNA microarray technique. Expression of claudin and other genes were confirmed by real time PCR, northern-blotting and/or immuno-blotting experiments. Results: We found 17 and 19 genes whose expression increased or decreased, respectively, by indomethacin. Among them, we focused claudin-4 and other tight junction-related proteins (claudin-1 and occludin), which were induced by indomethacin. The induction of these proteins was confirmed by several methods. Other NSAIDs (diclofenac and celecoxib) also induced these proteins. The induction of claudin-4 was inhibited by addition in the medium of neither prostaglandin E2 (PGE2) nor inhibitor of MEK-ERK pathway or PPAR-alpha. We recently found that NSAIDs induce endoplasmic reticulum (ER) stress response. Thapsigargin and tunicamycin (inducers of ER stress response) also induced the expression of caludin-4. Conclusions: Results suggest that the function of tight junction is positively altered by NSAIDs. We consider that this action of NSAIDs may be involved in their anti-tumor activities, because it was recently reported that over-expression of claudin-4 in tumor cells suppressed their invasion activity. Results also suggest that ER stress response but not COX (PGE2), MEK-ERK pathway and PPAR-alpha is involved in the induction of these proteins by NSAIDs.

W1323

Activation of PPARgamma and Modulation of the Expression of PPARgamma Target Genes by NSAIDs in Gastric Epithelial Cells

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(Background and Aim) Although most of the pharmacological actions of NSAIDs arise from inhibition of COX, recent evidence also suggests the presence of possible targets of NSAIDs other than COX. Lehman et al. (JBC 272; 3406) showed that indomethacin and some other NSAIDs can serve as ligands for PPARgamma. Since PPARgamma plays an important role in the regulation of cell growth and cell death in gastrointestinal epithelial cells, we examined the effect of various NSAIDs on the PPARgamma activity and the expression of PPARgamma target genes in gastric epithelial cells. (Methods) MKN45 and AGS, cell lines derived from human gastric cancer, were used. NSAIDs used in this study were indomethacin, ibuprofen, piroxicam, naproxen, phenylbutazone, NS-398, aspirin, sodium salicylate, and 5-ASA. To monitor PPARgamma activity, a reporter vector which contained multiple copies of PPRE (peroxisome proliferator responsive element) upstream of the luciferase gene was made (PPRE-Luc). pSV-beta-galactosidase reporter vector was always co-transfected for standardization. DNA synthesis of the cells was assessed with Cell Proliferation ELISA (Roche). Real-time quantitative RT-PCR was performed to analyze the expression of PPARgamma target genes, TSC-22 (JBC 278;7431), adipophilin (JBC 276; 29681), TFF2 (APT 18;119). GW9662 was used as a specific inhibitor of PPARgamma. (Results) (1) Among NSAIDs tested, 24h-incubation with indomethacin (100 microM), ibuprofen (100 microM), NS398 (100 microM), aspirin (5 mM), and sodium salicylate (5 mM) significantly up-regulated the transcription of PPRE-Luc, which was sensitive to GW9662 (30 microM). (2) These effects of NSAIDs were dose-dependent and maximum effect was observed at 125-250 microM in the case of indomethacin and at 5-10 mM in the case of aspirin. (3) Indomethacin (1-250 microM) and aspirin (0.5-10 mM) suppressed DNA synthesis of the cells in a dose-dependent manner which was partly sensitive to GW9662. (4) 24h-incubation with indomethacin (100 microM) or aspirin (5 mM) caused more than several-fold increase in the expression levels of adipophilin, TSC-22, and TFF2, which were also sensitive to GW9662. (Conclusions) In addition to indomethacin and ibuprofen, we found that NS-398, aspirin, and sodium salicylate also activate PPARgamma at high concentrations. These results suggest that some of the pharmacological effects of NSAIDs are mediated by PPARgamma in gastric epithelial cells.

W1324

Nitric Oxide Induced by Aspirin Mediates Leukocyte Recruitment Within Mesenteric Venules

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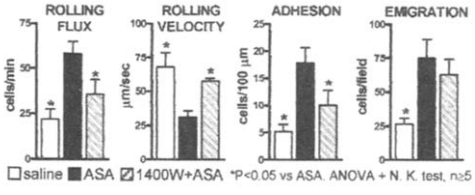
NSAIDs induce pro-inflammatory events and modulates nitric oxide synthase expression in the gastrointestinal tract. The role of iNOS during inflammation is controversial and opposite effects depending on the type, location and phase of the inflammatory response have been reported. AIM: To analyse the role of iNOS-derived NO on the inflammatory process induced by gastrolesive doses of aspirin (ASA). METHODS: Fasted rats were administered orally with vehicle (CMC 1%, 1ml/rat) or ASA (150 mg/kg). Some rats received the iNOS selective inhibitor 1400W (5 mg/kg, s.c.) 10 min before. Six hours later, rats were anaesthetised with pentobarbital (65 mg/kg, i.p.) and leukocyte rolling flux, velocity, adhesion and emigration were measured in mesenteric venules by intravital microscopy. iNOS mRNA expression in gastric corpus was analysed by quantitative RT-PCR. RESULTS: Oral ASA increased leukocyte/endothelial cell interactions. The increase in rolling and adhesion was prevented by pre-treatment with 1400W while emigration was not altered (graph). A significant increase (3.2 ± 0.2 fold) in iNOS mRNA expression compared to vehicle was observed 6h after ASA, ($P<0.05$, Student's t-test, n=3). CONCLUSION: iNOS-derived nitric oxide is involved in leukocyte/endothelial interactions induced by ASA treatment and may contribute to gastric damage associated to this circumstance



W1325

Identification of the TPO1 in Yeast and Its Possible Human Homologue, Tetran, Which Cause Phenotype Resistant to NSAIDS

Hyun-Jung Hwang, Tohru Mizushima

Background & Aims: Non-steroidal anti-inflammatory drugs (NSAIDs), such as indomethacin, have a serious gastrointestinal side effect and the individual variation of sensitivity to this side effect makes the use of this type of dugs more difficult. Howeve r, genes that affect this sensitivity have not been identified. We recently reported that not only cyclooxygenase-inhibition by NSAIDs but also the direct cytotoxicity of NSAIDs are involved in NSAID-induced gastric lesions. The purpose of this study i s to identify genes that affect this direct cytotoxicity of NSAIDs. Methods: Genomic expression library of S. cerevisiae was prepared and genes whose over-expression in yeast causes phenotype resistant to indomethacin were screened. A BLAST search was used to identify the human homologue of the screened yeast gene and the effect of its over-expression in cultured gastric cells on NSAID-induced cell death was examined by MTT method. Results: The TPO1 gene, which encodes a major facilitator superfamily (MFS) transporter located at plasma membranes, was identified as such a gene. Over-expression of TPO1 in yeast made cells resistant to other NSAIDs. On the other hand, deletion of TPO1 from chromosomes made yeast cells sensitive to NSAIDs. TPO1 mRNA wa s induced by indometahcin. TETRAN, tetracycline transporter-like protein, that was predicted to be MFS drug transporter based on its amino acid sequence, was identified as a possible human homologue of TPO1. Over-expression of TETRAN in human gastric cel l s made cells resistant to indomethacin and other NSAIDs. TETRAN mRNA was also induced by indometahcin. Conclusions: There results suggest that TPO1 and TETRAN are efflux pumps for NSAIDs. This is the first report as for NSAID-resistant genes. As a r es ponse to NSAID-treatment, yeast and human cells may make themselves resistant to NSAIDs by inducing TPO1 and TETRAN, respectively. We consider that activity and expression of TETRAN is one of factors determining the sensitivity of each patient to gast roi ntestinal side effect of NSAIDs. n

W1326

Differential Effects of Selective or Non-selective Inhibitors of Nitric Oxide Synthase on the Expression and Activity of Cyclooxygenase-2 During Gastric Ulcer Healing

Guo Jin Sheng, Wang Ji Yao, Cho Chi Hin, Koo M. Wing Leung

Two isoforms of nitric oxide synthase (NOS), i.e., endothelial and inducible nitric oxide synthase (eNOS and iNOS), and cyclooxygenase-2 (COX-2) are important enzymes that were involved in gastric ulcer healing. Close relationship may exist between these two

enzyme systems. The aim of this study was to investigate the effects of selective or nonselective inhibition of NOS on the expression and activity of COX-2 during the healing of gastric ulcers. Gastric kissing ulcers were induced in male Sprague-Dawley rats by luminal application of acetic acid solution. A potent selective iNOS inhibitor, N-[3-(aminomethyl)benzyl] acetamidine (1400W, 0.1 mg/kg/day), or a nonselective NOS inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME, 10mg/kg/day), were intraperitoneally administrated to the rats at the day onwards of ulcer induction. The ulcer size was measured at day 1, 3, and 7 after ulcer induction and the gastric ulcer tissues were collected. The protein levels of COX-2 in the ulcer tissues, and activated nuclear transcription factor κB (NF-κB) in the nuclear protein extract of the ulcer tissues were analyzed with Western blotting. NOS activity was measured as the ability of tissue homogenates to convert L- [^{3}H]-arginine to L- [^{3}H] citrulline. COX activity was measured as the concentration of PGE_2 in the tissue homogenates. It was found that 1400W accelerated ulcer healing while L-NAME induced a visible healing impairment. The expression and activity of COX-2, the activation of NF-κB in the gastric ulcer tissues were down regulated with the administration of L-NAME, but not 1400W. We concluded that iNOS might contribute to ulcer formation while COX-2 and eNOS might promote ulcer healing. There is a positive regulation of eNOS on COX-2 expression and activity in the healing of gastric ulcers, possibly through modulating the activation of NF-κB.

W1327

Effect of *H. Pylori* Eradication on Development of Gastric and Duodenal Ulcers (GDU) in NSAID Users: A Meta-analysis

Chang-Cheng Wang, Yuhong Yuan, Richard H. Hunt

Background: *H.pylori* infection and NSAIDs are the most important risk factors for the pathogenesis of peptic ulcers and serious GI complications. However, controversy exists regarding the effect of eradication of *H.pylori* on prevention of GDU and ulcer complications in NSAID users. We aimed to study this issue through meta-anlaysis. Methods: A comprehensive English language literature search for clinical trials addressing this issue was conducted using Medline, Pubmed and Cochrane databases up to September 2003. 52 papers were retrieved; 10 met inclusion criteria (randomized studies reporting raw data on eradication of *H.pylori* in adult NSAID users). Results: ITT analysis of NSAIDs-naive patients (n=192) showed an incidence rate of GDU in the eradication group significantly lower than that in the non-eradication group (8.33% vs 28.13%, $p<0.01$). The incidence of DU (2.08% vs 10.42%) and symptomatic ulcer (3.13% vs 15.63%) were markedly lower in the eradication group ($p<0.05$), while the incidence rate of GU (6.25% vs 15.63%, and bleeding ulcer (0% vs 4.17%) was slightly lower in the eradication group ($p>0.05$). In low-dose aspirin (≤325mg/day) users (n=373), the ulcer rebleeding rate in the eradication group was markedly lower than that in the non-eradication group (1.06% vs 4.84%, $p=0.04$), while the relapse rate of GU (1.60% vs 4.84%, $p=0.09$) and DU (0% vs 0.54%, $p=0.05$) was slightly lower in the eradication group. In current NSAID users, the incidence of GU (2.16% vs 2.90%), DU (0.9% vs 3.73%), and erosions (19.91% vs 22.82%) was not different between the eradication (n=231) and placebo (n=241) groups ($p>0.05$), but less dyspepsia was reported in the eradication group (24.68% vs 33.20%, $p=0.04$). There was no difference in the healing rate of GU (51.75% vs 65.49%) and DU (51.4% vs 44.25%) at 8 wks between the eradication (n=221) and omeprazole (n=236) groups ($p>0.05$). Conclusion: In NSAIDs-naive patients, eradication of *H.pylori*infection before NSAID therapy significantly reduces the occurrence of NSAID associated GDU and symptomatic ulcer but not bleeding ulcer. In patients taking low-dose aspirin, eradication of *H.pylori* significantly reduces ulcer rebleeding but not recurrent ulcer. Eradication of *H.pylori* does not significantly increase the ulcer healing rate in current long-term NSAID users. *H.pylori* infection should be tested for and treated in patients who require long-term NSAIDs.

W1328

Influence of Zaprinast (Type V Phosphodiesterase Inhibitor) on the NSAID-Induced Experimental Enteropathy

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Enteropathy is a frequent toxic effect of NSAID. Their pathogenic mechanisms are practically unknow and we lack effective treatment. Reciently. it has been reported that NSAID-NO have a good intestinal tolerance. They do not induce enteropathy or they make it in smaller grade. This effect seems to be mediated by cGMP, second messenger of NO. If this possibility is true, zaprinast (ZAP) administration should have a preventive effect on the NSAID-induced enteropathy. We have researched the influence of ZAP (100 ppm) and 8Br-cGMP (1mg/kg s.c. + 50 ppm dayly) on the increase of the intestinal permeability induced by oral administration (in mixture with meal) of three NSAID: acethylsalycilic acid (500 ppm), sodium diclophenac (200 ppm) and piroxicam (1000 ppm). After seven days of treatment, the food is supressed and 10 mcCi of EDTA-51Cr are orally administred. After this, each animals is placed in a metabolic box and the urine is collected during 24 hours. ZAP and 8Br-cGMP prevent the increase of intestinal permeability induced by NSAID and they diminish the control group permeability to EDTA-51Cr (data not show in table). Then, ZAP and 8Br-cGMP are effective to prevent one of the main signs of the NSAID enteropathy, the increase of intestinal permeability.

Influence of zaprinast and 8Br-cGMP on NSAID-induced increase of intestinal permeability (% absortion)

Control (n=12)	**14.14+/-1.2**
ASA (n=10)	34.88+/-2.11a
ASA + 8Br-cGMP (n=10)	7.71+/-1.67a,b
ASA + ZAP (n=10)	19.70+/-3.98a,b
DIC (n=7)	68.56+/-9.69a
DIC + 8Br-cGMP (n=10)	25.25+/-3.41a,b
DIC + ZAP (n=10)	33.73+/-4.26a,b
PIR (N=10)	46.65+/-5.62a
PIR + 8 Br-cGMP (n=10)	16.10+/-2.20a,b
PIR + ZAP (n=10)	16.04+/-1.70a,b

a) $p<0.05$ versus control group. b) $p<0.05$ versus NSAID groups

W1329

NO Releasing Aspirin (ASA) Fails to Impair Ulcer Healing in Diabetic Rats due to the Reversal of the Fall in Ghrelin, Leptin and COX-2 Expression

Peter C. Konturek, Tomasz Brzozowski, Vitalyy Kukharskyy, Karolina Bazela, Robert Pajdo, Wieslaw W. Pawlik, Eckhart G. Hahn, Stanislaw J. Konturek

Our previous studies demonstrated that experimental diabetes mellitus dramatically impairs ulcer healing (Konturek et al; EJP 2003). The aim of the present study was to compare the effects of classic non-steroidal antiinflammatory drug, such as ASA, with NO releasing ASA (NO-ASA) on the course of ulcer healing in diabetes. For induction of diabetes, rats received streptozotocin (STZ, 70 mg/kg i.p.). Four weeks after induction of diabetes, experimental gastric ulcers were induced using acetic acid method. The rats with or without diabetes (control group) received daily vehicle, ASA (40 mg/kg-d i.g.) or NO-ASA (64 mg/kg-d i.g). At day 10 after ulcer induction, the rats were sacrificed and the gastric ulcer area and gastric blood flow (GBF) at ulcer margin were determined by planimetry and H2 gas clearance method. In addition, gastric mucosal expressions of leptin, ghrelin, cyclooxygenase-2 (COX-2), peroxisome proliferator receptor gamma (PPARγ) and IL-1β were assessed by RT-PCR and Western blot. In diabetic rats, a marked prolongation in ulcer healing was observed. This delay in ulcer healing in diabetics was associated with a significant decrease in GBF and gastric mucosal expression of ghrelin, leptin, COX-2 and PPARγ, but significant increase in IL1β expression. ASA caused further significant delay in ulcer healing in diabetic rats, while NO-ASA treatment failed to affect ulcer healing. The treatment with NO-ASA led to a significant rise in expression of PPARγ, ghrelin and leptin and reduction in IL1β in diabetes. We conclude that in contrast to ASA, NO-ASA, does not delay ulcer healing in diabetic rats possibly due to its antiinflammatory properties and the rise in PPARγ and GBF at the ulcer margin as well as the partial reversal of the fall in expression of COX-2, ghrelin and leptin in ulcerated mucosa

W1330

Cross-Talk Between Inducible Nitric Oxide Synthase and Cyclooxygenase-2 in the Gastric Mucosa of *in vivo* Rat Endotoxemia Model

Kaname Uno, Katsuaki Kato, Naohiro Dairaku, Shuichi Ohara, Yoshihito Iuchi, Junichi Fujii, Tooru Shimosegawa, Tetsuhiko Yoshimura

Backgrounds & Methods: Nitric oxide (NO) and prostaglandin-E2 (PGE2) play important roles in the maintenance of gastric mucosal integrity. Inflammatory responses increase in their production through inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), respectively. Recent studies have provided evidence for a cross-talk between these enzymes. This issue is of importance in relation to the mechanism of NSAIDs-induced gastric mucosal injury. However, little is known of their interaction in the gastric mucosa. The present study investigated the interaction between iNOS and COX-2 in the gastric mucosa using in vivo rat endotoxemia model and their specific inhibitors. **Materials & Methods:** Male SD-rats were intravenously administrated bacterial lipopolysaccharide (LPS) after pre-treatment with an iNOS-specific inhibitor, 1400w, and non-specific or specific COX-2 inhibitor, indomethacin or NS-398, respectively. The specific NO production in the gastric mucosa was directly determined by an electron paramagnetic resonance (EPR) spectrometry. The intramucosal PGE2 levels were measured by an enzyme immunoassay. Changes in levels of mRNA and proteins of iNOS and COX-2 were assessed with RT-PCR, Western blot and immunohistochemistry. **Results:** The levels of NO and PGE2 in the rat gastric mucosa of non-treated control were 0.35 +/- 0.16 nmol/g-tissue/30 min and 288 +/- 16.6 pg/g of tissue, respectively. These significantly increased in LPS-administrated rats (NO: 13.1 +/- 0.8, PGE2: 806 +/- 15) with corresponding increase in mRNA and protein of iNOS and COX-2. Immunohistochemistry showed that both enzymes were induced in the gastric mucosal cells. The intramucosal NO levels was significantly lower in the group with 1400w-treatment (4.0 +/- 0.4) than the group without this treatment, although PGE2 contents did not decrease in this group (788 +/- 26). In contrast, both indomethacin and NS-398 significantly and dose-dependently suppressed intramucosal PGE2 contents and NO production to the same levels as the untreated control. However, both inhibitors showed no significant effects on the induction of iNOS and COX-2 by LPS at their mRNA and protein levels. **Conclusions:** The present results suggest that COX-2 and its products may play an important role in the activation of iNOS, but not its induction in the gastric mucosal cells. The cross-talk between iNOS and COX-2 may play an important roles in the mechanism of NSAIDs-induced gastric mucosal injury.

W1331

NSAIDS Induce Apoptosis Through Endoplasmic Reticulum Stress Response

Shinji Tsutsumi, Tohru Mizushima

Background & Aims: Various gastric stressors induce not only necrosis but also apoptosis in gastric mucosal cells, resulting in gastric mucosal injury associated with gastropathy. We have been able to reproduce gastric stressor-induced apoptosis in vitro using primary cultures

of guinea pig gastric mucosal cells. Recently, much attention has focused on the endoplasmic reticulum (ER) stress response. In the mammalian ER stress response, three types of ER transmembrane proteins are important: protein-kinase and site-specific endoribonuclease (IRE1), eukaryotic translation initiation factor 2 kinase (PERK/PEK) and activating transcription factor 6 (ATF6). Since the relationship between this response and gastric stressor-induced apoptosis has not yet been elucidated, we here examine this relationship, using our in vitro system. Methods: Induction and activation of endoplasmic reticulum stress response-related proteins were monitored by immuno-blotting and northern blotting analyses. Transcriptional activities of CHOP and ATF6 were monitored by luciferase assay. Expression of the dominant negative form of CHOP and cells from CHOP knockout mice were used to test their requirement for apoptosis. Results: Among the various gastric stressors tested (indomethacin, ethanol, hydrogen peroxide and hydrochloric acid), indomethacin induced not only GRP78 but also CHOP, a transcription factor involved in apoptosis. ATF6, ATF4 and XBP-1 positively regulate CHOP expression. p90-ATF6 (the inactive form) was cleaved into p50-ATF6 (the active form) in the presence of indomethacin. ATF4 was induced and XBP-1 was activated in the presence of indomethacin, suggesting that PERK/PEK and IRE1, respectively, were activated. Non-steroidal anti-inflammatory drugs (NSAIDs) other than indomethacin (diclofenac, ibuprofen and celecoxib) also induced CHOP. The transcriptional activities of ATF6 and CHOP were stimulated in the presence of indomethacin. Expression of the dominant negative form of CHOP suppressed indomethacin-induced apoptosis. Furthermore, cells from CHOP knockout mice showed resistance to indomethacin-induced apoptosis. Conclusions: These results suggest that endoplasmic reticulum stress response-related protein, particularly CHOP, is involved in NSAID-induced apoptosis.

W1332

Effect of Vitamin C-Releasing Aspirin on Gastric Mucosal Damage Before and After *H. Pylori* Eradication Therapy

Peter C. Konturek, Joanna Kania, Eckhart G. Hahn, Jan W. Konturek

The interaction between *H.pylori* (Hp) and aspirin is still controversial. The aim of the present study was to compare the effect of aspirin (ASA) and vitamin C-releasing aspirin (ASA-VitC) on the gastric mucosal damage before and after eradication of Hp. Methods: 10 Hp-positive volunteers were given ASA (1.6 g/day) or ASA (1.6 g/day) releasing VitC (0.96g/day) for 3 days. The treatment was performed before and 4 weeks after eradication of Hp. For evaluation of gastric mucosal damage, the gastroscopy was perfomed in all subjects on day 0 and 3 after start of treatment. Gastric mucosal samples were taken during endoscopy for the determination of the gene and protein expression of superoxide dismutase (SOD), catalase, inducible and constitutive nitric oxide synthase (iNOS, cNOS), cytokines IL1β, TNFα by RT-PCR and Western blot, respectively. The activity of NFκB was evaluated by highly sensitive ELISA-based assay. Results: ASA induced significantly more gastric lesions than ASA-VitC. After eradication therapy, the lesion index increased significantly in patients receiving ASA. In contrast, under ASA-Vit C the gastric lesion index was significantly lower and the eradication therapy did not aggravate it. At baseline, a significant upregulation of antioxidant enzymes and iNOS expression in Hp-infected mucosa was observed. ASA stronger than ASA-Vit C reduced gastric mucosal SOD and catalase expression. After eradication, a significant decrease in the expression of SOD and catalase expression was observed and this reduction was enhanced further by ASA therapy, but not by ASA-VitC. ASA led to the increase in iNOS expression before and after eradication, whereas ASA-VitC did not enhance iNOS levels in the gastric mucosa. In patients receiving ASA, the mucosal expression of IL1β, TNFα was increased before and after eradication and this effect was accompanied by increased NFκB activity. ASA-VitC significantly inhibited the mRNA expression of TNFα and IL1β and this was accompanied by decreased NFκB activity. Conclusions: 1) ASA-VitC in comparision with ASA induces less gastric mucosal damage and this protective effect is mainly due to the attenuation of oxidative stress in gastric mucosa, and the stronger inhibitory effect on NFκB activity and the mucosal gene expression of proinflammatory cytokines. 2) Eradication of Hp increases the susceptibility of gastric mucosa against damaging effect of ASA and this is accompanied by the increased activity of NFκB and production of proinflammatory cytokines.

W1333

Dexamethasone Produces Damage in the Stomach but not Small Intestine Under Inhibition of Cyclooxygenase (COX)-1

Aya Yokota, Akiko Tanaka, Yoshiaki Kubo, Koji Takeuchi

Background/Aim: We have recently reported that the inhibition of both COX-1 and COX-2 is required for gastrointestinal ulcerogenic properties of NSAIDs. Inhibition of COX-1 up-regulates COX-2 in these tissues, and the prostaglandins (PG) produced by COX-2 contributes to maintaining the mucosal integrity under inhibition of COX-1. We have recently found that rofecoxib, the selective COX-2 inhibitor, provokes damage in the gastrointestinal mucosa only when hypermotility and COX-2 expression in the intestine, resulting in damage when COX-2 is inhibited. Dexamethasone is known to have a negative influence on inducible type of enzymes. In the present study, we investigated whether dexamethasone induces damage in the rat gastrointestinal mucosa under inhibition of COX-1, in order to verify the idea that COX-2 expression is a key process for the ulcerogenic action of NSAIDs. Methods: Male SD rats were used with (for gastric damage) or without (for intestinal damage) 18 h fasting. The animals were given dexamethasone (3 mg/kg) PO in the absence or presence of SC-560 (a selective COX-1 inhibitor: 10 and 30 mg/kg) PO, and the stomach or intestine was examined 8 h or 24 h later, respectively. PGE2 content was measured by EIA while COX-2 or iNOS expression was examined by RT-PCR. Results: Neither dexamethasone nor SC-560 alone caused damage in the gastric and intestinal mucosa. In the presence of SC-560, however, dexamethasone induced damage in the stomach but not the small intestine. SC-560 up-regulated the expression of COX-2 in the gastric and intestinal mucosa, and this response was inhibited by dexamethasone. SC-560 also decreased PGE2 content in both tissues, followed by a gradual recovery from 6 h later, and this PGE2 recovery was inhibited by dexamethasone. On the other hand, SC-560 also up-regulated iNOS expression in the small intestine but not the stomach, yet this iNOS expression was inhibited by co-administration of dexamethasone. The gastrointestinal hypermotility response induced by SC-560 was not affected by dexamethasone. Conclusion: These results suggest that the administration of dexamethasone under inhibition of COX-1 induces damage in the stomach but not small intestine, supporting the idea that COX-2 expression is a key process for gastric ulcerogenic action of NSAIDs, and suggested a risk for steroid therapy of ulceration in the stomach when COX-2 is expressed. Failure of dexamethason to produce damage in the small intestine is explained by the inhibition of iNOS expression.

W1334

Factors Involved in Up-Regulation of iNOS Expression in Rat Small Intestine Following Administration of NSAIDs

Koji Takeuchi, Aya Yokota, Yujiro Hayashi, Katsuhide Yoshii, Akiko Tanaka

Nitric oxide (NO) produced by inducible NO synthase (iNOS) plays a pathogenic role in the intestinal ulcerogenic response induced by nonsteroidal anti-inflammatory drugs (NSAIDs). Indeed, it has been shown that indomethacin up-regulated the iNOS mRNA expression and NO production in small intestine and that dexamethasone prevented the iNOS expression and intestinal damage in response to indomethacin. However, it remains unknown how iNOS is up-regulated following indomethacin treatment. In the present study, we investigated the regulatory mechanism for iNOS expression in the rat small intestine after administration of NSAIDs and correlated this process with the ulcerogenic action of NSAIDs. Methods: Male SD rats were given PO with various NSAIDs (indomethacin, dicrofenac, naproxen, flurbiprophen), SC-560 or rofecoxib, and the intestinal mucosa was examined 24 h later. PGE2, atropine or aminoguanidine was given SC twice 30 min and 6 h after indomethacin while ampicillin was given PO twice 24 and 1 h before indomethacin. Intestinal motility was determined by balloon method. Mucosal PGE2 contents was measured by EIA while the iNOS mRNA expression was analized by RT-PCR. Results: All NSAIDs tested caused a decrease in PGE2 production, intestinal hypermotility and bacterial invasion as well as expression of iNOS mRNA, resulting in hemorrhagic lesions in the small intestine. The intestinal hypermotility response to indomethacin was prevented by both PGE2 and atropine but not ampicillin, yet all these agents inhibited not only the bacterial invasion but also the expression of iNOS in the intestinal mucosa, resulting in preventing the intestinal lesions. Although SC-560 the selective COX-1 inhibitor, but not rofecoxib the selective COX-2 inhibitor, also decreased PGE2 production, intestinal hypermotility, bacterial invasion and iNOS expression, this agent did not provoke any damage because of the recovery of PGE2 production due to COX-2 expression. In addition, food deprivation totally completely both iNOS expression and lesion formation in response to indomethacin. Conclusion: We confirmed the iNOS expression in the small intestine after administration of NSAIDs. The up-regulation of iNOS results from COX-1 inhibition caused by NSAIDs and is functionally related with intestinal hypermotility and bacterial invasion, the latter process playing a major pathogenic role in the intestinal ulcerogenic response to NSAIDs.

W1335

Aspirin Induced Gastric Mucosal Cell Death: Evidence Supporting an Apoptotic Mechanism

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Background: Although aspirin (ASA) is known to induce apoptosis in colonic cells and may, through this mechanism, mediate its chemoprotective effects against colon cancer, its effects on gastric mucosal cells have been less conclusive. The present study was undertaken to more clearly define the role that apoptosis may play in the induction of cellular injury and death by ASA in a gastric carcinoma cell line (AGS). Methods: AGS cells were treated with varying concentrations of ASA (1 to 50mM) and apoptosis was measured by DAPI and Annexin V staining, DNA gel electrophoresis, TUNEL assay and DNA-histone associated complex formation. Proteins of the caspase family and PARP were analyzed by Western blots from whole lysates. PKC expression and translocation were analyzed by Western blots from whole lysates, and from cytosol and membrane fractions using antibodies against specific PKC isoforms. Results: ASA induced profound apoptosis as early as 2 hrs after exposure in concentrations > 25mM. At lower concentrations (from 1 to 25mM), longer incubation with ASA was required to observe apoptotic death. An important role for caspase activation was demonstrated in ASA-induced apoptosis, because pretreatment with a pan-caspase inhibitor (z-VAD-fmk) suppressed formation of the DNA-histone-associated complexes, and cleavage of caspases -3, -7, -9 and PARP consistently occured. To determine PKC involvement in ASA-induced apoptosis, antibodies to PKC isoforms were tested. ASA (40mM) induced an increase in the expression of PKCα and PKCβ2 in whole extracts and in membrane fractions, indicating their translocation from cytosol as a response to ASA treatment. In contrast, PKCβ1 expression was substantially inhibited. It was also noted that pretreatment of cells with the transcription inhibitors actinomycin D and cycloheximide potentiated ASA-induced caspase-3 and caspase-7 cleavage and apoptotic death. Conclusions: Our findings clearly show that ASA induces apoptosis in AGS cells in a time and concentration related fashion using both morphologic and biochemical criteria. We further observed enhancement of PKCα and β2 expression and inhibition of PKCβ1 expression are associated with this process, and that selective transcription inhibitors can promote ASA-induced apoptosis, through what is proposed to be the inhibition of synthesis of anti-apoptotic proteins.

W1336

Nitric Oxide (NO)-releasing Aspirin Counteracts the Aggravation of Stress-induced Gastric Lesions by Aspirin or Coxib in Diabetic Rats

Tomasz M. Brzozowski, Peter C. Konturek, Pajdo Robert, Stanislaw J. Konturek, Slawomir Kwiecien, Wieslaw W. Pawlik, Eckhart G. Hahn

Gastric mucosa of diabetic rats is highly vulnerable to acute gastric injury but the influence of classic and NO-releasing NSAID on stress-induced gastric lesions under diabetic conditions has been little studied. In this study streptozocin (70 mg/kg injected intraperitoneally) was used to induce diabetes mellitus (plasma glucose concentration greater than 380 mg/dL)

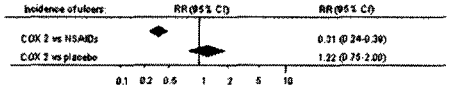
and diabetic or non-diabetic rats received 1) indomethacin (2 mg/kg i.p.) and native aspirin (ASA; 40 mg/kg i.g.); 2) COX-2 inhibitor, rofecoxib (5 mg/kg i.g.); and 3) NO-ASA (64 mg/kg i.g.) 30 min prior to exposure to 3.5 h of water immersion and restraint stress (WRS). The number of gastric lesions, gastric blood flow (GBF), plasma level of proinflammatory cytokines IL-1β and TNFα, reactive oxygen metabolites (ROS), superoxide dismutase (SOD) activity and the malonyldialdehyde (MDA) concentration as an index of lipid peroxidation, were determined in the gastric mucosa. WRS caused gastric lesions and this was accompanied by the significant fall in GBF (by 35% from basal) and an increase in ROS and MDA content and these effects were significantly augmented in diabetic rats. Pretreatment with ASA or indomethacin, which suppressed mucosal PGE_2 generation, and rofecoxib, which by itself had no significant effect on PGE_2 generation, aggravated significantly WRS-induced lesions, especially under diabetic conditions being accompanied by the fall in the GBF, the overexpression of mRNAs for TNFα and IL-1β and the significant rise in their plasma levels. NO-ASA attenuated WRS-induced gastric erosions while enhancing the GBF and causing downregulation of IL-1β and TNFα mRNAs in rats with or without diabetes. The mucosal ROS chemiluminescence and MDA content was significantly elevated but SOD activity was attenuated in diabetic animals exposed to WRS and these effects were significantly augmented by native ASA, indomethacin and rofecoxib but markedly reduced by pretreatment with NO-ASA. We conclude that 1) diabetes aggravates WRS-induced gastric damage and enhances the susceptibility of stressed mucosa to the damage induced by classic NSAID and coxib via the fall in the GBF, suppression of SOD activity, an increase in lipid peroxidation and the overexpression of IL-1β and TNFα, and 2) the beneficial effect of NO-ASA in diabetes could be attributed to increase in the GBF mediated by NO, preservation of SOD activity and the attenuation of ROS-mediated lipid peroxidation and IL-1β and TNFα expression and release.

W1337

Gastrointestinal Toxicity and Tolerability of COX-2 Selective Inhibitors: A Metaanalysis

Jerry McGrath, Richmond Sy., James Gregor

Purpose: To determine the gastrointestinal safety and tolerability of cyclo-oxygenase 2 (COX 2) selective inhibitors compared to non-steroidal anti-inflammatory drugs (NSAIDs) and placebo.
Methods: Randomized trials that compared at least 12 weeks of a COX 2 selective inhibitor with NSAIDs or placebo were systematically reviewed. Studies that meet predetermined criteria were combined using meta-analysis. The primary outcome measure was the incidence of gastroduodenal ulcers. Gastrointestinal side effects and withdrawal rates were secondary outcomes.
Results: Twelve randomized controlled trials that enrolled 22,307 patients were included. Patients taking COX 2 selective inhibitors were statistically less likely to develop endoscopically confirmed gastroduodenal ulcers than NSAID users (RR = 0.31, 0.24-0.39 95% CI). No statistically significant difference was found when COX 2 selective inhibitors were compared to placebo. The incidence of serious upper gastrointestinal events (bleeding, perforation and obstruction) was also statistically less likely with COX 2 therapy compared to NSAIDs (RR = 0.48, 0.33-0.69 95% CI). When subjective side effects were analyzed, in general COX 2 agents demonstrated a significantly superior profile.
Conclusion: COX 2 selective inhibitors are less likely to induce endoscopically confirmed gastroduodenal ulcers than NSAIDs and have a significantly less likelihood of developing serious upper gastrointestinal events.



W1338

NSAID-Enteropathy with Normal Mucosal Prostaglandins

Christoph M. Hotz-Behofsits, Matthew J. Walley, Gudmundur Sigthorsson, Robert Simpson, Ingvar T. Bjarnason

Background and Aims: Dual inhibition of COX-1 and 2 causes NSAID-enteropathy, but the precise role of the topical effect remains uncertain. The aim of this study was to assess the relative contribution of these 3 factors in the pathogenesis of NSAID-enteropahty. Methods: We dissociated and recombined the effect of selective COX-1 (SC-560 10 mg/kg) and 2 (celecoxib 30 mg/kg) inhibition and the topical effect (R-flurbiprofen 10 mg/kg) in rats and compared it with a classical NSAID with all 3 properties (Indomethacin 10 mg/kg). We also assessed normal, COX-1 and COX-2 deficient mice after dosing with R-Flurbiprofen (topical effect but no effect on COX-1 or 2), S-Flurbiprofen (conventional NSAID)), R- 2-Phenylpropionic acid 100 mg/kg (topical effect but not an NSAID and no COX inhibition). Intestinal permeability (51CrEDTA), inflammation (faecal granulocyte marker protein) and macro- and microscopic appearances as well as PGE2 (ELISA) were assessed at baseline and after the dosing of drugs. Results: Selective inhibition of COX-1 or COX-2 inhibition and the topical effect alone (using R-Flurbiprofen at doses that did not affect intestinal prostaglandin levels) did not increase intestinal permeability or cause inflammation in rats. Dual inhibition of COX-1 and COX-2 and in separate studies the simultaneous inhibition of COX-2 and the topical effect resulted in increased intestinal permeability and inflammation similar to that caused by Indomethacin. R-Flurbiprofen did not cause damage in normal and COX-1 knockout mice while S-Flurbiprofen was consistently toxic. However, administration of R-Flurbiprofen to COX-2 knockout mice resulted in identical damage as S-Flurbiprofen to normal or COX-2 knockout mice. R-2-Phenylpropionic acid led to GI damage in COX-2 knockout mice without a decrease in mucosal PGE2 levels, but did not cause damage in COX-1 knockout or normal mice. Conclusions: Short term selective inhibition of COX-1 or COX-2 or the topical effect does not damage the small intestine at the drug doses given. The combined inhibition of COX-1 & COX-2 or COX-2 inhibition & the topical effect (without a concomitant decrease in PGE2 levels) leads to NSAID-enteropathy. The latter combination shows that NSAID-enteropathy can occur without COX-1 inhibition or decreased mucosal prostaglandin levels which up to now has been thought to be of pivotal importance in the pathogenesis of the damage.

W1339

Does Adjuvant Chemotherapy (CT) to Combined Chemotherapy and Radiation Therapy (RT) Improve the Outcome in Patients with Locally Advanced Esophageal Cancer?

Frederic di Fiore, Stephane Lecleire, Jacques Jacob, Sok Hun Seng, Brigitte Vie, Bernard Paillot, Pierre Michel

Conventional medical treatment for locally advanced esophageal cancer is based on a combined CT and RT. To date, the benefit of an adjuvant CT to the combined CT and RT has not been demonstrated. Aim: To compare median survival, progression free survival, local recurrence and distance metastasis of patients treated by combined CT and RT followed by CT or by combined CT and RT alone. Patients and methods. Patients were retrospectively included between 1994 and 2000 in two different hospitals and were assigned in two groups (I and II) according to the hospital therapeutic practices as first line medical treatment of locally advanced esophageal cancer. Patients included in group I were treated by combined CT and RT including two courses of 5-fluorouracil (5FU) (750 mg/m2/d) and cisplatin (75 mg/m2) with concurrent 50 Gy radiation followed by 4 courses of CT by 5FU (1000 mg/m2/d) and cisplatin (100 mg/m2). Patients of group II were treated by two courses of 5FU (750 mg/m2/d) and cisplatin (75 mg/m2) with concurrent 50 Gy radiation. Exclusion criteria were a metastatic disease and a previous or synchronous history of cancer. Results. A total of 129 patients were included, 55 in group I and 74 in group II. Epidemiological characteristics (age, sex-ratio, performance status, dysphagia Atkinson score, weight before treatment) and main tumor characteristics (T3-T4: 75.6% and 78.4% in group I and II ; tumor's radiological and endoscopical size and diameter) were not statistically different between the two groups. Histological complete response after combined CT and RT was similar in group I and group II (38.1% vs 40.5%). Frequency of local recurrence was not different in the two groups (33.3% vs 46.6%). In contrast, frequency of metastatic recurrence was statistically lower in group I than in group II (23.8% vs 56.6%;$p=0.03$). Median overall survival and median progression free survival were 16.7 months and 12.4 months in group I versus 17 months and 11 months in group II (NS). Median survival in patients with histological complete response after combined chemotherapy and radiation therapy was higher in group I than in group II (23.3 months vs 18 months;NS). Conclusion. Adjuvant CT to combined CT and RT improve the outcome of patients with locally advanced esophageal cancer with a significant decrease in metastatic recurrences. This adjuvant CT also improves the median survival of patients with histological complete response after combined CT and RT without significativity.

W1340

Endoscopic Esophageal Mucosal Resection (EMR) of Early Esophageal Cancer and High Grade Dysplasia

Canard Jean Marc, Antoine de Leusse, Palazzo Laurent, Jian Raymond, Cellier Christophe

Background : EMR may be considered as a curative treatment for early esophageal cancer and high grade dyplasia. Aims : to assess EMR in the treatment for early esophageal cancer regarding technical feasability, results, course after treatment and recurence rates. Methods From september 1997 to july 2003, 35 patients (19 men, mean age 67 years) presenting a total of 39 superficial neoplastic lesions of the esophagus : high grade dysplasia (28% and superficial squamous cell carcinoma (72%). The lesion size was almost less than 20 mm (79%). All lesions was classified as T1 using endoscopic ultrasonography (72%) or T1 Sm1 using minisonde (28%). After lugol coloration, EMR was performed by aspiration section technique using a transparent plastic cap (43%) or traction section technique (31%) with double chanel endoscope or by combine technique (26%). For the firsts patients, EMR was associated with APC destruction of residual tumor. Endoscopic assessment was performed at 3 months and than every 6 months. Results EMR (en bloc = 17, piece meal = 22) was performed in all patients and was completed by APC in 5 patients (14%). EMR was considered macroscopicaly complete in 33 pt (94%). Early complication consisting of pseudo perforation was observed in 2 patients, and was treated medicaly. No bleeding needing endoscopic treatment or transfusion occured. In 3 patients, bleeding occured during EMR and was treated by clipping but was not considered as a complication. One 90 old patient died from accute respiratory fealure without direct link to EMR. Esophageal stenosis was observed in 3 patients after EMR of a large lesion (size greater than 20 mm in 2 patients). Histopathological examination showed an infiltrating lesion in 8 patients (23%). After curative resection (n = 25, 71%) et after mean endoscopic follow up of 18 month (3-39 months), a recurrence was observed in 1 pt (4%). Conclusion EMR of early esophageal cancer and high grade dysplasia seems an effective curative treatment in the majority of cases with a low incidence of complication and recurrence. Histopathological examination of tumor parietal extension permit to justified a complementary treatment (surgery or radio chemotherapy). This technique may be considered as the first line treatment in selected patients.

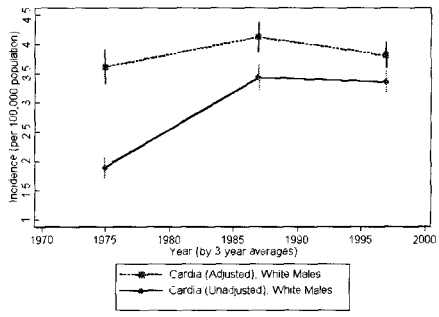
W1341

Improved Classification Largely Accounts for the Recent Increase in Gastroesophageal Junction Carcinoma Incidence

Douglas Corley, Ai Kubo

BACKGROUND: Recent reports suggest marked increases in the incidence of GE junction/cardia carcinomas, particularly in white males. Prior studies, however, did not evaluate the influence of improved site classification. METHODS: We evaluated the incidence of gastric cancer in the United States' SEER database between 1974-98. We contrasted the unadjusted incidence figures traditionally reported by SEER with incidence figures adjusted for improvements in site classification; adjusted numbers assigned carcinomas with an unknown location based on known cancer distributions. RESULTS: The proportion of gastric cancers not

assigned a location decreased markedly from 38% in 1974-6 to 14% in 1996-8. Accounting for these cancers, in white males the adjusted CARDIA cancer incidence did not increase between 1974-6 and 1996-8 (5.3% change, from 3.6 to 3.8 per 100,000 population/year, respectively, $p=0.59$); this contrasts with a 77% increase in SEER's unadjusted cardia incidence during this period (from 1.9 to 3.4 per 100,000/year, $p<0.001$). The adjusted NONCARDIA gastric cancer incidence significantly decreased by 3 per 100,000 population/year between 1974-6 and 1996-8 (from 6.8 to 3.8 per 100,000 population/year, $p<0.001$); a decrease more than twice as large as that reported using SEER's unadjusted numbers (from 4.5 to 3.2 per 100,000 population/year, $p<0.001$). Similar findings were observed for black males. CONCLUSIONS: Improved reporting of the gastric cancer sites accounted for almost all of the apparent increase in GE junction cancer incidence between 1974 and 1998 in this model. Noncardia gastric cancer incidence decreased more rapidly than previously appreciated.



W1342

Self-Expanding Metal Stents in the Palliation of Malignant Dysphagia: Outcome Analysis in 100 Consecutive Patients

David Elphick, Beverley Smith, Julie Bagshaw, Stuart Riley

BACKGROUND: Most patients with esophageal cancer present with incurable disease and palliation of dysphagia is often the principal goal of treatment. Self-Expanding Metal Stents (SEMS) have become a popular method of palliation in recent years, but some have expressed concerns that quality of life may be less good following SEMS insertion than following ablative methods of palliation. Our aim, therefore, was to identify factors associated with a less favorable outcome following SEMS placement. METHODS: A retrospective analysis of hospital records was undertaken to identify 100 consecutive patients in whom SEMS had been placed. Clinical, pathological and stent details were collected and correlated with post-stent outcome. RESULTS: 69 men and 31 women (age range 40 to 96 years) underwent SEMS placement. Tumors were predominantly distal, with 45 originating in the lower third of the esophagus and 26 at the gastroesophageal junction (GOJ). 65 were adenocarcinomas. 54 stents straddled the GOJ. All patients reported improvement in dysphagia. Marked pain was reported in 53, 15 suffered bleeding post-insertion, 1 suffered stent migration and 23 food bolus obstruction requiring repeat hospital admission. Reflux, food regurgitation or vomiting was reported as a new symptom following stent insertion in 40 patients and occurred more commonly if the stent straddled the GOJ. All patients have now died. Median survival from stent insertion was 12.5 weeks (range 0 - 97). Survival was longer if the tumor was in the middle or upper esophagus than if in the lower esophagus or at the GOJ. Survival was similar in patients with adenocarcinoma and squamous carcinoma. Survival was worse in patients with adenocarcinoma who had a stent deployed across the GOJ than in those with adenocarcinoma whose stent lay above the junction ($p=0.03$). CONCLUSIONS: SEMS provide good palliation of malignant dysphagia although pain, bleeding, food bolus obstruction and reflux commonly occur. Reduced survival in patients with adenocarcinoma whose stent enters the stomach has not previously been recognized, which raises the possibility of regurgitation through the stent leading to aspiration pneumonia in these frail patients. There is a need to compare different treatment modalities, including the use of stents incorporating an anti-reflux valve, in patients with this devastating disease.

W1343

FDG-PET as a Staging Method for Esophageal Cancer

Burkhard H. A. von Rahden, Hubert J. Stein, Hinrich Wieder, Wolfgang A. Weber, Katja Ott, Mario Sarbia, J. Ruediger Siewert

Background: Positron emission tomography with Fluoro-deoxyglucose (FDG-PET) is increasingly used as a staging method for esophageal cancer, but its diagnostic impact is not completely defined. Patients and Methods: We evaluated 117 consecutive patients who had received an FDG-PET scan during pretreatment staging for histologically proven esophageal cancer (30 adenocarcinomas and 84 squamous cell cancers, 3 rare histological tumor types). "Blinded" reevaluation of the PET results was performed by two nuclear medicine specialists. Data were correlated with morphological, clinical data and the histopathology reports, based on the treatment concept (14 primary resection, 39 resection after neoadjuvant treatment; 63 palliative strategy). FDG-PET was evaluated concerning demonstration of 1.) the primary tumor, 2.) lymph node metastases 3.) systemic metastases. Results: The primary tumor was delineated by FDG-PET in 93.2% of the cases. Lymph node staging with PET (evaluated based on the histopathology report in patients after surgical resection) had a low sensitivity (50.0%) and specificity (57.2%). FDG-PET was suggestive for pulmonary metastases in 8 cases (all true positive). Tumor spread to the liver was suspected in 2 cases (one true positive, one false positive result). Demonstration of tumor spread to the bones by FDG-PET was correlated with conventional x-ray as well as CT imaging. The results had to be regarded as true positive in all 6 cases. In 7 cases FDG-PET demonstrated additional tumors of the head and neck region (5 true positive and 2 false positive results). Conclusion: FDG-PET demonstrates the primary tumor correctly, when the tumor volume is large enough, but the method is poor in delineating early lesions. FDG-PET doesn't improve preoperative lymph node staging, but it is very well suited as an additional staging method (complementary to morphological imaging with spiral CT scan) for demonstration of distant metastases. The use of FDG-PET should be limited to the "diagnostic window" between early cancer and clearly systemically metastasized tumors.

W1344

The Effects of Esomeprazole Combined with Aspirin or Rofecoxib on Steady State Prostaglandin E_2 Production in Patients with Barrett's Esophagus

George Triadafilopoulos, Baljeet Kaur, Barry Traxler, Ngoc Chu, Douglas Levine, Alan Weston

Purpose: Tissue prostaglandin content reduction due to combined cyclooxygenase-2 (COX-2) inhibition and aggressive acid suppression appears to be a promising chemoprevention strategy in patients with Barrett's esophagus (BE). The aim of this pilot study was to determine the optimal treatment regimen to reduce the prostaglandin E_2 (PGE_2) content in BE mucosa. Methods: This multicenter, randomized, multiple-dose, open-label, 4-way crossover study was conducted in patients with documented non-dysplastic BE (segment length ≥2 cm). Patients were treated for 10 days with each of the following oral regimens: esomeprazole 40 mg twice daily, esomeprazole 40 mg twice daily plus aspirin 325 mg once daily, esomeprazole 40 mg twice daily plus rofecoxib 25 mg once daily, and rofecoxib 25 mg once daily. The treatment sequences were randomly assigned, with a 10- to 14-day washout period between each treatment. Endoscopic biopsies from BE and esophageal mucosa were obtained at baseline and after each treatment period. Extracted PGE_2 was measured using a Biotrak EIA assay kit, a competitive enzyme immunoassay (Amersham Pharmacia Biotech). The primary efficacy variable was the reduction of the product of the COX-2 enzyme, PGE_2, from baseline to day 10 for each treatment regimen. This variable was also analyzed using an analysis of variance model. Results: Of the 40 randomized patients, 35 completed the study. Mean baseline PGE_2 production was 98.6 pg/mg. Mean PGE_2 production at day 10 is shown in the Table. Only the reduction with esomeprazole plus aspirin was statistically significant ($p < 0.05$) compared to all other treatment regimens. Conclusions: The tissue prostaglandin content reduction combined with the anti-platelet properties of aspirin may make the combination of aspirin and esomeprazole 40 mg twice daily the preferred treatment combination for chemoprevention studies in patients with BE.

Prostglandin E_2 Production at Day 10

Treatment	Observed Mean (SD) (pg/mg)	Least Squares Mean Estimate (SEM) (pg/mg)
Esomeprazole 40mg bid and Aspirin 325mg qd	78.9 (54.1)	75.9 (5.4)
Esomeprazole 40mg bid and Rofecoxib 25mg qd	97.8 (55.1)	97.1 (5.1)
Esomeprazole 40mg bid	97.5 (63.8)	101.6 (5.3)
Rofecoxib 25mg qd	92.7 (47.6)	94.6 (5.1)

W1345

Aspirin Versus Coxibs for Chemoprevention in Barrett's Esophagus

Chin Hur, Norman S. Nishioka, Lee Simon, G. Scott Gazelle

BACKGROUND:
Aspirin therapy is widely accepted for secondary prevention in patients with documented cardiovascular disease, but there is a growing trend among healthy individuals to use aspirin as primary chemoprevention for both cardiovascular disease and cancers. Accruing evidence suggests that cyclooxygenase-2 selective inhibitors (coxibs) may be effective for esophageal adenocarcinoma chemoprevention, but would not provide the primary cardiac benefit that aspirin could. This study compared the cost-effectiveness of aspirin versus coxibs in patients with Barrett's esophagus for primary chemoprevention using a computer model.
METHODS:
A Markov model simulated hypothetical cohorts of 50 year old men who took either 325 mg of enteric-coated aspirin daily or celecoxib 400mg twice a day. Patients in both cohorts could develop drug-related complications which would lead to its discontinuation. The aspirin group was also modeled to have a decreased rate of coronary events, however decreased cancer mortality was not modeled in the base case analysis in either group based on the assumption that the two treatments were equally effective in this regard. Data sources included published literature and the Centers for Medicare & Medicaid Services. Endpoints used to compare the two strategies included quality-adjusted life years (QALYs), mortality and complication rates, as well as cost. The analysis was from a societal perspective with a time horizon of 10 years from age 50. Extensive sensitivity analyses were performed.
RESULTS:
Aspirin's cardioprotection and overall lower rate of complications led to 0.03 more QALYs and cost $23,000 less per person than coxib therapy over a ten year period. When compared to the aspirin group, the coxib group had 3.877% more complications and 0.17% more deaths. Alternatively stated, coxib therapy resulted in one patient complication or death for every 26 or 588 patients treated with coxibs, respectively.
CONCLUSIONS:
Assuming equal efficacy in esophageal cancer prevention over a ten year period, aspirin is both more effective and less costly than coxib therapy for primary chemoprevention in patients with Barrett's esophagus.

BASE CASE ANALYSIS

	ASPIRIN	COXIB
Effect (QALYs)	7.60	7.57
Cost*	$181	$34,403
Excess Complications (%)		
Total	3.539	7.416
MI	0	1.056
Bleed	3.539	0
Ulcer	0	6.360
Deaths (%)		
Total	8.702	8.875

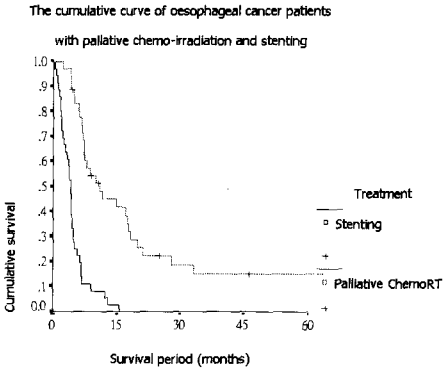
*Total average cost per patient.

W1346

The Clinical Efficacy of Intensive Chemoradiation for Inoperable Squamous Esophageal Cancer: Case-control Study

Simon K. H Wong, S. F. Leung, Angus C. W. Chan, Enders K. W. Ng, Sydney S. C. Chung

BACKGROUND: We evaluated the role of intensive chemoradiation regimen for patients with T4 inoperable or cervical nodal metastatic squamous esophageal cancer. METHODS: From 1996 to 2003, patients with histologically proven, inoperable, squamous cell esophageal carcinoma who received intensive chemoradiation therapy of two 3-weekly cycles of continuous infusion of 5-fluorouracil 200 mg/m2/day on days 1-21,22-42, cisplatin 60mg/m2 on day 1 and 22 and concurrent radiotherapy 50-60Gy in 25-30 fractions over 5-6 weeks were recruited for analysis. Efficacy of treatment was analyzed by endoscopy, biopsy, computerized axial tomography before and 8 weeks after completion of treatment. Median survival and the need palliative esophageal stenting were compared with the matched control group during the same period that decline chemoradiation therapy. Both groups were comparable in demographic characteristics, pre-treatment dysphagia score, comorbidity, tumor differentiation, location, staging and size. RESULTS: Thirty-six consecutive patients (33 male, mean age 63.2, S.D.9.5) with either T4 (81%) and/or cervical nodal metastatic (50%) tumor had received chemoradiotherapy. Treatment was completed in 32 patients (89%). Grade II neutropenia occurred in 8 patients (22%) and the incidence of febrile neutropenia was 11%. There was no therapy-related mortality. Tumor volume as measured by CT scan was significantly reduced after treatment with a major clinical response (> 50% reduction in tumor volume) observed in 18 patients (50%). Four patients (11%) received salvage esophagectomy for local recurrence 1 to 3 years after initial complete response. As compared to the matched controlled patients, chemoradiation therapy had significantly improved the 5-year survival rate (0% Vs 19%, $p=0.01$), median survival (4.0 months Vs 9.5 months, $p<0.005$) and the need of palliative stenting (100% Vs 22%, $p=0.005$). CONCLUSIONS: Although prognosis of patients with inoperable and cervical nodal metastatic esophageal cancer is extremely poor, this study suggests that intensive chemoradiation can both improve their overall survival and reduce the need of esophageal stenting among these patients.



W1347

The Cyclooxygenase-2 Inhibitors Rofecoxib and Celecoxib Protect Against Esophageal Carcinoma

Marc Bardou, Alan N. Barkun, Joumana Ghosn, Marie Hudson, Elham Rahme

Background: A rising incidence and a poor survival rate make esophageal cancer a major health issue; hence the need for chemoprevention. It has been suggested that selective COX-2 inhibitors (coxibs) may be protective against esophageal carcinoma. This study aimed to investigate the effects of the coxibs, rofecoxib and celecoxib, of the non-selective, non-steroidal anti-inflammatory drugs (NSAIDs) and of aspirin on esophageal cancer. Methods: We conducted a nested case-control study, using medical, pharmaceutical and demographic data obtained from the Government of Quebec Health Insurance Agency (RAMQ) database. We included patients 65 years and older who underwent esophageal imaging (esophago-gastro-duodenoscopy or barium swallow) between January 1999 and September 2002. Logistic regression models were used to determine the effect of chronic exposure (at least 30 days) to coxibs, non-selective NSAIDs or aspirin on the occurrence of esophageal cancer. Results: The study included 251 cases and 86,644 controls. Patients more likely to have esophageal cancer (odds ratio, 95% confidence interval) were men (3.42, 2.62- 4.48) and older subjects (75-84 years and ≥ 85 years, 1.40, 1.08-1.82 and 1.69, 1.05-2.72, respectively). Exposure to at least 30 days of coxibs or NSAIDs, but not to aspirin, significantly reduced the risk of esophageal cancer (0.63, 0.40-0.98; 0.47, 0.24-0.93 and 0.88, 0.64-1.20 respectively). When rofecoxib and celecoxib were assessed separately we found a trend that was more suggestive for rofecoxib toward a duration-response effect (Celecoxib, 0.51, 0.27-0.98; 0.30, 0.11-0.82; 0.39, 0.14-1.05, Rofecoxib, 0.39, 0.16-0.96; 0.37, 0.12-1.16; 0.33, 0.08-1.36 for exposures ≥ 30, ≥ 60 and ≥ 90 days, respectively). Conclusion: The coxibs, rofecoxib and celecoxib, and the non-selective NSAIDs appear to be associated with a decreased risk of esophageal cancer.

W1348

Cholecystectomy, Complications of Gastroesophageal Reflux Disease and the Risk of Adenocarcinomas of the Esophagus and Cardia

Edward A. Lew, Monir Hossain, Raj K. Goyal, J. Michael Gaziano

Background: Gastroesophageal reflux of acid and bile are believed to be important in the development of esophageal and cardia adenocarcinomas. Cholecystectomy increases duodenogastric bile reflux but it is controversial whether this surgery affects cancer risk. The purpose of this study was to examine the associations of cholecystectomy, complications of gastroesophageal reflux, and the risk of these cancers. Methods: Using a case-control study design, we examined patients from the Veteran Affairs (VA) hospitals throughout the US. Patients newly diagnosed with esophageal and cardia adenocarcinomas in 1999 to 2001 were eligible cases. Three controls were matched to each case by age, sex, geographic location (VA hospital network), and index date (year of cancer diagnosis for cases with year of VA visit for controls). Utilizing the VA Patient Treatment Files of all VA hospitalizations and procedures starting from 1986 to the index date, as well as Outpatient Clinic Files and available pathology records, we examined potential risk factors such as cholecystectomy, as well as prior diagnoses of esophageal reflux, esophagitis, and obesity before the index date. Odds ratios with 95% CI were calculated by logistic regression analysis. Results: We studied 827 patients newly diagnosed with adenocarcinomas of the esophagus and cardia with 2481 matched controls. Worsening complications of gastroesophageal reflux disease, including a history of esophageal reflux (OR, 2.72; 95% CI: 2.21 - 3.36), esophagitis (OR, 3.87; 95% CI: 2.69 - 5.56), as well as ulcer of the esophagus and Barrett's esophagus (OR, 4.18; 95% CI: 2.51 - 6.97) were associated with increasing risks of cancer. Twenty-nine cases (3.5 %) and 33 controls (1.3 %) underwent a cholecystectomy prior to the index date. Cholecystectomy was associated with an elevated risk of cancer (OR, 2.69; 95% CI: 1.63 - 4.47). Moreover, the cancer risk with cholecystectomy remained elevated (OR, 1.99; 95% CI: 1.13 - 3.51) while controlling for complications of gastroesophageal reflux disease as well as for obesity (OR, 4.52; 95% CI: 2.24 - 9.12). Conclusions: Worsening complications of gastroesophageal reflux disease are associated with increasing risks of esophageal and cardia adenocarcinomas. Cholecystectomy also increases the cancer risk independent of esophageal reflux, esophagitis, and obesity. These findings are consistent for a possible role for bile reflux in the carcinogenesis of esophageal and cardia adenocarcinomas.

W1349

High Expousure to Polycyclic Aromatic Hydrocarbons May Contribute to High Risk of Esophageal Cancer in Northeastern Iran

Farin Kamangar, Paul Strickland, Akram Pourshams, Reza Malekzadeh, Paolo Boffetta, Mark Roth, Christian Abnet, Mitra Sadaatian-Elahi, Nasser Rakhshani, Paul Brennan, Arash Etemadi, Sanford Dawsey

The northeastern region of Iran has some of the highest rates of esophageal squamous cell carcinoma (ESCC) in the world. Smoking and alcohol consumption, the two main risk factors of ESCC in western countries, are uncommon in northeastern Iran. Therefore high risk of ESCC should be attributed to other factors. High exposure to polycyclic aromatic hydrocarbons (PAHs) has recently been found in Linxian, China, another area with very high rates of ESCC but low tobacco and alcohol consumption. To investigate the role of PAHs in the etiology of ESCC in northeastern Iran, we measured urine 1-hydroxypyrene glucuronide (1-OHPG), a stable metabolite of pyrene, a common PAH, in 99 inhabitants of this area. Forty-two subjects (42%) had urinary 1-OHPG levels ranging from 1 to 5 pmol/ml, indicative of moderate PAH exposure typically observed in smokers, and 41 (41%) had levels above 5 pmol/ml, indicative of high exposure comparable to steel blast furnace or coke oven workers who also smoke. Median 1-OHPG was 4.2 pmol/ml, and 20th and 80th percentiles were 1.3 and 10.4 pmol/ml, respectively. Extremely high values of 1-OHPG (as high as 85.7 pmol/ml) were observed. Further analysis showed that 1-OHPG levels were high in all subgroups of our study subjects. We conclude that people of northeastern Iran are exposed to widespread and very high levels of PAH, which may contribute to the high rates of ESCC observed in this area.

W1350

Histopathologic Evaluation of Early Esophageal Adenocarcinoma; Implications for Endoscopic Versus Surgical Therapy

M. Westerterp, L. B. Koppert, Christianne Buskens, Herman van Dekken, Fiebo Ten Kate, Hugo Tilanus, J. J. B. van Lanschot

Introduction: Due to recent advances in diagnostic modalities the detection of early esophageal cancer is rising. Local endoscopic mucosal resection (EMR) is currently being applied for early carcinomas. However in patients with N1 disease, EMR would compromise long term survival. For these lesions surgical resection is considered the treatment of choice. Nevertheless, the extent of resection is still a matter of debate. To contribute to therapeutic decision making we analyzed which surgically removed early esophageal adenocarcinomas would presently be eligible for EMR. In addition, we analyzed if a limited transhiatal resection was sufficient to establish locoregional disease control and long-term survival in patients with early esophageal adenocarcinoma.
Methods: 120 patients with high grade dysplasia (n=13) or T1 adenocarcinoma (n=107), who underwent limited transhiatal resection between September 1980 and July 2002, in two academic medical centers, were included. These tumors were classified into six different categories, according to the depth of tumor invasion (mucosal; m1-m3, submucosal; sm1-sm3). Depth of invasion was correlated to N-stage; moreover long-term survival and pattern of recurrence were analyzed.

Results: Invasion was limited to the mucosa in 54 patients, while in 66 patients the tumor extended into the submucosa. Of the 54 m1-m3 and 25 sm1 cancers, only 1 m3 tumor had lymph node metastases in the resection specimen (N1,1%), whereas 6 out of 23 sm2 tumors (26%) and 12 out of 18 sm3 tumors (67%) showed lymph node involvement. N-stage was an independent prognostic factor for disease free survival (P logrank<0.001). Patients with m1-m3 or sm1 tumors had a 5-year disease specific survival of 97% as compared to 57% in the patients with sm2-sm3 tumors (P logrank<0.001). In the sm2-sm3 group, 6 patients developed locoregional recurrence, 7 patients developed distant metastases and 2 patients developed both locoregional and distant recurrence.
Conclusion: Our data indicate that m1-m3 and sm1 tumors are eligible for EMR, since they have only 1% chance of lymphatic dissemination. For sm2 and sm3 tumors, EMR is contraindicated, as indicated by high N1 percentages in our series, and surgical resection is the treatment of choice. The substantial locoregional as well as distant recurrence of the sm2-sm3 tumor patients after transhiatal resection is an argument in favor of additional therapy, e.g. more extensive locoregional and systemic therapy.

W1351

Association Between Breast Cancer (BC) and Esophageal Adenocarcinoma (EAC) In Women

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Background: While BC is one of the commonest cancers in women, EAC is distinctly uncommon in women. Objective: To evaluate if survivors of BC are at increased risk for developing EAC as a second primary cancer. Methods: The Surveillance, Epidemiology, and End Results (SEER) database of the National Cancer Institute was analyzed to identify a cohort of women newly diagnosed with primary, microscopically confirmed BC from 1973 to 2000. The study cohort was followed within the SEER system to ascertain subsequent primary EAC. Time of follow up for each patient was calculated from the date of diagnosis of the index BC to the earliest of date of diagnosis of EAC, date of death or last contact, or December 31, 2000. Person-years of follow up were stratified by 5-year age groups, race and year of diagnosis. Expected number of EAC in this cohort was obtained by multiplying the age, race and calendar year specific incidence rates of EAC in the SEER population. The standardized incidence ratio (SIR) was calculated as the ratio of observed vs. expected number of EAC and 95% CI were calculated using Byar approximation of the Poisson test. Results: 439,208 white women over a cumulative follow up period of 3,215,835 person-years developed 58 EAC versus 31.86 expected cancers (SIR, 1.82, 95% CI 1.40-2.37). Only 2 non-white patients with BC developed subsequent EAC, thus precluding further analysis. The median interval from index BC to EAC was 105. 5 months (inter-quartile range, 64 to 152). Majority (86%) of the patients were 65 years or older when they developed EAC. The median survival for time EAC in this cohort was 11 months (inter-quartile range, 3-20). Twelve percent patients had more than two primary cancers. Nineteen (32.8%) had localized disease, 14 (24%) had regional disease and 15 patients (26%) had metastatic disease and this distribution was similar to all EACs in the general population. Also, only 5 esophageal cancer were diagnosed within one year of diagnosis of the index BC, suggesting the association can not be entirely explained by increased screening after diagnosis of BC. For comparison, over the same follow up period the cohort of white women developed 187 cases of squamous cell cancer of esophagus versus an expected 139 cases (SIR, 1.35, 95% CI 1.17-1.56). Conclusion: White women with BC are at higher risk for developing a second primary EAC and epidemiological investigations are needed to identify shared risk factors, and potential benefit of screening.

W1352

Trend in Age Conditional Risk of Developing Invasive Esophageal Cancer (EC) in the 1990s

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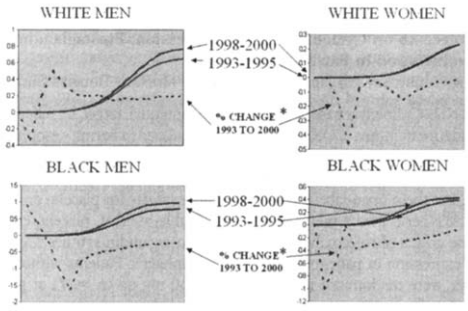
Background: Although it is generally agreed that incidence of EC has increased in the 1990s, no information is available regarding the lifetime risk of developing EC in different age groups based on gender and race. Objective: Using data from Surveillance, Epidemiology and End Results (SEER) program, we calculated probability of developing invasive EC (both squamous and adenocarcionma) in different demographic groups and assessed the trend in the age conditional probability of developing EC from 1993-1995 to 1998-2000. Methods: Using the DevCan 5.1 software, a algorithm was used to convert cross-sectional incidence rates to age-conditional probabilities on a hypothetical population. The incidence rates were derived from the SEER first incident cases and the population estimates from the census data. Mortality rates were obtained from the National Center for Health Statistics mortality counts. Results: The age conditional risk of developing invasive EC was highest in white men and for a 20-year-old white male, the risk increased from 0.0003% at the age of 25 years to 0.8% at the age of 95 years; black women had the lowest overall risk. For any 5-year age group, the highest increase in risk occurred in 70 to 75 year age group. Comparison of the risks in the 1993-95 and the 1998-2000 cohorts, revealed that the risk of developing EC increased in white men and women by an average of 18% and 8%, respectively across all age groups. However, in black men and black women, the risks were lower by an average of 37% and and 30% respectively (Figure). Conclusion: in the 1990s, age-conditional risks of developing invasive EC increased substantially in white men and women, while the risk has decreased in black subjects. Given that age-adjusted incidence rate of squamous cell cancers have declined in this period, most of this increase is due to esophageal adenocarcinoma.



Y axis: prob. of esophageal cancer; x-axis: age groups from 20 to 95 years
*Percentage change in probability of developing esophageal cancer

W1353

Endoscopy Policy and Risk of Missing Upper Gastrointestinal Malignancy in Patients Aged over 55 Years - Data from the Scottish Audit of Gastric and Oesophageal Cancer (SAGOC)

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INTRODUCTION: There is uncertainty concerning the management of new-onset uncomplicated dyspepsia in patients aged over 55 years. The British Society of Gastroenterology dyspepsia guidelines suggest that such patients should undergo an urgent endoscopy to exclude upper gastrointestinal (GI) malignancy. However, these guidelines are at variance with those produced by the Scottish Intercollegiate Guidelines Network, which advise an "H.pylori test and treat" strategy as initial management. What would be the risk of missing upper GI malignancy in patients aged over 55 years if urgent endoscopy was reserved for patients with alarm symptoms only? AIM: To determine the proportion of patients aged over 55 years with upper GI malignancy who present without alarm symptoms. METHODS: The Scottish Audit of Gastric and Oesophageal Cancer collected data prospectively for all upper GI malignancies diagnosed in Scotland between July 1997 and July 1999. We reviewed the data for all patients over the age of 55 years presenting without alarm symptoms. These were defined as dysphagia, odynophagia, weight loss, GI bleeding, anaemia, vomiting, history of gastric surgery and history of peptic ulcer disease. RESULTS: Of the 3293 patients diagnosed with upper GI malignancy during the 2 year period of the audit, 3003 (91%) were aged over 55 years. Of these, 206 (6.9%) presented without alarm symptoms. However, only 74 of these patients underwent potentially curative surgery and only 50 survived more than 1 year. These figures represent 2.5%, and 1.7%, respectively, of all the patients with upper GI malignancy over 55 years age. CONCLUSION: A small proportion of the patients with upper GI malignancy aged over 55 years may have their diagnosis delayed if urgent endoscopy was restricted to patients with alarm symptoms only. However, only a minority of these patients have potentially curable disease.

W1354

Rise of Esophageal Adenocarcinoma in USA Is Temporally Associated with the Rise in Carbonated Soft Drink Consumption

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BACKGROUND AND AIMS: Incidence rates for adenocarcinoma of the esophagus (ACE) in rose by 570% in American white males in last 25 years and is still continuing to rise. There has been a 40% increase for each 5-year increase in date of birth- a birth cohort effect (El-Serag HB et al. Gut 2002;50:368-372) The reason for this rise remains unexplained. Time-trends in rates of ACE have wide variations world wide. We aimed to identify potential new risk factors that could explain these observations. METHODS: US Department of Agriculture (USDA) data was searched for major changes in the diets of Americans in 5 decades. Per capita carbonated soft drinks (CSD) consumption rose by 450% in USA from 10.8 gallons in 1946 to 53 gallons in 2000. Rise in CSD consumption preceded the rise of ACE by 20 years. Temporal trends between 3-year average of per capita consumption of CSD and incidence of ACE were analyzed by linear regression. RESULTS. Highly significant correlation was obtained between 3-yearly incidence of ACE (1974-2000) and the 3-yearly per capita consumption of CSD 20 years before (1964-1980); $r = 0.99$, 95%CI 0.92-1.0; $p<0.001$ $r2 = 0.98$. We found strong biological basis to explain increased dose and duration of exposure to acid: 1. Consumption of 350 ml CSD per day corresponds to 53.5 minutes of pH <4 (Shoenut et al. Dig Dis Sci 1998;43:834-39), and 53 gallons per year would mean 32100 more minutes of acid exposure per year. 2. Excess CSD drinking started in childhood and American teenagers drank 2 cans of CSD per day (USDA) explaining the Birth Cohort Effect. 3. Prevalence of H pylori infection in the population fell during the same period to increase endogenous acid secretion. In general identical time trends were seen worldwide. Countries with per capita CSD below 10 gallons (e.g. East Europe, Japan, China, Taiwan, Korea, India, etc) had little increase in the incidence of ACE. Countries with per capita CSD more than 20 gallons are reporting a rising trend of ACE. Scotland with high rates of ACE over England had a 1.8 times higher consumption of CSD. CONCLUSION: The linear association between per capita consumption of CSD 20 years before and the incidence of ACE is very strong. A biological explanation exists for this association, which is seen worldwide. The rising rates may continue for another 20 years. These findings are strong enough to initiate good epidemiological studies to establish the true association between CSD consumption and rates of ACE.

W1355

Effect of Rofecoxib on Cyclooxygenase-2 Expression, Prostaglandin E2 Content, and Cell Proliferation in Barrett's Esophagus

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BACKGROUND: Cyclooxygenase-2 (COX-2) is a potential target for chemoprevention of esophageal adenocarcinoma. COX-2 expression is increased in Barrett's esophagus and animal studies suggest that COX-2 inhibition decreases the risk of developing esophageal cancer. Prior human studies suggest markers of cell proliferation are decreased by selective COX-2 inhibition, but these open-label studies could not evaluate for placebo or temporal effects. METHODS: We performed a pilot randomized, double-blinded, placebo-controlled trial to determine the effects of rofecoxib on markers of prostaglandin synthesis, cell proliferation, and COX-2 expression in patients with Barrett's esophagus. Patients without recent aspirin or NSAID use were randomized to take rofecoxib 50 mg qd (n = 7) or placebo (n = 7) for 4 weeks. Esophageal biopsies were obtained at baseline and after 4 weeks of treatment. Prostaglandin E2 (PGE2) content was analyzed utilizing mass spectroscopy, cell proliferation was evaluated by Ki-67 immunohistochemistry, and COX-2 protein expression was assessed by immunohistochemistry. Differences were evaluated with paired and unpaired t-tests, and with the nonparametric Wilcoxon rank test. RESULTS: There was no significant difference in PGE2 content between the groups after 4 weeks (P = 0.76), although there was a trend for BOTH groups to decrease their mean values compared with baseline (rofecoxib group 50% decline from 21.2 to 10.5 ng/g, P = 0.15; placebo group 61% decline from 22.9 to 8.9 ng/g, P = 0.13). Similarly, the Ki-67 immunohistochemistry did not differ between the two groups at 4 weeks (P = 0.93), but there were trends towards mean reductions for both groups compared with baseline (rofecoxib 386 to 305 cells/ 1000 cells, P = 0.1; placebo 504 to 419 cells/ 1000 cells, P = 0.03). The rofecoxib and placebo groups had similar levels of COX-2 expression at 4 weeks; both arms were unchanged compared with baseline. All patients completed > 91% of their medications. CONCLUSIONS: Compared with placebo, rofecoxib did not significantly reduce esophageal PGE2 content, Ki-67 levels, or COX-2 protein expression. There were substantial (though not statistically significant) decreases in PGE2 content and Ki-67 levels for BOTH the rofecoxib and placebo groups; this finding suggests open-label trials of these agents may be subject to bias. Larger controlled trials of COX-2 inhibitors are needed to determine their ability to affect these markers at the level of the esophageal mucosa.

W1356

Results at 6 Years of a Follow-up Programme for the Evaluation of the Risk of Neoplastic Lesions in Patients with Atrophic Body Gastritis (ABG)

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Background: ABG is considered a definite risk factor for gastric cancer and carcinoids. H pylori (HP) is involved in the induction of ABG and included among class I carcinogens for gastric cancer. The effect of HP eradication on atrophy and intestinal metaplasia is unclear and it is still debated whether cure of infection can ameliorate histological changes and eventually reduce the risk of neoplastic and preneoplastic lesions. Aim: To assess the occurrence of gastric neoplasms and to verify the progression of histological alterations in ABG patients included in a long-term follow-up programme. Materials&Methods: 95 ABG patients (28 M, 67 F, median age 56 yrs) were included. All patients had gastroscopy with antral (n=3) and corporal (n=3) biopsies for histology at baseline and follow-up. Gastritis was scored according to the updated Sydney System. 29 pts (Group A, HP pos. at histology and/or serology) were cured of infection (bismuth based regimens), 66 pts (Group B, HP neg. and/or pos. at serology) were not treated. In both groups, median follow-up time was 74 months (range 48-123) and each patient underwent medianly 2 (1-8) endoscopic/ histological follow-ups. Results: Macroscopical lesions: In Group A, 1 (3.4%) patient developed gastric cancer and 1 (3.4%) a body hyperplastic/dysplastic polyp. In Group B, 1 (1.5%) patient developed a gastric carcinoid, 1 (1.5%) a mild dyplastic adenoma, 1 (1.5%) a body hyperplastic/dysplastic polyp, and 8 (12.1%) hyperplastic polyps (5 body and 3 antrum). Histological findings: 3 (10.3%) Group A patients developed mild antral dysplasia vs 1 (1.5%) Group B patient (p=0.08). In Group A, body atrophy score did not change, whereas body intestinal metaplasia increased (1.12 vs 1.42, p=0.057). In Group B patients, both, body atrophy and intestinal metaplasia did not change. Conclusions: At long-term follow-up the large majority of ABG patients does not develop neoplastic lesions and their histological pattern of gastritis (atrophy and intestinal metaplasia) does not substantially change. The occurrence of neoplastic (2.1%) and dysplastic lesions (6.3%) is similar among patients treated for active HP infection and those not treated, and seems thus irrespective of successful HP cure.

W1357

The Relative Importance of the Different Sites of Gastric Biopsies in Detection of Intestinal Metaplasia in Clinical Practice

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Introduction: The Modified Sydney System has been used for evaluating the type, severity and topography of gastritis and to determine the presence of premalignant lesions such as gastric atrophy and intestinal metaplasia. This involves taking multiple biopsies from various predefined areas of the stomach. The usefulness of these individual biopsies in identification of premalignant lesions has not been studied in a large cohort of patients. Methods: A total of 4140 patients undegoing upper gastrointestinal endoscopy in a university teaching hospital had biopsies taken from antrum, incisura and corpus and were classified using the modified Sydney System. In addition, cardia biopsies were also taken and were assessed for evidence of carditis and cardia intestinal metaplasia. The relative contribution of individual biopsy sites in identification of intestinal metaplasia was calculated. Results: The median age was 53 years (range 15-97 years) and male to female ratio was 1:1. Eighty three percent of patients had endoscopy for evaluation of dyspepsia while other indications included bleeding, screening and investigation of anaemia. Gastroeosophageal reflux disease was the commonest endoscopic abnormality identified. Intestinal metaplsia was detected in 18.8 % of all biopsies, Antral, incisura and corpus intestinal metaplasia was found in 12.1%, 5.9% and 4.7% respectively. Of the patients with intestinal metaplasia in the incisura biopsy, vast majority had con-commitant intestinal metaplasia in other biopsies with only 8.3% only had this area as the sole site with this abnormality. Cardia intestinal metaplasia was identified in 7.4% of the patients. In contrast to the incisura biopsies, 56.66% of patients with cardia intestinal metaplasia had this feature detected solely in the cardia biopsy. Four patients had dysplasia and one of these had invasive carcinoma. Conclusion: Incisura biopsies may not add significantly to the antral and corpus biopsies in the detection of intestinal metaplasia and hence may not be useful in the clinical setting. Cardia intestinal metaplasia seems to exist without the presence of metaplasia in the antrum and corpus and hence need to be added in the biopsy protocol.

W1358

What Is the Appropriate Endoscopic Surveillance Interval for Patients with Helicobacter Pylori-Associated Gastritis and Intestinal Metaplasia in a Country with Moderate Risk of Gastric Cancer?

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Background: Helicobacter pylori (Hp)-associated chronic gastritis and intestinal metaplasia (IM) are potential premalignant lesions. However, the timescale of progression of these lesions to gastric cancer and the optimal interval for performing surveillance endoscopy to detect these lesions are unknown. Aim: This study analyses patients with endoscopy records prior to developing gastric cancer to provide information for decisions on appropriate surveillance intervals for patients with premalignant gastric lesions. Patients and methods: 20,985 patients underwent gastroscopy at a single center over a 10 year period. 402 (1.9%) were diagnosed with gastric cancer. Those patients who underwent gastroscopy with initial non-cancer findings but who subsequently developed gastric cancer were identified. Their records were matched against those of the National Cancer Registry to ensure completeness. Results: 24 patients developed de novo gastric cancer with records of prior endoscopy at least 18 months before. The endoscopic findings at first gastroscopy were: normal findings in six patients, gastritis in eleven, peptic ulcer in seven. Histology was obtained at the initial endoscopy in 9 of 24 patients, and this showed Hp in all of them and IM in four of them. The median time between gastroscopy with initial non-cancer findings and the subsequent discovery of gastric cancer was 51 months (range 18-121) for the series of 24 cancer patients; 50 months (18-82) for patients with gastritis (n=9); 39 months (18-81) for gastritis with Hp (n=6); 31 months (18-81) for gastritis with Hp plus IM (n=4) respectively. Surveillance at 18 and 24 months from the previous endoscopy would have detected 100% and 87.5% of cancer cases respectively. Conclusion: Patients with Hp gastritis and intestinal metaplasia are at increased risk of developing gastric cancer and endoscopic surveillance at 1.5 to 2 yearly intervals is appropriate to detect gastric cancer at an early stage. A prospective surveillance programme for cohorts at high risk of gastric cancer is in progress.

W1359

Chromoendoscopic Evaluation of Intestinal Metaplasia: An Update

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BACKGROUND: Studies have reported that intestinal metaplasia (IM) of the stomach can be detected chromoendoscopically using methylene blue (MB). However, wealth of information regarding the accuracy of this technique, morphologic features of intestinal metaplasia and its association with H. pylori infection is still lacking. OBJECTIVES: 1. To determine the accuracy of methylene blue staining for the detection of IM. 2. To determine the relationship of IM with age, sex and H. pylori infection either by rapid urease test or histology. 3. To report the endoscopic and histologic features of IM. METHODS: 93 consecutive dyspeptic patients, aged 40 years and above underwent elective EGD followed by MB staining of the stomach with emphasis on the antrum and distal third. Positive staining was interpreted as persistently blue staining mucosa despite water irrigation. Staining patterns were observed followed by 5 MB targeted biopsies. Similarly, 5 random biopsies were collected from subjects without demonstrable staining. H. pylori infection was documented both by rapid urease test and histologically. RESULTS: Sensitivity and specificity were 90.9% (PPV= 87%), 95.8% (NPV=97.1%) respectively. Accuracy rate was at 94.6% while the prevalence rate of intestinal metaplasia in the study population was 23.7%. Three staining patterns of intestinal metaplasia confirmed histologically were observed: focal- 70%, diffuse- 15%, multifocal-10%. Of the 22 cases of intestinal metaplasia, the following were documented histologically: focal- 90%, focal with dysplasia- 4%, marked intestinal metaplasia- 4%. According to age grouping intestinal metaplasia was detected in the following: 40-49 years- 13.6%, 50-59- 27.3%,60-69-27.3%, 70-79- 22.7%, 80 years and above- 9.1%. Using the Pearsons Chi- square test, there is no sufficient evidence to conclude that there is an association between sex and IM (0.010, df-1, p value-0.921), age and IM (1.906, df-1, p value-0.167) intestinal metaplasia and H. pylori infection either by rapid urease test (2.912, df-1, p value-0.088) or by histopath (0.409, df-1, p value-0.523). Conclusion:MB chromoendoscopy is accurate for the detection of intestinal metaplasia of the stomach. Based on our results, there is insufficient evidence to conclude that this lesion is associated with sex, age and H. pylori infection. In our study, 3 specific staining patterns were observed namely: focal, diffuse and multifocal staining. Dysplasia was noted in only 1 of the 22 confirmed cases of intestinal metaplasia.

W1360

Interobserver Variation in the Histopathologic Assessment of MALT/MALT Lymphoma: Towards a Consensus

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Background: Modern classification of MALT lymphoma is based on characteristic morphologic and immunophenotypic patterns as well as distinctive chromosomal aberrations. The most important and first step in diagnosis is based on evaluating H&E stained sections. Aim: To determine the interobserver variability among pathologists in evaluating gastric lymphocytic infiltrates. Methods: We organized an inter-observer study on MALT diagnosis designed to provide insight into current approaches to gastric MALT lymphoma diagnosis. A set of 41 gastric biopsies that ranged from simple gastritis to primary gastric lymphoma was reviewed independently by 17 participants. Participants included hematopathologists, pathologists with interest in gastrointestinal pathology, and general pathologists from the United States, Europe, and Japan. The resulting data were analyzed using kappa statistics and Monte Carlo simulation was used to correct for multiple biases. Then a team conjointly reviewed the slides and the results to devise a diagnostic approach with a comparative cost benefit analysis to identify the most cost-effective method for diagnosing gastric MALT lymphoma. Results: The initial blinded interobserver reproducibility was suboptimal. The kappa was 0.3 for simple gastritis, low grade MALT and for high grade MALT lymphoma. Further scrutiny using Monte Carlo simulation suggested that the degree of agreement was directly related to the pathologist's experience with gastric biopsies with MALT lesions. Even in this group the kappa was 0.3. The recommended cost-effective approach favored the combination of morphology, immunophenotype and molecular patterns. The suggested approach will be studied prospectively. Conclusions: The terminology presently used to classify gastric lymphomas is both controversial and confusing and interobserver reproducibility among pathologist is suboptimal. The group agreed on findings that would increase the reproducibility of diagnosis, especially for pathologists with limited experience with this disease. Important features include macroscopic data, extensive sampling, the presence of lymphoepithelial lesions, abnormal collections of B-cells, as well as molecular finding such as monoclonality and t (11; 18).

W1361

Capsule Endoscopy in Gasric Malt Lymphoma and Other Gastrointestinal Lymphomas

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Background: Until now the relevance of capsule endoscopy in the staging of gastrointestinal lymphomas has not been evaluated. The aim of this prospective trial is to assess the frequency of intestinal involvement in newly diagnosed gastric lymphoma and to define the appearance of gastrointestinal lymphomas obtained by capsule endoscopy. Methods: Commercially available capsule video endoscopes (Given M2A, Given Imaging Ltd.) were given to 21 patients (13 male and 8 female; age 27-77 years) with known or suspected gastrointestinal lymphoma. 3 patients were investigated twice. Evaluation of the capsule images was carried out by one of the authors. Results: 5 of 5 examined patients with primary intestinal lymphomas had pathological findings (jejunal or ileum ulcerations in 4/5, villous atrophy with erythema in 1/5, prominent nodes in 1/5 and plaques/microplaques in 1/5). One patient with severe diarrhea was examined twice after receiving 3 courses of chemotherapy and improvement of the lesions was evident going along with resolution of diarrhea. 16 patients with gastric lymphoma (12 low grade of MALT type and 4 high grade B cell lymphomas) were examined. In 2 patients the capsule did not leave the stomach indicating impaired motility as a possible reason. In one patient a second capsule was released in the duodenum by conventional endoscopy revealing no abnormalities. 4 of 16 examined patients had pathological findings (1 with villous atrophy and plaques in ileum and jejunum, 1 with microplaques in duodenum and jejunum, 1 with secondary histologically proven follicular lymphoma in duodenum and jejunum with jejunal plaques/microplaques and 1 with secondary histologically proven high grade lymphoma in ileum presenting with localized jejunal villous atrophy and prominent nodi and erythema in ileum). Conclusions: Capsule endoscopy is a valuable tool for patients with intestinal lymphoma for definition of the extend of bowel involvement and for evaluation of treatment efficacy. In this ongoing series, 25% of patients with gastric lymphoma had pathological findings in capsule endoscopy, 2 patients presenting with a secondary distinct lymphoma entity. Our results indicate that the role of capsule endoscopy has to be further evaluated since the results may have clinical relevance for management of the individual patient.

W1362

Results of the German Randomised Prospective Multicenter Study in 42 Patients with Localized Primary Gastric B-Cell Lymphoma

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Background: A stage- and histology-stratified therapy of primary gastric B-cell lymphoma has proven as an effective management offering a favourable prognosis (Fischbach et al., Gastroenterology 2000; 119: 1191-1202). There is an ongoing discussion if one can pass on a surgical approach in favour of a primary radio-/chemotherapeutical therapy to treat localized primary gastric lymphoma. We here present the first results of 42 patients participating in the randomised trial. Methods: 42 patients (18 female, 24 male) were diagnosed to have primary gastric B-cell lymphoma (28 high-grade, 14 low-grade) by central histological review. All underwent complete staging work-up revealing a stage EI or EII. All 14 patients with low-grade gastric lymphoma were non-responders to Helicobacter pylori eradication therapy or relapsed thereafter. They were referred to either gastric surgery or radiotherapy after central randomisation. All 28 patients with high-grade lymphoma received gastric surgery or chemotherapy randomly. The median follow-up time is 16.4 months (range 5-48). We reviewed the data with respect to therapeutical outcome, relapse at any time and overall survival. Results: 7 patients with low-grade gastric lymphoma (3 stage EI, 4 stage EII) underwent gastric surgery. All had complete lymphoma remission (CR). 1 patients relapsed after 8 months. Further 7 patients with low-grade gastric lymphoma (5 stage EI, 1 stage EII, 1 stage EI/II) were referred to radiotherapy. All but one acchieved CR, there was no relapse. 15 patients with high-grade gastric lymphoma (6 stage EI, 8 stage EII, 1 stage EI/II) were randomised to undergo gastric surgery. All achieved CR, there was one relapse. Further 13 patients with high-grade gastric lymphoma (8 stage EI, 4 stage EII, 1 stage EI/II) received primary chemotherapy. All patients were re-staged to have CR, nobody relapsed. Conclusion: The data of this prospective study underline that primary gastric lymphoma has a good prognosis after initial treatment (complete lymphoma resection followed by radio-/chemotherapy or primary radio-/chemotherapy). The follow-up time is too short to favour one therapeutic option. However, with respect to the quality of life even these preliminary data should lead to pass on a complete gastric resection. Long-term follow-up is necessary to substantiate this point.

W1363

High Frequency of Chromosome X Gains Is Characteristic of Gastric MALT Lymphoma: Comparative Genomic Hybridization Using Gene-Cluster Analysis

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Background: Mucosa-associated lymphoid tissue (MALT) lymphoma is a subset of non-Hodgkin's lymphoma that arises from lymphoid aggregates in the lamina propria. The cytogenetic abnormalities are incompletely known in part because karyotyping depends on the availability of fresh tissue that is rarely available in low-grade gastric MALT lymphoma, particularly in early cases. The current system of diagnosis is primarily based on morphologic criteria and is only weakly predictive of outcome. Methods: We used universal DNA-amplification on formalin fixed paraffin embedded tissue that ranged from simple gastritis to primary gastric lymphoma (low-grade and high-grade). This was followed by comparative genomic hybridization using gene-cluster analysis. Results: Comparative genomic hybridization was done on 25 low-grade MALT lymphomas, 25 high-grade primary gastric lymphomas, and 25 with reactive gastritis. CGH analysis was possible in all cases. CGH was able to identify aberrant regional changes. In low grade MALT lymphoma, genomic regional aberrations were seen in chromosome 18 (25% of cases), chromosome 21 (30% of cases), and chromosome X (30% of cases). In high-grade primary gastric lymphoma, genomic regional aberrations involved chromosome X (40% of cases), chromosome 16 (30% of cases), and chromosome 1 (30% of cases). In chronic gastritis, regional genomic aberrations were seen in chromosome 20 (10% of cases), chromosome 12 (5 % of cases), and chromosome 4 (5% of cases). Using cluster analysis with Bonferroni adjustment for multiple comparisons, differences in gene expression were limited to chromosomes 3, 11, 18, and X. Conclusion: Our data demonstrate the feasibility of Comparative genomic hybridization to visualize complete and partial chromosome gains/losses and thus allow gene amplification in archived MALT lymphoma samples. Hierarchical cluster analysis decreases the false detection rate that is common in microarray studies. These finding suggest gains on four chromosomes are frequent in gastric MALT lymphoma including chromosome X and chromosome 3 in both low grade and high grade MALT lymphoma. The association with the X gene might explain the gender differences in lymphoma prevalence.

W1364

The Clinical Course of Long Followed up Gastric Malt Lymphoma After Antibacterial Treatment and/or Radiotherapy

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Purpose: It is well accepted that most of gastric low-grade MALT lymphoma improved after Helicobacter pylori (HP) eradication. Moreover, radiotherapy has been reported to be effective in patients with no evidence of HP or who do not responded to antibacterial treatment. Data are lacking, however, with regard to the duration of the remission. This study was performed to evaluate the efficacy of antibacterial therapy and of radiotherapy for gastric low-grade MALT lymphoma in the series of long follow up. Method: Sixty patients with gastric low-grade MALT lymphoma after antibacterial treatment of HP observed for more than 2 years (median 50 months, range 28-94 months) between 1995 and 2003 were evaluated. HP was positive in 54 of the 60 patients. Patients were followed up by means of endoscopy and biopsy every 3-6 months. Histological diagnosis was classified according to grading scores of Wotherspoon. Result: In all of 54 patients positive for HP, eradication therapy was successful. Fifty patients (93%) showed complete remission (CR) of lymphoma (grade 0-2). One partial remission (grade 3) with endoscopic improvement have been watchful waiting for 26 months. Three non responded patients in histologically (grade 4-5) and endoscopically underwent radiotherapy. Three relapses were found 9, 11, and 25 months after histological remission by eradication therapy. Two of them showed protrudent features. None of them occurred t(11;18)(q21;q21) translocation. Subsequent histological remission was found in all of three without any additional therapy. One of HP negative 6 patients showed CR after antibacterial treatment. The other 5 patients showed no change and underwent radiotherapy. In consequence, 8 patients were treated with radiotherapy. Five of them occurred t(11;18)(q21;q21) translocation. The radiation dose was 30 Gy in a daily fractions of 1.5 Gy. All of them showed CR without major complication. No relapse was observed at a median follow-up of 25 months (range 13-72 months). Conclusion: Our results show the high efficacy of antibacterial treatment in HP positive cases and that of radiotherapy in HP negative cases or t(11;18)(q21;q21) positive ones for prolonged remission of gastric low-grade MALT lymphoma.

W1365

Introduction of Enhanced Antiretroviral Therapy Has Altered the Natural History and Risk Factors Associated with Gastrointestinal Lymphoma

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Background: Risk factors for developing gastrointestinal lymphoma (GI-L) such as HIV positivity, immunosuppression, and H. pylori have undergone variations in prevalence over the past two decades, which may have altered the incidence of GI-L. Aim: To find incidence, risk factors, histology and endoscopic presentation of GI-L in a large population sample from 01/00 to 09/03 and compare with a previous study from 1983-94. Methods: IRB-approved retrospective chart review of all histologically confirmed GI tract lymphoma cases from 01/00 to 09/03 using a database which captures all pathological diagnoses within a catchment area of over 1 million people in Western Canada. HIV positivity was cross-referenced from a provincial HIV database. Lymphomas were classified according to WHO criteria, and compared with a previous analysis of GI-L occurring between 1983 and 1994. Results: Twenty patients (11 female, 9 male; mean age 63 years, range 28-79) presented with lymphoma in the GI tract over the study period. None were known to be HIV positive, compared to a total of 13 GI-L documented in the previous study, although the prevalence of HIV infection has increased significantly in the region over the study periods. One patient had significant immunosuppression (post-transplant). Histology revealed 11 (55%) Diffuse Large B-Cell, 3 (15%) Extranodal Marginal Zone (MALToma), 3 (15%) Burkitt-like, 2 (10%) Follicular, and 1 (5%) Peripheral T-Cell (T/NK type) lymphomas. The GI tract was the primary site of lymphoma in 14/20 (70%) of cases; the remainder were metastasized from other sites. The most common site was stomach (10/20), followed by colon (5/20) and small bowel (including duodenum) (5/20). Age-standardized annual incidence for all GI tract lymphoma was 4.5 per million. Endoscopy was undertaken in 15/20 cases; 13 of these were diagnostic and the remaining 2 were normal or showed reactive changes only (both subsequently found to have lymphoma in small bowel at a non-endoscopically visualizable site). Endoscopic appearances were variable (mass, ulceration, mucosal wall thickening) and had no discernable correlation with lymphoma type. Conclusions: In a large Canadian population sample, incidence of HIV-related GI-lymphoma has dropped dramatically compared to the previous decade, which can be assumed to be due to implementation of highly active antiretroviral therapy. Endoscopic appearances are variable and do not correlate with lymphoma type.

W1366

Pancreatic Neuroendocrine Tumors: The Effect of Surgical and Medical Therapy on Survival

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BACKGROUND. There are few studies regarding the long-term results of the various therapeutic modalities used in pancreatic neuroendocrine tumors (NET). AIM: To evaluate the efficacy of the various treatment modalities in a large series of patients with pancreatic NET. PATIENTS. Of 275 patients with NET or MEN-1 followed by our Institution, 79 (43 M, 36 F, mean age 59.1 years, range 28-82) had pancreatic NET. The diagnosis of pancreatic NET was based on clinical history, evaluation of several specific and non-specific plasma markers and the findings of imaging techniques. MAIN OUTCOME MEASURES. The patients underwent a clinical check-up and abdominal ultrasound every 3 months during the first year after the diagnosis and every 6 months thereafter; surgical and medical procedures and survival rates were recorded. Kaplan-Meier curves were used to estimate the survival. RESULTS. Of the 79 patients studied, 2 were lost at follow-up; the mean follow-up of the remaining 77 patients was 55.7 months (range 2-252 months). Fifty patients (64.9%) had non-functioning NET, 15 (19.5%) had functioning NET and 12 (15.6%) had MEN 1 disease with pancreatic involvement. The tumor was localized in the pancreatic head in 27.3% of the cases, in the head and body in 10.4%, in the body in 10.4%, in the body and tail in 26%, diffuse throughout the gland in 5.2%. The size of the tumor was <30 mm in 31.1% of the cases and >30 mm in the 54.4%; the tumor size was not available in 14.5% of the cases. Forty-five percent of the patients had distant metastases at the time of diagnosis and 10% developed metastases during the follow-up period. Twenty-three patients had radical surgery, 42% had debulking surgery and 35% were treated medically (11 with chemotherapy, 13 with chemoembolization and 51 with somatostatin-analogs). The size of the tumor was not significantly related to the survival ($P=0.110$), whereas there was a statistically significantly longer survival in patients without metastases at diagnosis ($P<0.01$) and in those who did not develop metastases at follow-up ($P<0.01$). Patients who underwent curative surgery or debulking surgery had a statistically longer survival than patients who had had no surgery ($P<0.05$). Medical treatment did not affect survival. CONCLUSIONS. Radical surgery plays a central role in the therapeutic approach to NET of the pancreas and still represents the therapy of choice. Debulking surgery is recommended in cases with multiple liver metastases.

W1367

Hepatic Intra-arterial ^{90}Y-DOTA-lanreotide: A Novel Treatment for Gastroenteropancreatic (GEP) Neuroendocrine Tumours

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Somatostatin receptors (SSTR) are expressed at a high level on various tumor cells, providing the molecular basis for the successful use of radiolabeled somatostatin analogues as tumour tracers. The vast majority of GEP neuroendocrine tumours over-express at least one of the five SSTRs, most frequently SSTR 2. ^{111}In-DOTA-lanreotide has unique binding properties, binding SSTR 2 to 5 with high affinity. When labeled with beta-emitting radioisotope yttrium-90, this peptide can be utilised for receptor-mediated radionuclide therapy. The purpose of this study is to evaluate whether delivery of ^{90}Y-DOTA-lanreotide directly to liver metastases by hepatic intra-arterial injection is an effective treatment for patients with advanced GEP neuroendocrine tumours, who have very limited therapeutic treatment options available. Methods: Twenty-one patients with an established histological diagnosis of metastatic GEP neuroendocrine tumour were treated. All patients had radiological evidence of disease progression in the preceding 6 months, had at least 85% of their tumour load within the liver, and were SSTR-positive on scintigraphic imaging. The treatment consisted of selective hepatic artery intra-arterial injection of 1.2 GBq/m^2 ^{90}Y-DOTA-lanreotide, administered with or without polyvinyl alcohol particles. Radiological tumour response was determined by CT imaging three months after each treatment. Further cycles were given at 3 monthly intervals. Results: Tumour response was defined according to the WHO criteria. To date, regressive tumour disease was found in 14%, stabilization of tumour disease in 62%, and tumour progression in 14%. Concurrent particle embolisation made no difference to tumour response rate. Mean progression-free response was 9 months. No patient developed renal toxicity. Three patients developed haematological toxicity (NCI criteria): one grade 3 and two grade 4. Two recovered within 8 weeks, and one died from tumour progression. Median survival time of the 10 patients who died was 9 months. The study is ongoing. Conclusion: Treatment with hepatic intra-arterial injection of ^{90}Y-DOTA-lanreotide is an effective treatment for patients with advanced progressive GEP neuroendocrine tumours whose disease is predominantly in the liver. This novel therapy may potentially be indicated in patients with SSTR-positive advanced hepatocellular carcinoma

W1368

Long-Term Efficacy of Radionuclide Therapy in Patients with Disseminated Neuroendocrine Tumors Uncontrolled by Conventional Therapy

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Therapeutic options in patients with advanced-stage gastro-entero-pancreatic (GEP) neuroendocrine tumors are limited. Radionuclide therapy is possible but has never been evaluated in a controlled study. We compared the efficacy of radionuclide therapy with 111In-pentetreotide and 131I-MIBG in 20 patients (group A) to the outcome of similar patients who could not be treated for non-medical considerations (group B, $n=12$). All patients were planned to be treated because of uncontrolled tumor disease ($n=21$), contraindication to chemotherapy or surgery ($n=7$) and/or uncontrolled and badly tolerated clinical symptoms ($n=4$). Methods Group-A patients received monthly three times 3.7 to 7.4 GBq of 131I-MIBG ($n=5$) or 7 GBq of 111In-pentetreotide ($n=15$), according to the best tracer uptake. Clinical evaluation, biological tests and conventional imaging were performed at months 3, 6, 12, 18 and 24. Therapy was considered beneficial if clinical status improved, laboratory tests for secreting tumors improved by > 20%, tumor progression was halted, the size of the most significant localization had decreased by > 20%, the dosage of analgesic and cold somatostatin therapy could be lowered. Pejorative events were defined as: side-effect due to therapy, relapse in clinical symptoms, tumor progression, tumor laboratory marker increase, death. Results Overall survival rate at month 3 was significantly higher in group A ($p=0.05$). Radionuclide therapy was considered temporarily beneficial in 15 patients (79% of group A). The average time before relapse was 16.1 ± 7.8 months. Only one patient had a significant side-effect. Tumor progression within 3 months is significantly higher in group B than in group A ($p<0.01$). The cumulative event-free survival rate during the first 15 months was significantly higher in patients receiving radionuclide therapy ($p=0.019$). Conclusion Radionuclide therapy is feasible and safe and significantly defers the occurrence of fatal and non-fatal events in patients clinically uncontrolled by conventional therapy.

W1369

What Is the Influence of Bile Infection on the Early Postoperative Course After Pancreaticoduodenectomy ?

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Introduction - Aim : The effect of preoperative biliary drainage on the early postoperative course after pancreaticoduodenectomy (PD) is controversial. Among drawbacks of biliary drainage, bile superinfecton and its consequences are incompletely assessed. This study compared postoperative course of PD in patients with sterile and infected bile. Patients and Methods : From January 2002 to June 2003, 79 patients underwent PD for periampullary tumor with routine culture of gallbladder bile and antibiotic prophylaxis by cefazolin + metronidazole (C+M). Thirty-five patients with infected bile (group G1) were compared with 44 patients with sterile bile (group G2). Both groups were comparable regarding age, ASA score, tumor size and consistency of the pancreatic remnant. Results : Distribution of tumors was comparable in both groups except for ampullomas which were more common in G1 (26% vs 2%, $p=0.001$). Preoperative interventional biliary endoscopy has been performed in 80% of patients in G1 (vs 14% in G2, $p<0.001$), including 9 isolated sphincterotomies (20% vs 5%, $p<0.03$) et 20 endoprosthesis insertions (57% vs 0%, $p<0.0001$). Operative time and blood loss were similar in both groups. One patient died postoperatively (G1). Rate of pancreatic fistula was similar in both groups. Overall morbidity was increased in G1 (77% vs 59%, $p=0.05$. Postoperative infectious complications (all demonstrated bacteriologically) included wound infection (26% vs 5%, $p=0.005$), intraabdominal abcess (23% vs 7%, $p=0.035$), and pneumonia (14% vs 2%, $p=0.045$). To treat infectious complications, antibiotics (> 7 days) were more often given in G1 (71% vs 43%, $p=0.012$). In bile culture, the 3 most frequent germs were : Escherichia Coli, Enteroccocus Fecalis et Klebsiella Pneumoniae. Among G1 patients, bile contained at least 2 germs in 54%, and isolated germs were resistant to the association C+M in 94%. In patients with infectious complications, the same germ was isolated in bile and another sampling in 49%. In G2, bile sterility was definitively established after a 48h-culture in all patients but one. Conclusions : In patients undergoing PD, bile infection is related to previous interventional biliary endoscopy in 80% of cases. Bile germs are resistant to the association C+M in 94% of cases, and responsible for an increased rate of infectious abdominal complications. In patients undergoing PD for ampulloma and/or after interventional endoscopy, specific prophylactic antibiotics for 48h should be evaluated.

W1370

Heme Oxygenase-1 (HO-1) Inhibition Sensitize Pancreatic Cancer to Adjuvant Treatment

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Pancreatic cancer shows very poor survival rates mainly due its aggressive growth behavior and its exceptional resistance to all forms of adjuvant treatment. The so-called protective gene heme oxygenase-1 (HO-1), which plays a key role in the defense against all kind of cellular stress, is highly expressed in different human cancers. In several experimental solid tumor models the inhibition of HO-1 activity decreased tumor growth, by induction of apoptosis and/or inhibition of angiogenesis, and prevented the occurrence of metastasis. In this study we demonstrate that the cell specific down-regulation of HO-1 expression and activity sensitize pancreatic cancer cells to adjuvant treatment options. Methods: The expression of HO-1 was analyzed in human pancreatic cancer samples in comparison to normal pancreas by quantitative PCR, Western blot analysis and confocal microcopy. Influence of radio- and chemotherapy on HO-1 expression in pancreatic cancer cell lines was evaluated. Finally, HO-1 expression was specifically suppressed by siRNA transfection. Alterations of growth behavior and resistance to adjuvant treatment were tested. Results: Human pancreatic cancer showed significant over-expression of HO-1 in comparison to normal pancreas on mRNA and protein level. The cancer tissue revealed marked immunostaining in tumor cells and in some tumor associated macrophages. Pancreatic cancer cell lines demonstrated divergent expression levels, from high to not detectable. Treatment of the pancreatic cell lines with Gemcitabine or radiation strongly induced HO-1 expression. Targeted knockdown of this HO-1 expression led to pronounced growth inhibition of the pancreatic cancer cells and made tumor cells significantly more sensitive to radio- and chemotherapy. Conclusion: HO-1 seems to give pancreatic cancer cells a growth advantage and makes them resistant against radio- and chemotherapy. Specific inhibition of HO-1 sensitizes tumor cells to adjuvant treatment and may therefore be a new valuable agent in the therapy of pancreatic cancer.

W1371

Therapeutic Evaluation of a Novel Combined Therapy with the Oral Fluorouracil Preparation S-1 and Cisplatin in Patients with Inoperable Pancreatic Cancer

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S-1 is a novel oral fluorouracil antitumor drug that combines tegafur, 5-chloro-2,4-dihydroxypyridine, and potassium oxanate. Although efficacy of S-1 in patients with advanced pancreatic cancer has recently been reported, it is important to select patients whose survival time can be expected to increase. The aim of this study was to evaluate the clinical factors associated with the efficacy of S-1 in patients with inoperable pancreatic cancer. Method: Sixteen patients with locally advanced and metastatic pancreatic cancer were treated with S-1 combined with weekly cisplatin. Results: There were 5 SD cases and 11 PD cases, and median survival overall was 166 days. Hematotoxicity was grade 1 in one patient, grade 2 in two patients, and grade 3 in three patients. Four patients experienced grade 2/3 nausea and vomiting. Intestinal perforation occurred in one patient. Response could be evaluated in 16 patients, and we compared the clinical factors at the start of administration of S-1 and cisplatin between the eight patients who survived more than 100 days (group A) and the eight patients who survived less than 100 days (group B). Median survival was 304 days in group A, and 43 days in group B. There were no differences in age, tumor location, tumor size, or tumor stage between the two groups. Performance status was grade 0/1 in 75% in group A and 37% in group B. Although the serum CA19-9 concentration was elevated in all patients, the values were lower in group A (600 ± 243 U/ml) than in group B ($74{,}980 \pm 46{,}304$ U/ml). Conclusion: S-1 in combination with cisplatin prolonged mean survival in patients with low-grade PS and relatively low serum CA19-9 levels. PS and serum CA19-9 level may be important factors for predicting the efficacy of S-1 plus cisplatin in patients with pancreatic cancer. S-1 in combination with cisplatin is effective in the treatment of inoperative pancreatic cancer.

W1372

Effect of TNFerade Local Gene Therapy on Expression of Tumor Necrosis Factor Alpha (TNF-α) and Survivin in Tumors of Patients with Pancreatic Cancer: Can We Prove and Predict Response to Therapy?

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TNFerade is a novel gene transfer therapy using a replication-deficient adenovector containing human TNFα gene, regulated by a radiation-inducible promoter Egr-1. We recently treated 37 pts with locally advanced, unresectable adenocarcinoma of the pancreas (LAPC) with TNFerade delivered by EUS and percutaneous approaches. A substudy was undertaken to assess intra-tumor (serial weekly FNA biopsies, autopsy, surgery) TNFα expression. Expression of survivin, a novel inhibitor of apoptosis present in cancer cells, and a radioresistance factor in pancreatic cancer, was also assayed. Purpose: 1) Determine TNFα and survivin expression in pts with LAPC receiving TNFerade plus chemoradiation, including: expression at baseline, weekly injections, peak activity, durability, and relationship between TNFα and survivin. Methods: Five-week treatment consisted of weekly intratumoral injections of TNFerade (GenVec Inc.) via EUS-guided FNI ($4 \times 10^{10\text{-}11}$ particle units in 2 mL), continuous IV 5-FU (200 mg/m^2/day x 5 days/wk) and radiation (50.4 Gy). EUS-guided FNA biopsy was performed prior to TNFerade injection at weeks 1-5, and post-Rx week 4,8,12. TNFa and survivin determined by immunohistochemistry, as well as number of cancer cells, were quantified (0-3 score). Results: 4 pts were enrolled (3M/1F) see Table. TNFα expression was present in pancreatic cancer and inflammatory cells in all pts (serum TNF = nl), with up to a 3-fold increase expression within the tumor during the 5-week treatment (peak = week 3-5). TNFα expression was markedly reduced or absent by 2 mo post treatment. Survivin expression in cancer cells was present in all pts. One pt with a complete pathologic response to treatment had a very low expression of survivin at baseline and throughout treatment. Conversely, all other pts had absent or low baseline expression of survivin followed by a rise during treatment. Conclusions: 1) TNFα is expressed in cancer cells in patients receiving TNFerade gene transfer and chemoradiation 2) TNFα expression peaks between weeks 3 to 5 with durability of approximately 1 month post therapy. 3) Survivin may potentially predict patient response to therapy.

	Pt 1	Pt 2	Pt 3	Pt 4
Age	71	50	63	72
Tumor Size (cm)	3.2 x 2.8	3.8 x 3.5	4.0 x 3.0	5.8 x 5.0
Tumor Stage	Stage III	Stage III	Stage III	Stage IIB
TNFerade Dose	4x10 ^{10} pu	4x10 ^{10} pu	4x10 ^{10} pu	4x10 ^{11} pu
Tumor Response	Complete	Stable	Minor	Minor
Status	Alive	Died	Alive	Alive
Survival (months)	11	4	8	7

W1373

Ras Peptide Vaccination in Resected Pancreatic Cancer Patients; Safety, Immune Response and Survival

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K-Ras mutations are found in most adenocarcinomas of the pancreas, and induction of immunity against mutant Ras may be of clinical importance for patients with pancreatic cancer. The present studies were performed to determine safety and immunogenicity of intradermal vaccination with synthetic mutant RAS peptides in a phase I/II trial in patients who had undergone Whipple surgery. Patients were also followed for survival. The trial patients were divided into two groups, one for each of the two clinical studies, CTN98010 ($n = 13$) and CTN95002 ($n = 10$). Patients in CTN98010 were given a mixture of 7 mutant ras peptide, (700μg), corresponding to the most common mutations in pancreatic adenocarcinoma. The patients in CTN95002 were given a single mutant RAS peptide (100μg) corresponding to the RAS mutation identified in the patient's tumor. Patients were vaccinated in a 10 week regimen, using GM-CSF as an adjuvant, and were given boosters over an extended period of time. Immune responses were measured as skin reaction (DTH) and T-cell response. The majority of patients mounted an immune response to the vaccine. All evaluable patients (at least finishing week 4) in CTN98010 had a positive DTH reaction (100%), compared to 6 out of 9 (67%) evaluable patients in CTN95002. The vaccine was well tolerated; there were no signs of toxicity, or serious adverse events (no NCI grade 3-4 toxicity) related to the vaccine. The median survival for all patients included in the studies ($n = 23$) and the evaluable patients ($n = 20$) was 772 and 805 days, respectively. 25% of the evaluable patients survived for five or more years. The studies documented the immunogenicity and tolerability of the vaccination. Although a limited number of patients was included in this study, the data suggests that a clinical benefit of ras peptide vaccination may be obtained for this group of patients.

W1374

Allogeneic Reduced Intensity Stem Cell Transplantation for Advanced Pancreatic Cancer

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Background & Aim: Allogeneic reduced intensity stem cell transplantation (RIST) is a recently-developed treatment approach to obtain graft-versus-tumor (GVT) effect without the toxicity of myeloablative conditioning regimen. The feasibility of RIST in patients with solid cancers has been already shown in several studies, but not for pancreatic cancer. We evaluated RIST for advanced pancreatic cancer.

Patients & Methods: We started a prospective study of RIST against advanced pancreatic cancer after ethical approval in April, 2002. Only patients with pathologically proven pancreatic cancer, that was locally advanced or metastatic and not amenable to curative resection, were included. The preparative regimen consisted of gemcitabine, fludarabine, and busulfan. Graft-versus-host disease (GVHD) prophylaxis was performed with cyclosporine A (CsA) and short-term methotrexate. Mobilized peripheral blood stem cells were collected from an HLA-identical sibling donor and infused on day 0. So far seven patients underwent RIST after obtaining a written informed consent by October 2003, and we evaluated transplant-related complication and anti-tumor effect for 6 patients that passed after RIST more than two months.

Results: Complete donor chimerism was achieved in all of 6 patients on day 28. Grade II-III acute GVHD was observed in 3 patients before tapering CsA and in 2 after the rapid tapering of CsA to induce GVT effect. There was no transplant-related mortality within 100 days after transplantation. Three patients died with progressive disease on day 72, 262, 293, and one patient died with bacteremia on day 192. Two patients are alive at 137, 361 days after RIST, respectively. Objective minor response on CT scan was achieved in two patients associated with the development of GVHD two months after RIST. In one patient, tumor marker was decreased after the discontinuation of steroid. These suggested that the anti-tumor effect was brought by GVT effect. Three patients among 4 dead patients were not able to leave the hospital after RIST.

Conclusion: The anti-tumor effects appeared at least 2 months after RIST, suggesting the existence of GVT effect against pancreatic cancer. We need to accumulate more experiences to determine whether this treatment approach really improves survival of patients with advanced pancreatic cancer without impairing quality of life.

W1375

Pretreatment with Gemcitabine and Cyclooxygenase-2-Inhibitors Sensitizes Pancreatic Carcinoma Cells to Cytotoxic T Cell Responses Induced by Tumor-Lysate Pulsed Dendritic Cells

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Purpose: To investigate whether pre-treatment with chemotherapy or cyclooxygenase-2 (COX-2) inhibitors can sensitize pancreatic carcinoma cells to CTL-mediated cytotoxicity induced by tumor-lysate pulsed dendritic cells (DCs). Methods: DCs were derived from monocytes of HLA-A2+ donors according to a novel 2-day protocol, loaded with lysate from the HLA-A2+ pancreatic carcinoma cell line Panc-1 and cocultured with autologous T cells. Restimulations with autologous, tumor-lysate pulsed DCs were performed at weekly intervals. ELISPOT analysis was used to confirm induction of a tumor-specific T cell response. Lysis of Panc-1 cells left untreated or pre-treated with gemcitabine (50 nM) or celecoxib (50 μM) was determined in a standard chromium release assay after two restimulations of T cells with lysate-pulsed DCs. Results: Lysate-pulsed DCs primed tumor-specific, IFN-γ producing T cells. CTLs derived from cocultures with lysate-pulsed DCs specifically lysed Panc-1 cells. Pre-treatment of Panc-1 cells with gemcitabine or celecoxib prior to coculture with CTLs enhanced specific lysis by 45 % and 70 %, respectively. Combined pre-incubation of Panc-1 cells with gemcitabine plus celecoxib lead to a more than two-fold increase in MHC class I-restricted tumor cell lysis indicating an additive effect on tumor cell sensitization. Conclusions: Pancreatic carcinoma cells can be sensitized to CTL-mediated lysis by pre-treatment with gemcitabine and celecoxib. Thus, the efficacy of a DC-based vaccine for patients with pancreatic carcinoma may be augmented by concomitant treatment with gemcitabine and COX-2 inhibitors.

W1376

Barriers to Acceptance of a Pancreatic Cancer Surveillance Program in Familial High-Risk Individuals

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Familial pancreatic cancer (FPC) is said to represent about 10% of pancreatic cancers. The search for predisposition genes has been centered on registries of pts and family members of loosely defined high-risk FPC pedigrees. Surveillance programs for high-risk, healthy family members use various imaging and endoscopic modalities with the goal of detecting early, and possibly curable, pancreatic cancer. Methods. In December, 2002 we began an FPC Registry for pts with non-syndromic pancreatic cancer and their high-risk relatives. Eligibility criteria include young age of onset (50 yrs or less) and/or multiple 1st and 2nd degree relatives affected. Our protocol includes the family registry, an epidemiologic case-control study of environmental risk using spouses or in-laws as controls, a surveillance program of unaffected healthy high-risk family members using Magnetic Resonance Cholangiopancreatography (MRCP) and Endoscopic Ultrasonography (EUS) as surveillance tools arbitrarily performed every 2 years, and banking of serum specimens for molecular genetic evaluation. Results. During the first 11 months of our program, 51 pts and family members met our eligibility criteria and were approached for recruitment into the registry. 14 spouses/in-laws were approached as controls for the epidemiology study. 27 pts and family members agreed to participate in our registry. 9 were pts with pancreatic cancer (7 women, 2 men) mean age 54 yrs, and 18 well individuals (8 women, 10 men, mean age 47 yrs) were family members of high-risk families. 6 eligible pancreatic cancer pts (4 women and 2 men, mean age 43 yrs) and 14 well family members (5 women and 9 men) refused our registry and study. The reason for refusal of the registry and surveillance study was that the cost of surveillance with MRCP and EUS would not be covered by health insurance in 12 of the 14 family members (86%). 8 of the 18 (45%) well family members who accepted the study also refused the surveillance portion of the protocol for the same reason. Conclusions. Family registries and new biomarkers may provide a better understanding of pancreatic cancer risk, so that a universally accepted definition of FPC can be developed. While it is not clear whether surveillance for pancreatic cancer in healthy high-risk family members will identify early and potentially curable cancer, for many members of pancreatic cancer families, there are no worthwhile alternatives. Public policy should change so that surveillance for FPC family members is accepted and supported.

W1377

Rectal Aberrant Crypt Foci Identified Using High-Magnification-Chromoscopic Colonoscopy: Biomarkers for Right-Hemi-Colonic Flat and Depressed Neoplasia

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Background: Aberrant crypt foci (ACF) may represent pre-neoplastic lesions in the human colon. The prevalence of ACF detected using magnification chromoscopic colonoscopy is known to follow a stepwise progression from normal subjects to those with exophytic adenomas and colon cancer (CC). No studies have addressed the prevalence of rectal ACF in patients with flat and depressed colonic lesions that cluster within the right hemi-colon and may undergo de novo neoplastic transformation. Methods: All patients underwent total colonoscopy by a single endoscopist using the Olympus CF240Z magnifying colonoscope. Prior to extubation, pan high-magnification-chromoscopy using indigo carmine was applied to the rectum and the distal 10 cm of mucosa examined using forward and retroflexed views. ACF were defined as 2 or more crypts with dilated or slit-like openings that were raised above the adjacent mucosa. Univariate logistic regression was used to assess the ability of the number of ACF to discriminate between 3 groups (normal, adenoma, cancer). Results: N = 1000, mean age 53 years (range 18-98), male 561 (56%), caecal intubation 958 (96%). Logistic regression: Endoscopically normal vs. JRSC II adenoma $p<0.01$. JRSC type II adenoma vs. JRSC type II cancer $p<0.001$. Conclusions: The number of ACF in normal patients, patients with JRSC II adenoma and JRSC II cancer follow a stepwise incremental change as previously observed for exophytic adenomas and cancer. Detection of ACF in the rectum may be a useful biomarker for proximal colonic flat neoplasia and could be used at index flexible sigmoidoscopic screening to stratify risk of right-hemi-colonic neoplasia. Patients with dysplastic ACF of high density should receive total colonoscopy.

	n	Total no. ACF	Median no. ACF	% ACF	% dysplastic ACF
Endoscopically normal	574	602	1 (0-5)	15	3
JRSC II adenoma (LGD/HGD)	281	2796	9 (1-22)	82	18
JRSC II cancer (T2 or beyond)	14	594	38 (14-64)	100	61

W1378

Fibered Confocal Fluorescence Microscopy: A New Route for Ultra High Resolution Endoscopy of the Colonic Mucosa

Sacha Loiseau, Benjamin Abrat, Magalie Genet, Eric Peltier, Igor Charvet

Background and study aims: Fibered confocal fluorescence microscopy appears to be a key technology as a future diagnostic tool for non-invasive optical biopsies. The development of magnifying endoscopy used with dyes allowed the study of colonic microstructures aiming at improving the diagnosis of colorectal lesions. In the present study, an innovative and novel technology was evaluated on mice with cresyl violet acetate for endoscopic observations of the colonic mucosa. The objective is to prove its performances and uniqueness for research applications on small animals and to show its potential short term evolution towards a clinical use. Materials and methods: Fibered confocal fluorescence microscopy enables functional imaging on living biological tissues at the microscopic level. High-resolution images were acquired with optical flexible mini-probes with dimensions allowing colonoscopy in mice. Image sequences were recorded for cresyl violet acetate concentration from 0,5% to 2% and staining from 2 to 5 min for both duodenum and colon mucosa. Results: Staining with 1% cresyl violet acetate led to identification cytoplasm crypts and microvilli with an optimal contrast. A high specificity of cresyl violet was not obtained at lower concentrations. These results were confirmed by observations with conventional confocal microscope after resection of colon mucosa. Conclusions: Fibered confocal fluorescence microscopy used with cresyl violet offers unprecedented research possibilities for in vivo colorectal studies on small animals. The same technology can be used in the very short term on patients with potential new diagnostic capabilities of colorectal cancers or lesions.

W1379

Assessment of the Nature and Size of Colorectal Polyps Using Chromo-Magnifying Colonoscopy: A Prospective Study

Giuseppe C. Gizzi, Maria A. Pantaleo, Valeria Villani, Raffaele Pezzilli, Barbara Rizzi, Gianfranco Epifanio, Rosa Ceciliato, Guido Biasco, Roberto Corinaldesi

INTRODUCTION: Conventional colonoscopy (CC) is inadequate for differentiating neoplastic from non-neoplastic polyps and for assessing exact shape and size of non-pedunculated polyps (NPP). The chromo-magnifying colonoscopy (CMC) improves the evaluation of the shape and size of sessile and flat polyps (including laterally spreading tumours, LST) for endoscopic mucosal resection and permits to assess the mucosal crypt pattern (MCP) of NPP. AIMS AND METHODS: To assess the shape and size of NPP and predict the histological findings evaluating the MCP using CMC in comparison with CC. Between September 2002 and April 2003, 200 selected patients among people at average risk for colonic carcinoma were enrolled for the study. Patients with IBD, HNPCC or FAP, and those with previous polypectomy or cancer resection were not included. All patients were evaluated with a colonoscope (Olympus CF Q160ZI) providing both conventional and magnifying views. All procedures were performed by a single endoscopist; the mean time (SD) to reach the cecum was 6.8 (1.5) minutes; the mean time (SD) for the complete procedure was 23.5 (5.8) minutes. For each lesion, 10 photos (5 with conventional view and 5 with magnifying plus mucosal dye spraying) were recorded by an electronic digital system (Casti Imaging - Italy) which also permitted an accurate estimation of the size of the lesions. Macroscopically, the lesions were classified as polypoid (pedunculated, semipedunculated, sessile) and non-polypoid (flat elevated-including LST-,flat even or flat depressed). A total of 312 colorectal lesions were detected: 276 (88.5%) NPP and 36 pedunculated polyps (11.5%); the latter lesions were excluded from the statistical evaluation. Histopathological examination was performed after endoscopic or surgical resections. McNemar test was used for statistical analysis. RESULTS: Results are reported in the Table. CONCLUSION: The CMC gives better results than a CC for diagnosing colonic tumors (neoplastic from NNP) It also permits a more accurate evaluation of the size of the lesions. Work supported by grants of FIRB - MIUR - Rome

Total number, (percentage) of the colonic lesions with the two techniques

	Non-neoplastic lesions	Adenomatous polyps	Carcinoma	Lesion size 0-5 mm	Lesion size >5 mm
Conventional colonoscopy	71/89 (79%)	149/181 (82%)	4/6 (66%)	146/179 (81%)	81/97 (83%)
Magnifying colonoscopy	77/89 (86%)	163/181 (90%)	5/6 (83%)	163/179 (91%)	90/97 (92%)
P values	<0.001	<0.001	1.000	<0.001	<0.001

W1380

Detailed Morphological Features of Magnifying Endoscopically Normal Colorectal Mucosa

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(Background and aims) The initial morphological changes from normal crypt to aberrant crypt foci or micro-adenomas are currently unclear. Furthermore, whether endoscopically normal mucosa is histopathologically normal is unknown, as is the percentage of endoscopically normal mucosa that is in fact composed of morphologically abnormal crypts. We evaluated to determine the detailed morphological features of endoscopically normal colorectal mucosa. (Methods) We evaluated endoscopic specimens removed by forceps from normal colorectal mucosa as identified by magnifying colonoscopy. The specimens were carefully micro-dissected into single crypts or a few crypts under a stereomicroscope. Each specimen removed by forceps usually consisted of more than 100 crypts. Tissue was finally stained with 0.1 μg/ml propidium iodide in phosphate buffered saline for labeling of the nuclei. Using confocal laser scan microscope to examine the nuclei, whole crypt morphology was able to be observed without compression of the crypt. Serial confocal images were routinely taken at 4.0 μm optical increments through the crypts. The shape, length and width of the crypts were then determined (Results) In total, 512 crypts were evaluated, with more than 40 crypts evaluated for each patient. Morphologically, 500 crypts (97.6%) had only a single tubular structure, whereas the remaining 12 crypts (2.4%) were morphologically abnormal. Abnormal crypts displayed branching at the lower part and notching or budding at the middle part of the crypt. These abnormal crypts tended to crowd together. The width of the orifice of the crypts is 97.8+-18.4μm in morphologically normal crypts, 102.1+-22.8μm in morphologically abnormal crypt. No statistical difference was observed between morphologically normal and abnormal crypts. (Conclusions) We were able to evaluate whole crypt morphology without mechanical damage. Among normal rectal mucosa identified by magnifying endoscopy, 2.4% of the crypts were morphologically abnormal. These crypts are thought to be the earliest morphological change preceding aberrant crypt foci or micro-adenoma formation.

W1381

High Magnification Chromoscopic Pouchoscopy: A Novel *in vivo* Technique for Surveillance of the Anal Transition Zone and Columnar Cuff Following Ileal Pouch Anal Anastomosis

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Background: The residual rectal mucosa, anal transition zone (ATZ) and columnar cuff is a high-risk zone for dysplasia. Conventional endoscopic assessment of the ATZ is difficult and often accompanied by biopsy sampling error. High-magnification-chromoscopic pouchoscopy (HMCP) may improve endoscopic surveillance and biopsy accuracy. Methods: Patients with stapled J-pouches underwent HMCP using the Olympus CF240Z. Three discrete zones were identified:1. ATZ-appearing as a linear cellular matrix (LCM); 2. Columnar cuff-Kudo type I/II crypt; 3. Ileal pouch-villous projections. Each epithelial zonal interface was visualised as a matrix to type I crypt pattern and type I crypt to villous formation at the ATZ and the stapled ileal pouch-anal anastomosis respectively. Quadrantic biopsies of each zone were then taken using HMCP guidance. The anticipated endoscopic pouch zone was compared to histology. Results: N = 132. Median age 46yrs (range 22-78), 71 (54%) female, median pouch duration 6.5 yrs (range 2-12). Total no. of pouch years surveyed = 231. K coefficient of agreement between endoscopic zone 1 and squamous epithelium; zone 2 and columnar epithelium and zone 3 with villous histology was 0.78, 0.69 and 0.85 respectively. Sensitivity 91%, specificity 87% and accuracy 93%. There were no cases of dysplasia at the ATZ. Discussion: HMCP is a valid predictor of ATZ anatomy enabling accurate biopsy targeting of this high-risk mitotic zone. The absence of dysplastic yield may reflect the low numbers of pouches >10 post-op. years in this cohort. The identification of true columnar metaplasia and persistent severe villous atrophy using HMCP within the pouch reservoir may be useful when stratifying dysplastic risk and subsequent endoscopic surveillance intervals.

HMCP appearance	no. biopsies	Histology	Histology	Histology	Histology
		Squamous	Columnar	Villous	Other
zone 1: LCM	526	433	88	5	0
zone 2: kudo type I/II crypt	531	42	388	99	2
zone 3: villous	529	6	38	482	3

W1382

Indigocarmine Chromocolonoscopy Markedly Increases the Detection of Significant Polyps

Michael Miros, David Cohn, Neal Walker

Regular colonoscopy even in experienced hands can miss from 6-26% of polyps. AIM To asess whether indigocarmine chromocolonoscopy (ICC) can improve the detection rate of significant colonic polyps. METHODS All patients presenting for routine colonoscopy in one clinic were offered ICC. This was performed after each segment of the colon was initially observed with routine techniques with a variable stiffness Olympus colonoscope.0.1% indigocarmine with simethicone was then sprayed over each segment via a second water bottle through the air water channel.100mls of dye were used on average per patient. Withdrawal time increased by 5.4 mins.
All polyps were removed and retrieved for histology. Patients were excluded with colitis or poor bowel preparation. RESULTS. Over a 4 week period 225 colonoscopies were perfomed. Without ICC 85 patients (37%) had 113 polyps 3 with high grade dysplasia (HGD). After ICC a further 43 (19%) of patients had tubular adenomas. 175 new polyps were detected (all 1-12mm flat) 90 of which were adenomas (51%). 5 HGD polyps were found (1 flat depressed 4mm adenoma). CONCLUSION ICC can be used easily and routinely to increase the detection of flat adenomas in a clinic setting.

W1383

The Usefulness of Chromo-Magnifying Endoscopy in the Detection of Colorectal Dysplasia in Long-Standing Ulcerative Colitis

Giuseppe C. Gizzi, Raffaele Pezzilli, Maria A. Pantaleo, Valeria Villani, Bruno Misitano, Gianfranco Epifanio, Guido Biasco, Roberto Corinaldesi

INTRODUCTION: Patients with long-standing ulcerative colitis (LSCU) have an increased risk for developing malignancy through a dysplasia-carcinoma sequence. Dysplasia in ulcerative colitis (UC), categorized as either flat or dysplasia associated lesion or mass (DALM), is usually difficult to detect by means of conventional colonoscopic (CC) surveillance. Furthermore, polypoid dysplastic lesions (adenoma-like mass, ALM) may occur in these patients within the colitis mucosa and they need to be distinguished from inflammatory or hyperplastic polyps occurring along the inflamed mucosa and from coincidental adenomas (CA) arising in the colonic mucosa free of the disease. AIMS & METHODS: To evaluate the characteristics of polypoid lesions in patients affected by LSUC by means of magnifying plus dye spraying colonoscopy (CMC) and to compare these findings with the histological findings. Fifty patients (30 males, 20 females, mean age 48 years, range 28-65) with extensive LSCU were examined. The duration of the disease was 13.5 ± 2.5 years (mean ± SD). All patients underwent magnifying colonoscopy (Olympus CF Q160ZI, providing up to 140x) plus dye spraying with methylene blue (CMC). The size of the polyps was estimated by placing an open biopsy forceps (7 mm diameter) close to the lesion and measuring the lesion exactly by means of an electronic system which also provided the storage of colorectal photos (Casti Imaging System - Italy). In all patients, the time to reach the cecum was 7.1 ± 2,3 minutes (mean ± SD); the mean (± SD) duration of the examination was 43 ± 11.2 minutes (range 36-51). Irregular or polypoid lesions, occurring along the inflamed colonic mucosa or in the not-involved mucosa, were coloured with MB 0.2%, evaluated with magnification, assessed with the pit pattern of Kudo's classification system and resected or biopsied. RESULTS: A total of 65 polyps were detected at endoscopy in 22 patients; type of lesions at endosocopy and related histological findings are reported in the Table. CONCLUSION: In patients with LSCU, CMC permits an accurate detection of neoplastic lesions; it is also able to distinguish neoplastic from non-neoplastic polyps.

Morphology of the lesions at CMC and related histological findings

CMC Polyp morphology	Histology Hyperprplastic	Histology Inflammatory	Histology CA	Histology ALM	Histology DALM
Flat (N=24)	6	9	2	7	0
Sessile (N=29)	8	5	9	4	3
Pedunculated (N=12)	5	6	1	0	0

W1384

Adrenergic Agonist-Induced Calcium Oscillations in the Nucleus of the Solitary Tract: Laser Confocal Calcium Imaging in the *in vitro* Brainstem Slice

Richard C. Rogers, Jason Nasse, Gregory M. Holmes, Hans-Rudi Berthoud, Gerlinda E. Hermann

The medullary nucleus of the solitary tract (NST) receives substantial visceral afferent input and sends divergent projections to a wide array of CNS targets. The NST is essential to the maintenance of behavioral and autonomic homeostasis; especially gastrointestinal functions mantained by vago-vagal reflexes. The NST is the source, as well as the recipient, of considerable NE input. Our recent studies (Rogers et al AJP 2003) have shown that NE containing projections within the dorsal vagal complex are critical to the function of the esophageal-gastric relaxation reflex. The significance of NE projections from the NST to other CNS regions has long been appreciated, but the nature of NE action on NST neurons themselves is not well known. We applied calcium imaging methods to address adrenergic effects on NST neurons. 300 micron thick slices of the medulla were made from adult rats. NST neurons were loaded with calcium green 1-AM by incubation. Although alpha-2 agonists had no effect on NST cytoplasmic calcium levels, a 30 second exposure to the alpha -1 agonist phenylephrine (10um) induced dramatic cytosolic calcium oscillations. Pharmacologic studies showed that these oscillations are probably the result of an interplay between agonist-induced and IP3-mediated intracellular calcium release, PKC feedback regulation of IP3 production and calcium-ATPase control of intracellular calcium storage pumps. Extracellular calcium fluxes and calcium-induced calcium release mechanisms do not appear to play a critical role in generating oscillations in the NST. To verify that the oscillatory behavior seen in NST is neuronal, as opposed to glial, studies were repeated in a subset of cases where NST neurons were retrogradely labeled by microinjection of rhodamine dextran into the parabrachial nuclei and into the dorsal motor nucleus and hypoglossal nucleus. In both cases, prelabeled cells in the NST were also labeled with calcium green and elicited the oscillatory behavior in response to alpha-1 agonist. Calcium oscillations of this type occur in a wide variety of cells following activation of specific receptors. Cyclical activation of the NST may play a role in the powerful effects alpha-1 agonists have in the suppression of gastric motility and production of anorexia.

W1385

Narrow Band Imaging (NBI) for Improved Pit Pattern Imaging in Colonic Polyps

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High-resolution endoscopy (HR) combined with chromoscopy can recognize certain pit patterns in polyps that correlate with histopathology. Narrow-Band Imaging (NBI) is a new real-time endoscopic technique that uses a RGB-sequential endoscopy system in which the

band pass-range of the RGB rotation filters has been narrowed, and the relative contribution of the blue band pass-filter has been increased. As a result, NBI enables detailed inspection of the mucosal pattern with high levels of resolution and contrast, without the use of staining agents. The prototype NBI-system (Olympus Tokyo, Japan) uses a HR-videoendoscope (GIFQ240 Z) and is equipped with both a standard HR-mode and a HR-NBI-mode, with the possibility of immediate switching between both modes. From Sept to Nov 2003 we studied the feasibility of the NBI technique in imaging colonic polyps. The aim of this ongoing study is to investigate and compare the quality of pit pattern imaging in polyps using standard HR-endoscopy, HR-endoscopy combined with indigo carmine chromoscopy and NBI, and to study the correlation of these pit patterns with histopathological findings. Still images of detected pit patterns were taken with all three modes and the quality of each pit pattern image was evaluated by visual analogue scales (VAS), running from 0-10. Biopsy or polypectomy samples were taken for histological correlation. So far 33 polyps in 18 patients are evaluated. Using VAS, NBI-zoom mucosal images were found to be superior over standard HR images and comparable to HR-chromoscopy (see table). Future studies will elucidate the correlation between NBI-pit patterns and histopathology, as well as the clinical relevance of the improved pit pattern imaging with NBI. Conclusion: NBI improves the quality of pit pattern imaging of colonic polyps without the need for chromoscopy.

Visual Analogue Scales (VAS) for quality of pit patterns using standard High-Resolution (HR) endoscopy, HR-chromoscopy and NBI-endoscopy

	Mean	STD
VAS Standard HR	4.0	1.45
VAS Chromo	6.4	1.48
VAS NBI	6.2	1.39

VAS standard vs VAS NBI: p<0.001 (Paired sample t-test). VAS standard vs VAS chromo: p<0.001 (Paired sample t-test). VAS chromo vs VAS NBI: p=0.62 (Paired sample t-test).

W1386

Characterization of Normal and Adenocarcinoma of Human Colonic Mucosa on Frozen Sections by Laser Raman Microspectroscopy

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Among the spectroscopic techniques currently under investigation for tissue diagnosis, laser Raman spectroscopy provides the most detailed molecular information about tissue. This technique has the advantage of being non-invasive, non-destructive, and sensitive to the biochemical changes that a tissue can undergo during carcinogenesis. The purpose of this study was to characterize ex-vivo, using frozen sections, the normal and adenocarcinomatous colonic tissues by using the Raman microspectroscopy. Methods: Near infrared Raman microspectroscopy technique has been developed and optimized for characterization of colon tissues. This pilot study has been conducted on 52 frozen sections (26 normal mucosa and 26 tumor human fresh colonic samples). The spectral information was acquired from unstained tissue sections mounted on CaF2 slides. Each spectral map was generated by using molecular criteria such as band ratios, principal components analysis, and unsupervised hierarchical cluster. The mean cluster spectra were subsequently correlated with the histopathological structures in which these spectral features were observed. Results: Multivariate statistical analysis identified two spectral fingerprints regions (776-985 cm-1 and 1616-1700 cm-1), associated to molecular composition (lipids, proteins, and collagens) and specific of normal or malignant tissue. Least square minimization was then employed to display the distribution of each component in the tissues. Unsupervised hierarchical cluster analysis could classify all models extracted from tissues into two distinct clusters. These two groups were verified as normal and adenocarcinomatous by conventional histopathological examination. These discriminant spectral characteristics have been used to construct a spectral database of colon tissues, available for future classification of different pathological conditions. Moreover, at the level of a tissue sample, clustering Raman imaging analysis could be used to reveal detailed information on some areas of interest. Conclusion: Raman microspectroscopy appears to provide a highly sensitive and specific way for characterizing normal and adenocarcinomatous tissues. The spectral images thus obtained allowed good correlation between the spectral information and histopathological features. Research is ongoing to apply this technique to endoscopic approaches.

W1387

Intraoperative Fourier Transform Infrared Spectroscopy Can Guide Individual Resections in Patients with Gastric Cancer

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We have previously demonstrated that use of Fourier transform infrared (FT-IR) spectroscopy with an attenuated total reflectance (ATR) fiberoptic attachment can reliably distinguish neoplastic from normal gastric epithelia in vitro. We have also demonstrated its utility in examination of rectal and oral lesions. AIM: To determine the utility of intraoperative FT-IR spectroscopy with ATR fiberoptics in real-time guidance of the extent of gastric resection in a patient with gastric cancer on the lesser curvature. METHODS: With informed consent, a patient underwent operation and the extent of resection was estimated by obtaining biopsies 1,3, and 5 cm proximal to the visible edge of the tumor (J-1, J-3, J-5) and 1 and 3 cm distal to the tumor edge (Y-1, Y-3). First, frozen sections were taken and indicated that no malignant cells were present in the J-1, J-5, Y-1, and Y-3 samples. Then FT-IR readings were obtained at the same distances in vivo. Each reading took 5 min. Cancer and normal gastric tissues were easily distinguished. However, J-1, J-3, J-5, and Y-1 samples more closely resembled the cancer and differed significantly from normal epithelium in some or all of the seven major areas of comparison. The features for cancer were: **1.** amide I: peak <1642 cm^{-1} benign > 1642 cm^{-1}). **2.** amide II: weak (benign = strong). **3.** Intensity (1): $I_{1460}/I_{1400} < 1$). **4.** 1295-1320 cm^{-1}: strong (benign weak to absent). **5.** C-H stretch: weak-0 (benign:strong). **6.** C=O: weak-0 (benign:strong). **7.** $I_{N\text{-}H,O\text{-}H}/I_{amide\ I} > 1$ (benign <1). Readings from cancer were + for **1-7**. Readings from J-1, J-5, and Y-1 were + for **1-3, 5-7**. Y-3 was benign and agreed with **3,5-7**. J-3 was felt to show cancer and was positive 1-7. Three pathologists reviewed the histology blindly and concurred with each of the readings., Y-3, the benign biopsy, was felt to show inflammation and connective tissue. Thus, the frozen section biopsy did not detect dysplasia in 2 and cancer in 1 biopsy. The FT-IR readings did not detect cancer in 1 biopsy but was accurate in the remainder. CONCLUSION: Intraoperative FT-IR can lead to rapid tailoring of the operation with greater speed and accuracy than reliance on frozen sections. Histological review of traditional specimens is most sensitive and accurate, but is impractical for intraoperative guidance. Project supported by State Key Project 2002CCA01900 and NSF of China 30371604

W1388

Use of a Fourier Transform Infrared (FT-IR) Mid-IR Fiberoptic Attenuated Total Reflectance (ATR) Probe to Determine Malignancy in Submucosal Oral Tumors

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Mid-IR light penetrates through the mucosa or skin to the benign or malignant tumor below the surface. We used a mid-FT-IR spectrometer coupled with fiberoptic attachment equipped with an ATR probe to identify malignancy in submucosal oral tumors prior to operation. We examined 30 healthy subjects, three patients with benign parotid tumor, and three patients with parotid cancer using this method. The aim of this study was to determine if malignancy could be identified prior to operation. The results were in excellent agreement with our previous results obtained in resected specimens. We conclude that in vivo FT-IR ATR spectroscopy provides rapid, real-time information that will allow tailoring of operations on oral tumors. Project supported by State Key Project 2002CCA01900 and NSF of China 30371604

W1389

Long-Term Efficacy of Azathioprine (AZA) in Crohns Disease (CD) Beyond 4 Years - a European Multicenter Study in 818 Patients

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Introduction: AZA is the gold standard of immunosuppressive therapy for CD. In combination with steroids AZA is effective in the induction of remission and long term treatment is able to reduce the frequency of flares. However, the question as to how long treatment with AZA is meaningful, is still a matter of controvery. No long term prospective data are available. We therefore performed this retrospective analysis of 1200 patients on long term AZA treatment in 16 european centers. Methods: Charts of 1200 IBD patients from 16 European centers were screened, of whom 818 patients with clinically confirmed CD and a history of long term AZA therapy between 3 and 15y were selected for further analysis. Patients under azathioprine therapy were compared to patients that discontinued therapy after 3-15 y. The patients had a median age of 33y (24-41). Patients were stratified in a subgroup treated for at least 3 to a maximum of 4 y and a subgroup treated for more than 4y. The incidence of flares (#/month) and the steroid dosage required (prednisolone equivalent in mg/month) were calculated during and after azathioprine therapy and described by medians and quartiles. Differences were tested by Sign Tests with $p < 0.05$ considered as statistically significant. Results: AZA therapy significantly suppressed the incidence of acute flares from a median of 0.11/m (0.05-0.26) to 0.02/m (0.00-0.03) within the first 4 years after initiation of treatment ($p < 0.001$). Although the number of acute flares remained low after discontinuation of AZA therapy after 4 y (quartiles: 0.00-0.05), continuous therapy beyond 4y led to a further significant reduction ($p < 0.001$) of flares (quartiles: 0.00-0.01) in 179 CD patients. Furthermore, AZA therapy led to a significant suppression of prednisolone dosage in CD patients within the first 4 y after initiation of treatment (mean 315 mg/m versus 70 mg/m). While discontinuation of therapy after 4y or more did not cause an increase in prednisolone dosage (70 mg/ month), further continuous therapy significantly further reduced the required steroid dosage (median 0 mg/m). Discussion: Our data suggest that AZA therapy can be safely discontinued after 3-4y of therapy in a subgroup of CD patients that did not show evidence for flares or required steroids while on AZA therapy. In contrast, therapy beyond 4y showed additional highly significant benefit in CD patients that required steroids or had flares during the first 4 years of AZA treatment.

W1390

Safety of Repeated Cycles of Sargramostim in Patients with Moderately-To-Severely Active Crohn's Disease (CD): Experience from an Open Label Extension Trial

Joshua Korzenik, Christian Stone, John Valentine, Diana Hausman

Background: Sargramostim, a growth factor that stimulates cells of the innate immune system, has been shown to induce response and remission in patients with moderately-to-severely active CD. We report here the experience of patients participating in an open-label study to evaluate the safety of repeated cycles of sargramostim.
Methods: Patients with moderately-to-severely active CD (CDAI ≥220 and ≤475) who previously participated in a randomized, controlled trial of sargramostim versus placebo were eligible. Treatment consisted of sargramostim 6 μg/kg/day SC for 8 weeks. Concurrent antibiotics, 5-ASA compounds, and steroids were permitted. In this open label trial, cycles of 8 weeks of 6 μg/kg/day dosing were repeated for up to one year as long as the baseline CDAI score for each cycle was >220. Patients were evaluated for safety and changes in CDAI score.
Results: As of October 2003, 31 patients had initiated treatment, with a median age of 42 yrs and median pre-treatment CDAI score of 285. 18/31 of patients received sargramostim during the prior study and 13/31 received placebo. Efficacy data were available for 17

patients who had completed one cycle of treatment (11 prior sargramostim and 6 prior placebo patients). 9/17 (53%) patients responded (decrease in CDAI >100 pts) or achieved remission (CDAI <150). 7/11 (64%) prior sargramostim patients experienced response and/or remission at the end of retreatment, similar to their initial treatment. Adverse events (AEs) occurring in 10% or more of patients included mild to moderate injections site reactions (22/31, 71%), bone pain (7/31, 23%); and headache (4/31, 13%). Six of 31 patients discontinued drug due to AEs, the majority occurred among patients who had previously received placebo. This study is ongoing, and an updated interim data analysis is planned for presentation.
Conclusions: Repeated treatment with 8 week cycles of sargramostim for moderately-to-severely active CD was generally well tolerated. AEs may decrease with repeated dosing. While efficacy analyses require further follow-up, retreatment was not associated with decreased response rates with a second cycle of treatment.
Sponsor: Berlex, Inc, a member of the Schering AG, Germany Group.

W1391

Probiotics (VSL#3) in Arthralgia. Preliminary Results of an Ongoing Open Trial in Patients with Ulcerative Colitis and Crohn Disease
Ouafae Karimi, Amado Pena, Adriaan A. van Bodegraven

Background: Arthralgia is a common extraintestinal manifestation of inflammatory bowel disease (IBD) with a prevalence between 10% and 25%. Occurrence of arthralgia may be related to intestinal contents. NSAID are often efficacious, but may induce a flare-up of IBD. Aim: To study the safety and efficacy of VSL#3 in an open label trial in IBD-patients suffering from arthralgia of at least two weeks duration. Methods: Patients with quiescent IBD and arthralgia with stable medical therapy were given VSL#3 sachets b.i.d. for 3 months (sachet containing 450 billion viable lyophilized bacteria of 4 strains of Lactobacillus, 3 strains of Bifidobacterium, and one strain of Streptococcus salivarius subsp. Thermophilus). IBD activity was assessed by Truelove-Witts or Harvey-Bradshaw score. Arthralgia was assessed by the Ritchie score (joints assessed separately and graded for tenderness on a 0-3 scale) at baseline and at week 12. Results: Up to November 30, 2003, twenty-seven patients with a median age of 43 year (range 29 - 62) were included. There were 12 withdrawals (9 females and 3 males) due to distaste (n = 2), spontaneous improvement (n = 3), deterioration of general well-being (n = 2) which was not IBD related, and non-specific gastrointestinal symptoms (n = 5). Fifteen patients continued VSL#3 for more than one week. This group consisted of 12 female and 3 male patients with a period of disease varying from 3 to 36 years, of which 11 had Crohn disease and 4 ulcerative colitis. Per protocol analysis revealed a significant decrease of the Ritchie score from 20.0 to 7.0 (n = 9), (p = 0.03). Improvement of gastrointestinal symptoms, with concomitant increase of general well-being, was reported by 3 patients. Conclusion: These preliminary results suggest improvement of arthralgia in patients with quiescent IBD due to VSL#3. About thirty-five percent of patients refrained from drugs due to various adverse events, but only of minor seriousness. A randomised placebo-controlled trial is warranted to confirm these challenging results.

W1392

Rapid Improvement of Bone Metabolism in Crohns Disease After Infliximab Treatment
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BACKGROUND: Crohns disease (CD) is associated with osteopenia and osteoporosis. Several factors affect bone metabolism in CD, including chronic inflammation, impaired calcium and Vit D absorption, and steroid treatment. Infliximab, which is able to induce a rapid clinical, biological and mucosal response, may influence these factors. We therefore assessed the evolution of bone metabolism in CD patients treated with infliximab. METHODS: We studied 71 CD patients treated for the first time with infliximab for either fistulizing (n = 21) or non fistulizing (n = 50) refractory CD. Biochemical markers of osteoformation (type-I procollagen N-terminal propeptide (P1NP), bone alkaline phosphatase (BALP), osteocalcin (OSC)), and of osteoresorption (C-telopeptide of type-I collagen (CTx)), were measured in the serum before and 8 weeks after infliximab therapy (1 infusion in non fistulizing CD and 3 perfusions at weeks 0, 2, 6 in fistulizing CD). Serum levels of these markers were compared before and after treatment by a paired non parametric test. Demographic and clinical factors associated with clinically significant improvement of bone metabolism (increase of P1NP >30% or decrease of CTx >30%) were looked for by univariate and multivariate analysis. RESULTS: Globally in the whole group of patients, there was a significant increase in serum concentration of osteoformation markers (BALP (ng/ml): 7.3 (2.6-45.2) vs 8.2 (3.8-44.1), P = 0.0008; P1NP(ng/ml): 30.3 (9-190) vs 41.8 (7.5-190), P = 0.003; OSC (ng/ml): 15.0 (0.9-75.3) vs 17.2 (0.3-57.5), P = 0.001) and a significant reduction of osteoresorption marker (CTx (pg/ml): 256.6 (15-1314) vs 224.3 (15-1765), P = 0.04), 8 weeks after infliximab treatment. A clinically relevant increase in bone formation marker (P1NP increase >30%) and a relevant decrease in bone resorption marker (CTx decrease >30%) were present in 46.0% and 38.2% of patients respectively (60.0% with at least one of these two results) and were not associated with any demographic or clinical factor tested. CONCLUSION: Infliximab therapy in CD may influence bone metabolism by acting either on osteoformation or osteoresorption. A clinically relevant improvement in bone metabolism is present in 60.0% of the patients. This improvement seems to be independent of clinical response to infliximab, steroid weaning or other standard demogrphic or clinical data.

W1393

Improved Outcome Over Time in Patients With an Ilealpouch Anal Anastomosis (IPAA)
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Purpose: Since the introduction of the IPAA, modifications in surgical technique and patient care have been made. The objective of this study was to review a large cohort of IPAA patients to determine if outcome has improved over time.
Methods: Data were retrieved from the MSH IBD Registry. Further information was obtained through chart reviews and mailed questionnaires. Overall results of patients operated on between 1981-2003 are reported. In addition, results in the first 4 years (1981-1984) were compared with data from 1997-2000 to determine changes over time. The latter period was chosen so there was at least 2 years of follow-up for each patient. Differences were compared using chi square or Student's t test.
Results: Between 1981 and 2003, 1,449 patients (650 females, 799 males; mean age 34 years) had 1,559 procedures including primary and revision procedures. The diagnosis was UC in 1,278; FAP in 84; CD in 45; indeterminate colitis in 36 and other in 6. There were 1191 (82%) J pouches (53% vs 96%* 1981-84 vs 1997-2000); 1045 (72%) stapled IPAA (37% vs 96%* 1981-84 vs 1997-2000) and 906 (63%) had loop ileostomies (100% vs 31%* 1981-84 vs 1997-2000).
The outcomes overall and according to time periods are shown in the table.
Conclusions: Over time, there have been changes in surgical technique in the IPAA at our institution. Furthermore, outcome has improved significantly likely because of increased experience and modifications in surgical technique. Thus, IPAA is now the procedure of choice in most patients with UC who require surgery.

	Overall (n=1449)	1981-1984	1997-2000
Mean blood loss (ml)	545	1305	249*
Mean OR Time (hrs)	4.8	7.2	3.9*
Mean Hosp Stay (d)	12.1	15.8	9.7**
IAA Leak Rate	139	12(13%)	19(5.2%)*
Complications	509(35%)	40(43%)	64(18%)*
Re-operations	269(19.6%)	33(35%)	26(7%)*
Reconstructions	38(2.6%)	4(4.3%)	5(1.3%)
Failures	85(6%)	28(30%)	5(1.4%)*

* p<0.0001 (1981-1984 vs 1997-2000) ** p<0.02 (1981-84 vs 1997-2000)

W1394

Steroid Therapy in the Age of Infliximab (IFX): Immediate & 1 Year Outcome in Newly Diagnosed Children with Crohn Disease (CD): Experience of the Pediatric IBD Collaborative Research Group
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Steroids induce CD remission within 3 months in 58% of adults, but by 1 year only 32% are steroid/surgery free (Gastro 2001;121:255). The use of IFX may have changed patients' outcomes to steroid therapy. PURPOSE: To determine children's responses to steroids begun within 30 days of CD diagnosis (dx) and their need for IFX during the 1st year post-dx. METHODS: Since January 2002, 18 US/Canadian pediatric GI centers prospectively enrolled newly diagnosed children with IBD in an observational registry. All subjects were managed according to the dictates of their physicians, not by standard protocols. Subjects for analysis were identified by searching the database for children with CD followed for ≥1 yr who received po/iv steroids within 30 days after dx. Data on use of steroids, immunomodulatory agents (IA), IFX and surgery were prospectively compiled. Children were considered to have a complete response if steroids were successfully stopped by 3 months without requiring IFX or surgery. A prolonged response was defined as no steroids, IFX or surgery in the next 3 quarters (9 months). RESULTS: 47 children (mean age 11.5 yrs, 30 male, PCDAI at dx 32 ± 16) started steroids within 30 days of dx. 32 (68%) were on concomitant IA, including 20 who began IA in the 1st month. By 3 months after dx, 29 (62%) had a complete response and another 4 (9%) stopped steroid after adding IFX. Half of these responders also received IA. A prolonged response was seen in 21 (64% of initial responders, 45% of total population), but 11 required additional courses of steroids or IFX and 1 required surgery. Among the 14 subjects not off steroids by 3 months, 9 received steroids in the Quarter 2, 3 in the Quarter 3 and 2 throughout the year. Seven of the 14 were only weaned off steroids after starting IFX, and 1 failed IFX and required surgery. Overall, 16 (34%) subjects received IFX in the year and 18 (38%) required steroids (8), IFX (7) or both (3) in the 4th quarter of observation. CONCLUSIONS: 70% of children with CD receiving steroids within 30 days of dx can be weaned off steroids by 3 months, although at times only by adding IFX. While 77% of the cohort is off steroids at 1 year, 21% remain on IFX and 4% have undergone surgery despite widespread use of concomitant IA. Frequency and duration of steroid use is in large measure determined by when IFX is started. While steroid dependence can be decreased by use of IFX, studies are needed to see if IFX leads to better long term outcome in children with CD.

W1395

Cytomegaloviral Infection in Refractory Ulcerative Colitis
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Aims: This study was aimed to evaluate 1) the prevalence of CMV infection in steroid refractory ulcerative colits (UC), 2) the significance of endoscopic findings which suggest CMV infection in UC, 3) the clinical course and outcome of CMV superimposed refractory UC. Methods: The medical records of 77 patients with severe UC were reviewed. The patients

received i.v. hydrocortisone (>300 mg/day). In steroid refractory cases, cyclosporine A (4mg/kg/day for 7 days) was added and in CMV superimposed cases, ganciclovir (10mg/kg/day for 14 days) was administered. The diagnostic criteria of CMV infection were 1) positive enzyme immunoassay for IgM anti-CMV in serum, and/or 2) presence of cytomegalic inclusion bodies in pathology, confirmed by immunohistochemistry. Endoscopy and biopsy were performed on admission, after 2 or 3-week steroid therapy, and after cyclosporine A or ganciclovir treatment. Disease activity, clinical course and outcome were evaluated in 8 weeks after discharge. The endoscopic findings that assumed to be associated with CMV infection were deep ulcerations with discrete and sharp edge. Results: In a total of 77 patients, 47 were steroid responsive and 30 were steroid refractory. Of 30 steroid refractory cases, 18 patients had CMV infection (60%), and 11 patients had no CMV infection. 1 patient received therapeutic trial of ganciclovir and achieved complete remission. If 4 cases, in which only IgM anti-CMV was positive, were excluded from CMV infection cases, the prevalence of CMV infection by pathology in refractory UC was 46.7%. The characteristic endoscopic ulcerations were observed in 72.2% (13/18) of CMV infected cases and in 9.1% (1/11) of CMV non-infected cases, respectively ($p=0.002$. if 4 cases excluded, $p=0.003$). 28 of 47 steroid responsive patients were tested for IgM anti-CMV and 1 patient was positive (3.6%). None of 47 steroid responsive patients had the pathologic evidence of CMV infection and only one patient showed characteristic deep ulcerations. Of 13 CMV infected patients with ulcerations, 9 patients received ganciclovir. 8 of 9 achieved complete remission and 1 achieved partial remission in 8 weeks after discharge. Remaining 4 of 13 could not be treated with ganciclovir and all of them required colectomy. 5 CMV infected patients without ulcerations received ganciclovir. 3 achieved partial remission and 2 required colectomy. Conclusions: Cytomegalovirus has an important role in the refractoriness of UC and characteristic endoscopic ulcerations are strongly associated with CMV infection in refractory UC.

W1396

Rofecoxib and Early Relapse of Inflammatory Bowel Disease Patients with Associated Arthralgias: An Open-Label Trial

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Background. The safety and efficacy of selective cyclo-oxygenase-2 inhibitors in Inflammatory Bowel Disease (IBD) is under investigation. Aims. To assess, in a prospective open-label trial, the efficacy and safety of rofecoxib in IBD and controls. Methods. Thirty-eight inactive IBD patients (23 Crohn's Disease, CD; 15 Ulcerative Colitis, UC) with associated arthralgias were enrolled. Controls (C) included 20 dyspeptic patients. The efficacy and safety of rofecoxib (12.5 mg/day; 3 days-3 months) was recorded. Results. In IBD, 8/38 (21%) patients dropped out due to side effects. Arthralgias relief was complete in 6/38 (16%), partial in 19/38 (50%), while no benefit was reported by 13/38 (34%). In CD group, 5/23 (22%) patients dropped out due to diarrhoea ($n=1$) or abdominal pain ($n=4$), subsiding at drug discontinuation. Arthralgias relief was complete in 5/23 (22%), partial in 12/23 (52%) (4 drop out), while no benefit was reported by 6/23 (26%) (1 drop out) CD patients. In UC group, 3/15 (20%) patients dropped out due to bloody diarrhoea. Arthralgias relief was complete in 1 (6%), partial in 7/15 (47%) (1 drop out), while 7/15 patients (47%) (2 drop out) referred no benefit. No C dropped out to side effects, although 2/20 (10%) subjects referred epigastric pain subsiding after PPI treatment. In C group, arthralgias relief was complete in 4/20 (20%), partial in 10/20 (50%), while 6/20 subjects (30%) referred no benefit. Conclusions. Rofecoxib appears to control arthralgias in almost two/thirds of IBD patients. Side effects requiring drug discontinuation are however observed in about one/fourth of patients.

W1397

Rifaximin-Ciprofloxacin Combination Therapy Is Effective in Patients with Chronic, Active, Refractory Pouchitis

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Aim To evaluate the efficacy of Rifaximin-Ciprofloxacin combination therapy for chronic, active, treatment- resistant pouchitis. Patients and methods Conventional treatment of pouchitis is based on antibiotic therapy, either single or in combination. However, there are some patients who fail to respond to these therapies, which we have treated with a novel combination of oral Rifaximin 1gm bd plus Ciprofloxacin 500mg bd for two weeks. Symptomatic, endoscopic and histological evaluation was performed before and after therapy using the Pouchitis Disease Activity Index (PDAI). Improvement was defined as a decrease of at least three points in the PDAI score and remission as a PDAI score of 0. The Wilcoxon signed ranks test was used to compare pre-treatment & post-treatment PDAI scores. Results Eight patients with chronic, active, refractory pouchitis were given rifaximin- ciprofloxacin combination therapy. Seven out of 8 patients either went into remission ($n = 5$) or improved ($n=2$). The median PDAI scores before and after therapy were 12 (range 9-18) and 0 range (0-15) respectively ($Z-2.366$) $P= 0.018$ (2 tailed). All patients were compliant and no side affects were reported. Conclusion Rifaximin - Ciprofloxacin combination therapy is an effective treatment for chronic, active, refractory pouchitis and may salvage a significant percentage of at risk pouches.

W1398

Successful Apheresis Therapy in Active Steroid-Refractory Ulcerative Colitis (UC)

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There is a paucitiy existing in treatment options for patients with chronically active UC refractory to steroids. Early results of leukocyte apheresis therapy in selected patients have been promising. Patients and methods: A total of 34 patients with active UC (total colitis 22, left sided 12; median duration of the disease 6 yrs) was included into an open study by inclusion criteria as follows: CAI (Rachmilewitz) >6 and <8; a minimum total dose of 400mg prednisone within 4 weeks prior to the study; at least one unsuccessful attempt to wean steroids. In addition to steroids, 13 patients were on immunosuppressants. Assessments were CAI, endoscopic index (EI), histology, IBDQ, safety parameters and steroid consumption. The study treatment comprised 5 (1/week) aphereses using the AdacolumnTM system (Otsuka, Germany), an adsorption column which selectively binds granulocytes and monocytes. Results: Both, CAI and EI droped significantly ($p<0.05$) within 6 weeks. Clinical and endoscopic remission were reached in 38.9% and 29.4%, respectively. IBDQ increased from 138 to 162 points ($p<0.001$) and steroids were significantly ($p<0.01$) tapered. Without additional apheresis the remission rate increased 63.3% after 4 months of further follow-up. One patient had a significant drop in hemoglobin at the end of apheresis therapy, but no other relevant adverse events occurred. Conclusions: When applied in a chronically active refractory group of patients with UC leukocyte apheresis shows promising efficacy. Particularly to note is the continuing therapeutic effect after the initial treatment had been finished. Results on safety and improvement of quality of life are excellent and larger sham controlled studies seem waranted

W1399

Colesevelam for the Treatment of Bile Acid-Induced Diarrhea in Crohn's Disease Patients Intolerant of Cholestyramine

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Introduction: Bile acid-induced (choleretic) diarrhea frequently emerges in CD pts following terminal ileal resection due to malabsorption of bile acids. The bile acid sequestrant cholestyramine powder may provide effective treatment of choleretic diarrhea, but is often poorly tolerated due to gritty texture and abdominal bloating. Colesevelam is a non-absorbed lipid lowering polymer that binds bile acids in the intestine, impeding reabsorption. It is available as a pill and is often better tolerated than cholestyramine. We reviewed our open-label experience with colesevelam for the treatment of choleretic diarrhea in CD pts who were intolerant of cholestyramine. Methods: Retrospective review of all CD pts followed in a tertiary referral center from 2001 - 2003. Pts with history of small bowel resection with post-operative diarrhea and bile acid sequestrant therapy were identified. Results: There were 160 CD pts who had required terminal ileal resection. 61 had received cholestyramine for treatment of cholerhetic diarrhea. 16 pts were intolerant of cholestyramine. The 2 most common reasons for cholestyramine intolerance were unpalatability and abdominal bloating. These 16 pts were treated with colesevelam, and 9 remained on treatment for > 6 months. Of the 7 pts who discontinued; 3 stopped due to bloating or constipation, 2 felt no improvement, 2 sought alternative anti-diarrheals. Complete and reliable data was available for 8 pts with long term followup. Median colesevelam dose was two 625 mg pills per a.m. The mean ($\pm$ SE) number of daily liquid bowel movements decreased from 5.8 ± 1.3 to 2.0 ± 0.7 ($p<0.03$) following treatment. Colesevelam use resulted in an improvement in health related quality of life, as measured by the SIBDQ, which increased from 45 ± 5.4 to 51 ± 4.5 following treatment (mean $\pm$ SE). Conclusions: Choleretic diarrhea frequently complicates the course of CD pts following distal small bowel resection. In CD pts intolerant of cholestyramine, the bile acid sequestrant colesevelam may provide an acceptable alternative for choleretic diarrhea treatment.

W1400

The Use of Endoscopic Ultrasound (EUS) to Guide Combination Medical and Surgical Therapy for Crohn's (CD) Perianal Fistulas

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Aims: To assess if using endoscopic ultrasound (EUS) to assess and then guide combination medical and surgical therapy during fistula healing will improve the rate of durable fistula closure and lower the incidence of perianal abscess formation in patients with Crohn's perianal fistulas.

Methods: 20 patients (pts) who presented with a symptomatic CD perianal fistula were enrolled in a clinical practice protocol of serial EUS exams. All pts underwent an baseline rectal EUS and were placed on maximal medical treatment with 6-MP or azathioprine, Cipro and infliximab (5 mg/kg at 0,2,6 wks and q 8 wkly). Pts were also assessed at baseline by a colorectal surgeon who was aware of the EUS findings. Seton placement and incision and drainage were performed when appropriate. Serial EUS examinations were performed and the findings were then used to guide therapy (i.e. the presence of fistula healing by EUS was used to guide seton removal, discontinuation of infliximab and Cipro, etc.)

Results: Five pts were excluded for < 10 wks of follow-up and 2 pts for procto-colectomy, this left 13 patients (9 men) to analyze. The median duration of active perianal symptoms was 9 wks (1-28). 5 (38%) had previous perianal surgery. The fistulas treated included 6 trans-sphincteric, 2 superficial, 2 recto-vaginal, and 3 with multiple &/or complex fistulas. 8 (61%) had abscesses. The median length of follow-up was 30 wks (11-69).

Twelve of 13 patients (92%) had complete cessation of drainage. One patient who has Crohn's disease in an ileoanal pouch and has 2 horseshoe fistula tracts continues to have scant drainage 62 weeks later. Median time to cessation of drainage was 7.6 wks (4-24). No abscess developed during treatment in any pt. EUS evidence of persistent fistula activity was seen in 7 (58%). Of the 5 in whom EUS showed no persistent fistula activity, 5 (100%) have maintained fistula closure off of infliximab and Cipro. Median duration from last infliximab infusion was 16.4 wks (12-55). No pts with complex fistulas demonstrated complete healing on EUS.

Conclusion: Using EUS to guide therapy for Crohn's perianal fistulas is associated with improved fistula response rates. EUS may identify a subset of patients who can discontinue infliximab without recurrence of fistula drainage.

W1401

Cinnamon and Benzoate Free Diet as Primary Treatment of Oro-facial Granulomatosis

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Background: Oral-facial Granulomatosis (OFG) is a chronic inflammatory disorder characterised by inflammation in a variable number of sites in the oral cavity. Its cause remains unclear, but a possible role of sensitivity to dietary components, specifically benzoates (preservative in fizzy drinks) and cinnamon (common flavouring agent), has been proposed. The relationship between OFG and Crohn's disease remains unclear but a number of cases of OFG have co-existent gut inflammation. The aim of this study was to investigate the benefit of dietary exclusion of cinnamon and benzoates in patients presenting with OFG. Methods: 25 patients (11 female, median age 36 yrs) attending a joint Oral Medicine/Gastro clinic with a diagnosis of OFG were offered a cinnamon and benzoate free diet as their primary treatment. Patients were given verbal and written advice by a Dietitian to follow the diet for a minimum of 6 weeks. Response was assessed using an oral activity scoring system (sites affected and severity at each site) pre and post dietary therapy. Results: See table. Significant improvements in oral inflammation were seen on the diet at 3 months. Improvement in lip activity was less marked than oral activity. Gut inflammation did not predict a response to the diet. Conclusions: A cinnamon and benzoate free diet appears to offer genuine benefit in OFG and can be reasonably considered as initial therapy in mild or moderate cases.

Cinnamon and Benzoate Free Diet in OFG

		score before diet (s.e.)	score after diet (s.e.)	p value
Oral	site	7.52 (0.78)	5.30 (0.63)	<0.001
	activity	9.48 (1.41)	6.30 (0.84)	<0.005
	global	16.52 (2.02)	10.08 (1.51)	<0.001
Lip	site	1.30 (0.15)	0.96 (0.16)	<0.01
	activity	2.13 (0.35)	1.30 (0.25)	<0.005
	global	3.54 (0.41)	2.17 (0.38)	<0.001

W1402

Intentional Infliximab Use for Maintenance or Acute Crohns Disease (CD) Flare in Pregnancy

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Purpose: Inadvertent use of infliximab (INF) around conception is not associated with adverse maternal or fetal outcomes, (Katz, Gastroenterol 2003;124:A-7), and the drug is stopped once pregnancy is recognized. However, increasing use of maintenance INF raises the dilemma of managing disease and avoiding immunogenicity while accommodating the desire for pregnancy. We report the outcome of 6 pregnancies with intentional INF use at various times during pregnancy. Methods: A 29-page chart abstraction form was developed to retrospectively collect maternal and newborn data. Patients were contacted to collect missing data. This study was approved by each institutional IRB. Results: 6 patients were identified in 4 centers. Four additional patients are awaiting delivery. Patient characteristics are in the table. CD activity ranged from none to moderate in the first 2 trimesters, and none to severe in the third and postpartum. Five patients received INF in the first trimester, 4 in the second, 4 in the third and 4 in the postpartum period. Three patients breastfed while on INF. There were no maternal infections. There were 6 live births, all by Caesarean section (2 active luminal CD, 3 perianal CD, 1 preterm labor). Two infants were preterm. There were 4 male and 2 female infants with a mean gestational age of 37.4 weeks. There was 1 small for gestational age infant, 1 child with jaundice and 1 with respiratory distress requiring a 1 day ICU stay. There were no congenital anomalies. The mean age at follow up was 5.8 mos (3-17) with no lingering health concerns at the last follow up. Conclusions: We report 6 cases of intentional INF use for CD in all trimesters of pregnancy. No adverse fetal or maternal outcomes occurred. As maternal CD activity is a strong predictor of fetal outcome, maintenance INF use in selected patients during pregnancy may be indicated for the health of the mother and child.

Patient Characteristics

Mean Age (yrs)	**28.3 [24-33]**
Duration CD (yrs)	12 [1-24]
Prior surgery	5/6 pts
Tobacco use	2 former
Concomittant Meds	5ASA,steroid,AZA
Prior Maint. INF	4/6 pts
Mean INF dose	5 mg/kg
Mean no. doses	3.7
Mean dose interval	5.5 weeks

W1403

Meropenem Improves Complex Perianal Crohn's Disease Refractory to Infliximab

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Background: Though medical treatment options for perianal fistulizing Crohn's disease include antibiotics and immunosuppressive agents, infliximab is the only drug shown to close fistulas. However, up to 40% of patients may not respond to infliximab or will have recurrent symptoms off treatment. Experimental evidence in rodents suggests broad-spectrum antibiotics are effective for Crohn's disease-like inflammation. Aim: To assess the clinical benefit and safety of meropenem, a broad-spectrum antibiotic, for perianal fistulizing Crohn's disease in patients refractory to conventional medical treatment, including infliximab. Methods: Six outpatients with symptomatic complex perianal fistulizing Crohn's disease (≥5 tracts) who failed infliximab were given meropenem 500mg q8h through a peripherally-inserted central catheter. Clinical fistula improvement (closure of ≥50% of tracts) or fistula remission (absence of drainage from all tracts) was measured at 6 wk, and then at 8-wk intervals. Radiological response was measured by paired comparison of pre- and post-treatment magnetic resonance imaging (MRI), scored by a single radiologist using a MRI fistula severity score. Results: Clinical improvement occurred in all patients within 3 days of starting treatment, with decreased perianal drainage and perianal pain. At 6 wk, all patients showed fistula improvement, though no patient achieved complete fistula remission. The mean MRI Score decreased from 19.8 pre-treatment to 14.7 post-treatment. After 10-14 days of meropenem discontinuation, perianal drainage increased in all patients. Five patients therefore continued meropenem (mean 32 wk; range 10-70 wk) with sustained fistula improvement; one patient had a panproctocolectomy. Meropenem caused no adverse effects. Conclusions: Meropenem appears to be an effective and safe option for treatment of perianal Crohn's disease refractory to infliximab. As with other treatment modalities, symptomatic recurrence may accompany treatment discontinuation.

W1404

Long Term Efficacy and Toxicity of Thalidomide in Crohn's Disease

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Background. Data on thalidomide in Crohn's disease (CD) are scarce. Short term results from preliminary studies including a small number of patients have shown a response rate of approximately 70%. Toxicity of the drug may be a limiting problem for long term use. Methods. Charts of 58 CD patients (41 F; median age 33 yrs) treated with thalidomide in our two centers between February 2000 and March 2003 were reviewed. All patients had active disease with intestinal symptoms (n=37), perianal lesions (n=6) or both (n=15). The initial daily dose of thalidomide was 100 mg (n=57) or 50 mg (n=1). Prior treatments included steroids (n=57), azathioprine/6MP (n=55), methotrexate (n=36), infliximab (n=48), and intestinal resection (n=19) or anal surgery (n=23). At the start of thalidomide, 47 patients (81%) had received an infliximab infusion within the last 2 mo, 13 were on steroids (22%), 10 on AZA/6MP and 3 were on cyclosporin. The end-point was thalidomide withdrawal for lack (or loss) of efficacy (in patients who received at least 1 mo of treatment) or toxicity. Results. Two patients stopped thalidomide within the 1st mo and underwent a colectomy. Median duration of follow-up of the 56 other patients was 19.1 mo (0.9-42.2). Thalidomide was stopped in 43 patients (77%) because of lack of efficacy (n=16) or of toxicity (n=27). Probabilities of thalidomide withdrawal for lack of efficacy or toxicity were 16% at 1 mo, 36% at 3 mo, 45% at 6 mo, 70% at 12 mo, and 84% at 18 mo. Before withdrawal, thalidomide dosage was changed to 50 mg/d in 23 patients (41%) in order to reduce toxicity. Adverse events leading to thalidomide withdrawal included: neuropathy (n=16), rash (n=3), drowsiness (n=2), hepatic abnormalities (n=1), headache (n=1), vertigo (n=1), myocardial infarction (n=1), transient amaurosis (n=1) and sino-auricular block (n=1). Among the 14 patients in remission at thalidomide withdrawal due to side effect, 12 relapsed in a median time of 2.8 mo. Conclusion. This study confirms previous studies suggesting thalidomide efficacy in luminal or fistulizing CD. However, toxicity of the drug is frequent and limits its long term use in half of the patients.

W1405

Successful 1 Hour Infusions of Infliximab for Crohn's Disease Patients in an Outpatient Office Setting

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Background: Intravenous infusions (IVINF) of infliximab (INF) is becoming the standard as in-office infusions provide a safe and cost effective means to deliver treatment. Currently, most patients are infused over a 2 hour period. In a Phase IV study, RA patients who had tolerated 4 two hr infusions of INF recieved 2 more infusions given over a 1 hr period. This represents the first report of administering INF to Crohn's Disease (CD) patients in one hour in an in-office setting. Methods Patients who had a miniumum of 4 two hour infusions without an infusion reaction were then given subsequent infusions over one hour. Results: 80 patients were followed prospectively and have recieved a total of 173 IVINF in our office based infusion suite. IVINF were run at 100cc/hr for the first 10 minutes and then at 250-300cc/hr for the remainder of the infusion. 65 patients had a diagnosis of CD; 5 had a diagnosis of RA; 4 had a diagnosis (dx) of UC; 1 CD and Ankylosing Spondylitis; 1 with Psoriatic Arthritis; 2 had Polymyositis and 2 were dx with Indeterminate Colitis. The average duration of disease was 12.2 yrs (range 0.5-37 yrs, n=67). Patients ranged in age from 22-79 with the average age being 42.3 yrs. The range for the total number of infusions per patient was 5 to 18 (average 8.3 infusions, n=75). Patient body weight ranged from 44kg to 127kg and dose ranged from 3mg/kg to 10mg/kg. One of 80 pts was pre-medicated with Tylenol for headache that occurred at the end of infusions; otherwise, patients were not pre-medicated. All 173 infusions were successfully completed with 6 infusion reactions (3.5%). One patient experienced headache and dizziness Another experienced nausea and headache after the infusions were started. 2 others complained of severe headache, one during, and the other post infusion. 2 patients had moderate allergic reactions, 1 with hot flashes, nausea and dyspnea; the other developed hives with puritis. Both experienced these symptoms within 5 minutes of the increased rate of 250-300cc/hr. In 5 of the cases, excluding the patient who complained post infusion, the infusions were slowed. Tylenol was administered in all cases, and Benadryl was added for the two patients who exhibited allergic symptoms. Subsequent infusions for those 6 patients were given at the 2-hour rate. Conclusion: IVINF of INF in a office setting for patients with a minimum of 4 uneventful 2-hour infusions is safe and effective. Ongoing analysis will further determine safety and efficacy as well as cost effectiveness and patient satisfaction.

W1406

Cyclosporine in Crohn's and Indeterminate Colitis - a Five Year Experience at Cedars-Sinai Medical Center, Los Angeles

Andrew Ippoliti, Maria Abreu, Phil Fleshner, Kostos Papadakis, Stephan Targan, Eric Vasiliauskas

Intravenous cyclosporine (CSA) is a well-recognized treatment for patients with severe ulcerative colitis, unresponsive to oral and intravenous steroids. The use of iv CSA in patients with Crohn's colitis or indeterminate colitis is not as widely accepted. The purpose of this abstract is to review the experience with iv CSA in all in-patients with colitis due to inflammatory bowel disease treated at Cedars-Sinai Medical Center between 1998-2003. Method: All patients were admitted or transferred to CSMC for iv CSA, 2.0 - 2.5 mg/kg infusion, for 14 days. Dose was adjusted to achieve serum CSA levels between 250-400 ng/ml. Response was defined in clinical terms as completion of treatment and discharge on oral CSA. The parameters of response included bleeding, bowel frequency, and abdominal pain. Non-responders were patients who required colectomy within 2 months of treatment or received other medical treatments. Patients unable to complete CSA treatment due to side effects were considered non-responders. A total of 137 patients received 143 courses of iv CSA. There were 81 patients with ulcerative colitis, 40M/41F, mean age 37 yrs, range 9-72 yrs. There were 45 patients with Crohn's colitis and 11 with indeterminate colitis. The mean age of these patients was also 37 yrs, range 12- 66 yrs, 23M/33F. Results: Response to CSA treatment was similar in each group of colitis patients (see Table below). All eight patients with pyoderma gangrenosa (4UC, 2CC, 2IC) responded to CSA. Seven of eight patients with Crohn's colitis and fistula responded to CSA. Among non-responders with ulcerative colitis 88% had colectomy, while 76% of the Crohn's or indeterminate colitis non-responders had colectomy. Nine patients experienced side effects from CSA but in only 3% of the total group was CSA discontinued. 74% of Crohn's or indeterminate colitis patients had been previously treated with anti-TNF antibody. Conclusion: Intravenous cyclosporine at a dose of 2.0-2.5 mg/kg is as safe and effective in patients with Crohn's and indeterminate colitis as it is in ulcerative colitis. CSA should be viewed as an alternative to surgery in selected patients who have failed other medical treatments.

Colitis	No. Courses CSA	Response	No Response	Side Effect
Ulcerative	82	70%	30%	0
Crohn	48	75%	19%	6%
Indeterminate	13	69%	23%	7%

W1407

Prospective, Open, Pilot Study of Granulocytapheresis (GCAP) for the Treatment of Steroid-Dependant Inflammatory Bowel Disease: Preliminary Results

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BACKGROUND: Preliminary studies suggest that GCAP may be useful for the treatement of autoimmune disorders, such as IBD, psoriasis and Behcet's disease. AIMS: To assess 1) the clinical effectiveness of GCAP in inducing remission in steroid-dependant IBD patients; 2) the steroid-sparing effect of GCAP in these patients; 3) the effect of GCAP on endoscopic lesions in UC patients, and 4) the safety of this therapy. PATIENTS AND METHODS: 25 IBD patients (14 UC, 11 CD) with steroid dependance -defined as (a) the impossibility to discontinue systemic steroids because of disease relapse, or (b) the occurrence of 2 or more flare-ups requiring steroids in a 6-month period- were included. Failure of conventional immunomodulatory therapy (azathioprine, methotrexate) was not considered as an exclusion criterion. All patients started with 60 mg/day of prednisone at week 0, followed by a weekly 10 mg tapering if clinical improvement occurred. At week 1, a 5-session GCAP program (one per week) was started. The clinical response was assessed at week 6. Remission was defined as an inactive clinical index (modified Truelove index for UC, Van Hees or CDAI for CD) together with complete steroid withdrawal. In UC patients endoscopic activity was also assessed at baseline and week 6. RESULTS: More than a half of patients were refractory to conventional immunomodulators. 8 out of 14 UC patients went into remission and 2 additonal patients experienced some improvement. Moreover, endoscopic remission was documented in 9 of them, and some improvement in a further patient. Two CD patients were excluded because of protocol violation. Seven out of the remaining 9 patiens went into remission at week 6. Five adverse events (1 colonic CMV infection, 1 catheter-related sepsis, 1 pneumonia, and 2 headaches) were observed. CONCLUSIONS: GCAP seems to be safe and effective at short-term in steroid-dependant IBD patients. In UC, clinical effectiveness is often associated with a high rate of endoscopic remission.

W1408

Adsorptive Granulocyte and Monocyte Apheresis Therapy for Refractory Crohn's Disease: An Open Multicentre Prospective Study

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Factors which initiate or exacerbate Crohn's disease (CD) are not well understood yet and this might be a major factor in the difficulty to treat the disease. However, active CD is often associated with elevated blood platelets, granulocytes and monocytes/macrophages that are activated and resistant to apoptosis. Similarly, the level of neutrophils in the mucosa has been quantitatively related to the severity of intestinal inflammation and can predict CD relapse. We postulated that patients with refractory CD might respond to reduction of granulocytes and monocytes by adsorptive apheresis (GCAP). Twenty-one patients with active CD, CDAI (CD activity index) 200-399, all unresponsive to standard medication including nutritional therapy received GCAP therapy as an adjunct to their ongoing medication. GCAP was done by using an Adacolumn which adsorbs granulocytes, monocytes and a small fraction of lymphocytes (FcγR and complement receptors bearing leucocytes). Patients received one GCAP session/week over 5 consecutive weeks. One session was 60 minutes at 30mL/minute. Treatment efficacy was evaluated in week 7 by measuring changes in CDAI, IOIBD (international organization for the study of inflammatory bowel disease) and IBD questionnaire (IBDQ). During an initial course of conventional/nutritional therapy, no significant improvement in CDAI was seen in any of the 21 patients (CDAI>200). However, at week 7 of GCAP therapy, significant improvements of CDAI, IOIBD and IBDQ scores were observed. The CDAI, IOIBD and IBDQ scores (mean ± SD) before GCAP therapy were 276 ± 54, 3.4 ± 1.4, and 152 ± 22, respectively. The corresponding values after GCAP therapy were 215 ± 89 (P = 0.0005), 2.5 ± 1.5 (P = 0.0150) and 165 ± 29 (P = 0.0327), respectively. No severe adverse effects were observed. Our data indicate that GCAP is safe and effective for inducing remission and improving quality of life in patients with active CD that is refractory to conventional drugs and nutritional therapy.

W1409

Clinical Features Cannot Be Used to Predict Crohn's Disease in Patients with Ileoanal Pouches

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Background: A small subset of patients with presumed ulcerative colitis (UC) who undergo colectomy with ileal pouch-anal anastomosis (IPAA) will ultimately be diagnosed with Crohn's disease (CD). To date a single uncontrolled study has described clinical features of IPAA patients who were later diagnosed with CD (Colombel JF et al, *Am J Gastro* 2003; 98: 2239). No controlled study has yet identified risk factors associated with the development of CD in patients with ileoanal pouches. Aims: To determine risk factors predictive of CD after IPAA. Methods: We queried the University of Pennsylvania Health Systems database to identify 7 patients who were post-operatively diagnosed with CD after colectomy with IPAA for presumed UC. We also identified 9 controls who had undergone colectomy with IPAA and never developed CD. Mann-Whitney rank analysis and Fisher's exact tests were performed to compare clinical and demographic characteristics between the two groups. Results: Mann-Whitney rank analysis and Fisher's exact test showed that no clinical or demographic feature was associated with the development of CD in those patients with presumed UC who had undergone colectomy with IPAA. Variables analyzed include gender, age, body mass index, smoking, family history, extra-intestinal symptoms, indication for surgery, extent of colonic disease, duration of disease, and the use of 5-ASA compounds, corticosteroids, 6-mercaptopurine, methotrexate, infliximab, and antibiotics ($p>0.05$ for each variable). Conclusions: Our study is the first to show that no clinical characteristic can be used to predict the occurrence of CD after colectomy with IPAA for presumed UC. Perhaps further studies using other means such as serologic markers or pathology will be helpful to identify possible predictors of CD in this patient population.

W1410

Dimethylsulfoxide Using for Systematic Secondary Amyloidosis Complicated in Chronic Inflammatory Disease

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Background: Secondary amyloidosis is an obstinate disease complicated in chronic inflammatory disease, but there is few effective conventional therapy for this disease. This study evaluated an effect of oral dimethylsulfoxide (DMSO) on systematic secondary amyloid A amyloidosis. Methods: Fourteen secondary amyloidosis patients (M: F = 3:11, 26-70 years) with amyloid A were treated with DMSO during 1995-2003. DMSO was administered orally in all patients in 3 to 20 g/day. Clinical symptoms and renal + gastrointestinal functions were evaluated before and after treatment. Among the patients, 10 cases were complicated in rheumatoid arthritis (RA), 3 cases were in Crohn's disease and 1 case was in Adult Still's disease. Nine cases were mainly involved in renal amyloidosis with renal dysfunction and proteinuria, four cases were mainly involved in gastrointestinal tract with protein-losing gastroenteropathy and intractable diarrhea, and one case had both gastrointestinal and renal amyloidosis. Results: DMSO successfully treated 9 (64.3%) out of 14 patients (RA: 6 out of 10, Crohn's disease: 3 out of 3, and Adult Still's disease: 0 out of one). The effect of DMSO was appeared within 2 months. Regarding renal amyloidosis, 6 out of 10 patients were improved renal function and proteinuria, although DMSO was not effective in patients with severe and/or advanced renal dysfunction. Regarding gastrointestinal amyloidosis, all five patients were improved the gastrointestinal symptoms including diarrhea and protein-losing gastroenteropathy. No serious side effects of DMSO were encountered. Mild nausea, headache, anorexia and unpleasant breath odor were main concerns of the patients. Conclusions: Oral administration of DMSO is effective on systematic secondary amyloid A amyloidosis, especially on gastrointestinal involvement and early stage of renal dysfunction.

W1411

Efficacy of 6-Thioguanine in Patients with Crohn's Disease Intolerant to Azathioprine

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Background. Currently the role of 6-Thioguanine (6-TGN) as an alternative immunosuppressive therapy for patients with Crohn's disease (CD) is under investigation. Methods. Twelve patients (age 20-46) with chronically active luminal and/or perianal CD were included in this open prospective study. All patients had a history of AZA-intolerance: pancreatitis (4), fever (4), nausea (5), arthralgia (3), cephalea (1). Patients with AZA-induced leucopenia were excluded. For induction of remission 6 patients were treated with infliximab (4) or prednisolone (2); they received 6-TGN (40mg/d) for maintenance of remission. Six patients had moderately active disease, and they were treated with 6-TGN (40mg/d) without induction therapy. Patients were followed every 3 months. Disease activity was measured by using

the Crohn 's Disease Activity Index (CDAI) for luminal disease, and the Perianal Disease Activity Index (PDAI) for perianal disease. Relapse was defined as CDAI>200 or PDAI>4. Results. Patients with induction therapy were in remission after 3 months. The 6 patients with moderately active disease and 6-TGN monotherapy achieved remission within 7 months (range 6-9). All patients were followed for 11.7 months (range 6-27). One of 12 patients relapsed after 6 months, 11/12 maintained remission. 6-TGN was discontinued in one patient because of planned pregnancy (month 9), and in 2 patients because of adverse events: elevated liver enzymes and thrombopenia (month 18), severe symptomatic thrombopenia (month 14). In the patient with elevated liver enzymes liver biopsy revealed nodular regenerative hyperplasia. 6-TGN levels averaged 774 picomol/108 RBC (range 244-1404). Conclusion. 6-TGN appears to be an effective alternative to AZA for the treatment of patients with CD. Patients need to be montitored closely for hematologic and hepatic side effects.

W1412

Management of Pouchitis of the Ileoanal Pouch with Infliximab

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Background: Proctocolectomy with ileal pouch-anal anastamosis (IPAA) is the preferred surgical procedure in patients with ulcerative colitis (UC) and familial adenomatous polyposis (FAP). The most frequent complication of the IPAA is acute/chronic inflammation of the ileal reservoir known as pouchitis. Chronic pouchitis can evolve into refractory pouchitis, which leads to pouch excision in 3-5% of patients. Infliximab (chimeric monoclonal antibody to tumor necrosis factor) has been successfully used to treat patients with an IPAA performed for a presumed diagnosis of UC who developed Crohn's disease complications of the pouch. The use of infliximab in treating pouchitis has not been well described. Methods: We reviewed the records of eleven patients (eight females, three males) who received infliximab for pouchitis from April 2000 to November 2003 in our tertiary care hospital. Pouchitis was diagnosed by clinical, endoscopic and histologic criteria. Three patients who had a prior diagnosis of UC and underwent proctocolectomy with an IPAA later developed findings consistent with Crohn's disease and were excluded from this study. Patients received 1-5 doses of infliximab over the study period. The median number of infusions was 3.3 (1-5). Short term clinical response was classified as complete, partial or no response as classified by the frequency of stools, presence of blood, and/or abdominal pain. Long term response was assessed by the presence of an ileostomy or by pouch excision. Results: At short term follow-up all patients demonstrated clinical improvement. Seven patients (87.5%) had a complete response with one patient (12.5%) having a partial response. Long term follow-up information was available on six patients. None of these patients have required a surgical excision of the pouch or a diverting ileostomy. One patient had a possible infusion reaction (shortness of breath) 2 weeks after the first dose and had no further infusions. Symptomatically pouchitis was improved. Conclusions: These preliminary results suggest that infliximab has clinical benefit in treating pouchitis in patients who have had an IPAA for ulcerative colitis. Further studies evaluating the long term effects and safety of maintenance infliximab in patients with pouchitis is warranted.

W1413

6-MP Metabolite Interacts with Purine Analogs in Patients with Inflammatory Bowel Disease

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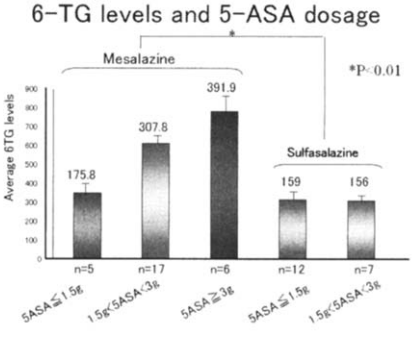
Background and aim: It has been reported that the measurement of erythrocyte 6-thioguanine nucleotides(6-TG) metabolite level is helpful to determine the dosages of purine analogs, 6-mercaptopurine (6-MP) / azathiopurine (AZA) and to avoid the dose dependent cytotoxicities and immunosuppression. It has also been noted that the enzymatic activity of metabolize 6-mercaptopurine: thiopurine methyl transferase (TPMT) is the result of genetic polymorphism. The purpose of the study is to assess the interaction between 6-TG level and 5-aminosalicylates (5-ASA) containing compounds, mesalazine and sulfasalazine. Material and method: 58 Patients with ulcerative colitis or Crohn's disease treated with 6-MP/AZA were measured erythrocyte 6-TG metabolite levels with reverse phase hige performance chromatography and examined for disease activity and 5-ASA dose. Results: 1) 6-TG levels were higher in patients in clinical remission with Crohn's disease than those who were in active phase. The same was found in patients with ulcerative colitis. 2) 6-TG levels were higher in patients given mesalazine than those given sulfasalazine. Conclusions: 6-TG levels correlates with clinical efficacy and interacts differently with 5-ASA containing compounds.



W1414

Maintenance of Long Term Response to Infliximab over 1 to 5 years in Crohn's Disease Including Shortening Dosing Intervals or Increasing Dosage

Christopher E. Shih, Theodore M. Bayless, Mary L. Harris

Background: Infliximab, at a dosage of 5 mg/kg, has been shown to maintain remission in Crohn's disease in up to 40% of responders at 54 weeks. Potential methods of increasing long term response include: a) avoiding development of antibodies to infliximab; b) optimizing immunomodulator dosage (Cuffari et al, *Gastroenterology* 2003;124:A11); c) shortening the interval between doses; and/or d) increasing the dosage of infliximab. Our five-year experience with 56 patients on long term therapy was analyzed.
Methods: Records of 56 patients who received between 10 and 37 infusions (total 731) from October 1998 through October 2003 were reviewed retrospectively. 46 patients (82%) were also receiving azathioprine or methotrexate. Complete response equaled sustained absence of symptoms. Partial response equaled: a) decrease in abdominal pain, b) decrease in number of bowel movements, c) reduction in fistulae drainage, d) weight gain, and/or e) increase in sense of well-being.
Results: 27 of the 56 patients (48%) required an increase in dosage and/or a decrease in the interval between infusions to maintain response. 18 (32%) required an increase in dosage to 10mg/kg to maintain response (same percentage of patients who crossed over to 10 mg/kg in the ACCENT I trial). The interval of administration was decreased because of loss of response in 15 patients (27%): q 6 weeks in 10 patients, q 5 weeks in 2 patients, and q 4 weeks in 3 patients. Currently, all 20 patients with luminal disease are in complete or partial response. Of the patients with perineal fistulae, 11 (31%) responded completely, 13 (36%) responded partially, and 12 (33%) had continued fistula drainage but partial improvement in their intestinal disease. All 34 steroid dependent or steroid refractory patients reduced or discontinued their steroid dosage. Adverse events occurred in 6 patients (11%), but no one discontinued therapy.
Conclusion: To maintain long term response to infliximab for Crohn's disease, the dosage was increased to 10mg/kg in one-third of patients. The interval between infusions was shortened in one-third. Long term responses were complete or partial for all patients without fistulae including steroid sparing. Fistulae persisted despite intestinal improvement in one-third. Use beyond 54 weeks and as long as 5 years is feasible, safe and effective.

W1415

Premedication and Infusion Reactions with Infliximab: Results from a Pediatric Inflammatory Bowel Disease Consortium

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Introduction: Infusion reactions (IR) are the most common adverse events associated with the use of infliximab use for inflammatory bowel disease (IBD). Antipyretics, antihistamines, and corticosteroids have been used to prevent the development of IR, but their efficacy is not known. We investigated the proportion of patients receiving infliximab for IBD that developed IR, and the potential effects of premedication on IR. Methods: Uniformly collected data from a cohort of patients with IBD enrolled between January 2000 and May 2003 at 6 pediatric centers were analyzed. All patients entered had IBD diagnosed before age 18. Data were entered prospectively into a secure, central computerized registry. Data were retrospectively reviewed and analyzed, including demographics, date of infusion, medications, IR (if present), and premedication (if given). Results: 1652 infusions given to 243 patients in 6 centers were analyzed (mean age 13.1, range 3-17). Overall, 60 IR were recorded in 40 patients (3.6% of infusions, 16.5% of patients). 30 of 243 patients received premedication beginning with the first infusion (group 1) while 213 patients did not receive premedication until the development of IR, if at all (group 2). IR were more common among patients in group 1 than group 2 (12/30 vs. 28/213, $p<0.01$). Of the 12 patients from group 1 with IR, 4 developed subsequent IR despite receiving premedications with each infusion. Of the 28 patients in group 2 with IR, 10 began receiving premedication with each subsequent infusion, 12 continued without premedications, and 6 had no further infusions recorded. 2/10 who began receiving premedication had a subsequent infusion reaction vs. 6/12 who did not receive premedication ($p=0.15$). 14 patients experienced multiple IR (34 IR, range 2-5). Conclusions: IR occur in a small proportion of infusions among patients receiving infliximab for IBD. Premedication does not appear to prevent the development of IR; however, once an IR has occurred, premedication may be indicated to prevent subsequent IR.

W1416

Seven Years on: Living with Inflammatory Bowel Disease

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Purpose: Chronic disease such as Inflammatory Bowel Disease (IBD) diagnosed in childhood will affect a young persons life and maturation. Few studies acknowledge how young people live beyond the confines of this illness. Therefore we investigated how young people deal with adolescent life issues alongside the demands of IBD, such as peer relationships, boy/girlfriends, education and work experience, and what they would like to tell others in that situation.
Methods: Sixteen adults aged 20-25 years, diagnosed with IBD before age 18 (9 female, 7 male, with combined 116 years experience of the illness) were interviewed using a semi-structured questionnaire. Participants related, to an independent interviewer, their experiences of growing up with IBD, providing examples of adolescent life issues.
Results: All participants related difficulties communicating disease-related matters to prospective sexual partners and 7 had begun to see their selves and bodies as separate entities. All spoke of negative feelings about physical changes in their bodies caused by IBD and steroid treatment. Half had concerns about taking medication for long periods of time, and 12 reported anxiety of possibilities of relapse at significant times (family holidays, school exams). On recall, 13 were dissatisfied with information available to them at diagnosis. However 6

participants described the value of attending non-clinical support groups for young people, as this facilitated discussion of symptom and social management.
Conclusions: In addition to reviewing information sources and developing support groups, these data suggest that a prospective investigation is needed to investigate how young people live with IBD, to promote awareness of the complexities of communication and adjustment in areas outside of the clinical setting.

W1417

Delayed Hypersensitivity Reactions Following Infliximab Infusion. Analysis of Risk Factors
Gregory Bonner, Marcia Cruz-Correa

INTRODUCTION. Reactions to infliximab include immediate infusion reactions and delayed hypersensitivity reactions (DHR). Prolonged delay between infusions has been felt to increase the risk but the relationship is not well described. We reviewed the history of adverse reactions in a cohort of Crohn's disease patients to determine if time interval to followup infusion was associated with increased risk of DHR or other infusion reaction. METHODS. Records from a computer database of CD patients receiving infliximab infusions were reviewed. Reactions to the first followup infusion after an interval of 8 weeks or longer from the initial infusion or series of infusions were analyzed. Time to followup infusion was analyzed using univariate and multivariate analysis; stepwise multiple logistic regression models were constructed to determine the association between time interval to infusion and DHR. RESULTS. 75 patients, 40 female, mean age 42.5 (±15) were reviewed. Most (56) received an initial series of 3 infusions on a 0,2, and 6-week schedule. 12 received 1 initial infusion and 7 received 2 initial infusions. 59 patients received followup infusions on a prn basis, varying from 3 to 55 months. 16 patients started immediately on routine q8 week infusions. 11 (14.7%) patients experienced DHR and 8 (10.7%) had infusion reactions. Risk of DHR increased with increasing time-interval between infusions. Risk of immediate infusion reaction did not change in relation to time-interval. Risk of DHR was: 5.3% at 2-11 months, 33% at 12-23 months, and 50% at ≥24 months ($p < 0.002$). When a cutoff time of ≥12 months is used the risk of DHR was 5.3% vs 44% ($p<0.001$). The risk of DHR also correlated with any infusion reaction (immediate or delayed) during the initial series of infuions. The risk of DHR was 9.1% vs. 55.6% among patients without or with prior reaction ($p<0.001$). Both time-interval of ≥12 months and prior reaction were independently associated with DHR. Patients who had ≥12 months time-interval between infusions were 21.4 (95% CI 2.9-154.1; $p=0.002$) times more likely to have DHR. Similarly, patients with prior reactions were 27.5 (95% CI 2.3-331.5; $p=0.009$) times more likely, after adjusting for immunosuppresives, number of previous infusions, gender, and age. CONCLUSIONS. Risk of DHR to infliximab increases significantly after a time interval of ≥12 months from the initial infusion(s). Prior infusion reaction also increases DHR risk significantly.

W1418

Centrifugal Leukocytapheresis Therapy Without Concurrent Corticosteroid Administartion for Ulcerative Colitis
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Purpose: Corticosteroid therapy is an important therapy of active ulcerative colitis (UC). However, long-term use of corticosteroid has been also known to cause serious complications such as osteoporosis, diabetes or growth retardation. On the other hand, the effect to perform combination therapy of corticosteroids plus leukocytapheresis has been reported mainly in Japan. However, as there have been few examinations concerning the effect of leukocytapheresis therapy without concomitant use of the corticosteroids, we performed a preclinical trial whether leukocytapheresis without the use of corticosteroids may be effective in the patients with active UC. Methods: Six patients (2 men and 4 women) with active UC (2 with severe and 4 with moderately severe), who did not respond to 5 aminosalicylate derivatives but refused corticosteroid use, were enrolled. Centrifugal leukocytapheresis was performed once a week with a total of 4 sessions per course. The treatment effect was defined to be effective when the patients experienced remission after one course. Results: Leukocytapheresis was effective in 5 of 6 patients (83 %). When being stratified by severity, all of 2 severe cases and 3 of 4 moderately severe cases responded effectively to this therapy. The clinical activity scores according to Lichtiger et al. in effective cases decreased to 6.6 at 1week after from 9.8 ($P<0.0001$), and the clinical activity score improved to 2.4 at the end of the course. There was no obvious complications of leukocytapheresis except for a decrease in hemoglobin level by 1 g/dl at the end of the course. Conclusions: This study showed that centrifugal leukocytapheresis was able to induce remission without the use of corticosteroids in patients with active UC. It may be considered benefical for patients who have difficulties in using corticosteroids because of the adverse effects.

W1419

Long Term Oral Cyclosporine Therapy in Severe, Steroid Refractory Ulcerative Colitis with a Daily Dose of 4 mg/kg - Result of the One-Year Follow-up
Tamas Molnar, Ferenc Nagy, Janos Lonovics

Cyclosporine therapy has proven to be an alternative to emergency colectomy in steroid-refractory ulcerative colitis. Oral cyclosporine has less toxic side effect than intravenous treatment, but therapeutic ability, optimal dosage, the long therm efficacy and safety of this formulation is not well studied. The aim of this study was to investigate the clinical outcome of all patients treated with Sandimmune Neoral (Novartis) oraly at a daily dose of 4 mg/kg for a year. Patients and methods: 16 of the 290 regularly controlled ulcerative colitis patients were treated with cyclosporine because of severe active colitis (12 female, 4 male; the extent of inflammation: 15 pancolitis, 1 left-side colitis; clinical course: 5 continuous activity, 7 intermittent activity and 4 first attack). All patients were treated with oral microemulsion cyclosporine as a first line therapy, and the administration of the drug was continous for a year. The dose of corticosteroid was gradually reduced, and 2 mg/bw/kg dose of azathioprine was initiated during the cyclosporine treatment. Disease activity, and blood level of cyclosporine were followed by using of a two-point sampling times to evaluate efficacy and safety of therapy. Primary objectives were the induction of remission, the frequency of relapse during the next twelwe month and colectomy-free survival. Results: All patient improved within 14 days and complete remission was achieved in 14/16 patients after 1 month. 13/16 patients have remained in remission during the one year of cyclosporine therapy. 1/16 patient was operated due to a reversible hepatotoxic side effect, while 2/16 due to a second relapse. Discussion: Oral cyclosporine seems to be effective in the treatment of severe steroid refractory ulcerative colitis not only for the induction but - in combination with azathioprine - also in the maintainance of the remission. Colectomy can be avoided in most of the patients with prolonged use of oral cyclosporine. Therapeutic drug monitoring by using of a two-point sampling times is the guarantee of the safety during a long term oral cyclosporine therapy.

W1420

Efficacy of Granulocyte and Monocyte Adsorption Apheresis in Chronic Active Crohn's Disease
Sebastian Petermann, Wolfgang Ramlow, Stefan Liebe, Joerg Emmrich

Background and aims: Recently published studies have suggested that leukocytapheresis is a useful adjunct to therapy of ulcerative colitis after failure of conventional treatments. Selective granulocyte and monocyte adsorption apheresis (GMCAP) can modify peripheral blood leukocyte function. The efficacy of GMCAP in Crohn's disease is not known. Methods: We recruited 8 patients with chronic active Crohn's disease (CDAI 200 - 400) treated with steroids (≥ 10 mg/die prednisolon) for more than 6 months. All patients did not respond to azathioprin as a maintenance therapy. Four patients did not respond to infliximab also. GMCAP was performed twice a week for three weeks and weekly for additional three weeks using the Adacolumn® system (Otsuka Pharmaceutical Co., Japan). With a flow rate of 30 ml/min for 60 min, blood was treated with cellulose diacetate beads of 2mm in diameter during each apheresis session. Endoscopies of the colon were made before and after the therapy and the endoscopic index CDEIS was calculated. After the treatment cycle steroids were tapered. Results: The procedure was well tolerated. After apheresis sessions we found a moderate reduction in circulating granulocytes with no significant alterations in red blood cells, monocytes and the total lymphocyte counts. CD4 and CD8 lymphocyte counts remained unchanged. Remission of disease (CDAI less than 150) was achieved in 4 of 8 patients after the therapy with GMCAP. Remission was accompanied by reduction of CDEIS as well as of CRP. After the apheresis sessions steroid treatment was finished in the four patients without disease activity. Three of these four patients are in remission since four months without steroids. One patient had a relapse after two months. Conclusion: GMCAP was successful to induce remission in patients with long-standing steroid dependent Crohn's disease who did not respond to azathioprine. There were no GMCAP side effects. Further studies are needed to evaluate the optimal treatment protocol and the influence of GMCAP on the gastrointestinal immune system.

W1421

The Metabotropic Glutamate Receptor 5 Antagonist MPEP Inhibits Transient Lower Esophageal Sphincter Relaxations in the Dog
Jorgen Jensen, Anders Lehmann, Malin Hulander, Anna Uvebrant, Anita Carlsson, Mia Umaerus, Karolina Nilsson, Claudine Frisby, L. Ashley Blackshaw, Jan Mattsson

Transient lower esophageal sphincter relaxations (TLESRs) are the major cause of gastro-esophageal acid reflux and are initiated by stimulation of gastric vagal afferents following postprandial gastric distension. The metabotropic glutamate receptors (mGluR) belong to family III of G-protein coupled receptors. Eight different mGluRs (mGluR1-mGluR8) have been identified and these can, based on sequence homology, signal transduction mechanisms and pharmacology, be divided into three groups (I-III). The aim of the present study was to investigate the effect of the selective mGluR5 antagonist 2-methyl-6-(phenylethynyl)-pyridine (MPEP) on TLESRs in the dog. Labrador retrievers equipped with esophagostomies were intubated with a multilumen Dentsleeve assembly and a pH electrode. Pressures were recorded from the stomach, lower esophageal sphincter (LES) and esophagus, as well as from the pharynx. In order to assess the affinity of MPEP for the canine mGluR5, saturation binding analysis of tritiated MPEP to dog brain membranes was performed. The expression of mGluR5 in nodose ganglion, containing the cell bodies of gastric vagal afferents, was investigated using RT-PCR. MPEP (1.4-8.7 μmol/kg; $n=3$-4) produced a dose-dependent reduction of TLESRs. The maximum inhibition obtained with the highest dose was 59 ± 11%. No significant effects were seen on basal LES pressure, swallowing or on esophageal peristalsis. The binding affinity of MPEP at dog mGluR5 was 16 ± 4.6 nM, i.e. similar to the affinity for the human mGluR5. RT-PCR analysis showed expression of mGluR5 mRNA in dog nodose ganglion. It is concluded that the mGluR5 antagonist MPEP has an inhibitory effect on TLESRs and that gastric vagal afferents may be one site of action for this effect. These results suggest that mGluR5 is a potential target for the treatment of gastroesophageal reflux disease.

W1422

Esophago-Upper Esophageal Sphincter Contractile Reflex (EUCR) and Secondary Esophageal Peristalsis (2° P) During NREM and REM Sleep
Jasmohan S. Bajaj, Kulwinder Dua, Shailesh Bajaj, Tanya Rittmann, Reza Shaker

During retrograde transit of gastric contents, 2° P and EUCR can protect the airways by clearing the esophagus of refluxate and by preventing esophago-pharyngeal reflux, respectively. However, the integrity of these reflexes during sleep, when the airways are most vulnerable to aspiration, has not been previously studied. AIM: To evaluate the integrity of 2° P and EUCR during NREM (Stage II) and REM sleep. METHOD: 5 healthy volunteers were studied in supine position during awake, NREM and REM sleep states using polysomnographic monitoring (REMBrandt 5.2, Medcare, Amsterdam). EUCR and 2° P were evaluated

during 2.7ml/min of water infusion into the proximal esophagus. At this rate the two reflexes are triggered separately. UES and esophageal motor function were recorded using a sleeve assembly (Dentsleeve, Australia) RESULTS: All 5 subjects were successfully monitored for 5 hrs during which several periods of Stage II sleep were observed. In addition, 2 subjects also reached REM sleep. UES pressure decreased precipitously with deeper sleep stages (Awake (n = 5), 99 ± 13mmHg; Stage I (n = 5), 34 ± 15mmHg; Stage II (n = 5), 18 ± 10mmHg; REM (n = 2), 19mmHg in one, 0 in another). EUCR was triggered in all subjects during NREM sleep and 2° P in all but one (Table). EUCR stayed active during water perfusion until the development of 2° P in NREM state. Both reflexes occurred in close temporal association and this coordination of activation of EUCR and 2° P during Stage II sleep was similar to that of the awake state. There were no statistically significant differences between threshold volumes to elicit EUCR and 2° P between awake and Stage II sleep. None of the subjects exhibited any change in the sleep stage or arousal during stimulation of these reflexes. EUCR and 2° P were also triggered during REM sleep without any change in sleep stage or arousal. CONCLUSIONS: 1) EUCR and 2° P are elicited during sleep. 2) The hypotonic UES during REM sleep can contract with triggering of EUCR despite continued hypotonia in other striated muscles.

TABLE (Mean±SD)

	Time to Activation (sec)			Volume Threshold (mL)		
	Awake	Stage II	REM	Awake	Stage II	REM
EUCR	93±42	127±71	58±20	4.2±1.9	5.7±3.2	2.6±0.9
2° P	153±44	171±62	87±18	6.9±2.0	7.7±2.8	3.9±0.8

W1423

Apnea During Deglutition and Gastroesophageal Reflux in Infants: It's All About the Bolus

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A temporal association between gastroesophageal reflux (GER) and apnea episodes has been demonstrated in infants (Wenzl et al., Pediatr Pulmonol 2001, 31: 144-9). Aim of this study was to analyze the coincidence of retrograde (GER) and antegrade (swallow) esophageal bolus movement with apnea. Methods: 4 infants (age 139 ± 66 days) with recurrent apnea episodes were investigated with simultaneous intraesophageal impedance measurement (IMP), pH-metry and polygraphy. IMP patterns, pH, oronasal airflow and chest wall movement were recorded and analyzed. Apnea was defined as a breathing arrest ≥5s. The occurence of an apnea episode during 30s preceding or following the beginning of a GER or swallow was defined as temporal association. Statistical analysis was performed using Fishers exact test. Results: 166 GER and 1482 swallowing-episodes were registered by IMP in all infants during a total measuring time of 32 hours. Most GER were non-acidic (pH≥4) and reached the hypopharynx. 136 episodes of apnea were documented by polygraphy. 7 GER (4.2%) and 72 swallows (4.8%) were associated with an apnea. The mean time spent apneic during both GER and swallowing was significantly greater than the mean time spent apneic without esophageal bolus movement ($p < 0.01$). However, there was no difference between the directions of bolus movement in this respect ($p = 0.85$). Conclusions: There is a temporal association between episodes of apnea and esophageal bolus movement in infants. This is true not only for acidic and non-acidic GER, but also for episodes of swallowing. Apparently, hypopharyngeal bolus presence is more important than direction or pH of the bolus regarding apneic events. Short apneas during bolus passage are considered as airway protection. Alterations of autonomic nervous control, e.g. due to immaturity, may induce prolonged pathological airway obstruction. The pH-independent intraluminal impedance technique in combination with polygraphy has proven to be a sensitive diagnostic tool for this approach.

W1424

Subtypes of the Capsaicin-Sensitive Vagal C-fibers in Guinea Pig Esophagus

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Background: The vagal sensory neurons in the guinea pig are located in two anatomically and embryonically distinct vagal ganglia: the jugular ganglion (neural crest in origin) and the nodose ganglion (placodal in origin). The exact contribution of these different ganglion to the innervation of the esophagus is not known.
Aims: To determine whether both vagal ganglia contribute to C-fiber (conduction velocity<1m/s) innervation of the guinea pig esophagus.
Methods: The neuronal tracer True Blue was injected into the cervical esophagus and retrogradely labeled vagal sensory neurons were evaluated by immunohistochemistry 7 days later. In separate experiments, the animals were euthanized, the esophagus was removed en bloc with intact vagal innervation including vagal ganglia. Extracellular single unit recordings were made in vitro from the jugular and nodose ganglion neurons with receptive fields in the wall of perfused esophagus. The barriers of drug access from the lumen to the nerve terminals were reduced by stripping esophageal mucosa.
Results: Retrograde tracing from True Blue dye injection into the cervical esophagus revealed esophagus-specific neurofilament-negative neurons (cell bodies of unmyelinated C-fibers) situated in both ganglia. In electrophysiological experiments, C-fibers projecting to the esophagus from jugular (n=9) and nodose (n=11) ganglia were identified by esophageal distention (100 mm Hg). The C-fibres responded reproducibly to step distention (20-100 mm Hg, 20s) with a non-adapting action potential discharge. The nodose C-fibers responded quantitatively stronger (distention pressure 60 mm Hg induced action potential discharge with peak frequency10+/-2 Hz, n=9) than the jugular C-fibers (5+/-1 Hz, n=4, $p<0.05$). Capsaicin (1μM) stimulated both both nodose C-fibres (5 of 5, number of action potentials counted in 2 min 120+/-18) and jugular C-fibres (7 of 8, 110+/-48). In contrast, the purinergic P2X receptors agonists (ATP, 30μM or α,β-methylene-ATP, 30μM) were effective to stimulate nodose (6 of 6), but not jugular (n=5) C-fibers.
Conclusion: Both jugular and nodose vagal ganglia project capsaicin-sensitive C-fibers to the guinea pig esophagus. The two types of C-fibers can be distinguished qualitatively based on their responsiveness to P2X receptor agonists, and quantitatively based on their responsiveness to esophageal distension. These C-fiber subtypes may have distinct embryological origins and therefore we speculate that they may subserve different physiological functions.

W1425

Rho Kinase Mediates Thromboxane A2 (TXA2)-Induced Sustained Contraction in Cat Lower Esophageal Sphincter (LES)

Weibiao Cao, Karen M. Harnett, Ling Cheng, Jose Behar, Piero Biancani

We have previously shown that spontaneous LES tone depends on arachidonic acid metabolism and formation of prostaglandin PGF2α. We have previously shown that spontaneous LES tone depends on arachidonic acid metabolism and formation of prostaglandin PGF2α, and TXA2. We have also shown that RhoA-Rho Kinase is involved in maintaining PGF2α-induced sustained contraction and LES tone by activating a PKC-dependent pathway. In the present investigation we examined the signal transduction pathway of the TXA2 analog U46619-induced contraction in circular smooth muscle of cat lower esophageal sphincter. In LES circular smooth muscle cells, isolated by enzymatic digestion, U46619 (10^{-13}-10^{-7} M) caused a relatively rapid contraction that achieved maximum shortening in approximately 30 seconds and persisted in excess of 20 minutes. U46619-induced initial contraction was inhibited by antibodies against G_q-type G proteins and a 35S-GTPγS binding assay showed that G_q G proteins were activated by U46619. In addition, initial contraction was inhibited by the phosphatidylinositol specific phospholipase C (PI-PLC) inhibitor U73122 and not affected by the selective phosphatidylcholine specific phospholipase C (PC-PLC) inhibitor D609 and by propranolol, which inhibits the phospholipase D (PLD) -dependent pathway. The initial contraction induced by maximal dose of U46619 was blocked by the calmodulin inhibitor CGS9343B, but not by the PKC inhibitor chelerythrine. The Rho kinase inhibitor Y27632 abolished the sustained, but not the initial contraction of LES circular muscle strips in response to U46619. Cells treated with Y27632 achieved the same maximum initial shortening as untreated cells, but did not remain contracted, and returned to their unstimulated length within approximately 10 minutes. In saponin-permeabilized cells, U46619-induced shortening was the same as in intact cells. In these permeable cells, G_{13} and RhoA antibodies almost abolished sustained contraction induced by U46619, confirming that the sustained contraction is mediated by RhoA-induced activation of Rho kinase. The data suggest a role of RhoA-Rho Kinase in thromboxane-induced sustained contraction, but not in the initial contraction where U46619 activates thromboxane receptors linked to G_q to activate a PI-PLC and calmodulin-dependent pathway. Supported by NIH RO1-DK-28614

W1426

A Measure of Achalasia Disease Severity

David Urbach, George Tomlinson, Julie Harnish, Rosemary Martino, Nicholas Diamant

Background: The lack of a measure of achalasia severity has limited the design of clinical trials comparing the effectiveness of alternative treatment modalities. Our objective was to develop a measure of achalasia disease severity with good clinical measurement properties. Methods: We generated a list of potential items to include on a measure by semi-structured interviews with 7 patients with achalasia, and by expert opinion. We then administered a long-form questionnaire to 70 patients with achalasia, and used factor analysis and item response theory methods to reduce the number of items to create a concise measure of achalasia severity. The severity measure underlying item responses was constructed using a Rasch model. We assessed the construct validity of the measure by comparing scores on the instrument with scores on items from the long-form questionnaire that were ultimately dropped from the final measure. Results: We reduced a 37-item prototype questionnaire to a 10-item measure of achalasia disease severity that was reliable (person separation reliability 0.79, Cronbach's alpha 0.83), had good data-to-model fit (mean infit and outfit statistics for items, 1.00 and 0.98 respectively), and had a wide effective measurement range (able to discriminate severity between 87% of subjects with achalasia). Questionnaire items probed concepts such as food tolerance ("Indicate which of the following foods you are able to swallow with no problem/a little difficulty/with great difficulty or not at all"), dysphagia-related dietary maneuvers ("How often in the past month have you needed to drink water while eating to deal with food caught in your esophagus?"), pain, heartburn, distress ("When you sit down to eat a meal, are you bothered by how long it takes you to finish eating?"), lifestyle limitation, and satisfaction. The construct validity of the measure was supported by evidence that scores on the final measure correlated well with scores on items from the long-form questionnaire that were omitted from the final measure. The measure was recalibrated onto a 0-to-100 interval-level scale, with a mean score of 48.9 and a standard deviation of 17.2. Conclusions: We developed a reliable measure of achalasia disease severity that has a broad effective measurement range, interval-level properties, and evidence of construct validity. This measure is appropriate for use as an outcome measure in clinical trials and other evaluative studies of the effectiveness of treatments for achalasia.

W1427

Deterioration of Airway Protective Reflexes Accompanied by Absence of Arousal Response to Pharyngeal Water Stimulation: A Recipe for Aspiration

Kulwinder S. Dua, Jasmohan S. Bajaj, Tanya Rittmann, Candy Hofmann, Reza Shaker

A number of aerodigestive reflexes have been described recently that, based on their functions, can potentially help prevent laryngeal and pulmonary aspiration of gastric contents. However, the aspiration-preventing functions of these aerodigestive reflexes have not been demonstrated directly. AIM: To determine the contribution of aerodigestive reflexes in prevention of aspiration in humans. METHODS: Four smokers and 3 non-smokers were studied during natural stage I-II sleep (monitored for 4 hours). Upper esophageal sphincter pressure was monitored using a specially designed manometry catheter (3mm diameter) that incorporated separate water injection and aspiration ports. Glottal functions were monitored using an ultra-thin (3mm diameter) endoscope (Pentax FNL10AP). Sleep was monitored by polysomnography (REMbrandt 5.2, Medcare, Amsterdam). All modalities were synchronized using

a timer. Pharyngo-UES Contractile reflex (PUCR), Pharyngo-glottal Closure reflex (PGCR), and Reflexive Pharyngeal Swallow (RPS) were evaluated during awake and sleep with slow colored water perfusion into the pharynx at 0.98ml/min. To abort imminent aspiration, when colored water reached the superior aspect of the inter-arytenoid, water was suctioned out through the aspiration port. RESULTS: All tested reflexes were absent in all 4 smokers during sleep (Table). Water perfusion into the pharynx did not elicit arousal in all smokers. Imminent aspiration was observed and aborted in all 4 smokers. CONCLUSIONS: 1) In smokers, PUCR, PGCR and RPS deteriorate further during sleep and are accompanied by absence of arousal response to pharyngeal water stimulation. 2) In smokers, absence of these reflexes and arousal response to pharyngeal water stimulation can cause laryngeal penetration of pharyngeal fluid.

Threshold volume (mean ml ± SD)

SUBJECTS	PUCR		PGCR		RPS		AROUSAL	
	Awake	Sleep	Awake	Sleep	Awake	Sleep	Awake	Sleep
Non-smoker	0.4±0.2	0.21±0.1	0.5±0.3	0.18±0.1	1.4±1.2	0.23±0.2	NA	0.23±0.1
Smoker	0.6±0.5	Absent in all	0.6±0.1	Absent in all	Absent in 2 of 4	Absent in all	NA	Water spill into larynx in all

W1428

High-Resolution Manometry (HRM) Improves the Detection of Incomplete Lower Esophageal Sphincter Relaxation (ILESR)

Annamaria Staiano, Ray E. Clouse

ILESR is an important manometric finding that identifies inhibitory nerve dysfunction typifying achalasia (Ach) and the spastic disorders. HRM using closely spaced pressure sensors, data interpolation, and 3D plotting methods shows that an elevated trans-sphincteric pressure gradient (TPG), a method of calculating increased barrier pressure independent of its axial location, accurately identifies ILESR in Ach. The relative contribution of the TPG to conventional point-pressure measurement of residual pressure (RP) has not been studied but is important in developing the most accurate manometric assessment tools. Methods: Presence of ILESR was determined in 400 subjects (49 ±16 yr, 245 female) who had undergone manometry for clinical indications. HRM had been performed using a 21-lumen perfused catheter system with 1-cm sensor spacing (DentSleeve; MMS). TPG was determined from topographically identified locations 2 cm above and below the LES. Mean RP value was calculated from the ≤5s post-swallow nadir using the sensor most centrally located within the LES. Manometric patterns were categorically classified as previously described. Results: Abnormal RP (>2.5 mm Hg) was present in 63 subjects (15.8%) and elevated TPG (≥5 mm Hg) was found in 57 (14.3%). There was incomplete overlap such that at least one finding was present in 79 (19.8%), TPG increasing the yield of ILESR by 25.4%. RP identified 21 subjects with Ach (aperistalsis + ILESR); TPG found within the aperistaltic group another 5 subjects with ILESR (23.8% increase in Ach dx). TPG increased the rates of ILESR within the spastic disorders from 31.2% to 36.8% for the nonspecific spastic disorders (NSSD) and from 22.2% to 33.3% for DES. When subjects having ILESR as an isolated finding were removed from the NSSD category, then ILESR was found in exactly one-third of patients with any other feature of hypermotility (DES or NSSD) and 9.1% of patients with no other finding. The TPG progressively increased from hypomotility (LES and body: -5.4 mm Hg; LES only: -2.5 mm Hg) through normal (-1.7 mm Hg) to hypermotility groups (NSSD: -0.3 mm Hg; DES: 2.1 mm Hg; Ach: 15.9 mm Hg). Conclusions: TPG extracted from HRM and RP collectively are required to identify all cases of ILESR and together reveal the spectrum of ILESR across the esophageal motor disorders. Tools for HRM that electronically emulate sleeve devices should be developed to avoid the pitfalls of point-pressure measurement yet detect barrier pressures responsible for elevated TPG.

W1429

Fundoplication Reduces Esophageal Longitudinal Muscle but not Circular Muscle Function

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Deglutitive esophagogastric junction (EGJ) axial motion is attenuated in fundoplication (FP) patients (Kahrilas et al, Am J Physiol. 275:G1386, 1998) suggesting either an impairment in longitudinal muscle contraction or relative immobility imposed by FP. Aim: This study aimed to determine if there was a differential effect on longitudinal (LM) and circular musclularis (CM) propria contraction post FP. Normally, these are closely correlated. Methods: 8 post FP patients (6M, median age 39, 17+/-4 months post FP), and 8 healthy subjects (3M, median age 27) had an endoscopic clip placed at SCJ. Subjects were studied with high frequency intraluminal ultrasonography (HFIUS), manometry, and fluoroscopy during swallowing. Axial SCJ movement from digitized fluoroscopic images and LM thickening from HFIUS (5cm above EGJ) during swallows were measured as indicators of LM contraction. Manometric contractile amplitude and CM thickening from HFIUS were measured as indicators of CM contraction. HFIUS data were presented as maximal percentage relative to baseline (100%). Results: During swallowing, total muscle (TM) thickening in the FP patients increased to 149% baseline (IQR 130-167), which was significantly less than in controls (215% (IQR 177-235), $p<0.01$. This difference was mainly due to reduced contraction of the LM layer (Table) as no significant difference was noted in either CM thickness or peristaltic amplitude between groups. The period of shortened period of shortening was also reduced in the FP patients (6.1s (IQR 4.2-6.9) vs. 7.3s (IQR 6.2-8.6), $p<0.005$. Conclusions: FP reduces esophageal LM but not CM contraction during swallows. This is likely due to physical restraint of the EGJ as a consequence of FP. Reduced axial motion may be an important factor in observed impairment of esophageal emptying (and dysphagia) post FP.

	Max shortening	Max Amplitude	Max TM increase	Max LM thickness	Max CM thickness
FP	10.7mm	49mmHg	149%	129%	164%
Normal	19.2mm	63mmHg	215%	196%	236%
p value	< 0.001	>0.5	<0.01	<0.001	>0.05

W1430

Increased Esophagogastric Junction (EGJ) Bolus Transit Time Is Related to Postfundoplication Dysphagia

Bob Scheffer, Melvin Samsom, Alexander Haverkamp, Jac Oors, Geoff Hebbard, Hein Gooszen

Although dysphagia is prominent among symptoms after fundoplication, its relationship with bolus transit across the esophago-gastric junction (EGJ) is incompletely defined. The aim of the study was to assess bolus transit across the EGJ in relation to EGJ dynamics and symptoms of dysphagia in patients before and after laparoscopic fundoplication. Ten (7 male, 3 female) patients were studied (mean age 47 (3.4) yrs) before and 3 months after fundoplication. Simultaneous EGJ manometry and videofluoroscopy were performed with patients swallowing 5 liquid (10 mL barium suspension) and 5 solid ($1cm^3$) bolus consistencies in upright position. Manometry was done with a perfused assembly with side holes in the pharynx, the esophagus, and 11 side holes (1 cm apart) straddling the distal esophagus, the EGJ and the proximal stomach. Symptoms of dyphagia were scored using a visual analog scale during the test. Videofluoroscopic images were analyzed for the total esophageal and EGJ transit time. Manometric tracings were analyzed for the nadir EGJ relaxation pressure, relaxation duration and distal peristaltic amplitude for each bolus. Fundoplication increased EGJ transit time significantly, from 7.4±1.0 s to 9.8±1.1 s for liquids ($P=0.018$) and from 2.9±0.6 s to 6.1±0.9 s for solids ($P=0.015$), whereas esophageal transit time was not affected by fundoplication. No relationship between EGJ transit time and dysphagia was observed before fundoplication. In contrast, a significant relation was observed for both liquids ($r=0.72$, $P<0.01$) and solids ($r=0,75$, $P<0.05$) after operation. Nadir EGJ relaxation pressure increased for both liquids ($P=0.018$) and solids ($P=0.019$) but did not correlate with dysphagia or EGJ transit time. Distal peristaltic amplitudes and duration of EGJ relaxation were both not affected by fundoplication and did not relate to dysphagia. Conclusions: 1) Fundoplication increases the EGJ transit time for both liquids and solids. 2) Postfundoplication dysphagia is related to increased transit time across the EGJ for both liquids and solids.

W1431

HCl-Induced IL-6 and H_2O_2 in Cat LES in an *in vitro* Model of Experimental Esophagitis

Ling Cheng, Karen Harnett, Weibiao Cao, Jose Behar, Piero Biancani

We have previously shown that induction of experimental esophagitis by repeated perfusion of HCl reduces in vivo LES resting pressure and in vitro spontaneous tone. The reduction in LES tone may arise from inflammatory mediators released by immune cells in response to acid-induced inflammation and cell damage. Mucosal biopsies from human esophagitis patients show increased concentration of the pro-inflammatory cytokine IL-6 in the inflamed tissue. We therefore examined HCl-activated mechanisms for production of IL-6 by LES mucosa and circular muscle. A tubular segment of normal mucosa was removed from the distal esophagus and tied at both ends, forming a mucosal sac. The sac was filled with 0.01N HCl (or normal Krebs' for control) and kept in oxygenated Krebs' solution at 37 °C. The mucosa supernatant (MS) outside the sac was collected after 3 hr incubation and applied to circular muscle strips. HCl increased IL-6 content in mucosa, and in mucosa supernatant, but not in circular muscle. IL-6 levels, however, were elevated in mucosa and muscle from esophagitis animals. IL-6 receptor mRNA, measured by RT-PCR was not detected in mucosa before or after HCl exposure, but was present in normal LES circular muscle, and was increased in LES muscle and mucosa from esophagitis animals. These data suggest that HCl causes production of IL-6, which is released from the mucosa, to act on the muscle, where IL-6 receptors may be present. After prolonged inflammation, as occurs in experimental esophagitis, however, IL-6 levels are elevated and IL-6 receptors are expressed both in mucosa and in muscle. In LES circular muscle strips spontaneous tone was decreased by application of supernatant from normal mucosa exposed to HCl, or from esophagitis mucosa. LES tone was similarly decreased by application of IL-6 and the decrease was abolished by the H_2O_2 scavenger catalase, suggesting production of H_2O_2 in response to IL-6. Direct measurement confirmed that IL-6, or application of supernatant from normal mucosa exposed to HCl, caused production of H_2O_2 in LES circular muscle. No production of H_2O_2 occurred in the mucosa. These findings were confirmed by direct visualization of H_2O_2 by dihydrorhodamine fluorescence under confocal microscopy. We conclude that initial exposure of mucosa to HCl causes production of IL-6 in the mucosa. Activation of IL-6 receptors in circular muscle results in production of H_2O_2 by the muscle and reduction in tone. Supported by NIDDK RO1 57030

W1432

Comparison of Esophageal Sensory Testing, Clinical Characteristics and Psychometric Profile Between Patients with Nonerosive Reflux Disease (NERD) and Abnormal pH Testing and Those with Functional Heartburn (FH)

Ronnie Fass, Joy N. Beeler, Isaac B. Malagon, Jimmy M. Bautista

Purpose: NERD has been traditionally defined as patients with typical heartburn and normal endoscopy. However, 2 large subgroups fall into this category, NERD and abnormal acid exposure and FH. The latter group has been suggested to explain the low symptom response rate to proton pump inhibitors (PPIs) of NERD patients. **Aims:** To compare the clinical characteristics, psychometric profile and sensory testing results of patients with NERD and abnormal acid exposure (NERD+) and those with FH (normal pH testing). **Methods:** Patients with typical heartburn, at least 3/week, were enrolled. Patients underwent an upper endoscopy to exclude esophageal inflammation and then underwent pH testing to determine

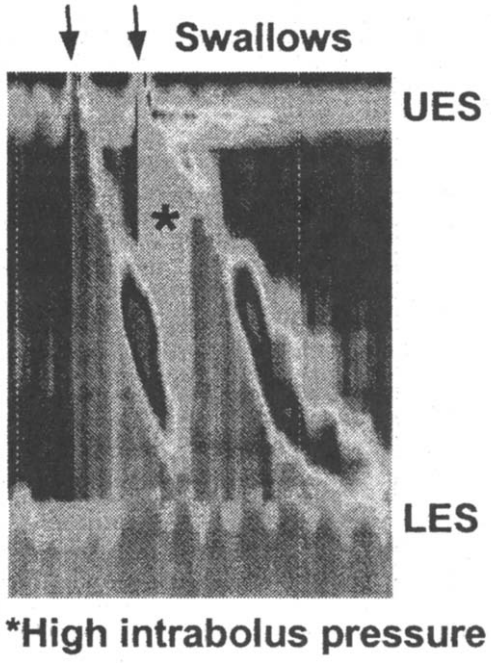
the percent total time pH <4 (normal <4.2% ≤ abnormal). The 2 groups were evaluated by the validated GERD Symptom Questionnaire (GSQ), demographics questionnaire, short form (SF) 36, SCL-90R (psychological profile) and a sensory testing using a modified acid perfusion test (t — time to heartburn, I — intensity and APSS - acid perfusion sensitivity score = duration of heartburn x I/100). **Results:** 20 FH were enrolled (mean age 44, range 24-64, M/F 12/8) and 13 with NERD+ (mean age 43.7, range 23-76, M/F 9/4). There was no difference in symptom characteristics between the two groups (severity, frequency, intensity) except duration of heartburn (FH 7.0 ±1.0 vs. NERD+ 4.1 ±1.0 years, p <0.01). There was no difference in all 8 domains of SF-36 (p>0.05) and there was no difference in psychological profile in all domains between the 2 groups (p>0.05). pH testing revealed that % total time, supine time and upright time between NERD+ and FH were significantly different (6±0.6 vs. 1.3 ±0.4, 5.1 ±1.1 vs. 1.2 ±0.3, 5.1 ±1.2 vs. 1.3 ±0.4, respectively, p<0.01). Sensory testing revealed that NERD+ patients were significantly more sensitive to acid in all parameters than those with FH (T — 198.8 ±63 vs. 232.6 ±75 sec, I —11.4 ±2.0 vs. 8 ±1.6, APSS — 56.8 ±11.3 vs. 42.3 ±9.0, all p <0.05]. Five (25%) patients with FH had a negative acid perfusion test. **Conclusions:** Patients with FH have longer duration of heartburn, than those with NERD+, but otherwise have similar clinical and psychometric characteristics. Patients with NERD+ are significantly more sensitive to esophageal acid exposure than those with FH, further supporting the hypothesis that FH is partially composed from patients with non-acid related causes for their heartburn.

W1433

High-Resolution Manometry (HRM) Reveals Bolus Entrapment in the Proximal Esophagus with Double Swallows in Patients with Spastic Motor Disorders (SMD)

Clinton T. Snedegar, Laura R. Haroian, Ray E. Clouse

HRM with 36 solid-state sensors (1-cm spacing) spanning the entire esophagus (Sierra Scientific Instr) was used to study deglutitive inhibition (DI) from repetitive wet swallows. Methods: 7 subjects with normal manometry and 9 with SMD (2 DES, 7 nonspecific) agreed to undergo HRM and take paired swallows separated by ≤3s, >3-≤5s, >5-≤7s, or >7s. Data were analyzed from contour maps created for each pair. Results: 134 pairs were available for analysis, and the effects of DI were easily visualized on the maps. Swallows spaced by <3s completely inhibited further transmission of peristalsis if contraction in smooth-muscle segments had not begun. Pairs with greater inter-swallow delay showed variable degrees of distal DI, and a consistent difference between subject groups could not be determined. A striking finding of isobaric pressure increase with swallow 2 (≥50 mmHg) was noted in some subjects consistent with bolus entrapment above ongoing distal motor activity from swallow 1 (figure). Systematic measurement of peak and 2-s mean intrabolus pressure 4 and 8 cm below the UES following swallow 2 revealed significantly (p<0.05) increased pressures in the SMD subjects in swallows paired at ≤3s or >5-≤7s and trend toward increases when paired at >7s (p<0.1). Explanations for entrapment varied and included ongoing prolonged distal contraction of swallow 1 and impaired inhibition as the 1st wave continued toward the LES. Conclusions: HRM demonstrates DI in a novel way using contour maps to show inhibition of the peristaltic sequence. Proximal bolus entrapment during repetitive swallows, in part from impaired DI, may serve as a stimulus for dysphagia and chest pain in patients with SMD.



W1434

Barrett's Esophagus After Pneumo-dilation For Achalasia

Pieter Scholten, Yvonne Leeuwenburgh, Rob Vaessen, Thomas Calje, Evelien Ong, Peter D. Siersema, Jelle Haringsma, Ernst J. Kuipers

Achalasia is a neuro-degenerative disease of the esophagus resulting in esophageal aperistalsis and defective relaxation of the lower esophageal sphincter (LES). Barretts esophagus (BE) is thought to be a premalignant condition of the esophageal mucosa caused by gastro-esophageal reflux in patients with an insufficient LES function. Because of this paradox, the co-incidence of achalasia and BE in the same patient is considered to be rare and so far, only 30 cases have been described in the literature. From these, 22 were after myotomy, 7 without former treatment and there is only one case of BE after pneumo-dilation. We studied the incidence of BE in achalasia patients in anatomy and function of the gastro-esophageal junction following dilation treatment. This is a single-center cohort study of 331 patients (160 male; mean age 51 yrs) with achalasia who presented at the Erasmus Medical Center in Rotterdam from 1975 to 2003. All patients were treated with pneumatic dilatation. Follow-up consisted of regular manometry, timed barium swallow-examination, and endoscopy with biopsy sampling from the distal esophagus. No patients had BE at baseline, but 28 (8.5%) developed this condition during follow-up. Hiatal herniation was present in 75 (23%) patients. Twenty-one (28%) of them developed BE compared to 7 out of 256 (3%) patients without HH (RR 13.8; 95% CI 5.6-34.2). Post-treatment LES pressures were slightly lower in patients developing BE than in those without BE (13.9 vs 17.4 mm Hg). Achalasia patients who are succesfully treated with balloon dilation are at risk for subsequent development of BE. This risk is related to reduced lower esophageal sphincter pressures and the presence of hiatal herniation. These findings support the concept of Barretts esophagus as a complication of gastro-esophageal reflux disease and has the consequence that the presence of either one of them warrants 24 h-pH metry and strict follow up.

W1435

Basal and Provocative Pharyngo-Esophageal Reflexes Are Impaired in Infants with Perinatally Acquired Neurological Injury

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BACKGROUND: Infants with perinatally acquired neurological injury commonly suffer with feeding difficulty or airway compromise. The pharyngo-esophageal motor mechanisms protective of aerodigestive tract are not known in infants at-risk. OBJECTIVE: To identify the (1) basal characteristics of primary peristalsis (PP) and (2) esophageal and upper esophageal sphincter (UES) reflex responses to mid-esophageal provocation, among controls and study infants. DESIGN AND METHODS:We performed esophageal motility study using a micromanometric pneumohydraulic water perfusion system, a specially designed catheter assembly (Dentsleeve) with 5-sideports, UES and LES sleeves, and a mid-esophageal infusion port to provide stimulus (air, water, or apple juice), and concurrent submental EMG, with MMS motility system, in 6 oral-fed controls at 40 ± 3 wk postmenstrual age (PMA) and in 8 tube-fed infants with perinatal neurological injury at 43.5 ± 9.8 wk PMA. RESULTS: 85 % of 152 infusions resulted in a response in controls vs. 68 % of 179 mid-esophageal infusions in study group. Esophageal body and UES relaxation characteristics were similar. Mean threshold volumes (ml) respectively, for air, water and apple juice were similar: 0.8, 0.6, 0.6 (controls) vs 0.9, 0.9, 0.9 (study). The magnitude of UES-pressure increase was 12.5 fold vs. 8.5 fold (control vs. study groups). CONCLUSIONS: In the study group, the 1) frequency and distribution of basal pharyngo-esophageal motility is impaired; 2) frequency occurrence of SP, E-D-R, and E-UES-CR are differently distributed; 3) air and liquid stimuli are handled differently. These findings may have an implication in aerodigestive tract clearance mechanisms.

Distribution of Basal and Provocative esophageal responses

Characteristics	Controls	Study group
Swallow propagation, complete:incomplete:failed, %	80:12:8 (n= 64 swallows)	31:39:30 (n=83 swallows) *
Swallow frequency, # /min, mean ± SD	1.9 ± 0.7	0.5 ± 0.8 *
All infusions with a response, SP:E-D-R: E-UES-CR, %	46:30:46	55:12:48 *
1.0 cc air stimulus with a response, SP: E-D-R: E-UES-CR, %	57:22:56	50:17:46
1.0 cc water stimulus with a response, SP: E-D-R: E-UES-CR, %	40:67:47	68:14:59 *
1.0 cc apple juice stimulus with a response, SP: E-D-R: E-UES-CR	42:47:37	58:16:47 *

SP-secondary peristalsis; EDR-esophago-deglutition-response; E-UES-CR-esophago-UES-contractile reflex; there were 1 or more responses to some infusions. * t-test or Chi-squared, P < 0.05

W1436

Evaluation of the Upper Esophageal Sphincter (UES) In Normal Volunteers and Human Cadavers Using Simultaneous High-Resolution Endoluminal Sonography (HRES) and Manometry

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Purpose: The aim of the current study is to characterize the motion, morphology, and pressure of the upper esophageal sphincter (UES) using simultaneous high-resolution endoluminal sonography and manometry. Methods: The UES and its surrounding structures were evaluated in seven normal subjects and four human cadavers, using a 20MHz ultrasonography transducer attached to a manometry probe. The morphology, anatomy, pressure profile and cross-sectional area (CSA), width, angle, and peak pressure, of the UES were evaluated at rest in the normal volunteers. Results: The UES musculature in normal volunteers is a C-shaped structure that forms an angle of 107 ±19 degrees between the center of the cricoid

cartilage and the attachment to the lateral aspects of the cricoid lamina. The mean peak resting UES pressure was 74 mmHg above the resting upper esophageal body baseline resting pressure with a total cross sectional area (CSA) of 0.87 ± 0.33 cm2. Both UES musculature CSA and peak pressure are significantly greater than total muscular (TM) CSA (P = 0.01) and pressure (P = 0.01) of the upper esophageal body at rest. During swallowing a simultaneous drop in pressure with the appearance of upper esophageal body morphology was observed on ultrasound. Following passage of the bolus, pressure increased in synchrony with the appearance of UES morphology on HRES imaging. The anatomy and morphology of the UES remained the same in the cadavers as in the normal volunteer. Peak pressure recorded in the area of the UES in the cadavers (localized on US) was a mean of 19.7 mmHg over baseline esophageal body pressures (UES: 33.4 ± 21.6mmHg; Esophageal body: 13.72 ± 12.09mmHg. P<0.0001). Taking 74.3 mmHg as the mean peak resting UES pressure above the upper esophageal body baseline resting pressure in normal volunteers, the peak UES pressure in the cadaver is approximally 26.5% of the mean peak resting UES pressure measured in the normal volunteer subjects. Conclusion: The sonographic anatomy of the UES was defined. In the cadaver studies a significant increase over esophageal body baseline pressure was observed. It was concluded that a significant proportion of the pressure in the area of the UES (approximately 26.5 % of the mean peak resting UES pressure) is due to passive mechanical conformational changes in this area and not due to active tonic muscle contraction of the UES.

W1437

Simultaneous Ultrasound (US) and Manometry During Pharmacologic Paralysis of the Crural Diaphragm Allows Spatial Localization of the Two Components of the Intrinsic Gastroesophageal Junction High Pressure Zone (GEJHPZ)

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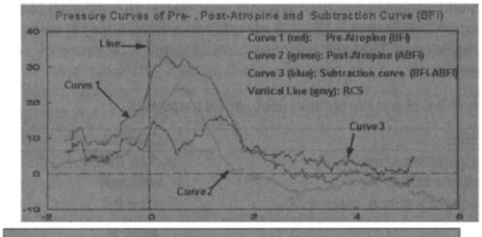
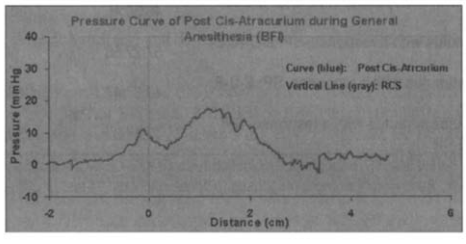
Purpose: To pharmacologically paralyze the crural diaphragm (CD) in order to define the components of the intrinsic GEJHPZ. Methods: Four patients undergoing general anesthesia for non-esophageal pathology were evaluated by placing a simultaneous 20MHz US/manometry probe into the stomach. Cis-Atracurium was given to cause CD paralysis. During inspiratory and expiratory pause on the ventilator. The probe was withdrawn at a constant velocity of 5mm/sec and US images and pressure tracings were recorded simultaneously. Ensemble averaging of the pressure curves was plotted against the geographic location of the beginning of the right CD (RCD). Tracings were compared with 15 normal subjects who were evaluated with and without atropine ablation of the intrinsic components of the GEJHPZ. Results: In the 15 normal volunteers, the difference between the pre- and post-Atropine pressure curves (the intrinsic sphincter components) displayed two peaks in both full inspiration and full expiration, 1.3 to 1.6 cm apart. The ensemble averaging of the four patients undergoing CD paralysis demonstrates residual pressure profiles from only the intrinsic sphincter components. This pressure profile demonstrates the same two peaks, 1.2 to 1.3 apart, in the same geographic location with respect to the RCD as seen in the other 15 volunteers. Conclusion: Skeletal muscle paralysis of the crural diaphragm allows spatial localization of the two intrinsic GEJHPZ sphincter components. These results are consistent with localization of the sphincter components using smooth muscle ablation of the intrinsic components with Atropine. The distal intrinsic high pressure zone represents the gastric sling fibers while the proximal component represents the intrinsic lower esophageal sphincter. Funded through NIH grant R01 DK59500-03.





W1438

Acid Reflux Therapy Improves Cardiac Symptoms in a Subgroup of GERD Patients with Idiopathic Arrhytmias and Altered Neurocardiac Function

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Background. We previously reported a link between acid reflux and cardiac autonomic reflexes in a subgroup of patients with idiopathic supraventricular arrhythmias and gastroesophageal (GER) symptoms. However, whether acid suppression is able to modify cardiac symptoms in these patients remains to be elucidated. Aim. To evaluate the effect of acid suppression on cardiac symptoms in patients with idiopathic supraventricular arrhythmias and symptoms of GERD. Subjects and methods: 31 patients (19 males, aged 20-69 years), with idiopathic supraventricular arrhythmias and GERD symptoms, underwent simultaneous 24-hours pH-metry and ECG monitoring. Power spectrum analysis of heart rate variability (PSHRV), with its low frequency (LF, influenced by sympathetic modulation) and high frequency (HF, influenced by vagal modulation) components, was obtained and LF/HF ratio calculated. Total time with esophageal pH<4 and mean pH, were considered during pH-monitoring, and compared with PSHRV data. A 4 months full-dosage PPI therapy was prescribed to each patient. Results. In 18 out of 31 patients (58%), a significant correlation between mean esophageal pH and LF/HF ratio was observed ($p<0.05$). In 12 of these patients the pH decrease was positively correlated with a decreased ratio ($r = 0.53 \pm 0.08$), which was dependent on the HF component increase. Conversely, in the remaining six cases, a negative correlation (r-0.57 ± 0.12), with an increase in ratio dependent on the LF increase was observed. Furthermore, time with esophageal pH<4 was higher in the 18 acid responsive patients than in non-responsive subjects (6.3 ± 1.7 % of time with pH<4 vs 4.8 ± 1.6; p n.s.), although the difference was not statistically significant. The PPI therapy significantly reduced GERD symptoms in the 22 patients who complied with treatment (there were 9 drop-outs). However a significant reduction of cardiac symptoms, as palpitations, was observed only in the subgroup of patients who experienced a significant correlation between mean esophageal pH and LF/HF ratio (12/15 vs. 1/7, $p<0.01$). Conclusions. This study identified a subgroup of patients with idiopathic supraventricular arrhythmias and cardiac autonomic reflexes elicited by the acid stimulus in the esophagus. In this subset of patients acid suppression improved GERD and cardiac symptoms.

W1439

Reversibility of Pharyngo-UES Contractile Reflex (PUCR) and Reflexive Pharyngeal Swallow (RPS) in Ex-smokers

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Earlier studies have shown that aerodisgestive reflexes that can potentially protect the airways against aspiration of gastric contents are defective in cigarette smokers. Reversibility of these deleterious effects of smoking after smoking cessation has not been previously studied. AIM: To study PUCR and RPS in ex-smokers and compare the results with non-smoker and chronic smokers. METHOD: 10 healthy non-smokers (4 men, 6 women), 10 healthy ex-smokers (5 men, 5 women; abstinent for 4.4 ± 2 months) and 5 healthy chronic smokers (3 men, 2 women) were studied. Smoking cessation compliance of ex-smokers was assessed using weekly CO breathalyzer at the smoking cessation clinic. PUCR and RPS were elicited by rapid and slow pharyngeal water injections using our previously described techniques of concurrent manometry and EMG. Water was injected posteriorly into the pharynx 2cm above the UES high-pressure zone. RESULTS: RPS and PUCR were elicited in all non-smokers by both, rapid and slow pharyngeal water injection techniques. RPS was also preserved in all ex-smokers and smokers but PUCR was absent in 2 of 10 ex-smokers and 2 of 5 smokers using both techniques. When present, the threshold volume to trigger PUCR during rapid water injection was similar between non-smokers and ex-smokers but was significantly higher in smokers compared to non-smokers or ex-smokers (Table). CONCLUSIONS: Threshold volume to trigger PUCR is similar between non-smokers and ex-smokers but significantly higher in smokers suggesting potential reversibility of smoking-induced deterioration of aerodigestive reflexes after smoking cessation.

Threshold volume to elicit PUCR and RPS (ml mean ± SD)

Subjects	[a]PUCR Rapid	[b]PUCR Slow	[c]RPS Rapid	[d]RPS Slow
Non-smokers	0.28±0.1	0.5±0.1	0.4±0.1	1.1±0.2
Ex-smokers	0.27±0.1	0.7±0.1	0.5±0.2	1.4±0.2
Chronic smokers	0.53±0.1	0.73±0.3	0.9±0.3	1.3±0.2

[a]PUCR Rapid: Non-smokers Vs Smokers P = 0.001; Ex-smokers Vs Smokers P = 0.001; Ex-smokers Vs Non-Smokers P NS. [b]PUCR Slow: Non-smokers Vs Ex-smokers P = 0.01. [c]RPS Rapid: Non-smokers Vs Smokers P = 0.03. [d]RPS Slow: P NS between groups

W1440

Asymmetrical Mechanisms Controlling Resting Tone, Relaxation and Contraction in Clasp and Sling Regions of Porcine LES

Ricard Farre, Emma Martinez, Xavier Sunyol, Pere Clave

Circular smooth muscle in the lower esophageal sphincter (LES) shows an anatomical asymmetry in humans and other species with clasp fibers on the lesser curvature and sling fibers on the greater. Scarce data are available on functional asymmetry in LES. Aim: To compare the neuromyogenic mechanisms controlling resting tone and responses to stimulation of enteric motor neurons (EMN) in the clasp and sling regions of porcine LES. Methods: Circular LES strips (25 clasp, 25 sling) of 3x10mm including both circular and longitudinal muscle layers and the myenteric plexus from 10 adult pigs were studied in an isometric organ bath. Experimental design: a) Control of resting tone by exposition of strips to calcium-free buffer, tetrodotoxin (TTX 1μM), ODQ 10μM, and atropine (ATR, 1 μM); b) Stimulation of EMNs by EFS 26V, 0.4 ms, 0.3-20 Hz) or by nicotine (NIC, 100 μM) at basal conditions, during sequential blockade of ODQ and apamin (APA 1 μM) sensitive neurotransmission, and blockade of excitatory EMN by ATR 1μM), and during axonal blockade by TTX. Results: * = $p<0.05$ clasp vs sling. a) Clasp and sling strips developed similar active tone (4.35 ± 0.5g vs 5.35 ± 0.7g). In clasp strips, tone was abolished (-122.2 ± 3.6%) by 30-min extracellular calcium depletion, slightly reduced by TTX (-24.77 ± 6.9%) or ATR (-17.7 ± 5.1%), and slightly enhanced by ODQ (29.9 ± 5.5%). In sling strips, tone was less rapidly affected by calcium depletion (-80.8 ± 8.5%*), more reduced by TTX (-75.0 ± 13.3%*) or ATR (-78.9 ± 10.3%*), and similarly enhanced by ODQ (17.7 ± 2.4%). b) The morphology of EFS on relaxation and off contraction, and NIC relaxation was similar among clasp/sling strips. Integrated amplitude of EFS on relaxation (-69.27 ± 16g vs -80.27 ± 26g 0.3-20 Hz) and NIC relaxation (-4.87 ± 0.5g vs -4.10 ± 0.4g) was also similar. In contrast, off contraction was stronger in clasp (209.7 ± 24.8*g 0.3-20 Hz) than in sling (120.2 ± 22.9g) region. ODQ reduced EFS and NIC relaxation and EFS contraction in clasp strips, and blocked EFS and NIC relaxation and unaffected EFS contraction in sling region. The APA-sensitive component of EFS relaxation was similar on both LES sides. TTX inhibited NIC relaxation in clasp strips and switched NIC relaxation to a cholinergic contraction in sling strips. Conclusion:

The porcine LES shows a strong functional clasp/sling asymmetry affecting the relative contribution of myogenic and neurogenic mechanisms controlling resting tone and the mediators of relaxation and contraction following stimulation of EMN.

W1441

Discoordination Between Circular and Longitudinal Muscle Contractions in Patients with High Amplitude Esophageal Contractions

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Background: The increase in muscle thickness and muscle cross-sectional area (CSA) during esophageal contraction seen on ultrasound images is a marker of longitudinal muscle (LM) contraction. On the other hand, pressure recorded by manometry is a measure of circular muscle (CM) contraction. In normal subject, there is a precise coordination between the two muscle layers, the peak muscle CSA occurs at the same time as the peak pressure. Aim: The goal of our study was to determine the coordination between CM and LM in patients with high amplitude esophageal contractions (HAEC). Method: Simultaneous and synchronized pressure recording and high frequency intraluminal ultrasound (HFIU) imaging were performed in 5 normal subjects and 10 patients with HAEC (contraction amplitude > 180 mmHg). Recordings were performed at 2 cm above the lower esophageal sphincter during a 5 ml water swallow. Data Analysis: HFIU images were digitized every 250 milliseconds and analyzed using a partially automated computer program for muscle thickness (CM + LM) and muscle CSA. The time between the peak muscle CSA and peak pressure (δt) was determined. Results: Normal subjects showed close temporal correlation between the peak contraction pressure and peak muscle CSA with median $\delta t = 0.5$ second (range, 0~0.5, 28 swallows). On the other hand, HAEC patients revealed dissociation between the peak CM and LM contraction, median $\delta t = 1.0$ second (range, 0.25~3, 52 swallows, as compared to controls); 42 (81%) of these swallows showed a $dt > 0.5$ second. HAEC patients also showed a greater baseline as well as peak muscle CSA as compared to normal subjects. There was a significant correlation in both groups between dt and the followings: a) peak contraction amplitude, b) upstroke of the pressure slope and c) baseline and peak muscle CSA. Conclusions: Discoordination between LM and CM appears to be another marker of esophageal motor dysfunction.

Comparison between Normal Subjects and HAEC Patients

	Peak Pressure (mmHg)		Peak Muscle Thickness (mm)		δt (second)	
	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
5 Normal (28 swallows)	111 ± 39	48 ~ 178	2.5 ± 0.5	1.9 ~ 3.8	0.4 ± 0.2	0 ~ 0.5
10 Patients (52 swallows)	227 ± 115*	60 ~ 701	3.7 ± 0.9*	2.0 ~ 5.3	1.1 ± 0.6*	0.25 ~ 3

*: $p<0.05$

W1442

Mechanisms of Incomplete Bolus Transit in Non-Obstructive Dysphagia Assessed by Combined Multichannel Intraluminal Impedance and Sleeve Sensor Manometry

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Background and aim: Patients with non-obstructive dysphagia (NOD) often have impaired esophageal bolus transit. Aim of this study was to assess the esophageal and LES motor patterns associated with incomplete bolus transit (BT) in NOD using combined multichannel intraluminal impedance and esophageal manometry. Methods: 22 consecutive non-achalasic patients with NOD (5 men/17 women, 26-83 yr) underwent esophageal and LES manometry combined with intraluminal impedance monitoring, using a single multilumen perfused manometry assembly with incorporated sleeve sensor. Series of 10 swallows of 5-ml saline followed by 10 swallows of a standard viscous solution were performed. Complete BT was defined as drop in impedance followed by recovery to baseline values in all recording segments. Results: Incomplete BT was associated with simultaneous and non-transmitted waves. Only 16% (7/45) of liquid and 0% (0/41) of viscous swallows associated with simultaneous contractions had complete BT (see table). Peristaltic contractions associated with incomplete liquid and viscous BT had significantly lower distal amplitudes than those associated with complete BT (38.5 vs. 43 and 30 vs. 55 mmHg, median values) ($p = 0.01$ and $p<0.001$). However, the overlap between the groups was large and only 12.5% of incomplete liquid and 18% of incomplete viscous BT had amplitudes below the 5th percentile of the amplitudes of complete liquid and viscous BT, respectively. Nadir pressure and duration of LES relaxation of peristaltic waves associated with incomplete liquid and viscous BT were similar to those with complete BT (nadir pressure: 1.1 vs. 1.7 and 1.3 vs. 1.4 mmHg in liquids and viscous swallows, respectively; LES relaxation: 7.6 vs. 7.3 and 8 vs. 7.5 sec. in liquids and viscous swallows, respectively). Conclusions: In patients with NOD, incomplete BT is associated with simultaneous, non-transmitted and low-amplitude peristaltic waves, but the associations are too weak to explain the majority of incomplete BT events. Likewise, incomplete BT cannot be explained by abnormalities in LES relaxation. Mechanisms beyond the scope of manometry must play a role in the pathophysiology of disordered bolus transport.

	Liquid swallows (N=226)		Viscous swallows (N=214)	
	complete BT (N=105)	incomplete BT (N=121)	complete BT (N=79)	incomplete BT (N=135)
Peristaltic waves	92 %	60 %	100 %	66 %
Simultaneous waves	7 %	31 %	0 %	0 %
Non-transmitted waves	1 %	9 %	0 %	4 %

W1443

Nonerosive Reflux Disease (NERD) in Patients with Non Cardiac Chest Pain (NCCP)

Amedeo Indriolo, Francesco Negrini, Sergio Signorelli, Maria Grazia Luca, Mario Strazzabosco

Abnormal gastro-esophageal reflux has been demonstrated in 20-66% of patients with non-cardiac chest pain (NCCP). The identification of NERD (nonerosive reflux disease) as a cause of symptoms in patients presenting with NCCP has not been sistematically investigated. AIM of the study was to investigate the occurrence of NERD in patients presenting with NCCP and to assess the pathophysiological differences between NERD, patients with erosive disease and patients without reflux. METHODS: 102 consecutive patients (52 males; mean age 47 yrs) diagnosed with NCCP after a complete cardiac evaluation underwent upper gastrointestinal endoscopy, ambulatory 24-hours esophageal pH-monitoring and esophageal manometry. Data obtained from pH-manometry performed on 22 healthy volunteers were used as control. RESULTS: in 42% of patients both endoscopy and pH-metry were normal (group 1), in 40% of patients endoscopy was normal but pH-metry showed pathological reflux (NERD) (group 2); in 18% an erosive disease was present at endoscopy and pathological reflux at pH-metry (GERD) (group 3). No difference was found between NERD and GERD patients in total time of pH below 4 (NERD 7.2% median value - 4.4-21.0 range; GERD 10.0% - 4.0-21.0) as well as in the pattern of acid reflux (NERD's % of time beelow 4 in supine position median value 5 - range 0-33; GERD's 6 - 0.3-21; in upright position 10 - 2.7-18.8 - in NERD and 10 - 3-29.3 - in GERD). Positive Symptom Index (SI) (more than 50% of episodes of chest pain temporary associated to acid reflux) was present in 20% of cases in group 1, 23% in group 2 and 11.7% in group 3 ($p<0.005$ vs group 3). Maior motility abnormalities were significantly more common in patients without reflux as compared to NERD and GERD (respectively 18.6% group 1, 4.8% group 2 and 5.5% group 3) ($p<0.003$). The prevalence of reflux-related aspecific motility disorders (AMD) was no significantly different in NERD as compared to GERD (13.9% in group 1, 39% in group 2 and 61.1% in group 3). CONCLUSIONS: (1) an esophageal abnormality was the possible cause of NCCP in 72% of patients (NERD, GERD, positive SI or major motility abnormalites). (2) Reflux disease accounted for 58% of patients with NCCP with an higher prevalence of NERD (40%) as compared to GERD (18%). (3) SI positivity was significantly increased in NERD as compared to GERD, in spyte of similar reflux pattern and AMD, suggesting that an increased visceral sensitivity may be responsable for symptoms in NCCP patients with NERD.

W1444

Longitudinal and Circular Muscle Contract Synchronously in the Esophagus during Peristalsis: A New Way to Look at the Contraction of Two Muscle Layers

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Objective: Increase in esophageal muscle cross sectional area (CSA), as seen on high frequency intraluminal ultrasound (US) imaging is a marker of longitudinal muscle contraction (LMC). On the other hand, pressure recorded by manometry is a marker of circular muscle contraction (CMC). Simultaneous US imaging and manometry reveal that LMC begins before and ends after CMC. We reassessed the relationship between contractions of two muscle layers. Method: US and manometry recordings were obtained at 5 and 10 cm above the LES in 4 healthy subjects during water swallows of 2, 5, 10 ml bolus volumes. US images were analyzed at a frequency of 4 Hz for muscle CSA (both LM and CM) during the entire swallow period. We also observed that during contraction esophagus assumed a perfect circular shape from a "slit like" shape. Therefore we calculated circularity index (CI: ratio of long axis to a perpendicular short axis of the cross sectional image of the esophagus) as a possible marker of CMC. Results: Each swallow resulted in distension of esophagus (by bolus) followed by lumen collapse. Pressure was recorded when the lumen collapsed completely around the manometry probe. Muscle CSA increased (LMC) prior to the onset of manometric contraction (CMC), however, it coincided with the onset of lumen collapse (an alternative marker of CMC). The time interval between the onset of lumen collapse and manometric contraction increased with bolus volumes but the lumen collapse and LMC always occurred together. The peak of the manometric contraction occurred within .05 to .9 s of peak LMC. The delay in the end of LMC after the end of manometric contraction was the time during which esophagus reverted from a circular to a "slit like" shape. The end of LMC was within .3 to 1.2 s of return of the CI to baseline (Table). Conclusion: The differences between the onset of CMC and LMC reported in the earlier studies may be related to the different techniques used for monitoring CM and LM contractions. Our data support that the two layers of the esophagus contract synchronously during peristalsis.

Various Time Intervals Observed during Swallows (Mean±SEM)

Level above LES	Bolus (ml)	LMC onset - MC onset (s)	Lumen collapse - MC onset (s)	LMC Peak - MC Peak (s)	CI end - LMC end (s)
5 cm	2	1.2±0.3	1.6±0.3	0.9±0.5	0.5± 0.8
	5	1.6±0.6	2.4±0.6	0.7±0.3	0.3± 0.3
	10	0.7±0.4	0.7±0.8	0.6±0.4	1.2± 0.1
10 cm	2	1.47±0.6	1.5±0.5	0.05± 0.2	0.9± 1.6
	5	1.77±0.3	1.5±0.4	0.6±0.3	0.6±0.6
	10	2.14±0.5	1.9±0.4	0.27±0.3	0.5±0.5

MC: Manometric contraction

W1445

Novel Solid-State Technology Simplifies High-Resolution Manometry (HRM) for Clinical Use

Ray E. Clouse, Thomas R. Parks, Laura R. Haroian

HRM offers advantages over conventional methods in both research and clinical studies of esophageal motility. Widespread use of HRM has been limited by technical complexity of water perfusion required for systems with large numbers of closely-spaced pressure recording sites. Re-positioning of the catheter is necessary to completely sample the esophagus and sphincters with equivalent high resolution. For this report, a solid-state system using novel pressure sensing technology was developed and tested in a clinical population (ManoScan; Sierra Scientific Instr, Los Angeles). Methods: 42 subjects (age 15-84 yr; 28 female) were recruited from patients referred for clinical manometry. A 4.2 mm OD catheter containing 36 solid-state circumferential sensors spaced at 1-cm intervals was positioned so that 1-2 sensors were proximal to the upper esophageal sphincter (UES). Sphincter locations were determined by viewing contour maps of HRM data. Lower esophageal sphincter (LES) basal pressure was determined during slow respiration from an intrasphincteric location before 10 wet swallows were offered. The catheter was withdrawn so that the sensors were offset 5 mm from the original position and wet swallows were repeated. Diagnoses were established at each position using a combination of conventional parameters (taken from locations easily determined from contour-map review) and subjective evaluation of the maps. Categorical diagnoses were established using previously reported methods. Results: 10 subjects had completely normal findings. Of those with abnormalities, 7 had hypomotility features, 21 hypermotility (14 non-specific spastic disorders, 3 DES, 4 achalasia), and 4 had mixed features. Diagnoses did not differ within subjects when analyses were performed at each of the 2 catheter positions. Subjective interpretation of the maps was simplified by the fact that the UES, entire esophagus, and LES were included on individual maps of each swallow, and features previously described when using HRM for interpretation of motor disorders were easily identified. Conclusions: HRM using the ManoScan, a novel solid-state system, eliminates water perfusion and simplifies the technical aspects of this manometric technique. Catheter position (with regard to 5 mm sensor offset) did not influence diagnosis — further reducing technical demands at the time of the study and allowing post hoc sensor selection for analysis. This approach should increase the acceptability of HRM for clinical use.

W1446

Combined Multichannel Intraluminal Impedance and Manometry Clarifies the Function Defect in Patients with Distal Esophageal Spasm

Radu Tutuian, Donald O. Castell

Background:
Combined multichannel intraluminal impedance and manometry (MII-EM) recently became available as an esophageal function test. Initial studies in healthy volunteers have shown that a proportion of simultaneous contractions actually have complete bolus transit.
Aim:
To evaluate esophageal bolus transit (by MII) in patients with a manometric pattern of distal esophageal spasm (DES).
Methods:
All patients referred for esophageal function testing underwent combined MII-EM studies including 10 liquid and 10 viscous swallows. DES was defined as 20% or more liquid swallows with simultaneous contractions defined as amplitude >30mmHg and onset velocity >8 cm/sec in the distal esophagus. Diagnosis of esophageal transit abnormalities was defined by MII as incomplete if ≥30% of liquid and ≥40% of viscous swallows had incomplete bolus transit.
Results:
Thirty-eight patients (19 female, mean age 52, range 16-84) with a manometric diagnosis of DES were identified out of a total of 450 combined MII-EM studies. Analysis of individual (380 liquid) swallows identified that distal esophageal amplitude and timing of distal contraction (i.e. antegrade vs. retrograde) influence complete bolus transit. Simultaneous swallows with complete bolus transit had higher ($p<0.001$) distal esophageal amplitude (186 +/- 23 mmHg; mean +/-SEM) compared to those with incomplete bolus transit (86 +/- 6 mmHg). 75% of antegrade contractions with velocity >8cm/sec had complete bolus transit compared to 38% of retrograde contractions ($p<0.001$). Based on transit of liquid and viscous swallows 49% of DES patients had complete bolus transit for both, 29% complete bolus transit for either liquid or viscous and 22% incomplete bolus transit for both liquid and viscous separating patients into mild, moderate and severe function defect respectively.
Conclusion:
The functional defect in patients with distal esophageal spasm can only be determined by combining manometry with a technique that evaluates bolus transit. Combined MII-EM provides a better assessment and proposes a grading scale of functional defects in these patients.

W1447

Prospective Follow-up of Patients with Nonspecific Esophageal Motility Disorders

Thomas Schmitt, Bjoern Goepel, Volker Eckardt

BACKGROUND: Nonspecific esophageal motility disorders (NEMD) do not fulfill the manometric criteria of either achalasia or diffuse esophageal spasm but exhibit non-peristaltic, repetitive contractions and isolated incomplete relaxations of the lower esophageal sphincter (LES). This study investigates whether progression or regression of this motility disorder may occur.
METHODS: Within a 20-year period, 78 patients, ranging in age from 16 to 84 years, were diagnosed to have NEMDs. During the initial examination, all patients underwent a structured interview, upper gastrointestinal endoscopy, barium swallow examinations and esophageal perfusion manometry using a previously described standardized protocol (Gastroenterology 1999;116:1300-4). An NEMD was diagnosed if more than 30% of esophageal contractions were non-peristaltic and/or triple peaked and if isolated incomplete LES relaxations occurred. All patients were invited to undergo a follow-up examination within two years or if progression of symptoms occurred. Patients who did not report for follow-up studies were contacted by phone and underwent the same structured interview as during the index examination.
RESULTS: Follow-up information could be obtained in 43 patients, 28 of whom underwent at least one follow-up esophageal manometric investigation. Of the latter group, 32% (n=9) of all patients exhibited similar findings during the second compared to the first examination, 11% (n=3) were found to have a normal manometric tracing and 57% (n=16) progressed to achalasia. Progression to achalasia was observed at a mean follow-up period of 1year± 1.8 (mean± 1SD). Among several risk factors (age, sex, symptom score and duration of symptoms), only young age was highly predictive for progression of the disease ($p=0.005$). Among patients who underwent only clinical follow-up (structured interview), 60% (n=9) reported an improvement in symptoms, 13% (n=2) became worse and 27% (n=4) remained unchanged.
CONCLUSIONS: A significant proportion of patients with nonspecific esophageal motility disorders progresses to achalasia. This risk is greatest for patients who are younger than 40 years.

W1448

Effect of Buspirone, a 5HT1A Receptor Agonist, on Oesophageal Peristalsis and Lower Oesophageal Sphincter Function in Healthy Volunteers

Michele Di Stefano, Rita Vos, Daniel Sifrim, Jozef Janssens, Jan Tack

Background: In the enteric nervous system, 5HT1A receptors mediate both pre-synaptic and post-synaptic neuronal inhibition. Recent data suggest that 5-HT1A receptor activation in man alters both gastric and small intestinal motility in man, but the effect on esophageal motility has not been studied. The aim of the present study was to investigate the influence of the 5-HT1A receptor buspirone on oesophageal peristalsis and lower oesophageal sphincter (LES) function in man. Methods: Oesophageal motor activity was studied in 20 healthy volunteers (mean age 26±4 yrs) using a water-perfused manometric catheter with 4 axial channels at 5 cm intervals proximal to a 6-cm sleeve sensor. After an overnight fast, the manometric catheter was introduced through the mouth and positioned with the sleeve straddling the LES. After a basal series of 10 swallows of 5 cc of water, placebo or buspirone 20 mg (n=10 each) was administered orally and another 3 series of wet swallows were performed after 10, 30 and 60 minutes. Mean distal esophageal peristaltic wave amplitude and duration, as well as LES pressure, LES pressure decrease and residual pressure during swallowing were calculated. Data were compared using ANOVA. Results: The protocol and drug administration were well tolerated. Placebo had no significant effect on motility parameters. Buspirone significantly increased mean oesophageal body wave amplitude (145.7±49 vs. 120.3±49 mmHg, $p<0.05$) and duration (7.7±0.3 vs. 8.0±0.3 sec; $p<0.05$). Buspirone increased basal LES pressure (26±5 vs. 21±5 mmHg, $p<0.05$) and mean residual pressure (6.99±1.0 vs. 2.06±0.3 mmHg, $p<0.0001$). Buspirone reduced mean % pressure decrease during relaxation (74.8±3.5 vs 89.3±1.7 %; $p<0.0001$) and mean duration of relaxation (7.7±1.5 vs 8.0±1.4; $p<0.02$). Conclusions: The 5-HT1A receptor agonist buspirone increases esophageal peristalsis and LES pressure and reduces the duration and the extent of LES relaxation. These observations suggest a potential for 5-HT1A receptor agonists in the treatment of impaired esophageal motility

W1449

Concurrent Video-Esophagram, Impedance Monitoring, and Manometry in the Assessment of Bolus Transit in Normal Subjects

Hala Imam, Mark Baker, Steven Shay

Barium esophagram and impedance monitoring both measure bolus transit. Aim. 1) Compare impedance and barium in measuring bolus transit and bolus composition, and 2) describe manometric findings with normal and abnormal bolus transit. Method. Simultaneous videoesophagram-impedance-manometry was performed for 2 minutes in 13 normal volunteers (M:F-4:9; median age-48). Combined impedance:manometry sites were 5,10,15,20 cm above the LES. Bolus' of 10 cc 45% barium mixed with 0.9% NaCl were swallowed at ≥ 20 sec intervals (5-6 swallows/subject). Normal bolus transit by impedance was an antegrade fall ≥ 50% of baseline in all 4 impedance sites (bolus entry), and subsequent return to 50% of the original baseline for ≥ 5 sec (bolus clearing). Residual barium in the esophagus (stasis) after a swallow was defined by impedance as failed bolus clearing ≥ 1 site. Retrograde barium flow ≥ 5 cm (REsc) was defined by impedance as retrograde fall ≥ 50% at one or more site after bolus clearing from that site. Results. See table. Barium and impedance bolus transit correlated in 97% (72/74) of barium swallows. 71% (53/74) of barium swallows had normal bolus transit by both methods. Stasis was seen in 10% (7/74), and in 5/7 there was a small barium residue at 15 or 20 cm above the LES. REsc was seen in 16% (12/74), and occurred because the barium bolus had not completely cleared before the next swallow 20-23 sec later. As a result, REsc occurred from the very distal esophagus to the site 5cm above LES. Neither impedance nor barium detected bolus entry between swallows. Impedance identified air in addition to liquid with 51% (38/74) of barium swallows. Video identified a "cushion" of air in the distal esophagus impeding barium bolus advance in 79% (30/38) which prolonged liquid bolus entry vs swallows without air present (4.1 sec ± 0.2 vs 2.3 ± 0.2; $p< 0.001$). Peristaltic contractions with amplitude ≥ 35 mmHg were seen with normal transit, stasis, and reflux in 100%, 86%, and 100%, respectively. Conclusions. There is an excellent correlation between barium and impedance in identifying bolus transit patterns in normal subjects. Minor bolus transit abnormalities were occasionally identified and can be minimized by waiting 30 sec between swallows.

Correlation of barium esophagram (Ba) and impedance (Imp) in detecting bolus transit patterns.

	Ba: Normal	Ba: Stasis	Ba: REsc	Ba: Not Seen
Imp: Normal	53	0	0	0
Imp: Stasis	1	7	0	1
Imp: REsc	0	0	12	0
Imp: Not Seen	0	0	0	0

REsc= Retrograde Escape.

W1450

Simultaneous Barium Esophagram (Ba), Impedance Monitoring (Imp) and Manometry (Ba-Imp-Manometry) in Patients with Dysphagia Due to a Tight Fundoplication

Hala Imam, Mark Baker, Steven Shay

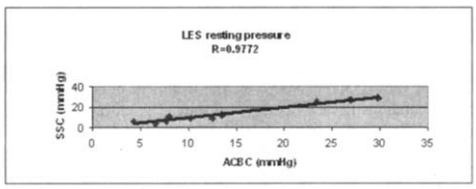
Simultaneous Ba-Imp-manometry in normal subjects revealed 72% of barium swallows with normal transit, proximal esophageal stasis in 10%, and retrograde escape (REsc) during a swallow in 16% (GE A:2004). Imp and Ba had excellent correlation (97%) in detecting these bolus transit patterns. Aim. Compare findings in normal subjects to those of five fundoplication patients with dysphagia (M:F-1:4). Method. A catheter with both an Imp electrode pair and a pressure transducer at four sites (5,10,15, and 20 cm above the LES) was passed, and boluses of 10 cc (45% barium mixed with 0.9% NaCl) were swallowed at ≥ 20 sec intervals. Four transit patterns were seen: 1) Normal bolus transit, defined by Imp as normal bolus entry (antegrade fall of impedance ≥ 50% from baseline) and bolus clearing (subsequent return to 50% of the original baseline). 2) REsc by Ba was retrograde barium flow ≥ 5 cm, and by Imp as a retrograde fall ≥ 50% at one or more site after clearing from that site. 3) Refilling by Ba was barium reentry into the distal from proximal esophagus after REsc, and by Imp as an antegrade fall ≥ 50% at ≥ 1 site after its clearing. 4) Stasis by Ba was residual barium after a swallow, and by Imp as failed bolus clearing ≥ 1 site. Results. 29/32 swallows had normal barium bolus transit to the distal esophagus where barium accumulated in the distal 5 cm (n=14), 10 cm (n=9) or 15 cm (n=6) of the esophagus. From this barium stasis, 29 episodes of REsc occurred with 26 having retrograde Ba transit >5 cm. Stasis and REsc occurred despite peristaltic contractions with amplitude >50 mmHg. Ba identified refilling on 5 occasions. Imp easily detected most episodes of REsc (90%) and refilling (80%) by Ba because they occurred between swallows while Imp baseline was normal. However, REsc and refilling between swallows did not clear, and resulted in low Imp baseline preventing bolus entry detection at 57/116 sites in the 29 swallows. In contrast, Imp and Ba had 100% correlation for bolus clearing at all 57 sites that lacked bolus entry by Imp, as well as bolus entry and clearing at the other 59 sites. 3/32 swallows had abnormal bolus transit with stasis throughout the esophagus. Conclusion. Tight fundoplication patients have a characteristic abnormal bolus transit pattern detectable by both Imp and Ba, and despite concomitant antegrade peristaltic contractions >50 mmHg. Imp and Ba show excellent correlation for bolus clearing, as well as bolus entry when Imp baseline is normal.

W1451

Comparison of Air Coupled Balloon Esophageal Manometry Catheters with Solid-State Esophageal Manometry Catheters

John Fang, Kristen Hilden, Ashok Tuteja, Kathryn Peterson

Purpose: Clinical esophageal manometry studies are currently performed with multilumen water perfused polyvinyl or strain gauge sensor solid-state catheters. A disposable catheter (Clinical Innovations Inc., Salt Lake City) incorporating air filled balloons that can be coupled to a standard manometry polygraph has been developed with the performance characteristics suitable for esophageal studies. Our aim was to compare esophageal pressure measurements using this newly developed catheter with measurements obtained using standard solid state catheters. Methods: Standard esophageal manometry studies were performed in ten healthy volunteers using a air filled coupled balloon (ACBC) and solid state esophageal catheters (SSC). Mean and individual measurements of resting LES pressure and esophageal body contraction amplitudes were obtained and compared. Linear regression and correlation analysis were performed for the above variables. Results: Mean LES pressure and esophageal body contraction amplitudes obtained with the different catheters were not significantly different. The mean resting LES pressure was 14.29 ± 2.88 mmHg using the ACBC's compared to 13.86 ± 2.94 mmHg using the SSC's. The mean distal esophageal body contraction amplitude was 78.2 ± 9.14 mmHg using the ACBC's and was 75.17 ± 9.14 mmHg using the SSC's. There was a very high and statistically significant degree of correlation between LES pressure and distal esophageal body contraction amplitudes measured with the ACBC's and the SSC's (r= 0.98 for LES pressure and r=0.92 for esophageal body contraction amplitudes) (Figure 1). Conclusions: Recently developed disposable air coupled balloon esophageal manometry catheters provide identical measurements of LES and esophageal body pressures compared to presently used solid state manometry catheters.



W1452

Bitter and Sweet Tastes Have Bidirectional Influences on Human Swallowing Cortical Motor Pathways

Satish Mistry, David Gow, John Rothwell, David G. Thompson, Shaheen Hamdy

Background/Aims: Human swallowing is a multidimensional experience, involving the integration of sensorimotor information with more complex behaviours such as taste. However, the interaction between taste and the cortical control of swallowing remains unknown. The aim of this study was to assess the effects of differing taste experiences on human cortical swallowing pathways.
Methods: In healthy adult volunteers (n=8, 7M/1F, mean age 29 years) we performed a 10-minute, swallowing task using previously titrated solutions; sterile water (neutral), 10% glucose (sweet) and 0.5mM Quinine Hydrochloride (bitter). Solutions were randomised to separate studies at least 24 hours apart. Transcranial magnetic stimulation was performed over swallowing motor cortex before and up to 1 hour after the swallow task, and pharyngeal motor responses were recorded from a swallowed intraluminal catheter. Pharyngeal responses were then compared using repeated measures ANOVA.
Results: Following neutral stimuli, pharyngeal responses were only increased in the period immediately after swallowing ($\Delta = 36 \pm 15\%$, $p \leq 0.04$), which was not sustained. By contrast, following bitter stimuli, responses were increased both immediately and were sustained throughout the 60 minutes post-intervention period (maximum $\Delta = 48 \pm 11\%$, $p \leq 0.01$). However, following sweet stimuli, no response changes were observed, implying suppression of activity.
Conclusions: Cortical swallowing pathways are modulated in a differential manner by sweet and bitter tasting stimuli. In comparison to neutral stimuli, bitter tastes enhance the cortical swallowing responses whereas sweet tastes may suppress these pathways. These bidirectional changes in excitability may be related to cellular mechanisms in CNS neurons or in peripheral taste receptor cells. This finding may help guide the use of taste stimuli to rehabilitate swallowing problems after cerebral injury.

W1453

The Study of Neuronal NOS Gene Polymorphism in Esophageal Motility Disorders

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Background: There are some evidence supporting the concept that diffuse esophageal spasm (DES), achalasia, and related motility disorders could be variants of a single entity rather than different diseases. Several study shows that patients with achalasia lack NOS in the GE junction. The nNOS gene knockout mice have LES hypertension with impaired relaxation resembling achalasia. Some studies have shown that production of NO is regulated by polymorphisms located in the genes that regulate their transcription. Aim: To assess whether some functional polymorphisms in some portion of nNOS (chromosome 12) gene are involved in the susceptibility to esophageal motility disorders. Methods/Materials: Total 163 persons (median age 49, 73 male) enrolled in the study; achalasia 24, DES 5, nutcracker esophagus (NE) 35, hypertensive LES (HLES) 3, low LES pressure 5, NEMD 17, GERD 36, normal 38. These diagnosis was confirmed by symptoms, endoscopy, esophageal manometry, and/or 24 hour ambulatory pH-metry. Genomic DNA was extracted from the peripheral blood. For the single nucleotide polymorphism (SNP) analysis of nNOS gene, SNP of promotor, splicing, and 3'UTR were selected. Single based primer extension assay using SNaPshot kit (ABI co, USA) and sequencing using ABI3700 automatic sequencer were performed. Results: 1) rs3741473 and rs2682826 (promoter region SNP), rs3782218 (3'UTR) shows polymorphism, but, rs7314856 (splice variant SNP) and rs4519169 (coding SNP) did not. 2) There is no differences in genotype among the various esophageal motility disorders (see table). Conclusions: Our results suggest that functional polymorphisms in the promotor region and 3'UTR of nNOS gene are not involved in the pathophysiology of esophageal motility disorders. Further study using other region of nNOS gene polymorphism will be needed

Distribution of genotype, n(%)

	rs3741473 (4607G/A)			rs2682826 (4575C/T)			rs3782218 (IVS1-2217)	
Genotype	GG(Wild)	GA(Hetrero)	AA(Homo)	CC(Wild)	CT(Hetero)	TT(Homo)	GG(Wild)	GA(Hetero)
Achalasia(24)	18(75)	4(17)	2(8)	12(50)	9(38)	3(12)	24(100)	0
DES(5)	3(60)	1(20)	1(20)	2(40)	2(40)	1(20)	4(80)	1(20)
NE(35)	28(80)	5(14)	1(3)	19(54)	12(34)	4(11)	33(94)	2(6)
HLES(3)	3(100)	0	0	3(100)	0	0	2(67)	1(33)
LLES(5)	4(80)	1(20)	0	1(20)	2(40)	2(40)	5(100)	0
NEMD(17)	10(59)	7(41)	0	6(35)	10(59)	1(6)	17(100)	0
GERD(36)	28(78)	5(14)	2(6)	23(64)	9(25)	4(11)	32(89)	4(11)
Normal(38)	29(76)	8(21)	0	17(45)	17(45)	4(11)	36(95)	2(5)

W1454

Gastroesophageal Reflux Disease in Diabetics

Lubomir Michalko, Boris Krahulec, Charles Antwi, Stela Hlinstakova

Although the gastroesophageal reflux disease is a comon condition in the general population, the prevalence in diabetics is not well established in diabetics. The aim of this study was to assess the prevalence of abnormal gastroesophageal reflux and reflux esophagitis in diabetics and analyze the influence of autonomic neuropathy in the development of this condition. Patients: We assessed 54 diabetics (15 type I, 39 type II), 28 males and 26 females, mean age 55,4 years with mean duration of diabetes 15,0 years. Methods: 1/ Patients completed a structured standardized questionaire regarding esophageal symptoms, a method that is generally accepted. 2/ Esophagogastroduodenoscopy performed by clinician who was unaware of the results of reflux questionaire. The presence of esophagitis was noted and evaluated according to the Los Angeles clasification. 3/ Cardiovascular auotonomic neuropathy was assessed by axamining cardiovascular reflexes and spectral analysis of the heart rate using a telemetric transmission of the electrocardiographis signal. The results were classified

as normal or as impaired (mild, moderate and severe). 4/ pH-metry using a single-channel glass electrode performed. Acid-supressing medication and prokinetics were discontinued 3-14 days before starting the study. Results: Reflux symptoms poorly correlate with the presence of the pathological gastroesophageal reflux. The duration of diabetes was the only important parameter associated with the development of reflux symptoms. Prevalence of endoscopic reflux esophagitis found in 40,7 % is about four times higher than in the general population. We noted a significantly lower total spectral power on analysis of heart rate variability and a low index of autonomic activity. Parameters of the heart rate variability do not differ essentially in the symptomatic and asymptomatic patients. The abnormal gastroesophageal reflux has been found in all patient having cardiovascular autonomic neuropathy. Essentialy, majority (75 %) of the patients having severe cardiovascular autonomic neuropathy developed esophageal mucosal damage. Conclusion: The prevalence of abnormal gastroesophageal reflux was 66,7 %, as detected by pH-metry. Endoscopic esophagitis was present in 40,7 % of diabetic patients. Presence of diabetic autonomic neuropathy is closely related to the development of gastroesophageal reflux disease. There is increasing severity of esophageal mucosal damage with the progression of autonomic neuropathy. Patients may be at risk of developing Barret's esophagus.

W1455

Esophageal Protective Reflexes Are Altered in Infants with GERD

Sudarshan R. Jadcherla, Erin Stoner, Colin D. Rudolph, Reza Shaker

BACKGROUND:The aerodigestive protective mechanisms in GERD are not clear. We have previously characterized the methods, and identified the esophageal protective reflexes in healthy infants (J Pediatr 2003; 143:31-8). Specifically, secondary peristalsis (SP), esophago-deglutition response (EDR), esophago-UES contractile reflex (E-UES-CR) can be evoked upon mid-esophageal provocation. OBJECTIVE: To characterize the: 1) responses during mid-esophageal infusions of graded volumes of air and liquids, and (2) esophageal motor responses during spontaneous GER events, in controls vs. GERD infants. METHODS: We recorded esophageal motility using a micromanometric pneumohydraulic water perfusion system, a specially designed catheter assembly with 5-sideports, UES and LES sleeves, and a mid-esophageal infusion port, using MMS motility system, in 6 healthy controls and 5 infants with GERD symptoms. Abnormal esophageal pH (n = 4) and upper GI barium studies (n = 5) were noted in GERD. Spontaneous GER events were recognized manometrically by the presence of LES relaxation, intra esophageal pressure increases (IPI), common cavity pressure changes, and retrograde peristalsis. RESULTS: Control and GERD infants were studied respectively, at 40 ±3 wk, and 55±15 wk PMA. Significantly, in controls and study group respectively, resting LESP was 20 ±13 vs 6±7 mmHg; distal esophageal amplitude 57 ±23 vs 20 ±28 mmHg, response time for liquid induced SP was 2.6 ± 0.9 vs 4.8 ±0.8 sec, mean ±SD. (table) CONCLUSIONS: Significantly different in GERD infants are the:1) distribution of swallow propagation, 2) esophageal responses to provocation, 3) failure to elicit a response to liquid infusions. These findings may have pathophysiologic significance in GERD infants.

Motor responses during mid-esophageal provocation and during spontaneous GER events.

Characteristics	Control infants	GERD infants
% Propagation of PP, complete:incomplete:failed	80:12:8, n=64 swallows	23:60:17, n=73 swallows *
Infusion related responses, % distribution of SP: EDR: E-UES-CR	46:30:46, (n=152)	36:19:48, (n=106)
% infusions that failed to evoke response, 1cc air	14 % (n=21)	43 % (n=12)
% infusions that failed to evoke response, 1cc liquids	3.4 % (n=34)	46 % (n=26) *
Responded to spontaneous GER events	79% (n=14 events)	96% (n=27 events)

PP-primary peristalsis; SP-secondary peristalsis; EDR-esophago deglutition response; E-UES-CR-esophago-UES-contractile reflex; there were 1 or more responses to some infusions. * Fisher exact-test P < 0.05

W1456

Effects of the SSRI, Fluoxetine (Prozac), on Regional Gastric Contractility

Arlene N. James, James P. Ryan, Henry P. Parkman

Selective serotonin reuptake inhibitors (SSRIs) can cause GI symptoms such as anorexia, nausea, and diarrhea. Surprisingly, the effects of SSRIs on gastric motility are not well understood. SSRIs have been shown to accelerate orocecal transit. SSRIs have also been reported to enhance the gastric accommodation reflex without affecting gastric emptying. Some SSRIs may also interact with muscarinic and adrenergic receptors. AIM: To determine the effects and mechanism of action of the SSRI, fluoxetine (Prozac), on fundic, antral, and pyloric smooth muscle contractility. METHODS: Guinea pig fundic, antral, and pyloric circular muscle strips were placed in *in vitro* muscle baths, and stretched to Lo, the length for maximal contractile response to ACh 100 μM. Fluoxetine hydrochloride was added in graded concentrations from 0.1 nM to 100 μM with washing between doses. Receptor antagonists were added to determine the neural pathways involved with the fluoxetine responses. RESULTS: Fluoxetine caused concentration dependent contractions, which were greatest in fundus and least in the pylorus. The maximal contractile responses to fluoxetine at 100 μM were 43±8%, 31±4%, and 23±6% of the maximal contractile response to ACh 100 μM for the fundus, antrum, and pylorus respectively. In some strips, fluoxetine at low concentrations caused relaxation of the fundic muscle strips. The antral contractile response of fluoxetine 10 μM was reduced by tetrodotoxin 1 μM (51±21% inhibition; $p<0.05$), atropine 1 μM (73±19% inhibition; $p<0.05$), phentolamine 1 μM (38±5% inhibition; $p<0.05$), and the 5-HT4 receptor antagonist GR 113808 10 μM (60±16% inhibition; $p<0.05$). The fundic contractile response of fluoxetine was reduced by atropine (83±7% inhibition; $p<0.01$), phentolamine (43±17% inhibition; $p<0.05$), and GR 113808 (34±20% inhibition; $p=0.08$), but not significantly by tetrodotoxin (2±20% inhibition; $p>0.10$). CONCLUSIONS: The SSRI, fluoxetine, affects gastric contractility. The contractile effect of fluoxetine demonstrates regional variability - contracting the fundus more so than the antrum and pylorus. The fluoxetine contractile effect is affected by tetrodotoxin, atropine, phentolamine, and 5-HT4 receptor antagonists implying an interaction of fluoxetine with muscarinic, alpha-adrenergic, and serotoninergic receptors. These results suggest an interaction of the fluoxetine molecule with these receptors and/or ongoing release of serotonin in the stomach.

W1457

Efficacy and Safety of Renzapride in Patients with Constipation-predominant IBS: A Phase IIb Study in the UK Primary Healthcare Setting

Nicholas L. Meyers, Richard M. J. Palmer, Alun George

BACKGROUND: Renzapride is a potent 5-HT_4 full agonist/5-HT_3 antagonist being developed for the treatment of IBS. AIMS: To investigate the efficacy of renzapride in alleviating abdominal pain/discomfort and the symptoms of bowel dysfunction in c-IBS patients. METHODS: A parallel group, placebo-controlled, double-blind study design was employed in which 510 patients (89% females) in 80 primary care centres in the UK, received either 1, 2 or 4 mg renzapride or placebo o.d. for 12 weeks, following a two-week screening period to identify symptomatic patients. Patients were diagnosed by the Rome II criteria, selecting c-IBS patients only. Patients recorded their symptoms daily in paper diaries and the primary efficacy end-point was the patients' own weekly assessment of adequate relief of abdominal pain/discomfort during weeks 5-12. Secondary end-points included the frequency of bowel movements and stool consistency score, as well as safety and ECG analyses. RESULTS: Renzapride 4 mg/day, but not 1 or 2 mg/day, increased the responder rate for the relief of abdominal pain/discomfort by up to 8% over placebo during weeks 5-12 (the average weekly placebo responder rate was 49% in the ITT population). Analysis of female patients only in the 4mg dose group increased the weekly responder rate during this period by an additional 5%, but had no effect on the responder rate in the placebo or the other two active dose groups. Renzapride increased the frequency of bowel movements in all three active dose groups during weeks 1-12. At the two higher doses, these effects were statistically significant ($p<0.001$ during weeks 1-4 and $p<0.005$ weeks 5-12). Renzapride also improved stool consistency, resulting in statistically significant differences over placebo ($p<0.05$, 2 and 4 mg/day during weeks 1-4, and 4 mg weeks 5-12). Renzapride was generally well tolerated and there were no clinically relevant effects on ECG. CONCLUSION: Renzapride 4 mg/day increased the responder rate for relief of abdominal pain/discomfort associated with c-IBS, and this effect was greater in female patients. Renzapride (2 and 4 mg/day) also significantly increased the frequency of bowel movements and improved stool consistency. There were no safety issues associated with renzapride in this study.

W1458

A Fixed Combination of Peppermint- and Caraway Oil Modulates Postinflammatory Visceral Hyperalgesia

Gerald Holtmann, Birgit Adam, Tobias Liebregts, Dennis Ruprecht, Marx Awasung, Jan Best, Lars P. Bechmann, Cam Tuan Tran

Background: Previous clinical trials suggest a beneficial effect of peppermint oil and caraway oil for the treatment of patients with functional gastrointestinal disorders (FGID). Development of symptoms in FGID patients is frequently preceded by acute gastrointestinal infections and linked to visceral hyperalgesia. Thus we aimed to analyze the effect of a fixed peppermint-caraway-oil (FPCO) treatment on visceral sensory function in a rat model of postinflammatory visceral hyperalgesia. Methods: Pressure-controlled colorectal distensions (CRD, 60 mmHg) were performed in male Lewis rats 4, 5 and 6 weeks after colorectal instillation of a mixture of trinitrobencoic acid and alcohol (TNB) (test group) or saline (controls). The visceromotor response (VMR) of the abdominal wall muscles to CRD were recorded by implanted electrodes. Animals were randomized to receive FPCO (Enteroplant®, *Dr. Willmar Schwabe Pharmaceuticals, Karlsruhe, and Spitzner Pharmaceuticals, Ettlingen, Germany, approx. 1 mg peppermint oil and 0.6 mg caraway oil per day) or placebo starting after the first CRD over a period of two weeks. Analysis of variance for repeated measures was used to compare treatment groups and asses the effects of the treatment. Results: Four weeks after intracolonic TNB instillation, the VMR to CRD was significantly ($p<0.01$) increased after TNB (1177±477μV) compared to saline controls (588±144 μV). In TNB-treated rats a significant decrease of the VMR to CRD was observed after two-week treatment with FPCO (492±185 μV, $p<0.01$ vs. placebo). However, the VMR to CRD was not significantly attenuated by FPCO in saline-treated rats (730±222μV, $p>0.5$ vs. controls). Summary: Administration of the peppermint-caraway oil preparation attenuates the TNB-induced visceral hyperalgesia. This may explain the beneficial effect of this preparation in patients with functional GI disorders.

W1459

A Meta-Analysis of the Clinical Efficacy and Safety of a New Class of Laxatives Containing High Molecular Weight Polyethylene Glycol in Patients with Chronic Constipation

Hans-Juergen Gruss, Marc Halphen

AIM: This meta-analysis concerns randomised controlled trials with high molecular weight polyethylene glycols (PEG3350/PEG4000) for the treatment of chronic constipation using the Cochran Collaborations Review Manager software. The efficacy and safety of this new type of laxative in the normalisation of stool frequency and consistency was analysed. METHODS: A total number of 16 controlled and randomised clinical studies, with a total number of 733 patients, were identified. The quality of the studies was graded according to the Cochran guidelines. There were 11 'high quality' studies comparing the therapeutic efficacy to placebo (9 studies) or reference therapies (2 studies). Statistical analysis used the odds ratio (OR) for assessment of dichotomous data or mean weighted differences (WMD) for continuous data (expressed as 95% fixed confidence intervals/CI and range). RESULTS: The assessment of the efficacy of high molecular weight PEG containing laxatives showed a significantly more effective response than placebo (OR: 4.45, 95% CI: 1.81 - 10.92 and

WMD: 2.76, 95% CI: 1.65 - 3.86). Similarly, PEG-containing laxatives were more effective than reference therapies (OR: 3.20, 95% CI: 1.46 - 6.99). The frequency of adverse events during the laxative treatment containing PEG was not significantly different to placebo. Higher daily doses of PEG plus electrolytes containing laxatives showed a favourable safety profile. CONCLUSION: PEG-containing laxatives are effective and safe for the treatment of chronic constipation. This type of laxative, particularly in combination with electrolytes, is significantly more effective than placebo, as well as standard reference therapies. The increased efficacy is not associated with an altered safety profile, which is mainly due to the physiological mode of action of this type of laxative, related to the high water binding of polyethylene glycol. Further analysis is required to assess if different forms of PEG-containing laxatives have similar, limited or broader clinical indications.

W1460

ATI-7505 Is a Novel, Selective 5HT$_4$ Receptor Agonist That Causes Gastrointestinal Prokinetic Activity in Dogs

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INTRODUCTION: The safety of 5HT$_4$ receptor agonists with gastrointestinal (GI) prokinetic activity has been limited due to cardiac effects (prolongation of QTc intervals, tachycardia) and adverse drug interactions due to hepatic P450 metabolism. A GI prokinetic agent of this class that lacks these liabilities may have important application in several therapeutic areas including GERD and gastric emptying disorders. ATI-7505 is a novel, potent analog of cisapride (C) being developed as a GI prokinetic.
AIM: We characterized the pharmacologic profile of ATI-7505, relative to that of C.
METHODS: A series of complementary in vitro and in vivo pharmacological studies were performed to define the efficacy and cardiac safety profile of ATI-7505. These included: 1) Binding affinity to 5HT$_4$ receptors, 2) 5HT$_4$ receptor agonist potency and intrinsic activity (IA, normalized to the full agonist, serotonin) in HEK-293 cells expressing human 5HT$_4$ receptors coupled to adenylyl cyclase (AC); 3) Patch clamp assay in HEK-293 cells expressing human I_{Kr} receptor; 4) 5HT$_4$ receptor-mediated heart rate acceleration in isolated piglet hearts; 5) Human microsomal P450 assay; 6) Pharmacokinetics and bioavailability in rats and dogs; 7) Canine model of GI motor activity (strain gauge transducers on antrum, small bowel and colon).
RESULTS: ATI-7505 properties were (respectively): 1) High affinity binding to 5HT$_4$ receptor, K_i = 1.4 nM (vs 25 nM for C), 2) Potent 5HT$_4$ receptor agonist to increase AC activity, EC_{50} = 4 nM and IA = 84% (vs 46 nM and 77% for C); 3) Negligible affinity for I_{Kr} channel, IC_{50} = 65,500 nM (vs 7.2 nM for C); 4) Minimal effect on heart rate (1-2% increase for ATI-7505 and C) at anticipated therapeutic levels for GI prokinetic effects (0.8 nM ATI-7505 vs 10 nM C); 5) Metabolism is p450-independent; 6) Orally bioavailable in both rats and dogs with an estimated terminal half-life of approximately 80 min in rats; 7) Dose-dependent (0.05 - 1.0 mg/kg IV) increase in coordinated antral and small bowel contractility with little effect on colonic motor activity.
CONCLUSIONS: ATI-7505, unlike cisapride, has significant prokinetic activity at plasma concentrations unlikely to cause cardiac or metabolic complications. Given its preclinical pharmacological profile, clinical studies are warranted to assess further its therapeutic potential for GI diseases.

W1461

The Selective Mu Opioid Receptor Antagonist, Alvimopan, Accelerates Gastrointestinal Transit in a Rodent Model of Postoperative Ileus

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Background: Postoperative ileus (POI) is a transient impairment of bowel motility following surgery, resulting in various symptoms and prolonged hospitalization and increased medical costs. In addition, POI is often exacerbated by opioid analgesic use during and following surgery, since mu opioid receptor activation results in a further delay of gastrointestinal (GI) transit. The effect of alvimopan, a selective and peripherally acting mu receptor antagonist, upon POI was investigated in rats with and without concomitant morphine administration. Methods: Under isoflurane anesthesia, POI was induced by laparotomy with intestinal manipulation. After laparotomy, terminal ileum (10 cm long) was eventrated and gently compressed for 10 minutes. Immediately after the surgery, the rats received 51Cr (0.5 micro Ci) by gavage. Three hours after the surgery, the rats were sacrificed and GI transit was estimated using the geometric center (GC) of 51Cr. Alvimopan (0.1-3 mg/kg) was administered by gavage 45 min before the surgery with and without morphine administration (1 mg/kg s.c.). Naltrexone (30 mg/kg), a non-selective opioid receptor antagonist, was administered by gavage 15 min before the surgery. Results: GC in control rats treated with isoflurane anesthesia only was 9.5+/-0.4 (n = 6). GI transit was delayed by intestinal manipulation (GC=3.0+/-0.9). Alvimopan (0.3-3 mg/kg) significantly reversed delayed GI transit induced by intestinal manipulation in a dose dependent manner. The stimulatory effect of alvimopan (3 mg/kg) on GI transit (GC= 4.5+/-0.2) was greater than that of naltrexone (GC=3.8+/-0.2, n=6). Morphine administration further delayed GI transit induced by intestinal manipulation (GC=2.0+/-0.1). Alvimopan (3 mg/kg) also significantly accelerated delayed GI transit (GC=3.2+/0.3) induced by intestinal manipulation plus morphine. Conclusion: These data suggest that mu opioid receptors play a role in the pathogenesis of POI induced by intestinal manipulation, and that the benefit afforded by alvimopan in this condition may be in part mediated via inhibition of an endogenous opioid release in addition to blockade of the unwanted GI actions of analgesic agents.

W1462

Short Chain Fatty Acids Induce Motor Responses in the Rat Isolated Gastrointestinal Tract Via the 7-TM Receptor GPR43

Narinder Dass, Gary Moore, Christopher Crumbley, Anna Bassil, Andrew Brown, Gareth Sanger

Short chain fatty acids (SCFAs) alter GI motility. It is not known if this affect can be mediated via GPR43, a GPCR activated by SCFAs with a distinct rank order of potency (Brown et al., 2003). This study determined GPR43 mRNA levels in the rat gut and the effects of SCFAs on motor responses in rat isolated GI tissue. *Method.* Adult, fed CD rats, 200-250 g were used. GPR43 mRNA levels in full-thickness GI regions were measured using quantitative real-time RT-PCR. Circular distal colonic and longitudinal forestomach strips were suspended between ring electrodes in Krebs solution (5 % CO_2 in O_2; pH 7.4, 37oC) for isometric recordings. Strips were electrically-field stimulated (EFS) for 10-20 s every 40-50 s at 3-5 Hz (0.5 ms, max-effective voltage) to evoke TTX (0.1 μM)-sensitive responses, during and immediately post-EFS. Data are means ± SEM. *Results.* GPR43 mRNA was expressed throughout the gut. The stomach and colon showed the lowest and highest levels, respectively. In colon, sodium formate (1 carbon, C1), acetate (C2), propionate (C3), butyrate (C4; 0.1-100 mM, 15 min contact, n=4-12 each concentration) concentration-dependently reduced the post-EFS contraction (pEC_{50} of 158±56, 29±16, 69±24, 50±23 mM and E_{max} of 70±2, 100±12, 98±27, 106±24%, respectively). The relaxation response during EFS was unaffected. In forestomach, C1, C2 and C3 concentration-dependently increased (1-30 mM, 15 min n=4-8; pEC_{50} of 16±2, 8±2, 12±2 mM and E_{max} of 50±1, 42±2, 55±2%, respectively) and decreased (at 100 mM, 41±15, 72±16, 78±6%) the nerve-mediated contractions. In both tissues, C2 30 mM, had no effect on the contractions induced by carbachol 10 μM, suggesting that C2 acts prejunctionally (colon: 7±5% change in amplitude cf. vehicle 2±5%, n=4, P=0.2; forestomach, 10±8% change cf. vehicle 7±5%, n=4, P=0.4). In the absence of EFS and in the presence of atropine and TTX (both 1 μM), C2 and C3 (3-100 mM, 10 min, n=4) concentration-dependently contracted then relaxed the forestomach, whereas C1 (30-100 mM) only induced relaxation. C1, C2 and C3 (30-100 mM) concentration-dependently relaxed the colon. *Conclusion.* The rank order potency in modulating nerve-evoked contractions in the colon (C2≥C3>C4>C1) and forestomach (C2≥C3>C1) suggests a GPR43-mediated effect. Similarly, at least in the forestomach, the direct muscle affect is consistent with a GPR43-mediated effect. The effects of SCFAs on GI motility may therefore be at least partly GPR43-mediated.
Brown, AJ. *et al.* (2003). *J Biol Chem.* 278 11312-9.

W1463

Prokineticin-2, a Potential Novel Protein Therapeutic, Increases Post-Operative Gastric and Intestinal Motility in Rats

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Post-operative ileus (POI) is a common, debilitating condition, characterized by decreased gastric emptying (GE) and delayed intestinal transit (IT). It occurs as a result of anesthesia, pain-relieving drugs and/or intestinal manipulation. There are very few available treatment options and traditional prokinetic agents have limited utility. We show here that the recently identified protein, Prokineticin 2 (PROK2; previously identified as Zven1), is efficacious in an animal model of POI. PROK2 is a small (81 AA), cysteine-rich protein that induces contractility of gastrointestinal smooth muscle ex vivo and in vivo at pM concentrations. It has also been shown to increase both GE and IT of a semi-solid meal in normal, unchallenged rodents (Neurogastroenterol. Motil.15:571;2003). Using a well-accepted rat model of POI, we evaluated the ability of PROK2 to attenuate POI-mediated decreases in GE and IT. Fasted, male Sprague-Dawley rats were subjected to a laparotomy (LA) with or without cecal manipulation (CM), followed by the measurement of GE and small IT of a non-nutritive, semi-solid tracer meal (1.5 % methylcellulose/0.05% phenol red). This study consisted of five independent experiments with the following treatment groups: vehicle+LA alone (n = 7), vehicle +LA+CM (n = 13), and varying doses of PROK2 +LA+CM (n=7-12 per dose). LA+CM- treated rats exhibited symptoms of POI, as noted by a 3-fold lower GE in vehicle +LA+CM rats compared to vehicle +LA rats (26.6±4.4 vs. 85.1±4.1% GE, respectively). PROK2 was effective in significantly attenuating symptoms of POI, as the PROK2 +LA+CM rats receiving an i.v. bolus injection of 0.8 μg/kg PROK2 (administered 20 min after the tracer meal and 35 min after CM) had a 2.5-fold higher %GE (65.7±4.4 vs. 26.6±4.4%; p<0.001) and a 3-fold higher %IT in the jejunum (47.6±5.7 vs. 15.6±3.5%; p<0.001) compared to vehicle +LA+CM rats. Furthermore, the %IT following this dose of PROK2 was not statistically different from vehicle+LA alone rats, indicating that PROK2 was able to alleviate POI symptoms that occurred as a result of intestinal manipulation in this model. Increased GE and IT were also observed at 0.1 and 5.0 μg/kg PROK2 (%GE ≈1.6 and 1.7-fold higher than vehicle at the 0.1 and 5.0 μg/kg doses respectively, and %IT=1.9 and 1.8-fold higher), suggesting a relatively broad therapeutic range. Taken together, these results demonstrate that PROK2 shows promising therapeutic potential in treating POI and perhaps other gut motility disorders.

W1464

Does Co-Morbid Irritable Bowel Syndrome Influence the Effectiveness of PPI Therapy for Gastroesophageal Reflux Disease?

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Aims: Patients with Irritable Bowel Sydrome (IBS) frequently suffer with symptoms suggestive of gastroesophageal reflux disease (GERD). IBS patients tend to be polysymptomatic and a subset have demonstrable visceral hypersensitivity. The aims of our study were to define the prevalence of co-morbid IBS and to assess whether co-morbid IBS influences the efficacy of PPI therapy in patients with GERD. Methods: Pts with heartburn at least twice per week referred for EGD were recruited. Pts who had taken a PPI within 2 weeks of EGD were excluded. Eligible patients completed validated questionnaires including: Digestive Health Symptom Index (DHSI) which assessed upper and lower GI symptoms, Reflux Disease

Questionnaire (RDQ) which assessed the severity and frequency of GERD symptoms, SF-12 which provided a generic assessment of QoL and QOLRAD a GERD specific QoL tool. IBS patients were defined by the presence of >3 Manning criteria on DHSI. All pts were treated with open-label raberazole sodium (Aciphex, Jansen Pharmaceutica Eisai, Inc) 20mg/d x 8 weeks. After therapy, follow-up surveys were completed. Compliance was assessed by pill count. Results: 80 pts have entered the study and thus far we have complete data for 68 pts. 25/80 (31%) GERD patients had co-morbid IBS by DHSI. Pts with co-morbid IBS had more severe GERD symptoms than those without IBS by RDQ (table). At baseline, pts with IBS had more psychosocial distress as evidenced by lower scores on the mental component of SF-12 (42.7 IBS vs. 48.5 non-IBS, p = 0.03). IBS patients also had significantly greater impairment in GERD specific QoL by QOLRAD (3.3 IBS vs. 4.4 non-IBS, p = 0.0001). Both pts with and without IBS experienced significant improvements in GERD symptoms and disease specific QoL after rabeprazole. Conclusions: GERD patients with co-morbid IBS have more severe GERD symptoms and more impairment in general and GERD specific QoL than those without IBS prior to treatment. Though PPI therapy significantly improved GERD symptoms in all pts, IBS pts may be left with more residual GERD symptoms than non-IBS pts after treatment.

Instrument	GERD with IBS (n=)	GERD without IBS (n=)	P-Value
RDQ-Baseline	17.6	12.4	0.013
RDQ-FU	8.3	5.3	0.151
P-value	0.0003	0.0001	
SF-12 Baseline	42.7	48.5	0.033
SF-12 FU	44.7 (NS)	49.2 (NS)	0.084
QOLRAD-Baseline	3.3	4.4	0.0001
QOLRAD-FU	4.7	5.2	0.068
P-value	0.0001	0.0001	

W1465

Types of Medications Used for Irritable Bowel Syndrome (IBS): Outcomes, Patient Satisfaction and Side Effects

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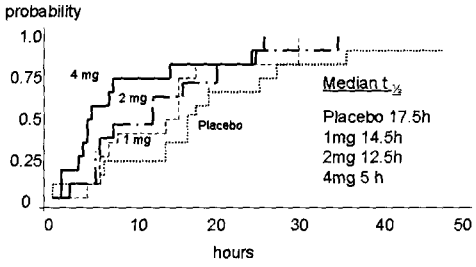
Background: Many medications are used to treat IBS, but their effectiveness in medical practice is unknown. Aim: To characterize patterns of medication use and side effects, and patient outcomes and satisfaction in IBS treatment. Methods: Patients with functional bowel disorders seen in primary care (PC) and gastroenterology clinics in a large health maintenance organization during the year 2002 were surveyed within 2 weeks of a clinic visit via mail (59% responded; n = 1767); responders were surveyed again 6-months later (76% responded). Questionnaires included the Rome Modular Questionnaire, the Irritable Bowel Symptom Severity Index (IBSS), the IBS-QOL, and rating scales for symptom improvement, medication effectiveness, satisfaction and side effects. Results: Analysis was limited to 641 patients (81% PC; 79% female; average age 53) meeting Rome II criteria for IBS who completed follow-up surveys. Patients described their usual bowel habits as diarrhea (17%), constipation (26%), and mixed constipation and diarrhea (39%). The most common drug treatments for patients with IBS were antidiarrheals (28% of patients), antispasmodics (26%), laxatives (24%), antidepressants (14%), and anxiolytics (12%). Satisfactory relief was reported by 57% on antidiarrheals, 51% on antispasmodics, 59% on laxatives, 49% on antidepressants, and 54% on anxiolytics. Satisfactory relief % tended to be lower in patients treated with medications than for patients who pursued dietary changes (62%), lifestyle changes (60%), or exercise (61%) on doctor recommendation. However, patients with severe IBS tended to use medications more often than those with less severe symptoms, and most patients required multiple treatments. Self-reported adherence to medication recommendations was 74.7% for antidiarrheals, 73% for antispasmodics, 61.7% for laxatives, 79% for antidepressants, and 75.6% for anxiolytics. Moderate to severe side effects were reported in 8.6% of patients advised to take antidiarrheals, 18.9% on antispasmodics, 16.3% on laxatives, 24.2% on antidepressants, and 11.8% on anxiolytics. Conclusions: Medications used in 2002 to treat patients with IBS were associated with satisfactory relief of symptoms for about 50-60% of patients, but none were better than simple advice about changes in diet, lifestyle, and exercise. Moderate to severe side effects were common and may have adversely affected satisfaction with treatment. [Supported by Novartis Pharmaceuticals Corp & RO1 DK31369]

W1466

Renzapride Accelerates Colonic Transit and Improves Bowel Function in Constipation-Predominant Irritable Bowel Syndrome (C-IBS)

Michael Camilleri, Sanna McKinzie, Jean Fox, Amy Foxx-Orenstein, Duane Burton, George Thomforde, Kari Baxter, Alan Zinsmeister

Background: Renzapride is a 5-HT_4 full agonist/5-HT_3 antagonist being developed for the treatment of IBS. Aim: To evaluate dose-related effects of renzapride on gastrointestinal (GE, SBT) and colonic (CT) transit and on bowel symptoms in C-IBS. Methods: 48 patients (46 F) with C-IBS and no evacuation disorder had normal to slow baseline CT ((colonic geometric center ≤2.65, where ascending colon (AC) = 1 and stool = 5)). After 1 week's recording of baseline symptoms, 12 patients per group were randomized in double-blind, parallel-group design to 2 weeks' treatment with renzapride (1, 2 or 4 mg) or placebo. Patients kept a daily diary of bowel function. After 2 weeks' treatment, GE, SBT and CT were measured by scintigraphy using a ^{99m}Tc-radiolabeled egg meal and delayed-release capsule containing ^{111}In-charcoal. The sample size was selected to identify clinically meaningful transit effects, rather than clinical symptoms. Results: A significant (linear) dose response to renzapride was detected for colonic transit ((GC8 h ($p<0.01$), GC24 h ($p=0.05$)) and for AC emptying $T_{1/2}$ ($p<0.01$, figure). The dose response was not significant for GE ($T_{1/2}$ ($p=0.09$) or SBT ($p=0.41$). The differences in AC $T_{1/2}$ between placebo and 4mg renzapride (median 17.5h vs. 5.0h,respectively) are clinically important. Improved bowel function scores (stool form and ease of passage) were significantly ($p<0.05$) associated with accelerated colonic transit. No significant adverse clinical, laboratory or ECG effects were observed. Conclusion: Renzapride causes clinically significant dose-related acceleration of colonic transit in C-IBS, and this is associated with improvement of bowel symptoms.



W1467

Physiological and Psychosocial Effects of Desipramine (DES) Treatment Response in Functional Bowel Disorders (FBD)

Yehuda Ringel, William E. Whitehead, Nicholas E. Diamant, Carolyn B. Morris, Yuming Hu, Brenda B. Toner, Shrikant Bangdiwala, Douglas A. Drossman

Background: Tricyclic Anti Depressants (TCA) are commonly used for treatment of IBS. The therapeutic effects may relate to central psychotropic effects and/or peripheral effects on gastrointestinal physiology. **Aim:** to investigate the association of changes in psychological and physiological factors with clinical response to DES treatment in patients with FBD. **Methods:** We studied 101 female patients with FBD. Patients were treated with DES, 50-150mg/day (mean 109 + 30 mg/day), for 12 weeks. Psychosocial assessment included *SCL-90* (Global Severity Index, Somatization, Anxiety, Depression), *Coping Strategies Questionnaire* (catastrophizing, degree of control over symptoms, and ability to decrease symptoms). Physiologic assessment included rectal *pain sensation threshold* (mmHg), *muscle tone*, and *frequency of bowel movements*. A composite score of general well being, 2 weeks pain scores, IBS health related quality of life (IBS-QOL), and overall satisfaction with the treatment was used to assess the clinical outcome. Pearson correlation analysis (SAS, Cary NC) was used to calculate the association of change in psychological scores, physiological scores, and the composite scores. **Results:** 1) Increased rectal sensation threshold and reduced change in bowel frequency correlated with greater clinical response ($r=0.31$, $p=0.01$, and $r=0.25$, $p=0.04$ respectively). 2) Improved depression scores and increased degree of control over symptoms correlated with greater clinical response (r-0.23, $p=0.03$ and $r=0.39$, $p=0.0002$ respectively). 3) There were no significant associations between changes in psychological and physiological scores. 4) Rectal muscle tone did not correlate with clinical response. **Conclusions:** This study provides clinical evidence for central and peripheral effects of DES treatment in FBD. These effects are associated with beneficial response to treatment. The psychological effects do not appear to be mediated through changes in GI physiology (no correlation between physiological and psychological changes). Supported by NIH RO1DK49334

W1468

Long-Term Safety and Tolerability of Tegaserod in Chronic Constipation (CC)

Stefan Mueller-Lissner, Michael Kamm, Peter Haeck, Ahmet Musoglu, Martin Huorka, Juliette Guillot, Julie Jones, Brigitte Nault

Aim: To assess the long-term safety and tolerability of tegaserod, a 5-HT_4 receptor partial agonist, that showed relief of symptoms in patients (pts) with CC. Methods: This was a 13-month single-blind, uncontrolled extension study, enrolling pts with CC who completed an initial 14-week (2-week baseline; 12-week treatment period) randomized, double-blind, placebo-controlled core study (Talley et al. 2003). In the core study, pts received tegaserod 2 mg bid, 6 mg bid or placebo. Pts who received tegaserod continued to take the same dose during the extension, while those who received placebo were switched to tegaserod 6 mg bid. Safety and tolerability were assessed by physical examination and by monitoring adverse events (AEs), serious adverse events (SAEs), laboratory parameters, vital signs and electrocardiograms. The results presented encompass the whole treatment period (core study + extension). Results: 842 pts (mean age 46 years, 87% women) were enrolled in the extension study and 451 (54%) completed. Most common reasons for early discontinuation were unsatisfactory therapeutic effect (19%), withdrawal of consent (11%) and AEs (6%). Headache was the most common AE (24% in the 2 mg bid group, 19% in the 6 mg bid group), followed by abdominal pain (15% and 11%, respectively). Diarrhea was reported by 8% of pts receiving 2 mg bid and 10% of pts receiving 6 mg bid, and was the most frequently reported drug-related AE (4% and 8%, respectively). Diarrhea rarely led to discontinuation (0% and 1%, respectively), was transient (lasting 3-4 days) and generally resolved without rescue medication or interruption of treatment. The highest incidence of AEs occurred in the first month of the initial and extension studies. SAEs were reported by 37 pts (15 in the 2 mg bid group, 22 in the 6 mg bid group). These were not related to tegaserod. No clinically relevant changes were seen in other safety parameters. Conclusions: Tegaserod (2 and 6 mg bid) is safe and well tolerated in CC. Overall, results were similar to those seen in studies of shorter duration, indicating that longer term treatment does not represent an increased safety or tolerability risk. Reference: Talley N et al. Am J Gastroenterol 2003;98(9):S269-70.

W1469

Tegaserod is Effective in Relieving the Multiple Symptoms of Chronic Constipation (CC): Pooled Data from Two Phase III Studies

Karna Bardhan, Ronald Schwarz, Maria Kanty-Okulewicz, Julie Jones, Sophie Hugot, Brigitte Nault

Background: Impaired GI motility and intestinal secretion are important in the development of CC. Tegaserod (T), a selective 5-HT_4 receptor partial agonist that normalizes GI motility and stimulates intestinal secretion in irritable bowel syndrome with constipation, has been investigated as a potential treatment for CC. Methods: Data were pooled from two randomized, double-blind, placebo-controlled CC trials of similar design. Eligible patients (pts) had a ≥6-month history of CC (<3 complete spontaneous bowel movements (CSBM)/week), with straining, incomplete evacuation and/or hard stools. After a 2-week baseline (BL), pts received T 2 or 6 mg bid (T2 and T6, respectively) or placebo (P) for a 12-week treatment period (TP). Efficacy parameters (analyzed using Cochran-Mantel-Haenszel tests) included response rate for CSBMs, time to first spontaneous bowel movement (SBM), bowel habits, constipation symptoms and laxative use as a rescue medication. Responders were defined by an increase of ≥1 CSBM/week vs BL. Results: 2,612 pts were randomized and 82.9% completed treatment. Response rate for CSBMs of T2 and T6 groups was statistically superior to P ($p<0.0001$ for both) across Weeks 1-4 (38.6%, 41.7% and 25.9%, respectively) and 1-12 (38.2%, 44.0% and 28.7%, respectively). The weekly responder rate for CSBMs was statistically superior ($p<0.05$) to P in T2 (11/12 weeks) and T6 (12/12 weeks) groups. The effect of T was rapid; the majority of T-treated pts experienced the first SBM within 24 hours. Statistically significant weekly improvements were observed in all T pts for stool frequency/consistency and straining ($p<0.05$ vs P). T pts had less bothersome constipation, abdominal pain/discomfort, bloating/distension, satisfaction with bowel habits and relief from constipation symptoms, vs P pts. Frequency of laxative use dropped during TP vs BL, being significantly lower in both T2 and T6 pts vs P ($p = 0.0038$ and $p = 0.0105$, respectively). Conclusions: T 6 mg bid, and to a lesser degree 2 mg bid, significantly relieves the multiple symptoms of CC. The effect is rapid and sustained over 12 weeks.

W1470

Tegaserod Significantly Improves Bloating in Female Irritable Bowel Syndrome Patients with Constipation (IBS-C)

Peter Whorwell, Peter Rueegg, David Earnest, Cornelia Dunger-Baldauf

Background: Bloating is a common, bothersome symptom of IBS for which no effective therapy has been previously available. Tegaserod (T) is a partial agonist at the 5-HT_4 receptor with promotile, secretory and sensory modulating effects. T is effective and well tolerated and relieves the multiple symptoms of IBS-C (abdominal pain/discomfort, constipation and bloating). Aims and Methods: To further examine the effect of T 6 mg bid on bloating in female IBS-C patients using pooled data from three double-blind, randomized, placebo-controlled studies (each with a 4-week treatment-free baseline followed by 12 weeks of treatment). Studies were conducted primarily in the US and Europe. The analysis included all females treated with T 6 mg bid ($n = 1,245$) or placebo (P) ($n = 1,227$). Bloating score was recorded daily using a 6- or 7-point scale (none to very severe). Response was defined as a ≥1-point reduction vs baseline. The monthly treatment effect of T on bloating was estimated in a meta-analysis using Mantel-Haenszel weights for each study. Comparison of treatment for weekly score and monthly response utilized Cochran-Mantel-Haenszel or van Elteren tests. Results: The mean baseline bloating score was 3.4 (moderate bloating). T reduced bloating from Week 1 ($p<0.0001$ vs placebo) and this effect was maintained throughout all remaining weeks of treatment ($p<0.01$). The mean reduction from baseline was 0.76 points in the T group vs 0.57 points in the P group at Week 1, and 1.14 points vs 0.91 points, respectively at Week 12. The treatment effect ranged from 0.13-0.2 points during Weeks 1-12. T significantly reduced bloating during Months 1-3 and at endpoint (last 4 weeks of studies; $p<0.009$). While response rates varied between studies, a consistent and significant advantage for T in improving bloating was observed. Conclusion: In female IBS-C patients, T 6 mg bid provides rapid and sustained significant relief of bloating. T is the first drug to demonstrate a significant positive treatment effect on bloating.

W1471

Cilansetron, a Novel 5-HT_3 Antagonist, Demonstrated Efficacy in Males with Irritable Bowel Syndrome with Diarrhea-Predominance (IBS-D)

Georges Coremans, Ray E. Clouse, Fred Carter, Guenter Krause, Steven Caras, Claus Steinborn

Cilansetron (C) is a novel 5-HT3 receptor antagonist being developed for the treatment of IBS-D for males and females. METHODS: A subset analysis of males was conducted in two double-blind, placebo-controlled studies, a 3-month US study (S1) and a 6-month multinational study outside the US (S2). Male and female patients were selected based on the Rome criteria for IBS-D and randomized to C 2mg TID or placebo (P) TID. Subject diaries collected weekly data concerning relief of IBS symptoms, abdominal pain/discomfort and abnormal bowel habits. RESULTS: The intent-to-treat population for S1 included 205 men ($n = 101$ for C and $n = 104$ for P); S2 included 358 men ($n = 186$ for C and $n = 172$ for P). Results from the two trials were similar and indicated significant efficacy for C in males with IBS-D (Table 1). Males showed statistically significant results favoring C over P in all the primary, main and key secondary parameters, except for adequate relief of IBS symptoms in S2, which showed a strong clinical trend favoring C over P with at least a 10% difference. The proportion of males in each group who reported adequate relief in at least 50% of their responses to the weekly diary questions of IBS symptoms, abdominal pain/discomfort, and abnormal bowel habits is reported for studies S1 and S2 in Table 1 and Table 2, respectively. C was well tolerated, with constipation being the most frequently reported adverse event: 8% with C and < 1% with P in S1, and 7% with C and < 1% with P in S2. No cases of ischemic colitis were seen for males in either S1 or S2. CONCLUSION: Cilansetron showed efficacy in males for the relief of IBS symptoms in IBS-D for up to 6 months. Treatment resulted in significant relief of overall IBS symptoms, abdominal pain/discomfort and abnormal bowel habits. Cilansetron 2 mg TID was well tolerated, with constipation occurring in only 8% of males.

Table 1: Percent of Males with Adequate Relief through 3 months in Study 1

	C %	P %	Δ	p-value
IBS Symptoms	41	18	23	<0.001
AB Pain/Discomfort	45	23	22	0.001
Abnormal Bowel Habits	39	17	22	<0.001

Table 2: Percent of Males with Adequate Relief through 6 months in Study S2

	C %	P %	Δ	p-value
IBS Symptoms	55	45	10	0.073
AB Pain/Discomfort	57	45	12	0.021
Abnormal Bowel Habits	60	44	16	0.002

Δ = treatment difference.

W1472

Alvimopan (ALV) Shortens Whole Bowel Transit Time in Adults with Chronic Constipation (CC)

William Garnett, Dennis L. Kelleher, Fiona Hickmott, William Barr, Alvin Zfass, Glendolynn Sanderlin, Shane Coots, LeaAnn Hansen, George Dukes

PURPOSE: This double-blind, randomized, placebo (PLA)-controlled, crossover trial was designed to determine the effect of ALV (3 mg twice a day for 7 days), a peripherally-acting mu opioid antagonist, on bowel transit of patients with CC. METHOD: Twenty-three adults, females (20) and males (3), with at least 6-month history of CC, not meeting Rome II IBS criteria, having ≤3 BM during a 7 day baseline period completed the study. Whole bowel transit (WBT) as a weighted sum of retained radio-opaque markers (WSM) by number and location at 48 h post ingestion; mean colonic transit time (MCTT), bowel movement frequency (BMF) as total bowel movements (BM) and spontaneous complete BM (SCBM), and BM symptoms of straining, discomfort, and satisfaction by patient self-report diary card, were performed for each test arm. Safety was monitored. RESULTS: Results are presented as mean (± SE) for ALV vs PLA respectively. Baseline BMF and SCBM were 2.6 ± 0.19 and 0.8 ± 0.21. ALV decreased WBT by 32% (WSM 109 ± 16.5 vs 160 ± 17.3; $p<0.05$). This was associated with a 19% reduction in MCTT (57 ± 6.6h vs 70 ± 6.9h), and with an increase in mean change above baseline for BMF (1.0 ± 0.34 vs 0.5 ± 0.28) and SCBM (0.6 ± 0.34 vs 0.3 ± 0.25). ALV improved stool hardness, straining, discomfort, and satisfaction compared to PLA. Both treatments were generally well tolerated. Incidence of GI-related AEs, mostly mild, was higher in the ALV arm. CONCLUSION: ALV (3 mg BID, 7 days) decreased bowel transit time, improved BM frequency, stool hardness, straining, discomfort and satisfaction of bowel movements in patients with CC. Further controlled trials of ALV for the treatment of CC are warranted.

W1473

Tegaserod Improves Gastric Emptying and Alters Myoelectrical Activity in Dyspeptic Patients

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Tegaserod, a 5-HT4-receptor agonist, is known to affect gastrointestinal motor function. Delayed gastric emptying and alterations in myoelectrical activity have been observed in patients with functional dyspepsia. The aim of the study was to investigate whether tegaserod influences gastric emptying and myoelectrical activity in patients diagnosed with functional dyspepsia and idiopathic delayed gastric emptying. Material & Methods: Eight patients (2 male, 6 female, mean age 40 years) with functional dyspepsia according to the Rome II criteria and delayed gastric emptying of a solid meal, as assessed by 13C-octanoid acid breath test, were included. Gastric myoelectrical activity was measured, using a three-channel surface electrogastrogram (EGG) (POLYGRAM NET, Medtronic, Denmark) with motion sensor, 30 minutes before and after a standard solid meal. Dominant frequency and power, frequency distribution, instability coefficient of dominant frequency and power, percentage of slow wave coupling, and power ratio were calculated. The 13C-octanoic acid breath test was performed simultaneously and gastric emptying expressed as t 1/2. Symptoms were assessed using a GI symptom questionnaire. This protocol was repeated after a 4-week treatment with 2 x 6 mg tegaserod. Results: The total symptom score at treatment was significantly lower than before treatment (23.1 ± 2.1 and 29.4 ± 1.1, respectively; $p<0.01$). Treatment with tegaserod significantly accelerated gastric emptying (t 1/2: before treatment 119.5 ± 5.9, at treatment 84.9 ± 9.4; $p<0.01$). The EGG power ratio was increased in all three channels during treatment with tegaserod (Ch1: 1.6 ± 0.3, Ch2: 2.5 ± 0.6, Ch3: 2.1 ± 0.8) compared to before treatment (Ch1: 1.6 ± 0.3, Ch2: 1.0 ± 0.2, Ch3: 0.8 ± 0.2; $p<0.05$). The percentage of slow wave coupling during the fasting period between channel 1 and 2 was significantly higher at treatment than before (70.9 ± 7.2 and 87.0 ± 3.1, respectively; $p<0.05$). No differences were detected in slow wave coupling between the other channels during the fasting or postprandial period. Conclusion: Clinical improvement in dyspeptic patients with idiopathic delayed gastric emptying at treatment with tegaserod is associated with a stimulation of gastric emptying and with an improvement of preexisting alterations in gastric myoelectrical activity. The data suggest that tegaserod is useful to treat the subgroup of patients with functional dyspepsia and concomitant idiopathic delayed gastric emptying.

W1474

Human Visceral Pain Hypersensitvity Is Independent of Cyclo-oxygenase-2 (COX-2)

Robert Willert, Claire Delaney, Anthony Hobson, David Thompson, Qasim Aziz

Introduction: Increased spinal cord neuronal excitability i.e. central sensitisation (CS), contributes to the development and maintenance of visceral pain hypersensitivity[1]. COX-2 is constitutively expressed in the spinal cord, is rapidly up-regulated following inflammation and contributes to CS in somatic pain hypersensitivity via prostaglandin production[2]. However, its role in mediating visceral pain hypersensitivity is unknown. *Aim:* To determine if selective COX-2 inhibition (with Valdecoxib) attenuates CS in our human model of oesophageal acidification induced hypersensitivity[3]. *Methods:* Healthy subjects were enrolled into 2 randomised, double blind, placebo controlled, cross-over studies. Pain thresholds (PT) to electrical stimulation were determined in the proximal oesophagus (PO) and foot (somatic control) before and after a 30-minute distal oesophageal infusion of 0.15M HCl acid. Study 1: Valdecoxib (40mg), or placebo was given orally for 4 days prior to the acid infusion and PT measured for 120 mins post acid ($n=10$). Study 2: 40mg IV Parecoxib (prodrug of valdecoxib), or saline was given 120 mins after the acid infusion and PT measured for 360 mins post acid in the PO, foot & chest wall ($n=6$). *Results:* Study 1: There was no effect of Valdecoxib on baseline PT in the PO ($p=0.8$), or foot ($p=0.4$). Valdecoxib did not attenuate the reduction in PT in the PO which is induced by a distal acid infusion ($p=0.71$). Valdecoxib had no effect on foot PT following acid (AUC: $p=0.8$). Study 2: Parecoxib did not reverse the reduction in PT in the PO following acid ($p=0.2$). Chest wall PT fell following acid but foot PT remained unchanged ($p<0.001$). Parecoxib had no effect on chest wall ($p=0.6$) or foot PT ($p=0.5$) following acid. *Conclusions:* The fall in chest wall and PO (but not foot) PT following distal oesophageal acid implies a mechanism of CS. Pre-treatment with Valdecoxib did not attenuate the acid induced fall in PT in the PO and IV Parecoxib post acid failed to reverse it. These results suggest that spinal COX-2 does not contribute to the development or early maintenance of visceral CS in our model. However, COX-2 induction can still occur several hours after the onset of inflammation and may therefore contribute to the long term maintenance of CS via prostaglandin production. Studies using COX-2 inhibitors to ascertain their role in chronic visceral hypersensitivity states are therefore still warranted. *References:* 1) Willert R et al. Gastroenterol ‹In Press«. 2) Seybould V et al. Pain 2003. 3) Sarkar S et al. Lancet 2000.

W1475

Omeprazole Slows Small Intestinal Transit (SIT) in Mice and Concomitant Tegaserod Antagonizes This Effect

Alan Cowan, David Earnest, Mikhail Rojavin

Introduction: Omeprazole (OME) 20 mg q.d. decreased the rate of gastric emptying in healthy human volunteers by 15-40% (Parkman et al. 1998; Rasmussen et al. 1999). Its effect on meal transport in the small bowel is unknown. This study was conducted to assess if (a) OME also decreases SIT, and (b) if this effect can be reversed by concomitant administration of tegaserod (TEG), a selective 5-HT_4 receptor partial agonist with intestinal promotile effects. Methods: Groups of 15 male Swiss mice received one i.p. injection (0.25 mL/25 g animal) on each of 5 consecutive days as follows: I - vehicle (1% Tween 80); II - TEG 0.1 mg/kg; III - OME 44 mg/kg; IV - TEG 0.1 mg/kg + OME 44 mg/kg. Immediately after the last injection, each mouse received a standard charcoal-containing meal (0.50 mL/25 g mouse) by gavage. 30 minutes later, each animal was sacrificed. The small intestine was excised and the distance traveled by the meal distal to the pyloric sphincter was measured and expressed as a percentage of the total small intestine length. Results: Dosing with OME for 5 days significantly ($p<0.05$) decreased the SIT compared with vehicle control ($59.89\pm3.58\%$ vs $70.59\pm1.86\%$) ($M\pm SEM$). TEG alone did not significantly affect SIT ($72.51\pm3.29\%$), but in combination with OME, TEG returned SIT back to normal ($68.57\pm2.51\%$). OME is an effective proton pump inhibitor widely used for treating acid-related gastroesophageal disorders (ARGD). However, its delay of food transit in the stomach and small intestine may be responsible for reports of continued upper GI symptoms in patients treated with OME (Nelson et al. 2000; Richter et al. 2001). Concomitant use of TEG normalizes SIT. Clinical trials are needed to establish if this combination is more effective than OME alone in treating ARGD. Conclusions: OME 44 mg/kg for 5 days caused a significant delay of SIT in mice. Concomitant use of TEG 0.1 mg/kg antagonized this unwanted effect.

W1476

A Phase IIb Clinical Study of Renzapride in Mixed-symptom (Alternating) Irritable Bowel Syndrome

Jane C. Henderson, Richard M. J. Palmer, Nicholas L. Meyers, Robin C. Spiller

BACKGROUND: Renzapride is a 5-HT_4 full agonist/5-HT_3 antagonist being evaluated as a treatment for IBS. METHODS: This exploratory Phase II study employed a randomised, double blind, placebo-controlled, parallel group design, and evaluated the efficacy and safety of 1, 2 and 4 mg doses of renzapride, administered once daily for 56 days in 168 patients (78% females) in 5 European countries. M-IBS patients were selected using modified Rome II criteria to exclude patients with either constipation- or diarrhoea-predominant IBS. The primary efficacy end-point was the average number of days of relief of overall IBS symptoms assessed by the patients, and recorded daily in electronic diaries. Additional analyses included number of days relief of bowel dysfunction, and responder rates (patients indicating a positive response on 50% of the days for which they recorded data). RESULTS: Mean values ($\pm$SEM's) for the number of days of relief of overall IBS symptoms over the 56 days, for the 1, 2 and 4 mg dose groups were 18.6 days $\pm$ 2.2 ($p=0.6$ for the between-group comparison), 21.5 $\pm$ 2.6; $p=0.9$) and 19.0 $\pm$ 2.8; $p=0.6$), respectively, compared to placebo (22.4 ± 2.7). The average daily responder rate during days 1-28 for satisfactory relief of the overall symptoms of IBS in the 2 mg dose group was 57% compared to 43% for placebo, a 14% difference (95% CI's for treatment difference -7%-35%; $p=0.09$). The difference in responder rate in this dose group over placebo increased to 18% when female patients only were analysed (64% *vs.* 46%; 95% CI's -5%-42%; $p=0.07$). The average daily responder rate for relief of bowel dysfunction was also higher in the 2 mg group compared to placebo (52% *vs.* 48%; $p=0.33$), which was similar in female patients only (54% *vs.* 51%; $p=0.40$). The proportion of responders was higher in all treatment groups, especially in the renzapride 2 mg group (59% for relief of overall IBS symptoms and 62% for relief of bowel dysfunction), during the second half of the study (days 29-56), although the differences between the active doses and placebo were smaller than in days 1-28. Renzapride was generally well tolerated and there were no clinically relevant effects on ECG. CONCLUSION: Treatment with renzapride 2 mg in this patient population resulted in a higher, but not statistically significant, average daily responder rate compared to placebo, for the relief of symptoms associated with IBS, particularly in female patients. These data, coupled with a good safety profile, warrant further clinical investigation of renzapride in m-IBS patients.

W1477

Spasmolytic Agents for the Treatment of Irritable Bowel Syndrome; a Meta-Analysis

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Background: Irritable bowel syndrome (IBS) is a chronic functional gastrointestinal disorder characterised by recurrent episodes of abdominal pain and altered bowel habit. Patients with IBS are primarily managed in primary health care. Though Family Physicians prescribe medication in 25-50% of the patients with IBS, data on efficacy in the literature are conflicting. In this systematic review, the efficacy of bulking agents was examined. Methods: this review was performed according to Cochrane guidelines. Randomized trials comparing bulking agents with a placebo were included. Trials were identified through a central literature search in the Cochrane Library, Medline, Embase and PsychInfo. Identification of additional papers was done in national databases of 9 European countries. Data extraction and quality assessment was done independently by two reviewers. Data was pooled for three outcome parameters: abdominal pain, global assessment, and symptomscore, for dichotomous and continuous outcomes separately. Results: 321 studies were identified concerning spasmolytic agents. Of the 25 studies with valid data, none were clearly executed in primary care, and 14 definitely in secondary care units. The duration of symptoms at inclusion could range from 2 months to almost 20 years. In 14 studies the mean age of patients was over 40 years, and 19 studies included more then 50% females. 6 studies did an outcome assessment at least 3 months after starting treatment. *Relief of abdominal pain:*12 studies comprising 1320 patients used a dichotomous outcome. The aggregated pooled RR was 1.34 (1.15;1.56) NNT 5.6 (12.5-3.6). 8 studies comprising 467 patients used a continuous outcome. The aggregated pooled SMD was -0.62 (-0.83;-0.43).*Global assessment:*17 studies comprising 1296 patients reported a dichotomous variable. The aggregated pooled RR was 1.37 (1.14;1.65).*Improvement of symptom score:*1 study with 34 patient reported a dichotomous outcome. The RR was 1.33 (0.96;1.86) 4 studies comprising 120 patients reported a continuous outcome. The pooled results comprise 66 patients with SMD -0.37 (95%CI-0.85-0.12). Conclusion: In line with other meta-analyses, spasmolytic agents, and more specific, pinaverium and scopolamine derivatives, appear to be effective regarding abdominal pain and global assessment of IBS improvement. Not enough studies use validated symptomscores.

W1478

The Effect of Severe Malnutrition and Subsequent Refeeding on Whole Body Metabolism and Protein Synthesis in Human Subjects

Trevor A. Winter, Stephen O'Keefe, Marie Callanan, Tessa Marks

Objective: To determine the consequences of severe malnutrition, and subsequent refeeeding, on whole body metabolism and protein synthesis. Methods: Respiratory quotient (RQ), resting energy expenditure (REE) and whole body protein synthesis (WBPS) was assessed in severely malnourished patients before and after a period of nutritional support. Patients were stratified into those with co-existent disease ($n=18$), and those with anorexia nervosa ($n=8$). Results were compared to a group of 17 normal healthy volunteers (controls). Results: Mean body mass index (BMI) of the patients was 13.39 ± 0.32, compared to 23.71 ± 1.01 in the controls ($p<0.001$). RQ was 0.80 ± 0.03, compared to 0.89 ± 0.06 in the controls. REE was lower in the patients than in controls (1216 ± 114.9 vs. 1807 ± 120.8 kcal/d; $p<0.01$), however when expressed as kcal/kg/d, it was significantly higher (33.02 ± 2.54 vs. 25.36 ± 1.38 kcal/kg/d; $p<0.05$). Whole body protein synthesis (g/d) was lower in the patients (127.4 ± 7.50 vs. 305.0 ± 21.64 g/d; $p<0.001$), but when expressed g/kg/d, the anorexia patients were similar to controls, whereas the patients with disease were lower (3.14 ± 0.24 vs. 4.27 ± 0.32; $p<0.01$). Refeeding resulted in a significant increase in BMI (15.84 ± 0.42 vs. 13.81 ± 0.36; $p<0.001$), RQ (0.93 ± 0.05 vs. 0.80 ± 0.03; $p<0.05$), and normalisation of the REE (28.47 ± 1.77 vs. 25.36 ± 1.38 kcal/kg/d). WBPS also increased in the disease patient group (171.9 ± 12.92 vs. 127.4 ± 7.50 g/d; $p < 0.01$). Conclusions: Malnutrition is associated with an increase in REE (kcal/kg/d), possibly as a consequence of altered body composition, with decreased muscle and fat. Reduction in protein synthesis (g/kg/d) was evident only in those patients with co-existent disease. Refeeding resulted in normalisation of RQ, REE (kcal/kg/d), and protein synthesis (g/kg/d).

W1479

Survival Rate and Prognostic Factors in Patients with Intestinal Failure

Italo Vantini, Luigi Benini, Fabrizio Bonfante, Giorgio Talamini, Carlo Sembenini, Giuseppe Chiarioni, Franco Benini, Franco Capra

Intestinal failure (IF) impairs nutritional status and survival expectance. In one half of patients artificial nutrition is needed. Our aim was to assess causes of death, survival rate, enteral independence over time and factors affecting the clinical outcome in patients with IF. Sixty-eight patients with IF due to major intestinal resection (60 short bowel syndrome: SBS; remnant intestine 101-150 cm in 31, 50-100 in 23, < 50 cm in 6) and to chronic idiopathic pseudo-obstruction (CIPO, 8 cases) were followed. In SBS patients the main causes of IF were ischemic bowel (28), major surgery complications (17), radiation enteritis

(10) and others. Seventeen variables referred to age, underlying disorders, lenght of remnant bowel, type of surgery, hospital stay, type of nutrition and its variations in time, causes of death, survival rate and time were considered. Statistical analysis was carried-out by Mann-Whitney U test, Pearson Chi square, Spearman correlation test, Kaplan-Meyer method and Cox's proportion hazards regression model. At hospital admission no patient had nutritional independence, 54 were on parenteral (PN) and 14 on enteral nutrition (EN). At discharge enteral independence was achieved in 23 cases, 39 were on HPN, 3 on EN+I.V. feeding, 1 on EN, 2 needed oral supplementation with hydroelectrolyte solutions only. After (median) 36 months, 30 and 2 patients were on HPN and EN+I.V feeding respectively, two on EN, two on oral supplementation, 26 reached enteral independence. A significant relationship among the lenght of remnant bowel and types of nutrition at both admission ($r = 0.38$; $p<0.001$) and discharge ($r = 0.48$; $p< 0.001$) was detected, being PN more frequent in patients with very short bowel. Twentyt-two patients died (4 for newly occurring malignancies,11 for conditions related to IF and/or HPN complications), 40 survived, 6 were lost to the follow-up. At 12, 24, 36, 48, 60 and 72 months survival rates were 95.4, 93.3, 88.1, 78.6, 78.6 and 65.5% respectively, but significantly lower in patients with < 50 cm of remnant bowel ($p < 0.05$) and in patients who started HPN above the age of 45 years ($p< 0.02$). Survival rate was higher in patients who reached enteral independence than in dependent ones ($p < 0.05$). Better survival rates were registered in patients with CIPO and MSC. Conclusions. Actuarial survival rate of patients with IF is 88 and 78% at 3 and at 5 years. It is influenced by the lenght of remnant intestine, age at starting HPN, enteral independence. Enteral independence can be achieved in time by about 40% of patients.

W1480

Contemporary Rates, Risk Factors and Outcomes of Parenteral Nutrition-associated Hepatic Dysfunction in Infants

Timothy A. Sentongo, Roopa Seshadri, Kishore Iyer, Robin Steinhorn

BACKGROUND: The efficacious use of Parenteral Nutrition (PN) therapy was initially reported three decades ago. However, hepatic dysfunction (HD) was recognized as a major clinical problem and a frequent indication for termination of PN therapy. Major changes in PN have including change of Intralipid from 10% to 20% solutions, increased use of Trophamine as protein source in infants, and growth in tertiary care. The objective of this study was to re-evaluate the contemporary incidence, risk factors and outcomes of PN-associated hepatic dysfunction in infants. METHODS: Retrospective study of infants (age <12-months) that received PN therapy during the 12-month period from 9/02 to 8/03 at a tertiary Children's Hospital. Infants on PN for ≥2-weeks) were reviewed for birth weight, diagnosis, duration and mortality during PN therapy. Medical conditions were categorized as low birth wt (≤1500g), sepsis, intestinal failure, and cardio-pulmonary failure. PN-associated HD was as rise in conjugated bilirubin (CB) to ≥1.5 mg/dl during PN therapy. Prevalence of HD was determined. Logistic regression analysis was used to determine the effect of duration of PN therpy and other risk factors on the various outcomes. Significance was determined as $p < 0.05$. RESULTS: 508 infants received PN during the 12-months; 102 received PN for ≥2-wk. The prevalence of HD in subjects receiving PN for ≥2-wk was 37.5% (95% CI: 27.9%, 47.4%). Duration of PN for ≥4-wk compared to 2 to 3-wk was associated with a four fold risk of HD (odds ratio 4.4 (95% CI: 1.75, 11). Duration of PN therapy was the greatest risk factor for onset and resolution of HD ($p = 0.0031$), along with cardiorespiratory failure ($p = 0.0042$). After controlling for duration of PN therapy, HD was 5.6 times more likely (95% CI: 1.7, 17.8) to resolve in infants with cardiorespiratory failure compared to those without. CONCLUSION: Despite the advances in PN constitution and delivery, duration of PN therapy continues to be the strongest risk factor for onset and resolution of HD in infants.

W1481

Effect of Intravenous Pamidronate in Home Parenteral Nutrition Patients wth Short Bowel Syndrome: Improvement in Bone Mineral Density

John K. Siepler, Reid Nishikawa, Stacy Siepler, Tom Diamantidis, Rod Okamoto

Rationale: Decreased bone mineral density (BMD) is often seen in patients receiving home parenteral nutrition (HPN). Short bowel syndrome often precludes the use of oral medications. Intravenous pamidronate (IV-P) has been associated with improvements in BMD in other disease states. We studied HPN patients to determine if the administration of IV-P would improve BMD. Method: All patients receiving HPN for short bowel syndrome who received IV-P for decreased BMD were included. Bone densitometry by DEXA was performed prior to start of IV-P and at yearly intervals. Lumbar sacral (LS) DEXA prior to start and 12 months after start of IV-P was recorded. Data are reported as T-score and % change in BMD. Statistical analysis was done by paired students T-test. Results: 20 patients (5M/15F, age 49+/-17years) qualified for inclusion in the study. Mean Ca++ dose was 15+/-7 mEq/d and mean IV-P dose was 40.6+/-3.3 mg/month. Mean duration of IV-P therapy was 1.05+/-1.7 years. The mean LS-T score pre IV-P was -2.72+/-1.0. The mean LS T-score after IV-P was 1.40+/-2. Increased LS-T scores were observed in 17/20 (85%) patients on following IV-P. (45.3% increase, $p=0.044$). No patient had a fracture during the study period. Conclusions: We demonstrated that the BMD using LS DEXA improved after initiating IV pamidronate in 17/20 HPN patients (Mean increase in BMD = 45.3%). We conclude that IV Pamidronate is efective in the management of decreased BMD in patients receiving HPN with short bowel syndrome. A randomized controlled study should be performed to confirm these results.

W1482

Intestinal Failure - Outcomes of Rehabilitation

Clarivet Torres, Debra Sudan, Simon Horslen, Wendy Grant, Jean Botha, Alan Langnas

Purpose: To analyze the outcome of children with intestinal failure referred for transplantation that were treated by our multidisciplinary Intestinal Rehabilitation Program (IRP). Methods: This is a retrospective study of all intestinal failure TPN dependent children referred for transplantation between May 2001-Sep 2003. 96 patients were evaluated for transplantation, 63 were listed for transplant, 7 were not listed and 26 were enrolled in the IRP. Indications for treatment in the IRP were 1) Infants with no liver disease who had more than 15 cm of bowel, 2) Children with more than 40 cm of bowel with elevated bilirubin but normal INR, 3) Children with advanced liver disease (hypersplenism, hyperbilirubinemia but normal INR) who had more than 60 cm of bowel and abnormal but potentially repairable intestinal anatomy. Height, weight Z score, serum albumin and bilirubin were obtained at the first day of evaluation and at the end of the study. (See table 1) Results. Of the 26 IRP patients, 19 had 26 surgical procedures including 11 Bianchi procedures, 5 enteroplasty (tapering/step), 2 ostomy takedowns, 4 fistula repairs and 4 intestinal stricture repairs.18 had elevated bilirubin; 15 normalized with surgical and dietary manipulation, 1 continue to improve. Of these 26 patients 1 patient died of sepsis, 1 patient received an isolated intestinal transplant due to venous access problems, 2 required combined liver/intestinal transplantation due to intestinal perforations. Of the remaining 22 patients, 16 (61%) patients were weaned from TPN and 6 are in the process of weaning. Survival of the IRP patients is 96%. Growth has continued along the same curve for most IRP patients, few have significant catch-up growth. Conclusion: A program that offers intensive medical and surgical intestinal rehabilitation can, in selected patients, avoid transplantation even if these patients have indications for transplantation. The IRP patients have better survival than either isolated or combined liver/intestinal transplantation patients. We have found that IRP patients have improved liver function, improved nutritional parameters with the ability to discontinue TPN while maintaining the growth curve. Early referral of these complex patients to specialized centers with a multidisciplinary team prior to the development of advanced liver disease is recommended.

Table 1

	Mean (Range)	
	First	Last
Bilirubin	5.0 (0.1 to 17.4)	0.7 (0.1 to 2.5)
Albumin	2.9 (2.4 to 3.9)	3.4 (2.5 to 4.5)
Height Z Score	-1.1 (-5 to 2.8)	-1.2 (-5 to 0.8)
Weight Z Score	-0.6 (-4.3 to 2.2)	-0.3 (-4.3 to 1.9)

W1483

When Should We Start with Total Parenteral Nutrition?

Michael Momma, Johann Ockenga, Gernot Sellge, Michael P. Manns, Stephan C. Bischoff

Patients with gastrointestinal disease such as IBD or pancreatitis often need total parenteral nutrition (TPN). However, the time of start of TPN has not defined yet. Patients were randomized in two groups (1) delayed TPN (5 days hypocaloric infusion of 4kcal/kg body weight, then regulatory TPN) (2) normocaloric TPN. After 5 days all patients receives a normocaloric TPN. To address this question, we performed a prospective, randomized, controlled study including 65 patients (40% pancreatitis, 24% IBD, 11% cancer, 8% cholangitis, 19% others). We found no significant differences between two groups regarding hospital stay (32 ± 21 vs. 32 ± 26 days), duration of TPN (460 vs. 456 days), catheter infection (7 vs. 6) and rate of rehospitalisation within 3 month. Moreover, we found no significant differences in body weight and serum electrolytes, albumin, lymphocytes. A significant increase of serum triglyceride in the first 6 days of TPN were found in the normocaloric group (from 115 ± 73 to 210 ± 125 vs. 112 ± 47 to 125 ± 63 mg/dl $p = 0,008$). In conclusion, our study indicates that a delayed start of normocaloric TPN within 5 days in gastroenterologic patients requiring TPN is not associated with malnutrition or negative effects on clinical outcome. Instead, such an approach reduces costs and risk of hypertrigyceridemia.

W1484

Survey of Nutrition Management Practices of Canadian Gastroenterologists

Harminder Singh, Donald R. Duerksen

Nutrition education is a required part of gastrointestinal (GI) fellowships. Involvement of gastroenterologists in clinical nutrition and perception of adequacy of nutrition training during GI fellowship is not known. Aims: To determine the attitude, practice pattern and adequacy of training of gastroenterologists in clinical nutrition. Methods: Canadian Association of Gastroenterology (CAG) mailed out a survey to all of its 463 Canadian clinician members and 88 trainee members. Components of the survey included: knowledge of nutritional assessment (NA) and TPN, involvement in a nutrition support service, physician involvement in nutrition assessment and nutrition support teams, obesity management, insertion of gastrostomy (G) tubes and management of tube related complications, and adequacy of training in clinical nutrition. Results: Initial response: 48% ($n=221$) of CAG clinicians and 25% ($n=22$) of fellows. Of the clinicians, 81% are practicing adult gastroenterologists with the following demographics: practicing full time in the academic centers (41%), community practice (44%), completed training in the last 10 years (31%), and completed training in USA (13%). Although only 3% have a primary focus of nutrition in their GI practice, 65% are involved in nutrition support (including TPN), 75% place G tubes, and 58% manage at least one of the major complications of G tube insertion. 52% practice in hospitals with a physician on the nutrition support team. 89% feel a gastroenterologist should be the physician consultant on nutrition support service. Underemphasized areas include: NA - 20% consider albumin or prealbumin as the best clinical indicator of nutritional status, 63% feel that NA should be carried out primarily by dieticians; Initiation of nutrition support - 34% recommend nutrition support at inappropriate time intervals; Obesity- managed by 6%; G tube complications - 39 % of those who insert G tubes manage all of the major tube related complications. Nutrition training in GI fellowship under emphasized: clinicians (65%), trainees (77%). Conclusions: The majority of Canadian gastroenterologists are involved in nutrition support. However most feel that nutrition training is under emphasized in the training programs. Areas of possible deficiency include NA, indications for nutrition support, management of G tube related complications and obesity. It is important for GI fellowship programs to develop standardized nutrition training that prepares trainees for their practice.

W1485

Enteral Nutrients Are More Efficiently Utilized for the Synthesis of Digestive Enzymes and Mucosal Proteins Than Parenteral Nutrients in Humans

Stephen J. D. O'Keefe, Ronzo B. Lee, Jing Li, Neeraj Kaushik

There is overwhelming clinical evidence that enteral feeding is superior to parenteral feeding in the nutritional support of hospitalized patients. Purpose: In order to determine whether this is due to better utilization of dietary nutrients for the synthesis of digestive enzymes and mucosal proteins, we studied 3 groups of healthy volunteers during conditions of isocaloric, isonitrogenous enteral (n=14) or parenteral (n=6) feeding (protein 1.5g/kg/d, 30kkcal/kg/d), or after a 12h fast (n=5) (control). Methods: Amino acid utilization was measured by stable isotope methodology over a 6-h period by labeling the enteral diet with deuterium-labeled leucine and the parenteral diet with 13C-leucine. Digestive enzymes secreted in response to the feeding were collected by duodenal perfusion technique and trypsin was isolated by affinity chromatography. At the end, mucosal biopsies were taken by endoscopy. Isotope enrichments of plasma, trypsin and mucosal protein were measured by GC-MS. Results: In comparison to fasting, enteral feeding increased whole body amino acid turnover and the rate of incorporation of labeled amino acid into trypsin and mucosal proteins, at the same time speeding up the rate of delivery of newly labeled enzymes into the intestine. In contrast parenteral feeding had no significant effect other than an increase in oxidation, suggesting that it better supported non-splanchnic protein synthesis (e.g. skeletal muscle). Conclusion: Our results provide evidence for the preferential uptake of enterally-administered proteins into intestinal and pancreatic proteins and parenterally-administered amino acids into skeletal proteins. Since critical illness is associated with a shift from skeletal to visceral protein metabolism, enteral feeding is better positioned to meet the altered nutritional needs.

	FASTING	ENTERAL	PARENTERAL
Whole body leucine turnover (umol/kg/min)	1.80(.16)	3.86(.30)*	2.28(.25)
Whole body leucine oxidation (umol/kg/min)	0.28(.03)	0.76(.36)*	0.50(.09)*
Whole body protein synthesis (umol/kg/min)	1.52(.23)	2.02(.19)	1.73(.27)
Trypsin secretion (u/h)	170(50)	746(126)**++	166(65)
Trypsin synthesis time (min)	71.8(9.6)	52.7(4.3)*+	97.2(16.6)
Trypsin stores (u)	995(230)	3877(826)*+	1016(532)
Mucosal protein turnover (%/d)	31.6(3.1)	211(34.3)**++	36.4(8.4)

Statistics: * p<0.05, ** p<0.005 vs. fasting; + p<0.05, ++ p<0.005 enteral vs. PN; Mann-Whitney NP test

W1486

Proton Pump Inhibitors Are Only Effective in the Short Term for Preventing PEG Site Excoriation

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INTRODUCTION Percutaneous endoscopic gastrostomy (PEG) is the most common route of long-term enteral nutrition. An overall morbidity rate of 10-20% is associated with PEGs. A significant component of PEG morbidity is insertion site excoriation that results from gastric fluid contact with skin around the PEG placement site. OBJECTIVE To assess the prophylactic use of multi-unit pellet system proton pump inhibitors (PPI) in PEG feeds to prevent acid reflux onto PEG placement site skin. METHODS A randomised, prospective, double blind, placebo-controlled trial. 42 patients undergoing PEG placement were randomly assigned to receive placebo or 40mg esomeprazole multi unit pellet system (MUPS) via the PEG from time of PEG placement to day 30. At day 3, 10 and 42 the PEG site was inspected and photographs taken. RESULTS 21 patients received drug and 21 received the PPI. At day 3, 20% of patients in the drug group had an event (excoriation > 1cm) compared with 30% in the placebo group (relative risk reduction 33% at 3 days, number needed to treat 10). At day 10, 47% in the drug group and 43% in the placebo group had an event. At day 42, 30% of the patients had been lost to follow up. Of the remaining, 2 patients in the drug group and 1 patient in the placebo group had events. CONCLUSION There was a positive benefit in excoriation reduction in the patients receiving esomeprazole 40 mg at day 3. Both groups had a similar event rate at 10 and 42 days. The main benefit of PPIs for PEG feeds is seen at 3 days and by day 10 this benefit is no longer observed. The use of PPIs following PEG placement is therefore associated with morbidity reduction over the short term but from our study does not appear to be advantageous for sustained use.

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Increased Plasma Arginine and Citrulline to Enteral and Parenteral Infusion of L-Alanyl-L-glutamine in Pre-operative Patients

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Background: Glutamine (Gln) is an important amino acid, especially during severe stress such as surgery. Many studies show clinical benefits of glutamine administration during stress. However, most enteral and parenteral feedings contain low or no glutamine because it is unstable in aqueous solutions. The dipeptide L-alanyl-L-glutamine (Ala-Gln), which is stable in watery solutions, is a good alternative. Since glutamine is the key substrate for arginine and citrulline and no enteral kinetic data are available of Ala-Gln on their plasma concentrations, we compared a 4-hour continuous parenteral and enteral administration of the dipeptide on the plasma arginine and citrulline response. Methods: Seven patients were admitted three days before operation. All patients received a self-migrating jejunal tube (Bengmark, Nutricia Nederland, Zoetermeer) for the enteral infusion of the dipeptide (day-2) and X-ray confirmed its location. Additionally, a venous line was given for the parenteral infusion (day -1) and an arterial line was placed in the forearm for blood sampling. During the 4-hour infusion of 20g/100ml Ala-Gln (Dipeptiven, Fresenius Kabi, Homburg, Germany) in 400 ml NaCl 0.9%, arterial blood samples were taken for amino acid determination by HPLC. ANOVA for repeated measures was performed with a paired T-test if significant. P<0.05 was significant. (mean +/- SEM, in μmol/l) Results: Baseline plasma Arg was 105.8+/-3.6 before the parenteral infusion and 110.0+/- 7.7 before the enteral infusion (n.s.). Baseline plasma Cit was 41.4 +/- 2.9 before the parenteral infusion and 43.6 +/- 3.6 before the enteral infusion (n.s.). Parenteral infusions had a significant increment in plasma Arg response and Cit response in time. During the parenteral infusion, plasma Arg was higher than baseline at t=45 until t=90 min, while plasma Cit was also higher than baseline from t=45 until t=270. Enteral infusion had a significant increment in Cit response in time while Arg was not. During the enteral infusion, plasma Cit was significantly higher than baseline at t=120. Plasma Cit was significantly higher at t=60 enterally as compared to parenteral. Conclusion: Both enteral and parenteral administrations of Ala-Gln resulted in increased plasma arginine and citrulline responses as compared to baseline values. Plasma citrulline was higher when given enterally; possibly due to a different utilisation of the dipeptide by the gut.

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Parenteral Nutrition Survival and Dependence in a National Referral Center

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Home parenteral nutrition (HPN) is an established treatment for the management of chronic intestinal failure, and patient survival appears to depend much more on the underlying etiology than on PN complications.
Aim: To report long-term survival rates and PN dependence in patients on HPN and to identify poor prognostic factors related to them.
Method: 188 patients on HPN followed in a national referral center from 1979 to 2003 were reviewed. The Kaplan-Meier method was used for survival and PN dependence rates. To identify prognostic factors a log-rank test and Cox regression model were used.
Results: The mean duration of HPN was 4.7 years (0.1-22.5). There were 115 females and 73 males. Crohn's disease (CD) was the diagnosis in 32% of the cases, mesenteric infarction in 16%, intestinal pseudoobstruction in 13%, radiation enteritis in 11%, and 4% had active malignancy. Short bowel syndrome (SBS) was present in 61%. Small bowel (SB) length was <50 cm in 14% patients, 50-99 cm in 21%, 100-149 cm in 20% and >150 cm in 43%. The survival rates at 1, 5, 10, 15 and 20 years were 87%, 72%, 70%, 56% and 30%, respectively. Multivariate analysis for survival demonstrates active malignancy (RR: 14 (95%CI: 5.6-38)), systemic sclerosis (RR: 3.2 (95%CI: 1.3-7.8)), radiation enteritis (RE) (RR: 3.3 (95%CI: 1.5-7.2)) and chronic cholestasis (RR: 2.7 (95%CI: 1.1-6.5)) as significant prognostic factors. 23 patients were weaned off PN. HPN dependence probabilities at 1, 5, 10 and 15 years were 90%, 86%, 86% and 78%. Multivariate analysis for PN dependece showed that SB length >150 cm (HR: 6.5 (95%CI: 0.83-50)) and surgical complications (HR: 2.8 (95%CI: 1.1-7.0)) were significant prognostic factors of PN independence.
Conclusions: Patients with surgical complications and SB length >150cm are more likely to come off HPN. Poor prognostic factors were active malignancy, systemic sclerosis, radiation enteritis and chronic cholestasis. Vascular complications do not appear to affect outcome in HPN. The only patients likely to have a better survival from intestinal transplantation are those with chronic cholestasis. In general, patient survival on HPN in a large specialist center is excellent.

W1489

Glucose Transport Is Upregulated in Distal Ileum Concomitant with Adaptive Small Intestinal Growth After Massive Small Bowel Resection in Mice

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Introduction: Massive small bowel resection (SBR) increases growth of residual ileum in mice, but nutrient transport and adaptation in other gut segments have been little studied in mouse models of short bowel syndrome (SBS). Adaptive mucosal growth ultimately increases total nutrient absorptive capacity; however, several studies in animal models show that glucose and amino acid transport are decreased during the early period after SBR. This study determined growth responses in jejunum, ileum and colon and L-glucose and L-glutamine (Gln) transport in distal ileum after SBR in mice. Methods: C57BL/6J mice (25 g) underwent 50% mid-jejunoileal resection (RX) or ileal transection (TX; control). Mice were pair-fed liquid diet until sacrifice at 7 days post-surgery. Segments of jejunum, ileum and colon were obtained for analysis of villus height (VH), crypt depth (CD) and total mucosal height (TMH). Electrophysiological studies using full-thickness distal ileal segments were performed using an automated volt-clamp Ussing chamber system. Baseline transepithelial resistance (TER) was determined as an indicator of epithelial barrier function and tissue viability. Potential difference across the ileum was maintained at 5 mV by voltage clamp and short circuit current (Isc) determined as a measure of net active ion transport after addition of L-glucose (10 mM) or L-Gln (10 mM). Net active ion transport served as the indicator of nutrient transport. Sodium-coupled glucose transporter-1 (SGLT-1) expression in ileum was determined by Western immunoblotting. Results: Jejunal VH and TMH were similar between the TX and RX groups, but jejunal CD increased significantly after SBR (TX 103 ± 17 vs RX 145 ± 11 μm). SBR significantly increased ileal VH (TX 229 ± 15 vs RX 294 ± 26 μm), CD (85 ± 5 vs 113 ± 11 μm), and TMH (298 ± 12 vs 385 ± 31 μm). Colonic CD was not different between groups. TER was similar between groups, suggesting that ileal barrier function was maintained following SBR. Ileal L-glucose transport was significantly increased by SBR (TX 63 ± 5 $\mu A/cm^2$ vs RX 84 ± 8 $\mu A/cm^2$) but L-Gln transport was unchanged (TX 31 ± 6 $\mu A/cm^2$ vs RX 35 ± 7 $\mu A/cm^2$). Ileal SGLT-1 expression tended to increase with RX (NS). Conclusion: Massive SBR in mice induces adaptive mucosal growth responses in both jejunum and ileum, but not colon, after 7 days. Transport of glucose, but not Gln, is adaptively upregulated in distal ileum concomitant with ileal mucosal growth in this murine model of SBS.

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Gut Mucosal Glutathione Status Differentially Regulates Ileal and Colonic Mucosal Growth in a Rat Model of Short Bowel Syndrome

Junqiang Tian, Naohiro Washizawa, Li Gu, Dean P. Jones, Thomas R. Ziegler

Background: Massive small bowel resection (SBR) results in adaptive small intestinal growth but colonic growth after SBR have been little studied. Current evidence suggest that glutathione (GSH) increases cell proliferation and decreases apoptosis, but effects of altered gut mucosal GSH status on gut growth after SBR are unknown. Our aim was to examine ileal and colonic mucosal growth after SBR, with or without blockade of cellular GSH synthesis by L-buthionine sulfoximine (BSO), a specific inhibitor of GSH synthesis. Methods: Male rats (N = 25, 200 g) underwent small bowel transection (TX; control) or 80% mid-jejunoileal resection (RX). Rats were pair-fed until harvest on day 7. On day 4 after operation, animals were given daily i.p. saline or BSO (0.5 mmol/kg) with BSO in drinking water (10mM). Crypt depth (CD), villus height (VH) and total mucosal height (TMH) and mucosal GSH redox status were determined in ileum and colon. GSH and glutathione disulfide (GSSG) were determined by HPLC. Results: In ileum, SBR significantly increased GSH concentration (TX-saline 8.9 ± 0.6 versus RX-saline 11.5 ± 0.4 nmol/mg protein), while BSO treatment markedly decreased mucosal GSH (TX-BSO 0.8 ± 0.2 and RX-BSO 0.8 ± 0.2 nmol/mg protein, respectively). BSO also significantly decreased mucosal GSSG (not shown). SBR significantly increased ileal VH (by 68%), CD (by 51%), and TMH (by 63%) versus control rats. However, adaptive ileal growth after SBR was significantly blunted in BSO-treated rats (the increase in VH was 36%, CD 14%, and TMH 29%). In saline-treated rats, there was a significant positive correlation with mucosal GSH content and CD, VH and TMH.. In colon, SBR significantly increased mucosal GSH (TX-saline 7.0 ± 1.3 versus RX-saline 11.6 ± 2.0 nmol/mg protein) and BSO markedly decreased mucosal GSH (TX-BSO 0.7 ± 0.2 and RX-BSO 1.4 ± 0.5 nmol/mg protein, respectively). SBR did not alter colonic CD; however, BSO resulted in a significant increase in CD in both TX and RX rats (+ 20 and +32%, respectively). Conclusions: Maintained or increased mucosal GSH is an important determinant of adaptive ileal mucosal growth following SBR in rats. In contrast, GSH depletion upregulates adaptive growth in colonic mucosa. Thus, alterations in GSH status during gut adaptation may differentially signal mucosal growth-related pathways in a site-specific manner.

W1491

Can Enteral Nutrition Provide Adequate Stress Prophylaxis for Patients on Mechanical Ventilation?

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INTRO: Risk for stress gastropathy and gastrointestinal bleeding (GIB) is associated with critical illness and placement on mechanical ventilation (MV). We designed this pilot prospective double-blind randomized trial to determine whether enteral nutrition (EN) can protect ICU pts on MV from mucosal injury and GIB. METHODS: Critically ill pts with NG feeding tubes eligible for the study were randomized within 48 hrs of placement on MV to receive EN alone (Peptamen 1.5 continuous infusion starting at 25 cc/hr, advanced as tolerated) or be made NPO (no stress prophylaxis). Neither group received antacids/acid-reducing agents. Over 72 hrs, a stress ulcer risk score (SU Risk) was calculated, and pts were monitored by daily Hgb/Hct, stool Hemoccult, and gastrocult guaiac testing (for occult GIB) with visual inspection (for overt GIB) of NG aspirates q 4 hrs. Pumps and tubing were covered to maintain blinding. At 72 hrs, endoscopy was done, videotaped (read later in blinded fashion), and a mucosal injury score from 0 (normal) to 4 (frank ulcers) was determined. Statistical analysis done by Chi Square and Student t test. RESULTS: Ten pts (mean age 50.0 yrs, 70% male, mean APACHE II score 27.8) were entered in the study, 5 study pts on EN, 5 controls NPO. Study pts received 38% of goal feeds over the 72 hrs (range 18-61%). No pts had increased residual volume, pneumonia or gut ischemia. There was no difference in control of pH between groups. Data shown below: CONCLUSIONS: Provision of EN incurred no deleterious effects. Despite slightly higher risk, based on older age and greater endoscopic mucosal injury scores, pts receiving EN showed evidence of less GIB than controls on no stress prophylaxis. This protective effect appeared unrelated to control of pH or meeting caloric requirements.

* p=0.07	Age (yrs)	SU Risk Score	Endo Score	Chg Hgb (gms)	Chg Hct (%)	%Occult NG GIB	%Overt NG GIB
Study pts (n=5)	54.4	2.0	1.4	-1.18	-3.24	3.8%	4.1%
Controls (n=5)	45.6	2.2	1.0	-1.94	-6.00	34.9%	28.2%*

W1492

Three Main Dietary Fibers, Cellulose, Resistant Starch and Pectin, Exhibit Major Differences in the Amounts Which Reach the Large Intestine

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BACKGROUND: If dietary fiber (DF) is defined as polysaccharides resistant to digestion in the small intestine, then only that DF found in the terminal ileum can give the "true" value of food-derived DF. Several physiological measurements of DF in the human terminal ileum have been reported to date, but with questionable accuracy. This study was designed to obtain an accurate value of three major DFs (cellulose, resistant starch and pectin) in the terminal ileum to evaluate the "true" value of DF. METHODS: Eighteen male volunteers aged 20-27 years were allocated to three groups according to the test meal ingested: 4.05 g pectin (n = 7), 10 g heat-moisture-treated high-amylose cornstarch (88 % resistant starch) (n = 7), and 5 g cellulose (n = 4). A double-lumen tube was placed in the terminal ileum using the endoscopic retrograde bowel insertion method, and the ileal contents were aspirated by gentle suction (less than 100 mmHg) through the tube for 12 h after the test meal intake, and collected samples were stored separately every 30 min. A solution of 0.03 % PSP with distilled water was infused continuously at the rate of 1.0 ml/min as a non-absorbable marker to calculate ileal flow. The amounts of cellulose, resistant starch and pectin orally administered and collected from the terminal ileum were estimated from the as glucose concentration for the first two DFs and from the galacturonic acid concentrations for pectin, and were compared with each other. RESULTS: The mean percentages of cellulose, resistant starch and pectin collected in the terminal ileum were 102.5 ± 8.5 %, 34.5 ± 9.7 % and 88.4 ± 10.5 %, respectively, of the amounts administered. Furthermore, there were large individual differences among subjects in the amounts of resistant starch and pectin recovered compared with cellulose. CONCLUSIONS: The "true" values of the three major DFs showed a marked difference when compared with each other. Therefore, the classical definition of DF i.e., "polysaccharides resistant to digestion in the small intestine" should be reconsidered.

W1688

New Prognosis Prediction System for Liver Transplantation in Patients with Fulminant Hepatic Failure

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Background: Liver transplantation (LTx) is effect for patients with fulminant hepatic failure (FHF), who die from cerebral edema and/or multiple organ failure. However, many patients die before LTx because the suitable timing of LTx is not identified. We established new prognosis prediction system useful to determine the timing of LTx. Methods: First sample consisted of 80 FHF patients (33 male and 47 female, median age 46 years), corresponding to hepatic coma grade ≥ II and PT < 40% within 8 weeks from first symptoms. By using stepwise regression analysis, we examined 9 parameters (age, gender, etiology, duration from first symptoms to diagnosis of FHF, hepatic coma grade, systemic inflammatory response syndrome (SIRS), total bilirubin (T.Bil), ratio of total to direct bilirubin (T/D ratio) and prothrombin time) at the diagnosis of FHF (day 1) and at day 4, 8 and 15. SIRS was determined on the criteria of the Consensus Conference on Sepsis and Multiple Organ Failure. The end point which were determined 2 weeks after the prognosis prediction date was defined as mortality of patients (patient receiving LTx was regarded as death). A 2-week prognosis prediction system consisting of parameters associated with 2-week fatal outcomes was made. In order to confirm the accuracy of this system, prospective validation was performed in second sample consisted of 26 FHF patients (10 men and 16 female, median age of 61 years). Results: In first sample, causes of FHF were HBV in 27 patients, indeterminate in 24 and other in 29. Overall, 27 patients survived, 48 died, and 5 received LTx. In the stepwise regression analysis, etiology, SIRS and T/D ratio were associated with 2-week fatal outcomes at day 1. Only hepatic coma grade was associated with 2-week fatal outcomes at all of day 4, 8 and 15. The four 2-week fatal prognostic parameters were elicited: etiology (HBV or indeterminate), hepatic coma grade (III or IV), SIRS (yes) and T/D ratio (>2.0). 2-week survival rate in patients corresponding to 2 or less of 4 parameters was more than 80%. In contrast, 2-week survival rate in patients corresponding to 3 or more was less than 30%. When this 2-week prognosis prediction system was applied to second sample, the sensitivity, specificity, and positive and negative predictive value were 87.5%, 90.0%, 93.3% and 81.8%, respectively. Conclusion: Our 2-week prognosis prediction system is considered useful to predict 2-week prognosis and determine the suitable timing of LTx in FHF patients.

W1689

Are Genetic Polymorphisms in Orthotropic Liver Transplant Recipients Responsible for Elevated Serum Homocysteine?

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C677T polymorphism in the methyltetrahydrofolate reductase (MTHFR) gene results in thermolability and decreased enzyme activity with subsequent decrease in cellular levels of 5-methyltetrahydrofolate and DNA-methylation. Frequency of the MTHFR in the general population is 45% for the wild type (CC), 45% for the heterozygote type (CT) and 10% for the homozygote type (TT). Both CT and TT are known to have decreased activity compared to the wild type (CC). Impaired activity of MTHFR leads to increased serum homocysteine (HCY) levels. HCY is metabolized to methionine in a 5-methyltetrahydrofolate (5-MTHF) depending reaction. Hyperhomocysteinaemia is a known risk factor in the development of various vascular diseases. Liver transplant recipients have an increased risk for cardiovascular disease because of a high incidence of obesity, arterial hypertension, diabetes mellitus, and hyperlipidemia. Aim of this study was to investigate the distribution of the MTHFR gene polymorphism in patients after liver transplantation (LTX) with elevated serum HCY. Methods: In 42 liver transplant recipients fasting serum HCY was analyzed with HPLC technique. According to the German-Austrian-Swiss HCY league normal HCY concentrations were determined as < 9 µmol/L. MTHFR polymorphism was detected by PCR amplification and digestion with HinFI restriction enzyme of a 198-bp DNA fragment in exon 4 of MTHFR gene. Results: 14 Patients were homozygote for the wild type (CC = 32%). In contrast, 21 Patients were heterozygote (CT = 49%) and 8 homozygote for the MTHFR-variant C677T (TT = 19%). HCY levels were: wild type CC 14 ± 4 µmol/L; CT: 22.5 ± 16 µmol/L and TT: 31.2 ± 16** µmol/L (**p<0.01). Discussion: About 70% of our patients were heterozygote and homozygote for the MTHFR-variant C677T and therefore have reduced MTHFR activity. Compared to normal population (about 55%) this distribution obviously differs. Furthermore, depending on the genotype, the lowest HCY levels were found in the wild type group (CC). In the homozygote variant group (TT), HCY levels were significant higher. Besides other well known determinants for hyperhomocysteinemia in this specific group MTHFR polymorphism is an additional explanation for impaired homocysteine metabolism.

W1690

Asymmetrical Dimethylarginine; a Possible Future Marker for Acute Liver Allograft Rejection?

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Asymmetrical dimethylarginine (ADMA) has been recognized as an endogenous inhibitor of the arginine-nitric oxide (NO) pathway. Its concentration is tightly regulated by urinary excretion and degradation by the enzyme dimethylarginine dimethylaminohydrolase, which is highly expressed in the liver. Considering the liver as a crucial organ in the clearing of ADMA, we hypothesized increased ADMA levels during hepatic failure and, consequently, a decline of ADMA concentrations after liver transplantation. Furthermore, high ADMA concentrations may lead to NO deficiency; an important factor in the pathogenesis of ischaemia-reperfusion injury which, at least partly, causes hepatic dysfunction. The aim of the present study was to investigate the role of the liver in the metabolism of ADMA in patients undergoing liver transplantation. In this prospective study, we investigated the course of ADMA concentrations in 42 patients undergoing liver transplantation and results showed that ADMA concentrations were higher in all patients on the day before transplantation (0.77 μM) compared to healthy volunteers (0.41 μM, $p<0.001$). In patients undergoing liver transplantation, ADMA decreased on the first postoperative day (ΔADMA: -0.22 μM, $p<0.001$). In patients with acute hepatic failure, preoperative ADMA levels were higher compared with patients who suffered from chronic hepatic failure (1.26 μM vs 0.69 μM, $p=0.003$). Furthermore, in patients who experienced acute rejection, ADMA concentrations were higher during the whole first postoperative month compared with non-rejectors ($p=0.012$). Moreover, in 11 of 13 rejectors (85%) a clear increase in ADMA concentration preceded the onset of the first episode of rejection as proven by liver biopsy. In conclusion, this study shows that the liver plays an important role in the metabolism of ADMA. In addition, increased ADMA concentrations in the posttransplantation period reflect dysfunction of the liver graft during rejection. Studies with larger populations must confirm that the course of ADMA may be used as a diagnostic marker for acute liver allograft rejection.

W1691

Predictors of Weight Change Following Liver Transplantation

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BACKGROUND: Obesity, as measured by body mass index (BMI) >30 Kg/m2, is common after liver transplantation. AIM: Our aim was to 1) perform a longitudinal analysis of weight change following liver transplantation and 2) determine which factors were predictors of posttransplant weight changes. PATIENTS AND METHODS: Data were prospectively collected on 277 consecutive patients undergoing liver transplantation between 6/1/98 and 12/1/01. Patients were excluded if they underwent a re-transplantation or expired before 1 year posttransplantation, did not have an annual follow up, or had incomplete data. 186 patients fulfilled inclusion criteria. Weight at transplant was adjusted by the amount of ascites removed intraoperatively. RESULTS: Median BMI was 28.12 Kg/m2 at time of transplantation and was 29.21 Kg/m2 post transplantation. Mean increase in BMI at 1 year was 1.09 Kg/m2. Pretransplant BMI was significantly associated with post transplant weight gain ($P=0.0055$). Patients with lower pretransplant BMI experienced the largest post transplant increase in BMI (mean 5.39 Kg/m2). Subjects with very low transplant BMI (30) were likely to maintain or experience a decrease in their BMI at one year. Underlying liver disease was also predictive of 1 year weight change ($P=0.0008$). Patients with alcoholic liver disease were the most likely to gain weight (mean BMI increase 4.34 Kg/m2) compared to patients with other etiologies. Patients with fulminant hepatic failure had a mean loss of BMI of 1.5 Kg/m2. CONCLUSION: Median BMI increases following liver transplantation. Patients with lower BMI at time of liver transplant tend to gain more weight during the first postoperative year compared to patients with higher BMI at the time of transplantation. Patients with alcoholic liver disease gain significantly more weight after liver transplantation compared to patients with other liver diseases.

W1692

When Should Extended Donor Criteria Grafts Be Considered For Liver Transplant Under The MELD System? A Decision Analysis

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Purpose: Extended donor criteria (EDC) liver grafts may be more likely to experience primary graft failure due to factors such as increased donor age and ischemia time. However, a standard donor criteria (SDC) cadaveric liver may not become available for patients on the transplant waiting list. Therefore, our aim was to compare 1-year survival for an immediate EDC liver graft with waiting for an SDC organ. Methods: We constructed a Markov decision analytic model for cadaveric liver transplant candidates to compare survival of waiting for a SDC transplant with immediate EDC transplant. Parameters for MELD-specific graft and patient survival were based on UNOS data, published literature estimates and expert opinion. Risk of EDC primary (1-month) graft failure was based on published literature relating donor clinical risk factors (RF) to graft failure rates, ranging from 11% (≤ 2 RFs) to 40% (≥ 4 RFs). Risk factors included donor age, ICU length of stay, ischemia time, hypotension, LFTs, and hypernatremia. Results: For patients with MELD scores of 11-20, survival was greater with EDC grafts only if the probability of primary graft failure was 15% or less (Table). For patients with MELD scores > 20, survival was higher with EDC transplant even when graft failure was set at 50%. In sensitivity analysis, the EDC survival benefit for patients with MELD scores > 20 was most influenced by the high mortality while waiting for a SDC transplant. Other parameters in order of importance included mortality post transplant, mortality post retransplant, and probability of retransplant. Conclusions: Our results suggest that immediate cadaveric transplant with EDC grafts for patients with advanced MELD scores may be underutilized. Despite the higher risk for primary graft failure, survival post EDC transplant should be substantially higher than survival while waiting for standard donor organs with advanced MELD scores. At less advanced MELD scores, the decision depends on the risk of EDC graft failure. Additional data on primary graft failure rates associated with specific EDC would improve the optimal use of these organs for patients waiting for a transplant.

Table: Estimated 1-year survival of waiting for a SDC transplant versus undergoing immediate EDC transplant

Strategy	MELD 11-20	MELD 21-25	MELD 26-30	MELD 31-40
Waiting For SDC	77%	58%	50%	38%
Immediate EDC with Primary Graft Failure Rate=				
15%	80%	79%	76%	61%
25%	76%	75%	72%	59%
50%	67%	67%	64%	54%

W1693

"Crane-neck" Shaped Biliary Stricture as an Important Complication after Right-Lobe Living-Donor Liver Transplantation with Duct-to-Duct Biliary Reconstruction

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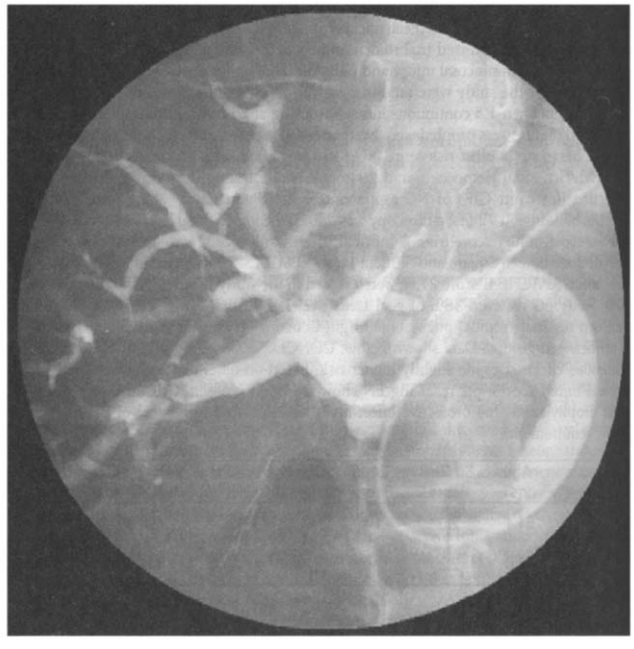
BACKGROUND: Biliary stricture is an important complication after living-donor liver transplantation with duct-to-duct biliary reconstruction (LDLT-DD). With increasing number of patients receiving right-lobe LDLT-DD, we encountered 3 characteristic cases of postoperative biliary stricture, whose common bile duct (CBD) was so severely bended that anastomoses positioned lower than the highest portion of CBD (Fig). We named this deformity "crane-neck" sign and assessed its cause and clinical feature. METHODS: Out of 128 consecutive cases with right-lobe LDLT-DD, 41 developed postoperative anastomotic biliary stricture, including 3 "crane-neck" cases. To elucidate the cause for such deformity, we compared the degree of graft regeneration between 3 "crane-neck" cases and the others. The success rate of endoscopic therapy was also compared. RESULTS: The extent of graft regeneration in the "crane-neck" cases was not different from that in other cases. Thus, the deformity could not be explained by excessive enlargement of the graft. Due to severely bended CBD, the success rate of endoscopic therapy in "crane-neck" cases was lower than that in all the cases with postoperative biliary stricture (33.3% vs 72.2%). CONCLUSION: "Crane-neck" shaped biliary stricture is not rare after right-lobe LDLT-DD and its endoscopic treatment is difficult. The cause for "crane-neck" deformity remains unclear.



W1694

Endoscopic Management of Biliary Strictures After Duct-to-Duct Biliary Reconstruction in Right-Lobe Living-Donor Liver Transplantation; The Second Report of the "Inside-Stent"

Shujiro Yazumi, Takanobu Yoshimoto, Hiroshi Hisatsune, Kazunori Hasegawa, Masanori Asada, Akiyoshi Nishio, Hiroto Egawa, Koichi Tanaka, Tsutomu Chiba

Background: We previously reported that most of the biliary strictures developed after right-lobe living-donor liver transplantation (LDLT) with duct-to-duct biliary reconstruction were "fork-shaped" type, and that placement of the multiple "inside-stents" above the sphincter of Oddi were temporally useful for the treatment of the biliary stricture (Transplantation 2003;76:810-5). This study aims to assess the long-term feasibility of the "inside-stent". Patients: The records of 128 recipients, who recieved right-lobe LDLT with duct-to-duct reconstruction from July 1999 to Jun 2003 and remained alive for more than three months after transplantation, were retrospectively reviewed. An average observation period was 791

days ranging from 155 to 1606 days. For the patients with biliary stricture, to prevent ascending cholangitis, the "inside-stents" ranging from 7Fr to 12Fr in size were endoscopically placed across the stricture without endoscopic sphincterotomy. The "inside-stent" was retrieved at more than 6 months after stenting when the patients agreed. Results: Of the 128 recipients, 41 (32.0%) suffered from posttransplantation biliary stricture. Of these 41 patients with the biliary stricture, 36 were referred for endoscopic retrograde cholangiogram (ERC), 13 were for percutaneous transhepatic cholangiogram (PTC), and accordingly 8 were for both ERC and PTC. Of the 36 patients who were referred for ERC, the "inside-stent" was placed in 28 patients. In 14 patients (50%), the "inside-stent" could be retrieved at an average of 223 days after stenting, and at present none of them have required reinsertion of the "inside-stent" for an average of 319 days ranging from 105 to 856 days. On the other hand, 12 patients (42.8%), who refused hospitalization to retrieve the "inside-stent" because they could return to their job without liver dysfunction, were observed as outpatients for an average of 532 days ranging from 207 to 1001 days. The "inside-stent" was not successful in 2 patients (7.2%). One patient suffered from acute cholangitis immediately after stenting and the other dyed from lymph node metastasis of hepatocellular carcinoma two months after stenting. Conclusion: The "inside-stent" was a favorable treatment option for the biliary stricture after right-lobe LDLT with duct-to-duct biliary reconstruction. Notably, the "inside-stent" could be retrieved at an average of 7 months after stenting.

W1695

Magnetic Resonance Cholangiopancreatography for the Noninvasive Evaluation of Liver Transplant Patients with Suspected Biliary Strictures: A Prospective Double-Blind Study

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Background: Currently, suspected biliary strictures are diagnosed by endoscopic retrograde cholangiopancreatography (ERCP). Magnetic resonance cholangiopancreatography (MRCP) provides a non-invasive alternative. However, it remains to be determined whether MRCP has sufficient accuracy to replace diagnostic ERCP and, accordingly, restrict ERCP to those patients requiring therapeutic intervention. The aim of this study was to determine the utility of MRCP in the evaluation of liver transplant patients with suspected biliary strictures. Method: Upon institutional review board approval, we enrolled a prospective cohort of orthotopic liver transplant patients referred for ERCP evaluation of suspected biliary strictures. MRCP was performed less than 24 hours prior to ERCP using a 1.5T MR Scanner. No sedation or contrast agent was given. Two radiologists (R1 and R2), both blinded to ERCP results, independently interpreted all MRCP studies. A single endoscopist, blinded to MRCP results, performed all ERCPs. ERCP was employed as the standard of reference and findings were standardized through the use of a scoring template. Results: Ten patients were enrolled in the study. Two of these patients were unable to complete MRCP due to claustrophobia. Eight patients underwent both studies. All patients had a duct-to-duct anastomosis. ERCP identified a stricture in four of eight patients: two at the anastomosis, one at the bifurcation and one in the right hepatic duct. All four strictures seen on ERCP were correctly identified by MRCP. In addition, R1 identified three false positive strictures and R2 identified one. The interobserver agreement κ for strictures was 0.39. There was 100% concordance between ERCP and MRCP with regard to the anatomic location of the strictures and absence of stones. Conclusion: In this pilot study, preliminary findings suggest that MRCP has high sensitivity but lower specificity for identifying post-transplant biliary strictures. As such, in liver transplant patients, a normal evaluation by MRCP may preclude the need for ERCP.

W1696

Early Biliary Complications Following Liver Transplantation Are Amenable to Endoscopic Intervention

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BACKGROUND: Our aim was to quantify the frequency of post transplant biliary complications and to characterize those that are more likely to need surgical intervention. METHODS: A single center retrospective study of all adult liver transplants 4/30/98 - 5/1/03. RESULTS: In 434 patients, 459 transplants were performed, 388 had duct-to-duct biliary anastomosis, the remainder had choledochojejunostomy, 60 had living donors, average patient age 51, 63% male, predominant transplant indication HCV (41%). Following transplant, 42 patients had PTC, 74 ERCP and 38 biliary surgery. Of the 459 transplants, a biliary complication was diagnosed in 102 (22.2%); 31 (6.8%) leaks, 60 (13%) strictures, 30 (6.5%) papillary stenosis or stones. Of the 388 patients with duct-to-duct anastomosis, 74 (19%) had at least 1 ERCP for a total of 174 ERCPs with 99.4% papillary canulation and no complications. Findings at initial ERCP were 39 (53%) strictures, 14 (19%) leaks, 16 (21%) papillary stenosis, 11 (15%) stones. Therapeutic interventions were performed in 53 of 74 (72%) patients, with 88 stents, 76 balloon dilations, 29 papillotomies. Fourteen of the 74 patients who had ERCP required subsequent biliary surgery. The odds of subsequent surgical intervention were lower when initial ERCP <90 days after transplant compared to > 90 days, OR 0.24 (95%CI 0.07 to 0.78, p=0.016). Leaks were found more often than strictures at initial ERCP <90 days after transplant; 13 of 14 (93%) leaks whereas only 27 of 40 (67%) strictures. There were higher odds of surgery for strictures identified at initial ERCP at any time, OR 3.9, p=0.04, but not for leaks, OR 1.2, p=0.79. Strictures identified at initial ERCP <90 days after transplant had lower odds of surgery than at ERCP > 90 days, OR 1.2 and OR 20.8, respectively, p=0.05. Balloon dilation had higher odds of surgery, OR 5.9, p=0.007 but not stents, OR 2.6, p=0.16 or papillotomy, OR 2.4, p=0.13. CONCLUSIONS: Biliary complications after liver transplant are common, 22.2%. Patients with initial ERCP at < 90 days after transplant have leaks more frequently than strictures, have findings that are more amenable to endoscopic intervention and are less likely to need surgical intervention than at >90 days. Strictures identified at initial ERCP >90 days from transplant are more likely to fail endoscopic therapy and require surgical intervention than strictures identified at < 90 days. The association between balloon dilation and subsequent surgery may be due to more severe strictures or from the intervention.

W1697

Activity and Neoplastic Potential of Ulcerative Colitis After Orthotopic Liver Transplantation for Primary Sclerosing Cholangitis

Pavel Drastich, Miroslav Ryska, Pavel Trunecka, Jan Martinek, Julius Spicak

BACKGROUND: The clinical outcome of patients with ulcerative colitis (UC) after orthotopic liver transplantation (OLTx) for primary sclerosing cholangitis (PSC) is still unclear and published studies provide contrasting data. AIM: To evaluate the course of UC after OLTx in our center (IKEM, Prague) including activity and possible dysplastic and/or neoplastic changes. PATIENTS AND METHODS: We analyzed a cohort of 31 consecutive patients with PSC (mean age 39.7 ± 12.6 years) transplanted at our center between 1994-2003 (9.8% of all OLTx). UC was diagnosed in 23 patients (74.2 %); no patients had history of Crohn's disease. Colonoscopy was performed in all patients before OLTx and annually after OLTx with extent biopsies. No patients had undergone colectomy before transplant. The analysis includes 18 patients as 3 died (at 0, 7, 8 months) and 2 patients were lost from follow up in our center. Median follow up post OLTx was 3.4 years (range 1.1-9.3). RESULTS: Despite the colitis was extent before OLTx, the clinical course of UC was mild or asymptomatic in 19/23 (83%) patients, 12/18 (66.7%) patients remained without relevant symptoms after OLT. Six patients (33.3%) were suffering from clinically active disease confirmed by endoscopy after OLTx, 2 cases had an active pretransplant course of UC and 4 patients who were quiescent beforehand developed severe exacerbation of colitis after OLTx, in one case the CMV disease was detected as triggering factor. 3 patients flared-up after corticosteroid withdrawal. Symptoms of all patients with active colitis were well controlled with 5'-aminosalicylates and oral prednisone without any surgical intervention. Low-grade dysplasia (LGD) of colonic mucosa was found in 6/23 patients before and in 7/18 patients after OLTx (NS). In 2 patients with LGD before OLTx it has not been confirmed after OLTx. On the other hand LGD newly developed in 4 patients after OLTx. High-grade dysplasia or colorectal carcinoma were not detected. CONCLUSION: The course of UC after OLT for PSC is frequently active despite the immunosuppressive treatment. The flare of disease developed also in patients with mild or asymptomatic course of UC in pretransplant period. No colectomy was necessary to control active UC after OLTx. We did not find increased frequency of dysplastic/neoplastic changes of colonic mucosa after OLTx.

W1698

Endoscopic Therapy for Biliary Complications Following Orthotopic Liver Transplantation with Choledochocholedochostomy Anastomosis

Ali Fazel, Henry Chiu, Shea Ross, Consuelo Soldevila-Pico, Koorosh MoezArdalan, Christopher Forsmark

Background: Biliary tract complications lead to significant mortality and morbidity after orthotopic liver transplantion (OLT) with choledochocholedochol anastomosis (CCA). Biliary complications occur with a frequency of 11.5% to 34%. These complications are increasingly diagnosed and treated through ERCP. Surgical intervention becomes necessary when endoscopic therapy fails. Aim: This retrospective study seeks to identify the frequency and type of complications seen and elucidate the effectiveness of ERCP in the treatment of biliary tract complications after OLT with CCA. Method: Between January 1994 and December 2001, 736 OLTs were performed at the University of Florida. ERCP was performed in 225 (30.6%) due to suspected biliary complication. Ninety-three (41.3%) cases were diagnosed with a biliary anastomotic stricture and 51 (22.7%) were found to have a bile leak. Endoscopic therapy consisted of dilation and stenting (10 Fr) for biliary strictures, stenting (10 Fr) alone for leaks, sphincterotomy for ampullary strictures and sphincterotomy with extraction for stones. Failure of endoscopic therapy was defined as the eventual surgical revision, retransplantation or death resulting from the post-OLT biliary complication. Results: The incidence of post-OLT biliary complication was 19% (142/736). More than one complication was seen in 13% (98/736). Table 1 shows the incidence of the different complications and the success rate of endoscopic treatment in each group. The most common complication was biliary stricture, followed by bile leak, biliary stone or sludge, and papillary stenosis. Endoscopic intervention was not successful in 34 (24%) out of 142 patients, leading to eventual surgical revision or re-transplantation. Endoscopic intervention precluded the need for surgical intervention in 76% of cases. Conclusion: ERCP should be considered the modality of choice for the diagnosis and treatment of biliary complications following OLT with CCA. Endoscopic intervention precluded the need for surgical interventions in over 2/3 of cases.

Table 1. Biliary tract complications after OLT in 736 patients between January 1994 and December 2001.

ERCP findings	No. (%) of patients	Success rate
Biliary stricture	93 (12)	75%
Bile leak	51 (7)	72%
Biliary stone or sludge	39 (5)	100%
Papillary stenosis	14 (2)	100%
Other biliary complications	16 (2)	94%
Unsuccessful diagnostic ERCP	11 (1)	-

W1699

Genomic Profiling Suggests That Barretta's Esophagus Is More Similar to Esophageal Adenocarcinoma Than to Normal Esophagus

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Background: Barrett's esophagus (BE) has long been designated as the precursor lesion for esophageal adenocarcinoma (ADCA). However, comprehensive insights into molecular events leading to ADCA is limited. Genomic and bioinformatic analyses have the power to perform this type of global analysis. We conducted a genomic profiling and scanning study to discover novel biomarkers for the early diagnosis of ADCA. Methods: cDNA microarray analysis was performed on ADCA and BE specimens, with corresponding normal esophagus from 10

ADCAs. This analysis enabled high-throughput data collection on 8,064 genes. Genes with significantly different expression levels between these three groups were identified using significance analysis of microarray data (SAM). Genes selected by SAM were verified using real-time quantitative RT-PCR. Results: 588 genes were differentially expressed in ADCAs relative to normal tissues (289 up- and 299 down-regulated). Similarly, 395 genes were differentially expressed in BEs relative to corresponding normal esophagus (196 up- and 199 down-regulated). 179 genes were up- or down-regulated in both BE and ADCA compared to normal esophagus (101 up- and 79 down-regulated). However, surprisingly, only 50 genes were differentially expressed in ADCAs relative to BEs (35 up- and 15 down-regulated). These results suggest that the global gene expression patterns of BE and ADCA are more similar to each other than they are to normal esophagus. Next, we further narrowed the group of 179 genes with similar expression patterns in BE and ADCA vs. normal using the following criteria: 1) highest levels of significance for differential expression, 2) at least a three-fold difference in mean expression levels for ADCAs and BEs vs. normal esophagus; and 3) potential functional links to growth control or apoptosis. Seven genes met these criteria and were verified with quantitative real-time RT-PCR: *CA9, GW112, A2M, MSLN, BBF-2, GATA6, and TSPAN-1*. Conclusions: Genomic profiling suggests that BEs have a molecular phenotype much more similar to ADCAs than to normal esophagus. Gene filtering identified seven genes commonly upregulated in both BEs and ADCAs relative to normal esophagus. These genes represent candidate early diagnostic markers in BE, as well as targets for future mechanistic studies.

W1700

Novel Expression of Vascular Cell Adhesion Molecule-1 (VCAM-1) in Human Squamous Esophageal Epithelium - Pathophysiological Implications

Jan Heidemann, Christian Maaser, Andreas Lugering, Thomas W. Spahn, Klaus P. Zimmer, Hermann Herbst, Wolfram Domschke, Parvaneh Rafiee, David G. Binion, Torsten Kucharzik

Introduction: Vascular cell adhesion molecule-1 (VCAM-1) is a crucial mediator of leukocyte adhesion and co-stimulatory functions in inflammation at various organ sites. Until recently, VCAM-1 expression was believed to be restricted to activated endothelial cells in inflamed tissues, directing leukocyte adhesion and diapedesis. Recent studies by our group focusing on the endothelial expression of cell adhesion molecules in the microvasculature of the esophagus revealed novel and unexpected expression of VCAM-1 by squamous epithelial cells of normal human esophagi. We therefore sought to characterize differential expression patterns of VCAM-1 in esophageal epithelial alterations. Material and Methods: Expression patterns of VCAM-1 in the esophageal epithelium were examined by immunohistochemistry and indirect immunofluorescence. Staining specificity was verified by preincubation of the monoclonal antibody with recombinant human VCAM-1 and by the use of preimmune mouse IgG. Ultrastructural expression patterns of VCAM-1 in esophageal epithelium were studied by immunogold electron microscopy. Specimens assessed included Barrett's columnar cell metaplasia, squamous cell carcinoma (SCC), and esophageal adenocarcinoma. Results: No VCAM-1 immunoreactivity was detectable in columnar cell epithelia in Barrett's esophagi and esophageal adenocarcinoma while in normal esophageal tissue, VCAM-1 was invariably membrane expressed in the basal squamous epithelial strata and gradually attenuated towards the luminal strata. In contrast, strong expression was detected in the epithelial cells of squamous cell carcinoma throughout the whole epithelial layer. VCAM-1 expression was focally accentuated at sites characteristic of microscopic tumor invasion in squamous cell carcinomas, pointing to a potential role of VCAM-1 in the development of metastasis. In low-grade SCC, VCAM-1 immunoreactivity was located within the cytosol. Conclusion: The altered cellular expression of VCAM-1 in the epithelial layer at sites of invasive growth and in low-grade differentiated SCC suggests a previously unrecognized role for VCAM-1 in the control of squamous epithelial cell growth.

W1701

Molecular Genotyping of Esophageal Neoplasms Pre and Post Photodynamic Therapy

Alyssa M. Krasinskas, Rehka Pal, Elizaburo Sasatomi, Laura Niehouse, Patricia A. Swalsky, Sydney D. Finkelstein

Purpose: To better understand the role of photodynamic therapy (PDT) in the treatment of esophageal neoplasia and to improve post-PDT biopsy diagnoses, we applied a combined molecular pathology approach to pre and post-PDT specimens. Methods: Microdissection genotyping was performed on 4 patients (2M, 2F, mean age 74 years) who had recurrent high-grade glandular dysplasia (n = 1), adenocarcinoma (intramucosal or invasive) (n = 2) and well differentiated squamous cell carcinoma (n = 1) post-PDT. Non-neoplastic and 1 to 4 neoplastic targets were selected per biopsy. A panel of 15 allelic loss (LOH 1p,3p,5q,9p,9q,10q,17p) and microsatellite instability (MSI BAT 25,26) mutational markers were analyzed on each cellular target using PCR/automated electrophoresis. Mutational and morphologic features were correlated. Results: Six pre-PDT biopsies were analyzed; all neoplasms showed a high rate of allelic loss (mean, 4.1 alleles, 18 microdissected foci); the mean percentage of cells containing mutations was 70.6%. Five post-PDT biopsies were analyzed; recurrent neoplasms showed a similar rate of allelic loss (mean, 4.3 alleles, 15 microdissected foci); the mean percentage of cells containing mutations was 73.0%. Two patients had highly discordant mutational profiles when their pre and post-PDT samples were compared; two patients had similar mutations pre and post PDT, but new mutations were also noted. Of note, one patient had 3 pre-PDT biopsies and another had 2 post-PDT biopsies analyzed; concordant results were obtained within each set of biopsies. Conclusion: When esophageal neoplasms recur post-PDT, the development of a genetically distinct clone implies that a new neoplasm has formed, while the development of a genetically equivalent clone suggests that the pre-PDT clone may not have been completely eradicated and the original neoplasm has recurred. Comparison of the mutational fingerprint of damage in patients who have recurrent disease with those who have not recurred (study in progress) will help to discriminate between reactive and neoplastic epithelium in post-PDT biopsies and will be able to optimize additional courses of PDT.

W1702

EGFR Inhibition in Patients with Esophageal Cancer: Assessment of Reduced Cellular Proliferation and Gene Expression Analysis by Microarray

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Background: The development of esophageal adenocarcinoma is characterised by progression along the Barrett's metaplasia dysplasia adenocarcinoma sequence. The epidermal growth factor receptor, EGFR, shows increased expression along this sequence. Specific inhibitors of the EGFR receptor have been developed and are being trialed as a therapy for esophageal adenocarcinoma. There are currently no effective treatments for metastatic diease. We sought to investigate the effect of the inhibitor ZD1839 (Iressa) on adenocarcinoma cells in patients enrolled in a phase II clinical trial.
Aims: To investigate the effect of EGFR inhibition on cell proliferation and gene expression in patients with esophageal adenocarcinoma.
Methods: Patients with inoperable esophageal adenocarcinoma were recruited to the trial and received 500mg daily of ZD1839. Biopsies were obtained from patients before, and one month after treatment commenced. Two biopsies were embedded in paraffin for histological assessment and immunohistochemistry. RNA was extracted from two further biopsies.
Cellular proliferation was assayed by nuclear Ki67 staining as assessed by immunohistochemistry. Gene expression was assessed using cDNA microarray with the 3DNA array 350 expression analysis system.
Results: Twenty four patients with terminal cancer were recruited to the trial and three showed clear clinical responses. From these twenty four patients, seven matched pairs of biopsies were obtained. A significant reduction in the number of Ki67 positive nuclei following one month of treatment was seen in 5 of 7 cases ($p<0.05$). Preliminary analysis of the microarray shows there is no consistent change in the expression of key proteins involved in apoptosis (eg. Caspase3, Caspase5, Bcl2, Bclx). None of the conventional targets of EGFR signaling (eg. CyclinD1, COX2) showed significant alterations in gene expression. Full analysis of the gene array data will be presented at the conference.
Conclusions: EGFR inhibition by ZD1839 causes a reduction in cellular proliferation rates. EGFR may be acting by other novel pathways and microarray analysis will highlight the genes that are altered by this therapy.

W1703

Gene Expression Profiling Analysis Reveals Calgranulin A and B as Potential Novel Tumor Markers for High-grade Dysplasia in Barrett's Esophagus

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Background Patients with Barrett's esophagus (BE) have a 30-125 times increased risk of developing esophageal adenocarcinoma. Carcinoma develops as a multistep process in which malignant degeneration is preceded by dysplastic changes of the metaplastic mucosa. Tumor markers for high-grade dysplasia (HGD) could enhance the detection of early lesions in Barrett's esophagus. The objective of this study was to identify these markers.
Methods Jumbo biopsy samples from BE without dysplasia and with HGD were obtained from a 53-yr old male patient during routine endoscopy. Biopsies were divided in two equal parts. One part was evaluated for the presence or absence of dysplasia and the other part was used for microarray analysis, using the Clontech Atlas human 12K array. The mRNA expression pattern of the sample without dysplasia was compared to that of the HGD sample. Microarray results were confirmed by semi-quantitative RT-PCR in biopsies from patients with BE without dysplasia (n = 22) and with HGD (n = 5) obtained during routine endoscopy from 22 patients (13 male, 9 female, mean age 68.1 years).
Results In HGD, 866 genes showed >2-fold difference in mRNA levels compared to normal BE. Of these 866, 33 displayed a >10-fold difference. For these 33 genes, semi-quantitative RT-PCRs were performed. The mRNA levels were normalized to β-actin mRNA levels. Two genes were significantly upregulated in the HGD, calgranulin A (relative expression in normal BE 0.79 versus 1.75 in HGD, p = 0.017 Mann-Whitney U-test) and calgranulin B (relative expression: 0.83 versus 1.81, p = 0.022). For the other 31 genes, differences in mRNA levels between HGD and normal BE were present, but not significant.
Conclusion The inflammatory proteins calgranulin A and B are potential new tumormarkers for high-grade dysplasia in Barrett's esophagus. These proteins, also known as S100A8 and S100A9 calcium binding proteins, are both subunits of the calprotectin complex that is involved in inflammation in Crohn's disease. They may play a role in the development of a more malignant phenotype of Barrett's esophagus, a process in which inflammation may be involved.

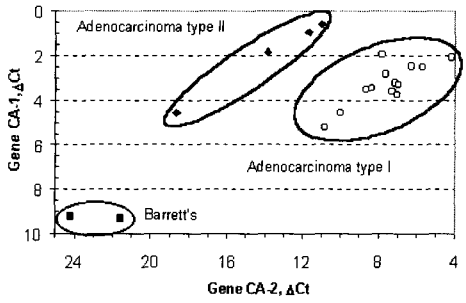
W1704

Real-Time RT-PCR Cluster Analysis from a 2-Gene Marker Panel Allows for Potential Molecular Diagnosis of Esophageal Adenocarcinoma

Michael Mitas, Kaidi Mikhitarian, David H. Robbins, Angela Collier, Peter King, David Cole, William E. Gillanders, Brenda Hoffman

While numerous reports suggest that Barretts esophagus (BE) is a precursor lesion for esophageal adenocarcinoma, not all patients with BE develop cancer. Surgical and endoscopic methods are available for erradicating BE epithelium but all have inherent risks. Understanding the molecular biology leading to malignant transformation in BE may lead to markers that identify high risk patients and allow early interventions that address this otherwise highly lethal neoplasm. In this study we used quantitative real-time RT-PCR and determined the expression levels of seven cancer-associated (CA) genes (1-7) in 17 endoscopically acquired adenocarcinomas of the esophagus and 2 BE samples. When expression levels of gene CA-1 were plotted as a function of gene CA-2, three unique clusters emerged (there is an inverse relationship between Ct value and gene expression, the axes are inverted for clarity). Two of the clusters were derived from adenocarcinoma samples (arbitrarily referred to as Type I and Type II), while two BE samples comprised a third cluster. For the respective adenocarcinoma clusters, the correlation between CA-1 and CA-2 expression levels were 0.996 and 0.651, providing evidence that expression of the two genes are coordinately

regulated. Although additional samples from BE and normal esophagus need analysis, our preliminary results suggest molecular techniques may distinguish premalignant changes from benign BE.



W1705

Effects of Aspirin and the Natural Cox-2 Inhibitor Quercetin on the Development of Esophageal Adenocarcinoma in a Rat Model

Andrew Hindmarsh, Edward Cheong, Virginia Sams, Lazlo Igali, Joanne Doleman, Nigel Belshaw, Elizabeth K. Lund, Michael Rhodes, Ian T. Johnson

BACKGROUND & AIMS: Non steroidal anti-inflammatory drugs have been widely reported to inhibit gastrointestinal neoplasia. These effects are mediated, at least in part, through COX-2 inhibition. We studied the effect of aspirin and the flavonoid quercetin, which is a natural inhibitor of COX-2, on the development of Barrett's esophagus (BE) and esophageal adenocarcinoma in a rat surgical model. METHOD: 48 male Sprague-Dawley rats were randomised to a chow containing quercetin or aspirin two wks before esophagojejunostomy, and continued on this diet after surgery. A further 105 male Sprague-Dawley rats were randomised to a chow containing quercetin, aspirin, or no treatment, 4 wks after esophagojejunostomy. Carcinogenesis was augmented with regular intra-muscular iron dextran. All animals were sacrificed 28 wks after surgery, and the esophagi examined for the presence of BE and adenocarcinoma. RESULTS: Animals in all dietary groups gained weight at similar rates. Aspirin significantly reduced the incidence of esophageal adenocarcinoma compared to controls (33% vs 60% $p < 0.03$). Fewer rats developed esophageal cancer in the quercetin treated group compared to controls (49% vs 60% NSD). The incidence of BE was similar in all groups. Within groups, no significant differences were observed in the incidence of esophageal cancer between rats given the test diets before or after surgery. CONCLUSION: Aspirin inhibits the development of esophageal adenocarcinoma induced by reflux in a rat model. No additional reduction in cancer incidence is observed if treatment is commenced prior to inception of reflux disease. We have not demonstrated a significant anti-cancer effect with quercetin, although fewer rats developed esophageal adenocarcinoma when treated with quercetin compared to controls. Further studies on the chemopreventive effects of natural COX-2 inhibitors seem warranted.

W1706

Cyclin D1 Polymorphism (G870A) and Risk for Gastroesophageal Reflux, Barrett's Esophagus and Esophageal Adenocarcinoma: A Case-Control Study

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Purpose: To investigate individual susceptibility to gastroesophageal reflux disease (GERD), Barrett's esophagus (BE) and primary esophageal adenocarcinoma (EADC), we studied the frequency of a polymorphism (G870A) in exon 4 of the cyclin D1 gene (CCND1), a cell cycle and DNA repair-associated gene recently implicated in esophageal carcinogenesis. Methods: Between 02/2001 and 02/2003, a total of 431 individuals were enrolled in a case-control study to evaluate lifestyle risk factors and molecular alterations in GERD (n = 142), BE (n = 130), and EADC (n = 57), defined according to strict clinicopathologic criteria. Controls comprised 102 healthy, asymptomatic individuals from the same geographic region. Blood samples were obtained, with informed consent, and genomic DNA successfully extracted from patients with GERD (n = 126), BE (n = 125), EADC (n = 56) and controls (n = 95). Polymerase chain reaction (PCR) was used to amplify exon 4 of CCND1 and after digestion with BsrI, acrylamide gel electrophoresis was used to identify wild-type and the common G870A polymorphic allele. The frequency of alleles (GG, GA, AA) was compared between cases (GERD, BE, EADC) and controls using logistic regression analysis to calculate age and gender adjusted odds ratios (OR) and 95% confidence intervals (CI). Results: The frequencies of each genotype are shown in Table 1. Compared to controls, only small differences were found for the GA genotype in patients with GERD (OR 1.33, CI 0.73-2.43), BE (OR 1.39, CI 0.76-2.54), and EADC (OR 1.37, CI 0.57-3.26). However, large and statistically significant increasing frequencies were seen for the AA genotype in patients with GERD (OR 2.83, CI 1.09-7.34), BE (OR 3.69, CI 1.46-9.29), and EADC (OR 5.99, CI 1.89-18.96). Conclusions: We conclude that the CCND1 polymorphism G870A is associated with increased risk for GERD, BE and EADC in this population. The contribution of this polymorphism to individual susceptibility for progression to EADC suggests potential clinical application in Barrett's surveillance programs.

Table 1: CCND1 genotype frequencies

Genotype	Controls (n=95)	GERD (n=126)	BE (n=125)	EADC (n=56)
GG (wild-type)	35 (37%)	34 (27%)	32 (26%)	12 (22%)
GA	52 (55%)	70 (56%)	66 (53%)	27 (48%)
AA	8 (8%)	22 (17%)	27 (21%)	17 (30%)

W1707

Polymorphism in Exon 4 of the p53 Gene in Barrett Metaplasia: Increased Frequency of the Pro/Pro Genotype in Hispanics

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Alterations in the p53 gene play an important role in malignant progression in Barrett metaplasia (BM). Although Exon 4 of the p53 gene encodes a region of the p53 protein that is important in DNA binding and apoptosis, Exon 4 alterations have not been studied in BM. DNA was sequenced to detect exon 4 alterations from microdissected sections of formalin-fixed and paraffin-embedded tissue obtained by endoscopic esophageal biopsy from 181 consecutive cases of BM. Genotype of codon 72 was examined by sequencing. Only one exon 4 mutation (missense) was detected, however codon 72 polymorphism was frequent. The genotype frequency was arg/arg (54%), arg/pro (38%), and pro/pro (8%). There were no association between the genotype and gender. However, the pro/pro genotype was more significantly associated with the Hispanic ethnicity (28%) than the either the arg/arg (4%) or arg/pro (6%) genotypes ($p = 0.0021$ and $p = 0.02$, respectively). We conclude that polymorphism in codon 72 of exon 4 of the p53 gene is common in BM, and that the pro/pro genotype is significantly associated with Hispanic ethnicity. Prospective follow-up studies to determine whether the three genotypes are associated with distinct risks for the development of esophageal adenocarcinoma are currently in progress in our laboratory. Supported by National Institutes of Health grant R01 CA81570-02, and in part by Ethicon/Janssen.

W1708

Cell Cycle Phase Distribution in Neoplastic Progression of Barrett'S Oesophagus

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Introduction: The loss of proliferative control is a feature common to all cancers. This may result from abnormal cell cycle entry or by rapid transition through the cell cycle. It has been demonstrated that there is an increased proliferation index as the Barrett's oesophageal (BE) mucosa progresses through the metaplasia-dysplasia-adenocarcinoma sequence. Understanding of the cell cycle dynamics (entry and distribution) in BE could shed light on the basic abnormalities leading to uncontrolled proliferation and cancer progression. The aim of this study was to determine whether the increased proliferation associated with progression of BE could be attributed to abnormalities in cell cycle dynamics. Methods: Paraffin embedded sections from 35 BE, 26 BE with low grade dysplasia, 11 BE with high grade dysplasia, 16 invasive adenocarcinoma, 10 duodenum and 20 gastric antrum were immunostained for a novel proliferation marker, mini-chromosome maintenance protein 2 (Mcm2), and Ki-67. The same slides were stained for putative cell cycle phase markers, cyclin D1 for late G1 phase, cyclin A for S, G2 and M phases, cytoplasmic cyclin B1 for G2 phase and phosphorylated histone H3 (pH3) for M phase. Results: The proliferation levels of non-dysplastic BE (29.9% ± 2.5) were similar to the other normal columnar tissues, gastric antrum (25.2% ± 2.5) and duodenum (34.7% ± 1.6) when stained with Mcm2 (similar results were obtained with Ki-67). Proliferation increased as BE progressed to dysplasia and cancer (Mcm2, $p<0.0001$; Ki-67, $p<0.001$) through expansion of the proliferative compartment towards the surface. There was a correlation between Mcm2, Ki-67, cyclin A and cyclin B1 expression levels and the degree of dysplasia ($p<0.001$). The expression levels of pH3 increased with progression but statistical significance was not reached. No clear trend was seen in cyclin D1 expression. When expressed as a percentage of the proliferating cells, the expression levels of cyclin D1, cyclin A, cyclin B1 and pH3 were constant as BE progresses to cancer. Conclusions: By immunohistochemical criteria non-dysplastic BE was not hyperproliferative when compared to other normal columnar mucosa. Proliferation increased during progression to dysplasia and cancer. However the distribution of the cell cycle phases is conserved throughout the metaplasia-dysplasia-carcinoma sequence. Therefore, the increase in proliferation as BE progresses to cancer is likely to be due to abnormal cell cycle entry rather than abnormal cell cycle phase distribution.

W1709

The Oesophageal Lumen Has the Chemical Conditions for Generating Carcinogenic N-Nitroso Compounds

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BACKGROUND: Bacteria in the oral cavity reduce 25% of the nitrate, absorbed from the diet and secreted into the mouth by the salivary glands to nitrite. Nitrite can be converted to carcinogenic N-nitroso compounds (i) by bacteria at neutral pH and (ii) non-bacterially at acidic pH catalysed by thiocyanate (SCN) which is also secreted in saliva. The concentrations of the chemicals relevant to N-nitroso compound generation have been studied previously in the mouth and the stomach but not in the nasal cavity, pharynx or different regions of the oesophagus. AIM: To study the concentrations of the chemicals relevant to N- nitrosation in the nasal cavity, pharynx, proximal and distal oesophagus under fasting conditions and following ingestion of nitrate. METHODS: Seven healthy volunteers were studied. A microdialysis probe was positioned at each of the four anatomical locations and samples collected for 40mins under fasting conditions for analysis of nitrite, nitrate and thiocyanate (SCN). Similar samples were obtained after intragastric instillation of 2mmol nitrate which is equivalent to that consumed in a portion of salad. RESULTS: No nitrite was detected in the secretions of the nasal cavity before or after nitrate administration. Moderate concentrations of nitrate, nitrite and thiocyanate were present in the pharynx and

high concentrations of each of these chemicals in the mid and distal oesophagus. Following nitrate administration there was a marked rise in the concentration of nitrate and nitrite in the pharynx and oesophagus. The concentration of nitrite and thiocyanate in the mid and distal oesophagus are equivalent to those in the mouth (Gastroenterology, 1999; 116: 813-822).

	Nitrate		Nitrite		SCN	
	Fasting	Post Nitrate	Fasting	Post Nitrate	Fasting	Post Nitrate
Nasal cavity	13 (7)	46 (9)	0 (0.1)	0 (0)	89 (15)	82 (14)
Pharynx	54 (7)	307 (70)	11 (6)	141 (61)	448 (154)	481 (147)
Mid oesophagus	97 (10)	817 (225)	31 (9)	278 (94)	758 (225)	826 (230)
Distal oesophagus	90 (11)	732 (214)	31 (8)	270 (98)	728 (222)	758 (233)

All values are umol/l presented as mean (SD)

W1710

Gene Expression in Early Barretts Esophagus Related Cancers

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Early cancers in Barretts mucosa are increasingly recognized. Endoscopic therapy has been utilized for early cancers using techniques such as endoscopic mucosal resection (EMR) but adjunctive therapies are often needed for the remaining Barretts mucosa.
Aim: To determine if there are differentially expressed genes in early cancers from Barretts mucosa. Methods: Patients that presented to our Barretts Esophagus Unit were evaluated with endoscopic ultrasound examination with a radial instrument scanning at 7.5 and 30 Mhz. Any regional lymph nodes were targeted for fine needle aspiration. Early cancers were defined as confined to the mucosa based on EMR of the lesion. Specimens were reviewed by two experienced pathologists to determine margins and depth. Tissue was obtained from the cancer, nearby Barretts mucosa without cancer, normal squamous mucosa, and gastric mucosa and flash frozen. These specimens were used for gene expression arrays that targeted known pathways of cell growth, apoptosis, metastasis, and angiogenesis. These included the c-erb-b2 family, growth factors, Ras family of oncogenes, p21, catenin/cadherin pathway, bcl-2 pathway, COX 1 and 2, cyclin D1, matrix metalloproteinases, integrins, MAPK, MEK, and angiogenesis factors. We also obtained tissue from patients with Barretts esophagus and high-grade dysplasia without cancer and from Barretts mucosa without dysplasia to serve as controls.
Results: We found that the Barretts mucosa surrounding early cancers expressed similar genetic profiles as those of the cancer. We found that COX-1 was increased in the squamous mucosa. COX-2 was overexpressed in the superficial cancers and not in either the squamous mucosa or Barretts mucosa without cancer. IL-8 was increased in Barretts esophagus, which could be the result of induction, by COX-2. E-cadherin also was found to be increasingly expressed from non-dysplastic Barretts mucosa to cancer. Specific collagenases were found to be over expressed in tumors as compared to dysplastic Barretts esophagus indicating that tumor invasion could be identified by expression of specific MMPs. Cell growth and angiogenesis factors were expressed similarly in both dysplastic and non-dysplastic Barretts mucosa.
Conclusions: Gene expression in early esophageal cancers is similar to that of surrounding histologically non-cancer containing Barretts mucosa. Secondary chemoprevention using COX-2 selective agents might be of value in decreasing cancer formation after localized treatment for early Barretts cancers.

W1711

Curcumin, a Natural Agent Found in Turmeric, Induces Growth Inhibition of Barrett's Associated Esophageal Adenocarcinoma Cells

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Purpose: Curcumin (diferuloylmethane), the bright yellow dietary pigment, derived from the rhizomes of the popular spice, turmeric, has been recently shown to have anti-neoplastic, anti-oxidant, and anti-inflammatory properties with potent chemopreventive activity against a wide range of cancer cell lines and tumors. Paradoxically, curcumin is present in Asian curries that are often felt to predispose to gastroesophageal reflux disease, which is often complicated by development of pre-malignant Barrett's metaplasia. We examined the anti-cancer effect of curcumin on Barrett's associated esophageal adenocarcinoma cells. Methods: Barrett's associated human esophageal adenocarcinoma cells, SEG-1, were treated with various concentrations of curcumin while untreated cells served as control. Cell survival, colony formation, and mode of cell death after curcumin treatment was examined. Results: Treatment with 50, 100, and 150 microM curcumin caused complete cell death at 48 hours. A marked arrest of cell proliferation was also observed in SEG-1 cells treated with lower concentrations of curcumin. Fifty percent SEG-1 cells were killed by 20 and 30 microM curcumin in a period of 7 days. By 3 weeks, all cells treated with 40 microM curcumin and 90% cells treated with 30 microM curcumin were growth inhibited. Dramatic reduction in colony size and number as compared to controls was seen at 1 and 3 weeks after curcumin treatment. Curcumin treatment resulted in induction of early apoptosis in SEG-1 cells as detected by Annexin V labeling at 48 hours. Control cells stained negative for apoptosis and necrosis. Conclusions: We demonstrate that curcumin treatment causes suppression of colony formation and proliferation arrest of Barrett's associated human esophageal adenocarcinoma cells by induction of apoptosis, making this common household spice, a potential chemotherapeutic agent in the treatment of esophageal adenocarcinoma.

W1712

MUC4 Is Increased in High-grade Dysplasia in Barrett's Esophagus and Is Associated with a Pro-apoptotic Bax/Bcl-2 Ratio

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Background MUC4 is a member of the membrane bound mucin family that protects the mucosa from damage by foreign bodies. It also acts as ligand for the receptor tyrosine kinase ErbB2 and has been reported to repress apoptosis. MUC4 is upregulated in several tumor types. The objective of this study was to determine the MUC4 mRNA level during the course of neoplastic progression of Barrett's esophagus and determine the association with the mRNA levels of pro-apoptotic Bax and anti-apoptotic Bcl-2.
Methods 95 Samples from 37 patients diagnosed with a Barrett's esophagus (mean age 65 years, 20 male and 17 female, mean length of Barrett's segment 5.7 cm) were obtained during routine endoscopy from esophageal squamous epithelium (n = 37), Barrett's epithelium without dysplasia (BE, n = 37), Barrett's epithelium with high-grade dysplasia (HGD, n = 6) and adenocarcinoma (ADC, n = 15). Histological evaluation was performed independently by two experienced pathologists. The mRNA levels of MUC4, Bax and Bcl-2, and as a reference β-actin, were determined by semi-quantitative RT-PCR.
Results The MUC4 mRNA level was significantly lower in BE than in normal squamous epithelium (mRNA level relative to β-actin 0.63 in BE versus 1.08 in squamous epithelium, p = 0.01), whereas levels were significantly elevated in HGD (relative mRNA level 1.27) compared to BE (p = 0.037). In ADC the MUC4 mRNA level remained high. An association between MUC4 and inhibition of apoptosis was not found. In contrast, in HGD the Bax/Bcl-2 ratio showed a two-fold increase compared to BE (ratio 1.71 versus 0.83, p = 0.04).
Conclusion The MUC4 mRNA level is elevated in Barrett's esophagus with HGD and in ADC. In contrast to animal studies in which MUC4 overexpression was associated with decreased apoptosis, upregulation of MUC4 in Barrett's esophagus with HGD is associated with increased apoptosis. This indicates that MUC4 has only a minor role in regulation of apoptosis in Barrett's esophagus with high-grade dysplasia.

W1713

Association of Interleukin 1 Beta Gene -511 Single Nucleotide Polymorphism with Esophageal Cancer

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Purpose: To estimate the distribution of IL-1B gene -511 C - T biallelic single nucleotide polymorphism (snp) in patients with cancer of the esophagus and a control population of Irish origin. Methods: In a retrospective case-control study design we selected 216 cases with cancer of the esophagus, and 223 controls. DNA was extracted and genotyping was performed using PCR-RFLP and TAQMAN chemistry. The study had local IRB approval and informed consent was obtained from all study subjects. Results: The distribution of CC homozygotes (53.2% in cases, 35.9% in controls) and CT heterozygotes (32.9% in cases, 51.6% in controls) was significantly different in the two study groups ($p<0.001$). In squamous cell cancer ($n=88$), a significant difference ($p<0.001$) was found in CC homozygotes (59.1% in cases, 35.9% in controls) and CT heterozygotes (26.1% in cases, 51.6% in controls). For adenocarcinoma ($n=117$), a significant difference between the distribution of alleles in controls versus cases was observed ($p=0.04$). This was reflected only in the male patients ($n=86$, $p=0.008$, CC homozygotes 54.7% in cases vs. 35.8% in controls and CT heterozygotes 33.7% in cases vs. 51.6% in controls), with no difference in female adenocarcinoma cases ($p=0.579$). Conclusion: An acidic pH in gastroesophageal reflux disease may trigger inflammation and metaplasia in the esophagus. Interleukin 1 beta (IL-1B) is a potent suppressor of gastric acid secretion, and the T allele of IL-1B gene -511 snp is associated with higher levels of IL-1B. We have found a significantly higher frequency of C allele in esophageal cancer patients as compared with the control population. The C allele, in comparison with the T allele, may be associated with lower levels of IL-1B production and more acid secretion, and we would suggest that this mechanism may link the existing data with the cancer risk.

W1714

Human Papillomavirus-16 and -18 DNA and Epstein-Bar Virus DNA in Esophageal Squamous Cell Carcinoma

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Background/objectives: Esophageal squamous cell carcinoma (ESCC) demonstrates wide regional variation in incidence and causal associations. An association between viral infection and the development of esophageal carcinoma has been reported, particularly the human papilloma virus (HPV) and Esptein-Bar virus (EBV). However, geographic variation in carcinogenesis is realized. The aim of this study was to determine whether HPV and EBV associate with ESCC, one of the most common malignancies in Iran. Materials and methods: To elucidate the association of HPV and EBV infection with ESCC, 120 consecutive samples of paraffin-embedded surgically resected specimens were gathered from a non-high-incidence of Iran, Tehran, and the DNAs were extracted from the specimens. Fragments of HFE gene that served as internal controls were successfully amplified from 94 of 120 specimens. Two sets of type specific primers for HPV -16 and -18 and 1 set of primer for EBV genome used to amplify HPV and EBV DNA sequences in the tumor samples using polymerase chain reactions (PCR). Results: Our PCR analysis detected no specific HPV-16 and -18 or EBV DNA in ESCC specimens. Conclusion: The results of our study suggest that HPV-16, HPV-18 and EBV are not generally associated with esophageal squamous cell carcinoma.

W1715

In situ Demonstration of Reduced Monocyte Migration in Mouse Pancreatic Cancer by Intravital Microscope

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BACKGROUND: Most cases of pancreatic cancer are inoperable when diagnosed. In the processes of the expansion or invasion of cancer, the escape of cancer cells from host immune systems has been supposed to be one of the possible mechanisms. Although the reduction of an infiltration of host antigen presenting cells (APCs) such as dendritic cells (DCs) has been reported, migration of monocytes as one of the precursors of DCs in cancer tissues has not been fully understood in vivo. Therefore, in this study, we demonstrate in vivo migration of monocyte in mouse orthotopic pancreatic cancer and examined the effects of immunotherapy by DCs as a supplement of infiltrated APCs. METHODS: 3x105 mouse pancreatic adenocarcinoma cells (pan02 originated fromC57/BL6) were orthotopically inoculated in C57/BL6 mice 4 weeks of age. Tumor take ratio was 100% at 6 weeks. Monocytes were isolated as CD14 positive cells using MACS. After labeling with CSFE, 1x106 monocytes were injected via tail vein and dynamic behavior of labeled monocytes were observed under intravital microscope. We quantified rolling and adherent monocytes in tumor vessels and compared with those in peritumor area and normal pancreas at 6 weeks after cancer cell inoculation. In addition, we investigated the effects of immunotherapy by DCs with or without tumor-lysate pulsation (control, TL-, TL+) and compared tumor volume, vascular density, and vessel diameter, and infiltrated lymphocytes among control, TL-, and TL+. RESULTS: Monocyte-endothelial cell interactions (rolling and adherence) were significantly decreased in tumors compared with those in peritumor area and surrounding normal pancreas. Immunotherapy by DCs attenuated tumor volume and tumor vascular density both in +TL and in -TL compared with that in control at 6 weeks. In addition, infiltrated CD4 and CD8 positive lymphocytes were significantly increased in +TL group compared with and -TL group. CONCLUSION: In situ monocyte migration in mouse orthotopic pancreatic cancer was demonstrated using intravital microscope. Data suggested that he reduced monocyte-endothelial cell interactions may be one of the possible mechanisms of the escape of cancer cells from host immune systems. The immunotherapy with tumor-lysate pulsed DCs was effective for mouse pancreatic cancer possibly by the suppression of tumor angiogenesis and induction of lymphocyte infiltration.

W1716

Implication of Chromosome 18 Abnormalities in the Progress of Pancreatic Cancer

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The events that mediate tumor progression in pancreatic carcinoma are still poorly understood to date. Cytogenetic, allelotype, and somatic cell hybrid studies in human pancreatic adenocarcinoma have suggested that chromosome 18 may carry tumor suppressor genes (TSG) including *SMAD4*. We previously identified that LOH of 18q at the *SMAD4* locus, along with LOHs on 17p and 12q, positively associated with poor prognosis of pancreatic cancer patients. However, introduction of the *SMAD4* gene did not suppress the growth of pancreatic cancer cells that harbor homozygous deletion of this gene. IPMT (intraductal papillary-mucinous tumor) is thought to be one of the premalignant lesions of the pancreas, which would transform into carcinomas. Although there were frequent LOH (7/14, 50%) at the *SMAD4* locus in IPMT samples, *SMAD4* protein was observed in tumor cells immunohistochemically, and no mutations of the *SMAD4* gene were observed, suggesting the existence of TSG in 18q, other than *SMAD4*, that suppresses cell growth. To functionally assess the activity of chromosome 18 in pancreatic cancer, we introduced it as a normal copy into some pancreatic ductal carcinoma cells with and without completely inactivated *SMAD4*. In this study, *in vitro* growth of the hybrid cells was significantly suppressed compared with the parental cells, regardless of the initial *SMAD4* status. To estimate the metastatic ability of the hybrids, we used a lung colonisation model. At the end of experiment, we recorded a significant suppression in the number of surface metastases developing in mice injected with hybrids as compared with those injected with parental cells. In order to identify and characterize gene(s) that is involved in the progress of pancreatic cancer, we used microarray expression analysis using 20k oligo-array system. It is revealed that the expression of 4 genes relating to apoptosis was increased in the 18 chromosome hybrids cells as compared with the parental cells. We are now analyzing the function of these genes.

W1717

ZD1839 (Iressa), a Selective Epidermal Growth Factor Receptor Tyrosine Kinase Inhibitor, Inhibits Pancreatic Cancer Cell Growth, Invasion, and Colony Formation

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Background: Pancreatic cancer is a devastating malignancy, characterized by low responsiveness to conventional chemotherapies. ZD1839 is a tyrosine kinase inhibitor of the epidermal growth factor receptor (EGFR) that has shown clinical activity against EGFR-expressing tumors. Since pancreatic cancers frequently overexpress EGFR and its ligands, our aim was to investigate the potential role of ZD1839 in this disease. Methods: The GI50 of ZD1839 as well as the effects of ZD1839 on growth factor actions in pancreatic cancer cell lines was analyzed using MTT assays. FACS analysis using Annexin and PI staining was performed to study cell cycle, apoptosis, and cell death. Western blot analysis was carried out to investigate EGFR expression levels in pancreatic cancer cell lines, as well as MAP kinase and EGFR phosphorylation. Soft agar assays were used to measure colony formations. Invasiveness of cancer cells was analyzed using Matrigel coated filters. Results: ZD1839 inhibited cell proliferation of pancreatic cancer cell lines with GI50 concentrations ranging from 2.5 to over 10microM. ZD1839 completely inhibited EGF induced cell proliferation, but did not significantly influence IGF induced mitogenesis. ZD1839 also completely abolished EGF induced phosphorylation of EGFR and MAP kinase. Furthermore, ZD1839 inhibited basal and EGF induced anchorage-independent cell growth and invasion. Conclusion: Our data demonstrate that ZD1839 inhibits pancreatic cancer cell growth through EGFR dependent pathways. ZD1839 also inhibits anchorage-independent growth and invasiveness, suggesting that ZD1839 may offer a new approach for the treatment of pancreatic cancer.

W1718

Human Insulin-Like Growth Factor Binding Protein 3 (IGFBP-3) Gene Transfer Dramatically Inhibits the Growth of Established Colonic Tumors in a Murine Model

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IGFBP-3 regulates the growth of various tumor cell lines. We have found that recombinant human IGFBP-3 (rhIGFBP-3) inhibits the growth of several human colon cancer cell lines as well as murine CT26 colon tumors. The in vivo effect of exogenous rhIGFBP-3 is restricted because of its susceptibility to proteolysis. The aim of this research was to determine the impact of IGFBP-3 gene transfer on the in vivo growth of experimental colon tumors. Methods: A genetically modified attenuated salmonella typhimurium (VNP20009), shown to inhibit tumor growth, was the delivery vehicle. An empty vector (pRC/CMV) or a plasmid containing IGFBP-3 cDNA (pRC/CMV-IGFBP-3) were used for transformation. Tumors were established in female BALB/c mice via s/c injections with syngeneic CT26 colon cancer cells. Two weeks after establishment of tumors, mice were randomized into 4 groups that received peri-tumor injections of either: 1) solvent alone (control group) 2) VNP20009 alone, 3) VNP20009 carrying the empty pRC/CMV vector (pRC/CMV group) or 4) VNP20009 harboring pRC/CMV-IGFBP-3 (IGFBP-3 group). Two weeks later the mice were sacrificed, their tumors weighed and IGFBP-3 expression evaluated by flow cytometry following the intracellular staining of tumor cells with an IGFBP-3 antibody. Results: IGFBP-3 protein was expressed in tumor cells in the IGFBP-3 group only. As previously shown, VNP20009 inhibited tumor growth (mean size 362+/-404 mg) when compared to controls (1362+/-1011 mg, $p<0.01$). In regards to the VNP20009 group, the tumor mass was significantly smaller in the IGFBP-3 group, 18+/-44 mg ($p<0.01$) but not in the pRC/CMV group, 99+/-81 mg. The difference between the IGFBP-3 and the pRC/CMV groups was also significant ($p<0.03$). Conclusions: IGFBP-3 DNA transfer via modified salmonella inhibits the growth of murine colon carcinoma significantly more so than the Salmonella alone. More studies are needed to determine the duration of the effect and to identify why the empty vector inhibited tumor growth.

W1719

Functional Isolation of Pancreatic Cancer Tumor Suppressor Activity at 12q21-23

Young-Mi Park, Ryan George, Kay Pogue-Geile

Purpose: The purpose of this work is to isolate novel, functional pancreatic cancer-tumor suppressor (TS) gene(s) located at 12q21-23. This region represents one of the most frequently deleted genomic regions in pancreatic cancer for which no known TS gene has been identified. This region has been finely mapped to two minimal regions of deletion at 12q21.31-.32 and at 12q23.3 (Kimura et al. Cancer Research 58:2456-60, 1998). Methods: To achieve this goal we have preformed genetic complementation studies, using yeast artificial chromosomes (YACs). YACs contain large regions of human DNA (up to 2 Mb) which eliminates the need for subcloning because all of the signals required for gene expression are likely to be included in a single clone. This allows for the genes to be expressed at physiological levels and may provide a better understanding of gene function, than genes introduced with artificial control signals. To carry out this study: 1) a positive control for TS activity (Y807E4-containing the p16 gene) and YACs that map to the two minimal regions of LOH at 12q were retrofitted with a neomycin resistance gene (NeoR), a mammalian cell selectable marker. 2) Panc-1 and A9 cells were fused to yeast cells containing YACs. 3) NeoR resistant colonies were selected. 4) To help in the identification of potential TS genes we made a custom cDNA composed of 28 genes localized to this region. Results: Evidence for TS activity at 12q21.31-32 include 1) The inability to isolate NeoR colonies when YAC 790F8 was fused with A9 cells and 2) Three A9 NeoR colonies were isolated with YAC 909H1 but all three showed selective loss of human DNA based on microsatellite analysis. Evidence for TS activity in region 12q23.3 is the observation that two NeoR colonies were isolated with fusions with a YAC which contains only a portion of 12q23.3 (721G9), but not with 948E9, which contains all of region 12q23.3. In parallel we analyzed gene expression pattern of 28 genes that had been localized to this region of the genome. We found that stabilin-2, which is localized to 23q23.3 showed significant down regulation in pancreatic cancer. Electronic mapping places stabilin-2 in the YAC with tumor suppressor activity (Y909H1) but not in the YAC with no tumor suppressor activity (Y721G9). This provides additional support for possibility that stabilin-2 is a tumor suppressor. Conclusions: Functional evidence for tumor suppressor activity at 12q21.31-.32 and at 12q23.3 was detected. Stabilin-2 is a potential candidate for tumor suppressor activity at 12q23.3.

W1720

Gene Expression Profiling of Periampullary Carcinomas: Why Is There Such Heterogeneity in Survival?

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Periampullary carcinomas arise within 2 cm of the major duodenal papilla and comprise carcinomas of the ampulla, distal common bile duct, pancreas, and duodenum. Though clinical features, anatomic locations, and therapeutic approaches are similar, long-term outcomes vary. Overall survival after pancreaticoduodenectomy is highest for patients with ampullary and duodenal cancers, intermediate for patients with bile duct cancer, and lowest for patients with pancreatic cancer. Moreover, survival for each tumor stage is greater for nonpancreatic periampullary cancers than for pancreatic cancers. Recent data suggest that

inherent differences in tumor biology rather than embryologic, anatomic, or histologic features probably account for differences in survival. The aim of this study was to compare the gene expression profiles of the three tumors with the goal of identifying genes that can predict or provide insights into the differences in clinical outcome. Frozen tumor and normal adjacent tissues were collected from 64 pancreaticoduodenectomy specimens at Samsung Medical Center, Seoul, Korea. Total RNA was extracted using Trizol (Invitrogen), and amplified mRNA was isolated from total RNA by the MessageAmpTM aRNA kit (Ambion). We used a cDNA microarray containing 44,500 cDNA clones, representing 30,300 unique genes. Unsupervised hierarchical clustering revealed two major clusters. The first contained periampullary carcinomas (Ampulla of Vater cancer, distal common bile duct cancer, pancreatic head cancer), and the second contained of normal adjacent tissues. The Ampulla of Vater cancer specimens clustered separately from pancreatic cancer specimens, but we found intermingling on the dendogram between distal common bile duct cancers, Ampulla of Vater cancers, and pancreatic head cancers. Significance of microarray (SAM) and prediction analysis for microarray (PAM) programs revealed serine protease inhibitor Kazal type 4 (SPINK 4), BCL2-associated athanogene (BAG 1), and mucin 2 intestinal/tracheal (MUC 2) genes were significantly overexpressed in Ampulla of Vater cancers relative to distal common bile duct and pancreatic head cancers. The data suggest that overexpression of BAG 1 and MUC 2 in Ampulla of Vater cancers are potential markers for the favorable prognosis of these tumors.

W1721

Marker Development for Early Detection of Pancreatic Cancer

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Purpose: Identification of gene expression patterns diagnostic and prognostic for specific pancreatic pathologies could aid in early diagnosis and treatment of pancreatic cancer. Methods: We have developed the Pittsburgh Pancreas gene-Enriched ARray, PittPEAR, - composed of 5,763 pancreas and pancreatic cancer expressed genes. In an effort to identify critical genes whose expression is changed early in pancreatic cancer development, we have profiled gene expression in pancreatic adenocarcinoma and in pre neoplastic tissues. The sources of pre neoplastic tissues are normal adjacent tissue (pancreatic tissue adjacent to pancreatic adenocarcinoma but with normal histology) and a pancreas tissue with PanIN-3 lesions. The PanIN-3 tissue was unique in that it came from an individual from Family X, 70% of who develop pancreatic cancer due to a susceptibility locus that we mapped to 4q32-34. The genetic background of this tissue makes it virtually certain that these PanIN-3 lesions would have developed into cancer. Results: We generated 3 lists of the most significantly differentially expressed genes by comparing pancreatic adenocarcinoma, normal adjacent tissue and a PanIN-3-Family X tissue to donor normal pancreas tissue. Comparison of the gene list from pancreatic adenocarcinoma to the normal adjacent and preneoplastic lists has revealed considerable overlap. The most significantly differentially expressed genes were similar when pancreatic cancer and when normal adjacent tissues were compared to the donor normal pancreas. At least 50% of these genes were identical in the two lists although the rank ordering of significance varies somewhat between the two lists. This supports the hypothesis that normal adjacent tissue has an expression pattern that is similar to the adjacent cancer even though the tissue appears pathologically normal. In addition we have identified the most significantly differentially expressed genes when a pancreas tissue with PanIN-3 lesions from Family X was compared to a donor normal pancreas. Some of the genes that were over expressed in all 3 data sets include ribosomal proteins S7, and S27a, nucleolin, villin 1, EST 36618, DMX-like 1 gene, and transcription factor-like 1. Conclusions: Normal adjacent tissue contains many gene expression changes indicative of the neighboring cancer. Numerous pancreatic cancer over expressed genes were also over expressed in a PanIN-3 tissue and in pathologically normal tissue adjacent to tumor. Such genes may be useful as markers for the early detection of pancreatic cancer.

W1722

Pancreatic Cancer Associated Gene Expression from FNA Samples Differentiates Neoplastic Pancreatic Masses from Normal Pancreas and Chronic Pancreatitis

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Background: Detection of pancreatic cancer is difficult and imaging studies have trouble distinguishing between cancer and chronic pancreatitis. In the current study we hypothesized that the expression level of specific pancreatic cancer genes (SPC-1,-2,-3) can be detected by Q-PCR from samples taken using ultrasound-guided fine needle aspiration (EUS-FNA) and can accurately distinguish chronic pancreatitis from pancreatic cancer. Methods: All samples were analyzed using Taqman probes in Q-RT-PCR. Microdissected frozen samples from pancreatic adenocarcinoma (5), normal pancreas (5) and chronic pancreatitis (5) served as a training set. For analysis of the test set, a diagnostic cut-off value was chosen as the mean plus one SE of the chronic pancreatitis training set values. Samples taken with EUS aspiration needles from resected pancreatic lesions were used as the test set. Ribosomal protein S6 was used as an internal control. Results: All three biomarker genes showed diagnostic levels of expression in all pancreatic adenocarcinoma specimens (6). Furthermore, these biomarkers were elevated in other pancreatic neoplasms including mucinous cystadenoma (2), mucinous cystadenocarcinoma (1), and ampullary adenocarcinoma (1). In contrast, the levels of these biomarkers were not elevated in any non-neoplastic condition, including chronic pancreatitis (1) and normal pancreas (2). In samples from diseases requiring surgery, SPC-1, SPC-2 and SPC-3 levels ranged from 2 to30-fold (mean=8), 5 to 1897-fold (mean=455), and 1 to 40-fold (mean=16) the cut-off level, respectively. Whereas, in samples from conditions not requiring surgery SPC-1, SPC-2, and SPC-3 levels ranged from 0.0006 to 0.022-fold (mean=0.086), 0.002 to 0.09-fold (mean=0.059), and 0.0001 to 0.087-fold (mean=0.031), the cut-off level, respectively. There was no overlap in values between these two groups. Conclusion: The selected biomarkers accurately distinguished all cases of pancreatic cancer. Furthermore, the biomarkers also predicted those conditions which would require surgical intervention. Based on these preliminary studies, measurement of these biomarkers in samples from in vivo EUS-FNA may be useful in the management of patients with indeterminate pancreatic lesions.

W1723

The Long-Term Impact of Deglutitive Aspiration in Acute Stroke: A 7 Year Prospective Study

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Background: Dysphagia after stroke is a major clinical problem, however, the association between oropharyngeal dysphagia, aspiration pneumonia and long-term outcome remains unclear. The aim of this study was to define the precise relationship between aspiration and clinical outcome after hemispheric stroke. Methods: A prospective cohort was assessed using videofluoroscopy within 14 days of stroke, aspiration severity was determined from 6 boluses using a validated Penetration-Aspiration Scale. Lesion location, volume of stroke injury, motor disability, and cognitive status were also recorded. Pneumonia incidence and mortality were reviewed annually for 7 years. Results: 99 patients (56 male, mean age 70 years, range 29-92 years, 52 non-aspirators) were followed up. Regardless of lesion location, volume and motor disability scores, aspirating patients developed more pneumonia (22 vs.15, $p=0.01$) and serial episodes of pneumonia than their non-aspirative peers (14 vs. 6 cases, $p=0.03$). The time to development of the first pneumonia, however was similar (aspirating 10+/-2 vs. non-aspirating 13.5+/-3.7 days, $p=0.5$). Critically, survival analysis indicated that aspiration was associated with a dramatic increase in mortality, but only in the first 6 months ($p=0.002$). Longer-term data showed that after this crucial period, mortality between groups became similar. Conclusions: Aspiration after stroke is a major risk factor for pneumonia, serial pneumonia and short-term mortality. However, after 6 months, stroke patients with aspiration show no further detriment, implying that recovery mechanism may have compensated for the loss of function. Appropriate management of aspiration and pneumonia is therefore crucial after stroke, and should be directed to the early months of rehabilitation.

W1724

Age, Hospitalizations, and Case-Fatality Rates Due to Complicated Gastric and Duodenal Ulcers in the United States: How Old is Old?

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Background and Aims: Gastrointestinal bleeding, perforation, and obstruction of gastric (GU) and duodenal ulcers (DU) are a significant cause of morbidity and mortality, particularly in the elderly. We examined hospitalizations and mortality due to complicated peptic ulcer disease (PUD), GUs, and DUs in the US to clarify the contribution of age as a risk factor. Methods: The Nationwide Inpatient Sample (NIS) is a stratified random sample of all US community hospitals. It is the largest inpatient care database in the US and the only database that has information on all inpatient care regardless of payer or insurance, including persons covered by Medicare, Medicaid, private insurance, and the uninsured. NIS's large sample size and data sampling techniques allow calculation of national estimates for particular diagnoses and analysis of secular analysis. We studied all inpatient hospitalizations with a primary diagnosis code of complicated ulcers (GU, DU, PUD with hemorrhage, perforation, and/or obstruction) in 1998 (before introduction of COX-2 specific inhibitors and widespread use of gastroprotective PPIs). Age-specific population estimates for 1998 were obtained from the US Census Bureau, and used to calculate age-specific incidence and case fatality rates. Results: During 1998 there were 34,874,046 hospitalizations among 271.4 million individuals resident in the US. Of these, 145,594 (0.42%) hospitalizations were for a primary diagnosis of a complicated GU, DU, or PUD, for a population rate of 53.65 per 100,000. A total of 873,548 people died in the hospital; of these, 11,782 (0.6%) deaths had a primary diagnosis of GU, DU or PUD. A correlation analysis with age showed that hospitalization rates for a primary diagnosis of GU, DU, or PU are fairly stable up to 50-55 years of age and then rise in an exponential fashion. Analysis of case fatality revealed that patients less than 40 years of age have a low rate (<0.5%). Case fatality rates double to 1% at 40-45 years of age and then increase rapidly to 2.5% at 70-75 years of age. The case fatality rate jumps to 4% at over 80 years of age. Persons over 80 years of age living in the US have a 6 per 1,000 risk of a hospitalization for a complicated GU, DU or PUD; 4% of these hospitalizations will be fatal.

Conclusions: Increasing age (≥55 years) exponentially increases the case fatality rate from complicated ulcer disease and should be an important factor for a more cost-effective implementation of prevention strategies and aggressive therapies.

W1725

EphB/Ephrin-B Signaling Enhances Intestinal Restitution: A Novel Protective Mechanism

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Eph receptor tyrosine kinases and their membrane bound receptor-like ligands, the ephrins, represent a bi-directional cell-cell contact signaling system that directs epithelial movements in developmental processes including the morphogenesis of intestinal crypts. The meaning of this system in the adult intestine is unknown. We show by real-time TaqMan PCR profiling that a unique and complex pattern of Eph-receptors and ephrins is still expressed in human adult intestinal epithelium. Beside the known co-expression of EphA2 (Eck) and ephrinA1 (B61), we found co-expressed EphB2 and ephrinB1/2 as the most abundantly expressed family members. Since, Eph/ephrin signal exchange between cells organizes coordinated movements, we further investigated possible consequences of EphB/EphrinB co-expression in wound healing models. In rat IEC6-cells, which also express ephrin-B1/2, the stimulation of ephrin-B-signaling with nM-doses of recombinant Eph-B-Fc receptor domains versus controls results in a significant dose-dependent acceleration of wound healing in in vitro scratch wound assays. We further demonstrate by fluorescence microscopy that this is associated with a coordinated migration activity of the wound edge cells, formation of lamellipodial protrusions into the wound, increased actin stress fiber assembly, production

of laminin IV at the wound edge, and phosphorylation of FAK. Immunoblotting revealed that the phosphorylation kinetics of pY397-FAK is paralleled by activation of Erk1/2 MAP kinase stress pathways. Consequently, further pleiotrope effects of Eph-B stimulation on gene transcription could be observed using the Affymetrix platform. The responding genes include several candidates assumed to be protective for the barrier and promote epithelial healing (e.g. c-fos, Cox-2, PAI-1, metallothionein 1). Interestingly, array technology, validated by real-time PCR and immunohistochemistry, also showed that ephrin-B2 is leading the list of differentially up-regulated genes in both perilesional and lesional IECs of samples from IBD patients, suggesting a possible role in epithelial maintenance and defense in IBDs. We conclude that Eph-B/ephrin-B intercellular signaling represents a novel possibly protective mechanism that promotes intestinal epithelial wound healing. Targeting of these neo-morphogenic pathways may help to establish novel molecular therapies that promote intestinal repair

W1726

Vascular Endothelial Growth Factor (Rat $VEGF_{164}$) Promotes Intestinal Epithelial Restitution *in vitro*: Evidence for a TGF-β_1 Mediated Effect

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Background/Aims: Vascular epithelial growth factor (VEGF) is a homodimeric 34-42 kD heparin-binding glycoprotein with angiogenic, mitogenic and vascular permeability-enhancing capabilities. VEGF receptors (VEGF-R) are localized on colonic mucosal epithelial cells and in permanent epithelial cell lines IEC 6, IEC 18 and Caco-2. Furthermore an increased expression of VEGF binding sites was detected in the colonic mucosa of patients with inflammatory bowel disease indicating an important role for VEGF in mucosal restitution and repair. The aim of our study therefore was to evaluate the role of VEGF on intestinal epithelial cell (IEC) restitution in an in-vitro wounding model. Methods: Confluent IEC-18, IEC-6 and Caco-2 monolayers were wounded with a razor blade as described previously. Cells were subsequently incubated in serum-deprived medium w/o VEGF in various concentrations. Epithelial cell migration was assessed by counting the number of cells migrating across the wound edge after an incubation period of 24 h. Furthermore, epithelial cell migration was investigated in the absence and presence of a neutralizing TGF-β_1-antibody. Proliferation of epithelial cells was assessed using the MTT test as described before. The mRNA expression levels of TGF-β_1 in the exposed epithelial cells was evaluated using real time RT-PCR. Statistical analysis was performed with ANOVA and Dunnet post-hoc test. Results: VEGF significantly increased the migration of Caco-2 (91% increase vs. control, $p = 0.0012$), IEC-6 and IEC-18 cells (97 % increase vs. control $p < 0.0001$). Addition of neutralizing anti-TGF-β_1 completely abolished VEGF induced migration in all cell-lines. VEGF significantly inhibited proliferation of Caco-2 cells, ($p < 0.01$ vs. control) while no effects on proliferation of the small intestinal epithelial cell lines were detected. A significant dose dependent increase in TGF-β_1-mRNA expression was detected in epithelial cells exposed to VEGF compared to control. Conclusion: Besides its well known angiogenetic effects VEGF significantly enhanced intestinal epithelial cell restitution in vitro. We show for the first time that VEGF induced wound repair in vitro is mediated by TGF-$\beta_1$in epithelial cells of intestinal or colonic origin. These results are similar in small intestinal and colonic epithelium. Presence of VEGF-R on intestinal epithelial cells might represent a novel target in intestinal wound repair.

W1727

Regulation of Post-Surgical Fibrosis by the Programmed Death-1 (PD-1) Pathway

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Surgical adhesions are a common and often severe complication of abdominal or pelvic injury that cause bowel obstruction, pelvic pain and infertility in women. Current treatments are of limited effectiveness because little is known about the cellular and sub-cellular processes underlying adhesiogenesis. Recently we showed that Th1 **AB** CD4+ T cells mediate the pathogenesis of adhesion formation in a rodent model of this disease process. Here, we demonstrate that in mice these T cells home directly to the site of surgically-induced adhesions and control local chemokine production in a manner dependent on the CD28 T cell costimulatory pathway. Conversely, the inhibitory Programmed Death-1 pathway plays a central role in limiting adhesiogenesis, as PD-1 blockade was associated with increased T cell infiltration, chemokine production, and a concomitant exacerbation of disease. Through use of confocal microscopy we also show that CD4+ T cells homing to the site of surgically-induced adhesions express CD25, the activation marker CD69, interferon Γand the chemokine receptors CCR5 and CXCR3, consistent with a Th1 phenotype. Our results reveal for the first time that the development of post-surgical fibrosis is under the tight control of positive and negative T cell costimulation and suggest that targeting these pathways may provide promising therapies for the prevention of adhesion formation.

W1728

Chronic Anal Fissure: Efficacy of Chemical Sphincterotomy

Martin J. Utzig, Anton J. Kroesen, Heinz J. Buhr

Introduction: Most patients with chronic anal fissure have high resting anal pressure. Treatment is directed at reducing the sphincter tone (MARP). Surgical sphincterotomy successfully lessens the anal resting pressure but may irreversibly damage the anal sphincter and cause anal incontinence. Topical application of both, glyceryl trinitrate (GTN) and botulinum toxin (ToxA) has been shown to reduce MARP and thus is considered to be an effective alternative to surgery («chemical sphincterotomy»). The aim of this analysis was to assess the efficacy of surgical and chemical sphincterotomy in healing chronic anal fissures. Methods: Using the terms «anal fissure», «surgery», «glyceryl trinitrate» and «botulinum toxin», a Medline database was used to perform a literature search. Moreover, all patients who presented to our surgical outpatient department with chronic anal fissure from January 2000 to June 2002 were treated with 0.2 percent GTN ointment and assessed at two weekly intervals. Results: 52 studies including 7606 patients in total were studied: 24 trials with 948 subjects analysed GTN ointment, 14 reports enrolling 623 patients ToxA - injection and 14 studies (6035 patients) surgical sphincterotomy. After topical GTN treatment, fissure healing was complete in 574 of 948 patients [30% - 93%, mean 60.6%] by eight weeks. Adverse effects, i.e. severe headaches were seen in 43.6% [0% - 84%], whereas no severe side effects were observed in those patients treated with ToxA-injection. Temporary incontinence (< two weeks) for mucus or flatus was seen in 17 / 531 [3.2%] after injection therapy; healing rates ranged from 43% to 96% [mean 72.6%]. Following surgical sphincterotomy, fissures were less likely to remain unhealed [0 - 11%, mean 2.8%], however incontinence for gas and faeces were observed in 10.4% and 2.8%. In our experience, forty patients were treated with 0.2% GTN ointment. After eight weeks, initial healing rate was 62.5% [25/40]. Following consecutive endosonography-guided ToxA-injection therapy, seven out of eight persisting fissures healed [87.5%]. Moreover, seven fissures were successfully treated by surgical fissurectomy [100%]. Discussion: Chemical sphincterotomy with GTN has replaced surgery as first-line treatment for chronic anal fissure. In those patients with persisting or relapsing fissure, injection of botulinum toxin offers an effective alternative. Only those patients with ineffective chemical sphincterotomy should be recommended surgical treatment.

W1729

Global Gene Expression Analysis Reveals a Role for the Rho/ROCK Pathway in Intestinal Radiation-Induced Fibrogenesis

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Background & Aims: Radiation enteritis is associated with accumulation of contractile fibrosis-activated myofibroblasts, increased CTGF expression (1) and cDNA array analysis revealed alteration of the genes coding for the Rho family of proteins (2). As Rho proteins are involved in the regulation of stress fiber formation and cell contraction, and may regulate CTGF expression, we postulated that alteration of the Rho Pathway is involved in maintenance of radiation-induced myofibroblastic differentiation. Methods: Radiation enteritis and normal ileum biopsies were dissected into mucosa and muscularis layers to establish primary culture of subepithelial myofibroblasts and smooth muscle cells. Structure and composition cytoskeleton, as well as CTGF and type I collagen secretion in mesenchymal cells derived from radiation enteritis were studied. The involvement of the Rho/ROCK pathway in the fibrosis-activated myofibroblastic differentiation was assessed using Y-27632, a specific inhibitor of Rho kinase. Results: The functional link between the Rho pathway and radiation-induced fibrogenic differentiation was investigated using primary culture of subepithelial myofibroblasts and smooth muscle cells isolated from radiation enteritis. These cells retained their fibrogenic differentiation in vitro and exhibited a typical cytoskeletal network. CTGF and pro-Collagen I secretion was enhanced in cells derived from radiation enteritis as compared with their normal counterparts. It was concomitant to alteration of the Rho pathway. Specific inhibition of the Rho kinase with Y-27632, induced a simultaneous decrease in the number of actin stress fibers, in the a-sm actin and HSP27 protein level. Y-27632 also decreased CTGF and Col1A1 mRNA levels. Conclusions: The present study suggests that the Rho/ROCK pathway is involved in the control of actin network and in regulation of CTGF expression associated with radiation-induced fibrogenic differentiation. Furthermore, they suggests that inhibition of the Rho/ROCK pathway could reverse the radiation-induced myofibroblastic differentiation. Involvement of NF-kB in these regulations is currently under investigation. 1) Vozenin-Brotons et al. Int J Radiat Oncol Biol Phys, 56, 561-72, 2003. 2) Vozenin-Brotons et al. Rad. Res. In press.

W1730

The Membrane-Bound Disintegrin Metalloprotease ADAM15 Is Expressed in Intestinal Epithelium

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Background: Disintegrin metalloproteases (or ADAMs) are membrane-anchored glycoproteins that have been implicated in cell-cell or cell-matrix interactions and in proteolysis of molecules on the cell surface. ADAMs play important roles in ectodomain shedding, membrane fusion, cell adhesion, fertilization, muscles development and cell fate determination. The expression and the pathophysiological implication of ADAM are not known in intestinal epithelial cells. Aim: To investigate the expression and the potential roles of ADAM in intestinal cells, especially ADAM-15 that is a membrane-bound disintegrin metalloprotease containing an RGD integrin binding sequence. Results: Reverse transcription polymerase chain reaction experiments showed that ADAMs-10, -12, and -15 are expressed in the enterocyte-like human cell line Caco-2 BBE as well as in the human colonic cell line HT29-Cl.19A. A Caco2-BBE ADAM-15 cDNA clone, encompassing the entire coding region, cloned from Caco2-BBE poly(A)+ RNA contained 69 single base deletions in the cytoplasmic domain, corresponding to 23 amino acid deletions, compared with human ADAM-15 from other tissues. In Caco2-BBE cells, ADAM-15 protein was found in the apical, basolateral, and intracellular compartments. Immunohistochemistry, performed on mouse intestine showed that there is a gradient of ADAM-15 protein expression along the crypt/villus axis in the small intestine and along the crypt in the colon: ADAM-15 staining was increased as the cells reached the villus/crypt tip, suggesting that ADAM-15 expression correlated with cell differentiation. Using the Electric Cell-Substrate Impedance Sensing (ECIS) technology, we demonstrated that an anti-ADAM-15 antibody, raised against the ectodomain, reduced cell migration in a wound-healing assay in Caco2-BBE monolayers. In addition, we have demonstrated that ADAM-15 up-regulates the interaction between Jurkat T-cells and Caco2-BBE monolayers. Conclusions: Altogether, these results suggest that 1) ADAM-15 are expressed in intestinal epithelia 2) a new variant of ADAM-15 is expressed in intestinal epithelial cells 3) ADAM-15 is involved in epithelial intestinal cells migration and 4) upregulates intestinal epithelial-lymphocyte interactions. Together, these results suggest that ADAM-15 may have important pathophysiological role in intestinal cell biology and inflammation.

W1731

Matrix Metalloproteinase Inhibitor Maintains Post-Operative Colon Anastomotic Strength in an Experimental Model

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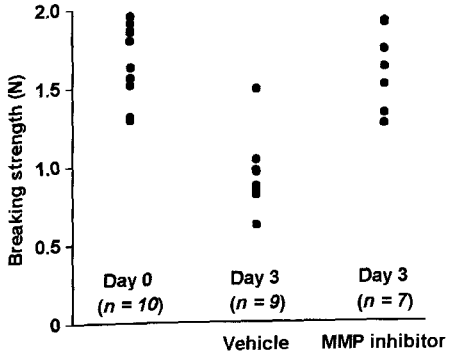
Background: Anastomotic leakage after colo-rectal resection is associated with high morbidity and mortality. The early biomechanical strength of the anastomosis relies on the existing submucosal collagens. A minimal breaking strength of a colonic anastomosis has been found on post-operative day 3. Excessive matrix metalloproteinase (MMP) activity may be responsible for the local collagen degradation. We have tested the efficacy of the broad-spectrum hydroxamate MMP inhibitor GM6001 compared to vehicle treatment on the initial breaking strength of left-sided colon anastomoses.
Methods: Male Sprague-Dawley rats (250 g) were treated daily with GM6001 (100 mg/kg) or vehicle (control) by subcutaneous injections starting 2 days before operation. The breaking strength of the anastomoses was measured using a materials testing machine (Alwetron TCT 5) immediately after construction of anastomoses in non-treated rats and on post-operative day 3 in treated rats.
Results: GM6001 concentrations in whole blood and in resected anastomotic wound segment were 1.5 μM 24 hours after the last GM6001 injection. Systemic GM6001 treatment effectively blocked MMP activity, measured by the collagen degradation marker ICTP (carboxyterminal telopeptide of human type I collagen) in serum. The breaking strength (figure) was doubled in GM6001-treated rats compared to controls on day 3 ($p < 0.001$). Furthermore, the anastomotic breaking strength in GM6001-treated animals did not differ significantly ($p = 0.58$) from the strength of the anastomosis immediately after surgery while it was lowered by 50% in controls.
Conclusions: Pharmacological inhibition of MMP activity prevented the decline in biomechanical strength of colon anastomoses in the early critical post-operative phase and doubled the anastomotic strength compared with no MMP inhibition. Our results warrant further exploration of this therapy to prevent anastomotic complications in patients undergoing colonic or rectal resection.



W1732

Ectodomain-Shed EGFR Ligands Promote Intestinal Epithelial Restitution: Role of TACE/ADAM17

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Re-epithelialization of intestinal tract ulcers by movement of wound-edge epithelial cells into the ulcer bed, known as epithelial restitution, initiates the wound healing process. Prior studies have indicated that the epidermal growth factor receptor (EGFR) and its ligands are important for intestinal epithelial restitution. However, the mechanisms of EGFR activation in wounded intestinal epithelium are not known. We tested the possibility that EGFR activation in wounded intestinal epithelium depends on the ectodomain shedding of pre-formed EGFR ligands by tumor necrosis factor α converting enzyme (TACE). Methods: Ulceration and restitution were modeled *in vitro* using scrape-wounded RIE-1 cell monolayers. EGFR activation was evaluated by phospho-tyrosine Western blotting. Transforming growth factor (TGF)-α was measured by RIA. The rate of intestinal epithelial restitution was quantified by measuring the re-epithelialization of RIE-1 monolayer circular wounds by image analysis. Results: Immunohistochemistry and Western blotting revealed expression of TACE in cultured RIE-1 cells and in human colonic epithelial cells *in vivo*. Intestinal epithelial cells stimulated with conditioned medium collected from scrape-wounded RIE-1 monolayers phosphorylated EGFR, which could be blocked with neutralizing anti-EGFR antibody but not control Ig. TGF-α, an EGFR ligand, was detected in scrape-wound conditioned medium. The release of EGFR ligands after scrape wounding could be dose-dependently blocked by TAPI-1, a specific inhibitor of TACE. TAPI-1 also blocked scrape-wound, but not epidermal growth factor-induced EGFR phosphorylation in RIE-1 monolayers. These results indicate that scrape-wounded RIE-1 monolayers release EGFR ligands by TACE-mediated ectodomain shedding. To test the functional significance of this process, epithelial restitution was quantified in the presence of TACE inhibitor TAPI-1. In control RIE-1 monolayers, a mean of 58% of the original ~10^6 μm^2 wound area was re-epithelialized after 16 hours. TAPI-1 dose-dependently reduced re-epithelialization to a mean of 30% of the original wound area. Conclusions: TACE mediates wound-induced ectodomain-shedding of EGFR ligands which promote intestinal epithelial restitution. Therapeutic strategies aimed at increasing the TACE-dependent release of EGFR ligands such as TGF-α, might aid the healing of intestinal tract ulcers.

W1733

FAK Protein Levels Regulate Caco-2 Intestinal Epithelial Wound Closure

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Intestinal epithelial migration is an essential factor in mucosal wound healing. We previously reported a dramatic decrease in FAK protein in intestinal epithelial migrating cells with an increase in the proportion of FAK that was autophosphorylated. This led us to hypothesize that FAK protein levels, as distinct from the proportion of FAK that is activated by phosphorylation, might be important in the regulation of intestinal epithelial migration. Caco-2 cells were transfected with SiFAK to inhibit FAK expression and migration was assayed as closure of a circular wound in a monolayer on a collagen I substrate. FAK-397 phosphorylation and FAK protein levels were measured by Western blot. Since transactivation of FAK has been implicated in some effects of the EGFR, we also studied the effect of epidermal growth factor receptor-related protein (ERRP) on migration as well as on FAK phosphorylation and protein levels. Wound closure at 24 hours was significantly inhibited in 6 separate studies by both SiFAK transfection (22-33%, $P<0.001$ each study) and ERRP treatment (10-17%, $P<0.05$ each study). Similar results were observed at 48 hours. Both siFAK transfection and ERRP treatment each significantly decreased levels of total FAK (U70% and 25% respectively, $n=3$, $p<0.01$ each) as well as phosphorylated FAK despite increases in the proportion of phosphorylated FAK to total FAK in each case (U2.3 fold and 20% respectively, $p<0.05$ each). Thus, ERRP appeared to act on FAK at the protein level rather than inhibiting phosphorylation. (ERRP also inhibited EGFR phosphorylation.) In 3 separate studies, FAK protein levels were not further decreased by the addition of ERRP to siFAK transfection and the combination of siFAK transfection and ERRP treatment did not more significantly inhibit wound closure at 24 hours than either treatment alone. For instance, in one typical study, siFAK inhibited wound closure by 32.5 ± 4.6% while ERRP inhibited wound closure by 10.24 ± 0.57. ($n=6$, $p<0.01$ for each) The combination of siFAK and ERRP in this experiment resulted in 32.97 ± 3.1% inhibition, not different from the effect of siFAK alone. Again, 48 hour data were similar. These results suggest that changes in cellular total FAK protein sufficient to affect the amount of phosphorylated FAK can regulate intestinal epithelial motility, and that some effects of chronic stimulation of the EGF receptor may be mediated by its effects on FAK protein levels.

W1734

The Novel PKC Isoforms, PKCδ and PKCε, Enhance Epithelial Cell Spreading and Lamellipodia Formation: Role of FAK and Paxillin

J. Cecilia Song, Peter Kim, Joshua Mammen, Karl Matlin, Jeffrey Matthews

Modulation of actin dynamics is a key regulatory process in restitution. Formation of lamellipodia (LAM) and focal complexes during cell movement requires coordinated actin rearrangement, yet signaling pathways are not well described. Phosphorylation of Src, focal adhesion kinase (FAK) and paxillin is associated with increased motility, though the mechanism is not well understood. We have shown that protein kinase C (PKC) regulates actin remodeling in T84 epithelial cells. Little is known of its role during cell spreading. Aim: To determine the effects of PKC on epithelial movement and examine the targets of PKC that modulate cell spreading. Methods: Subconfluent T84 monolayers were used to measure cell spreading +/- PKC agonists in serum-free media. Phosphorylation of FAK and paxillin was assessed by Western blot. Results: Phorbol ester PMA accelerated cell spreading in a time-dependent manner. Cells initially seeded at 38 % of total surface area (TSA) rapidly expanded to cover 53 % of TSA upon 1h PMA. By 4h, cells covered 76 % of TSA while untreated remained unchanged. This effect was associated with enhanced formation of LAM. Number of nuclei was unaffected. PMA activated the conventional cPKC isoform PKCα and the novel nPKC isoforms PKCδ and PKCε. The cPKC and nPKC inhibitor Gö6850 completely inhibited PMA-induced spreading and LAM formation while the selective cPKC inhibitor Gö6976 enhanced PMA-elicited cell spreading. The PKCδ-specific inhibitor rottlerin blocked the PMA-elicited spreading but did not prevent enhanced LAM formation. This pattern of inhibitor sensitivity suggests a role for PKCε in LAM formation. Bryostatin-1, which activates PKCα, PKCδ, and PKCε silmilarly enhanced spreading and LAM formation. PKCε-specific agonist carbachol stimulated only LAM formation with no effect on spreading. The Src-inhibitor PP2 attenuated PMA-elicited cell spreading, implicating Src as a downstream effector of PKC. Tyrosine phosphorylation of FAK and paxillin were increased by PMA, an effect attributed to PKCδ and Src since it was blocked by Gö6850, rottlerin, and PP2, but not Gö6976. Conclusion: Activation of PKCδ and PKCε stimulated cell spreading in intestinal epithelia, possibly via phosphorylation of FAK and paxillin by PKCδ and stimulation of LAM formation by PKCε. Activation of PKCα may oppose the effects of PKCδ and PKCε during cell spreading. These data implicate specific PKC isoforms in distinct aspects of events that contribute to cell spreading.

W1735

Distinct Temporal and Spatial Roles for Rho-Kinase (ROCK) and Myosin Light Chain Kinase (MLCK) in Purse-String Wound Closure

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Healing of epithelial wounds is essential for recovery from mucosal injury. Small wounds of <10 cells heal by purse-string wound closure due to contraction of a circumferential actomyosin ring that assembles at the wound edge. Actomyosin contraction is regulated by phosphorylation of myosin II regulatory light chain (MLC). MLC phosphorylation can be enhanced by MLC kinase (MLCK) or by rho-associated kinase (ROCK) inhibition of MLC phosphatase. However, the specific roles of ROCK, MLCK and MLC in purse-string wound closure remain ill-defined. We therefore characterized the kinetics of ROCK and MLCK recruitment and MLC phosphorylation at wound edges as well as the effects of ROCK or MLCK inhibition on wound closure. METHODS: Small wounds were created in monolayers of Caco-2 cells expressing GFP-β-actin and multidimensional video images collected during wound closure. At select times individual wounds were fixed and immunostained for ROCK, MLCK and phosphorylated MLC. RESULTS: Live cell imaging showed actin polymerization

at the wound edge within 2 min after injury. This progressed to a circumferential ring after 8-10 min. Subsequent ring contraction sealed the wound within 30-60 min. ROCK recruitment was temporally and spatially linked to the initial phase of actin polymerization. However, ROCK was lost from the wound edge after ring assembly was complete, e.g. <8 min after injury. In contrast, MLCK did not accumulate at the wound edge until actin polymerization was established, e.g. by 8 min. This MLCK recruitment was accompanied by MLC phosphorylation and coincided with actomyosin ring rounding, indicating the development of circumferential tension. Consistent with the differential kinetics of recruitment, pharmacological inhibition of ROCK and MLCK had distinct effects on wound closure. ROCK inhibition prevented actomyosin ring assembly as well as subsequent wound rounding and closure. In contrast, actomyosin ring assembly was not prevented by MLCK inhibition and contractile wound closure began successfully, albeit with delayed kinetics. Then, midway through wound closure, the actomyosin ring fragmented and the wound retracted to its original size. CONCLUSIONS: Small wounds close by coordinated actomyosin ring contraction. ROCK is recruited early and is crucial for local actin polymerization, while MLCK is recruited later and is necessary to maintain tension and effect wound contraction. Therefore, ROCK and MLCK serve distinct roles in purse-string wound closure.

W1736

Organ Transfection of Adenoviral Vector After Intraduodenal or Intravenous Gene Therapy in Experimental Duodenal Ulcer in Rats

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After demonstrating the effectiveness of gene therapy with adenoviral vectors of VEGF and PDGF in duodenal ulcer healing, we recently identified the localization of the intraduodenally or intravenously administered adenoviral vector in multiple organs in cysteamine-induced duodenal ulcer rats. Unfasted Sprague-Dawley female rats were given cysteamine-HCl (25mg/100g x3 by gavage at 4 hr intervals) and laparotomy was performed to evaluate ulcer severity on the 3rd day. Rats with equally severe duodenal ulcer were randomly given either saline (controls) or 5 x 10^8pfu/rat of adenoviral vector by intraduodenal or intravenous injection and the animals were killed 24 or 48 hr after injection. Sections of the duodenum, stomach, jejunum, liver, spleen, kidneys, and lungs were harvested and the expression of adenoviral transgene was examined by staining for beta-galactosidase using X-gal in both whole mount tissues and histologic sections. The results demonstrated that the duodenum after intraduodenal administration of adenoviral vector was stained much stronger than following intravenous injection of adenoviral vector, and the staining was concentrated around duodenal ulcer margin. The stomach and jejunum were also stained in both intraduodenal and intravenous groups but the staining after intravenous injection of adenoviral vector was stronger than that after intraduodenal administration. The spleen and kidney were only stained after the intravenous injection, while the liver and the lung lacked staining after either intraduodenal or intravenous injection of the adenoviral vector. These findings were confirmed by using the adenoviral vector with the green fluorescent protein reporter gene. Conclusions: 1) Extensive gene delivery of adenoviral vector was detected in the duodenal ulcer vicinity after either intraduodenal or intravenous administration. 2) Broad expression of the adenoviral transgene in distal organs was seen only after intravenous administration. 3) Thus, local (intraduodenal) administration seems to be a localized and safe delivery of gene therapy with adenoviral vectors of VEGF or PDGF into the ulcer in order to accelerate duodenal ulcer healing.

W1737

Impaired Mucosal Healing in IBD Patients-Role of Peripheral Blood Mononuclear Cells (PBMN)

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Background: Mucosal damage is a hallmark of IBD. Impaired intestinal wound healing may play a relevant role in the pathogenesis of mucosal damage observed in IBD. As peripheral blood mononuclear cells (PBMN) have been demonstrated to modulate epithelial cell restitution, the initial step of intestinal wound repair, in vitro, we aimed to characterize the role of PBMN in intestinal epithelial healing in patients with inflammatory bowel diseases (IBD). Methods: PBMN were separated from whole blood, obtained from healthy volunteers (n = 20) and patients with active and inactive Crohn's disease (n = 12 for each group) and ulcerative colitis (n = 12 for each group), using a density gradient. The effects of PBMN on intestinal epithelial restitution and proliferation were assessed using an in vitro coculture wounding model and colorimetric MTT assays with non-transformed small intestinal epithelial IEC-6 cells. Results: Coculture of PBMN from healthy volunteers with IEC-6 cells caused a significant 50% stimulation of epithelial cell restitution in vitro, while PBMN from patients with both CD and UC caused on average a 30% inhibition of epithelial restitution. The inhibitory effect of PBMN from IBD patients on epithelial restitution was independent of disease activity and was not further modulated by LPS stimulation of PBMN. In addition, coculture of PBMN from patients with active UC and both active and inactive CD caused on average a 35% stimulation of IEC-6 proliferation, while PBMN from healthy volunteers and patients with inactive UC did not significantly modulate epithelial proliferation. Functional blocking studies with immunoneutralizing antibodies indicated that the cytokines TGF-beta, IL-2, TNF-alpha and IFN-gamma may mediate PBMN effects on IEC-6 cells in vitro. Conclusions: These findings suggest that PBMN may impair intestinal epithelial repair in IBD patients by inhibition of intestinal epithelial restitution and modulation of proliferation through cytokine-dependent pathways. This study lends further support to the notion that intestinal wound healing in health and various inflammatory disorders may be modulated by a complex network of cellular and cytokine interactions.

W1738

Shiga Toxin 1 Traffics into the Nucleoli of Intestinal Epithelial Cells

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Shiga toxin 1 (Stx1) consists of a catalytic A subunit and pentamer of B subunits, the latter being necessary for toxin binding to its receptor glycosphingolipid Gb3 and intracellular trafficking. The purpose of this study was to examine the intracellular distribution of Stx1 in rabbit ileal epithelial cells infected with RDEF-H19A bacteria, which produce Stx1 holotoxin, using immunofluorescence microscopy. In addition to the well-documented Stx1 trafficking into the Golgi/ER, we found Stx1 accumulated in the nucleoli (NL) in ~ 40% of the cells. Fluorescently labeled B-subunit of Stx1 (Stx1B) also was accumulated in the NL in ~ 100% of living Caco-2 and T-84 intestinal epithelial cells after permeabilization of the plasma membranes (PM). The nucleolar trafficking of Stx1B was Gb3 independent, because opossum kidney (OK) epithelial cells, which do not express Gb3 and normally do not bind Stx1B, transported Stx1B into the NL after PM permeabilization. Simultaneous exposure of cells to Stx1B and B-subunit of Cholera toxin (CTB) showed that CTB did not follow the Stx1B nucleolar pathway and always remained outside the nucleus. This indicates that trafficking into the NL is specific for Stx1/Stx1B. Stx1B nucleolar trafficking was inhibited in a concentration-dependent manner by Wheat Germ Agglutinin, which blocks active protein transport across the nuclear pore, by low temperature and energy depletion. These data showed that Stx1B nucleolar uptake is not due to diffusion, but rather is an active carrier-dependent process. Accumulation of Stx1B in nucleoli leads to nuclear DNA degradation in intestinal epithelial Caco-2 and T-84 cell models. We conclude that: 1) Stx1 is transported into the NL of intestinal epithelial cells; 2) Stx1B is responsible for Stx1 nucleolar accumulation; 3) Stx1/Stx1B nucleolar trafficking is carrier-dependent; 4) Stx1B nucleolar accumulation causes apoptosis; 5) the B-subunit of AB toxin initiates a signal transduction, a new concept of enterotoxin actions.

W1739

Functional Involvement of SNARE Proteins in the Recruitment of Adenosine 2b Receptor to the Apical Plasma Membrane

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Background and significance: Adenosine, acting through the A2b receptor, induces vectorial chloride and IL-6 secretion in intestinal epithelia and may play an important role in intestinal inflammation. We have previously shown that apical or basolateral adenosine receptor stimulation results in the recruitment of the A2b receptor to the plasma membrane. In this study we examined the domain specificity of recruitment and the functional importance of soluble N-ethyl maleimide attachment receptor (SNARE) proteins in the agonist-mediated recruitment of the A2b receptor to the membrane. Methods: Model intestinal epithelial cell lines, T84 were used as they only express the A2b-subtype adenosine receptor. Confocal imaging, cell surface biotinylation, co-imnuprecipation and N-ethyl maleimide (NEM) were used to study the role of SNARE protein in A2b receptor trafficking. Results: Using confocal imaging and domain specific labeling, we show that the A2b receptor is recruited to the apical membrane in cells stimulated with apical or basolateral adenosine. VAMP-2, vesicle SNARE, is enriched in vesicular fraction in the sub-apical domain while SNAP-23, a membrane target SNARE is present in the membrane fraction both in the apical and lateral membrane. Upon agonist stimulation, the A2b receptor is enriched in the vesicle fraction containing VAMP-2 and VAMP-2 traffics to the apical domain upon adenosine stimulation. Further, in cells stimulated with apical or basolateral adenosine VAMP-2 associates with SNAP-23 within 5 minutes and is no longer detected in 15 minutes. Inhibition of SNARE complex with NEM inhibits cAMP synthesis induced by apical or basolateral adenosine by 98% and 90% respectively. NEM does not affect cAMP synthesis by direct stimulation of adenylate cyclase with foskolin. Conclusions: Collectively, our data suggest that i) A2b receptor is recruited to the apical membrane ii) the SNARE proteins, VAMP-2 and SNAP-23, participate in the recruitment of the A2b receptor and iii) the SNARE-mediated recruitment of the A2b receptor is essential for its signaling. This is the first demonstration of intracellular localization of a G-protein coupled receptor and agonist-specific recruitment of the receptor to the membrane.

W1740

Contribution Made by the mPar-1b Kinase to Maintenance of Polarity in Gut Epithelium

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Cell polarity is required for maintaining epithelial cell integrity in the gut. The Par-1 protein kinases are key polarity determinants that have been conserved throughout evolution. In mammals there are four family members denoted mPar-1a, b, c and d. In MDCK cells, mPar-1b localizes to basolateral membranes beneath epithelial cell tight junctions and may contribute to maintenance of cell polarity. Recent studies suggest that the mPar-1 kinases may be regulated by LKB1, a protein kinase mutated in Peutz-Jeghers syndrome (Spicer et al. Oncogene, 2003). We analyzed the expression of mPar-1b in the gastrointestinal tract of mice. mPar-1b expression was not detected in the small intestine or in the colon but rectal expression was readily observed. Interestingly, mice disrupted for mPar-1b develop rectal prolapse (Hurov et al. MCB, 2001). A thickening of the rectal wall and occasional ulcerations at the site of prolapse proximal to the anorectal junction were observed in longitudinal sections of distal colon and rectum. Crypts were elongated with evidence of reactive hyperplasia but no dysplasia. In severe cases, distorted crypt architecture and occasionally crypt abscesses were observed. Microscopic exam of H and E stained specimens did not reveal any defects in the muscularis propria or myenteric neurons. These findings suggest that mPar-1b contributes an important function in maintaining the structural integrity of the rectum.

W1741

Heat Stable Enterotoxin and cGkinase 11 Regulate CFTR Membrane Traffic in Rat Small Intestine

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Introduction: Pathogenic strains of E. Coli secrete Heat Stable Enterotoxins (STa) and stimulate intestinal fluid and chloride secretion that result in massive secretory diarrhea. In the small intestine, STa binds to the apical membrane of enterocytes, activates the STa-receptor guanylyl cyclase C and increases intracellular cGMP to signal cGKinase 11-dependent phosphorylation and opening of the CFTR chloride channel on the apical membrane of enterocytes (Gastro 1997; 112:437-443). cAMP regulates membrane traffic and CFTR mediated fluid secretion in the intestine (AJP 2003; 284:C429-438), but a role for STa and cGMP in regulating CFTR membrane traffic has not been defined. We hypothesize that STa and cGMP regulate CFTR membrane traffic and fluid secretion in the intestine. Methods: Male Sprague Dawley Rats (250gm) were anesthetized and STa (0.5uM) +/- PKG inhibitors or saline were administered into small intestinal loops. Thirty minutes following STa administration fluid accumulation was determined from the ratios of loop weight before and after fluid removal. Enterocytes were isolated, surface proteins biotinylated and CFTR and lactase detected by Western Blots. Biotinylated proteins were quantified by densitometry. Tissues were embedded in O.C.T embedding medium, frozen in liquid nitrogen and cryostat sections immunolabeled to detect CFTR, lactase and F-actin. Results: Fluid accumulation was as follows: (1) STa: 2.528 (SD, 0.17) (2) NS: 2.01 (SD, 0.22) (3) STa+H8: 1.994 (SD, 0.23). The difference between STa and NS or STa+H8 was statistically significant ($p<0.05$). Surface biotinylated CFTR in enterocytes from STa treated loops was 4-fold higher than saline controls ($n = 6$ animals, $p<0.05$). The STa-induced increase in surface CFTR was reduced to control levels in the presence of the protein kinase G11 inhibitor H8 (0.4mM). Surface biotinylated lactase remained unchanged among groups. Immunofluorescence labeling of cryostat sections revealed a specific STa-dependent shift in CFTR distribution to the apical domain of enterocytes that was reduced in the presence of H8. Conclusion: STa regulates CFTR membrane traffic and fluid secretion in rat small intestine by cGkinase 11.

W1742

Regulation of Plasma Membrane CFTR Stability: A Proteomics Approach

Christopher R. Marino, Ghanshyam D. Heda, James Mahan

The ΔF508 mutation of CFTR has a dramatic effect on the plasma membrane stability of this important epithelial chloride channel. We hypothesize that the surface instability of ΔF508 CFTR results from its selective targeting away from a recycling pathway and toward a pathway of degradation following internalization. If so, the proteins that regulate these distinct trafficking pathways should be identifiable through comparison of the proteome of vesicles isolated from ΔF508 and wild-type cells. AIM: The aim of the current study is to identify putative regulatory proteins within the endocytic compartment by comparing the proteome of vesicles in cultured cells expressing either ΔF508 or wild-type CFTR. METHODS: Polarized LL-CPK$_1$ epithelial cells transfected with either human ΔF508 or wild-type CFTR were pre-treated with sodium butyrate and low temperature to up-regulate surface expression of CFTR. Cells were then "chased" at 37°C in fresh media containing cycloheximide for 3 hours. A CFTR-enriched vesicle fraction was then obtained by discontinuous sucrose gradient centrifugation and the proteome of the vesicles from mutant and wild-type cells was imaged by 2-dimensional gel electrophoresis. Differentially-expressed proteins were identified for subsequent mass spectrometry analysis using MALDI-ToF technology. RESULTS: The 2-dimensional gel profile from both cell types was quite similar, due to the identical cell lineage from which they came. In replicate ($N = 3$) experiments, however, two proteins were reproducibly shown to be expressed to a greater extent in vesicles from ΔF508 cells. Both proteins are low molecular weight (~23 kDa). One has a pI~5.8 and the other has a pI~7.0. We are currently in the process of analyzing these proteins by mass spectrometry. CONCLUSIONS: Vesicles isolated from cells expressing mutant and wild-type CFTR contain a small number of differentially-expressed proteins. We speculate that these proteins may be regulators of distinct endocytic trafficking pathways that control CFTR stability at the cell surface.

W1743

Folate Status and Risk of Colorectal Polyps in African Americans

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Background: Low folate status may increase risk for colorectal cancer (CRC) by increasing DNA hypomethylation. Polymorphism of Methylenetetrahydofolate reductase (MTHFR; 677C>T and 1298A>C) determines the enzyme activity. The frequency of polymorphisms in the gene varies extensively in different population. Objectives: To determine in African Americans (AA) 1) the pattern of MTHFR polymorphisms. II) the association between folate status, tobacco, alcohol consumption, pattern of polymorphism and risk of colon polyp. III) the folate status and APC methylation profiling of DNA in peripheral blood. Methods: Among fifty seven patients who underwent a clinically indicated colonoscopy, 22 patients with histology confirmed colon polyps and 35 patients without colon polyp were recruited for a case control study. Blood samples were collected for determination of serum and RBC folate, serum B12 and homocycteine. Methylation specific PCR was used to analyze APC promoter methylation and PCR RFLP technique was performed to determine MTHFR 677 C>T polymorphism. Results:Among 22 cases 49 polyps (adenomatous, $n=41$ and hyperplastic, $n=8$) were identified. Twenty eight (57%) of the polyps were on the left side of colon and 21 (42%) on right side. Forty eight individuals (84%) showed homozygous for 677 CC. However, eighteen of these individuals (37.5%) had colon polyp while thirty individuals (62.5%) did not have polyp. There was highly significant association between smoking and alcohol consumption with colon polyp using chi-square Test ($p = 0.0006$ and $p = 0.05$, respectively). There was no statistical significance association between folate blood level (serum, RBC), B12 and homocysteine with colon polyp. Age, gender, body mass index (BMI) and use of folate based vitamin supplement were not significant different among the cases and controls using student t- test. Nine individuals showed heterozygous for 677 CT, four (44%) of these individuals had colon polyp. Eighty eight percent APC promoter tested using peripheral blood DNA from patients were unmethylated. Among the individuals with adenomatous polyps 66% show APC methylation vs 33% of controls using their DNA from the blood. Conclusions:The lack of the 677 TT may be a significant risk factor for colon neoplasm in the AA population. Smoking and alcohol were also the risk factors for the colon polyp. APC promoter methylation found in peripheral blood may be an indicators of risk of polyp formation and an important screening tool.

W1744

Identification of a Vitamin D_3 Responsive Region in the Murine PHEX Gene

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The PHEX gene (Phosphate encoding gene with Homologies to Endopeptidases on the X chromosome) has been implicated in the regulation of intestinal Pi absorption in juvenile mice, highlighting its importance in Pi homeostasis. 1, 25$(OH)_2$ vitamin D_3 (D_3) also influences Pi absorption in the intestine, and is known to regulate PHEX. The goal of this project was to determine the mechanism by which D_3 regulates PHEX gene expression. PHEX promoter constructs in pβGal basic (Clontech), were transfected into UMR-106 cells, singly or along with pSG-5 (empty vector) or E240A (pSG-5 expressing dominant-negative vitamin D_3 receptor (VDR)). Cells were then treated with D_3 or vehicle and assayed for β-galactosidase activity. Electrophoretic mobility shift assay (EMSA) was conducted using nuclear protein (NP) from UMR-106 cells treated with D_3 or vehicle for 48 hours or with purified VDR and RXR protein. NP was fractioned by SDS-PAGE and transferred to nitrocellulose for southwestern blot. Proteins were renatured in 1X EMSA binding buffer w/ 5% milk. Blots were hybridized with a ^{32}P labeled, ds oligos, washed with 1X EMSA buffer and exposed to x-ray film. Analysis of promoter constructs indicated that activity from the -133/+104 construct decreased 50%, while the -74/+104 and shorter constructs did not respond to D_3. The D_3 decrease in activity of -133/+104 was seen with cotransfection with pSG-5 but not with E240A. EMSA analysis of the -133/-74 region revealed that purified VDR/RXR was unable to bind in this region, however NP binding was seen in the -133/-83 region. Binding in this region was localized to a 17 bp poly A stretch (-116/-99) and binding decreased with D_3 treatment. Deletion of this region in the -133/+104 promoter construct abolished D_3 down regulation. Southwestern blot analysis revealed that a 110 kDa protein bound a probe from -133/-83. We previously showed that PHEX is regulated by D_3 in UMR-106 cells, and that promoter constructs of -133/+104 and longer responded similarly. EMSA indicated possible involvement of the -133/-83 region in the D_3 response. Here we show that a D_3 response element is located between -133 and -74 bps of the PHEX promoter and that the response is mediated through the VDR. This is likely not a direct effect of the VDR on the -133/-74 region of the PHEX gene, but rather can likely be attributed to a secondary effect of VDR on the binding of a potentially novel 110 kDa protein to the poly A stretch in the PHEX gene promoter. Thus, it seems likely that D_3 regulates PHEX gene expression by a novel mechanism. Supported by NIH grant R37 DK33209.

W1745

Treatment of Hyperhomocysteinemia in Liver Transplant Recipients with a Naturally Occurring Form of Folate (L-5-Methyltetrahydrofolic Acid) Versus Folic Acid.

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Background: Hyperhomocysteinemia is frequently associated with folate deficiency. Homocysteine (HCY) is an amino acid which is metabolized to methionine in a 5-methyltetrahydrofolate (5-MTHF) depending reaction. Hyperhomocysteinemia is a described risk factor of cardiovascular diseases. Liver transplant recipients have an increased cardiovascular risk because of obesity, arterial hypertension, diabetes mellitus, and hyperlipidemia. We previously described HCY as an additional risk factor in these patients. The aim of this study is (1) to evaluate the treatment of hyperhomocysteinemia in liver transplant recipients with L-5-methyltetrahydrofolate (L-5MTHF) (1mg) vs. folic acid (1mg) vs. placebo in a double blind placebo controlled study and (2) to compare the relative responsiveness of these patients to L-5MTHF and folic acid. Methods: 48 liver transplant recipients were randomized in this study. Over 8 weeks the patients were administered orally with L-5MTHF or folic acid or placebo. During week 0 (pre treatment),1, 2, 4, 8 and 10 (post treatment) fasting serum homocysteine levels were analyzed with HPLC as well as routine parameters of clinical chemestry were obtained. According to the German-Austrian-Swiss HCY league normal HCY concentrationens were determined as < 9 μmol/L. Results: 18 patients (group A) received L-5MTHF, 15 (group B) folic acid and 15 (group C) placebo. HCY concentrations (μmol/L) at week 0 (pretreatment) were: A = 15.0±6, B = 15.2±4, C = 13.9±7; at week 1: A = 12.4±3*, B = 13.3±4, C = 13.4±4; at week 2: A = 11.2±2**, B = 11.3±3, C = 13.9±5, at week 4: A = 11.2±3**, B = 12.6±2, C = 13.1±7, at week 8: A = 9.2±2***, B = 11.1±2*, C = 13.6±7, and at week 10 (post treatment) A = 12.2±4, B = 14.1±3, C = 15.2±8 ($p<0.05$*, $p<0.01$**, $p<0.001$***, ANOVA). Serum vitamin B12 and folate concentration were normal in these patients. Conclusion: Treatment with folates lowers serum HCY levels in liver transplant recipients. The effects of L-5MTHF are significantly more potent than folic acid itself. L-5MTHF acts as an direct substance in HCY degradation to methionine without prior metabolization in the folic acid pathway. After L-5MTHF ingestion no further transformation is needed. In contrast, folic acid has to be reduced and methylated during and after absorption. Lowering serum HCY in liver transplant recipients is more effective with L-5MTHF and therefore may be beneficial in decreasing hyperhomocysteinemia associated risks in these patients.

W1746

Ontogenic Regulation of Intestinal Thiamin Uptake: Molecular Mechanisms in Wild Type and Transgenic Mice

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Background: The water-soluble vitamin thiamin (B_1) is an essentiál micronutrient for normal cellular functions, growth and development. We have previously shown that two thiamin transport systems, THTR-1 and THTR-2 (the products of the *SLC19A2* and *SLC19A3* genes, respectively) are the major contributors involved in the normal thiamin uptake process in the human intestine (BBA 1561 (2):180-7, AJP:GI In Press). It has been well established that the transport of a variety of nutrients undergo marked ontogenic changes during early development, but nothing is known about ontogenic regulation of the intestinal thiamin absorption process. The aim of this study was therefore to address this issue. Methods: Wild type and transgenic mice that express the human *SLC19A2* and *SLC19A3* promoters (fused to the luciferase reporter gene) were used as models (mice have orthologues to *SLC19A2* and *SLC19A3*). Results: Carrier-mediated thiamin uptake by mouse jejunal brush border membrane vesicles (BBMV) was found to decrease with maturation (suckling > weanling > adult). This decrease was found, by quantitative real-time PCR, to be associated with a parallel decrease in levels of the mouse endogenous mTHTR-1 and mTHTR-2 mRNA (suckling > weanling > adult). Analysis of luciferase activity in the jejunum of transgenic mice carrying the *SLC19A2* promoter showed a similar decrease in activity with maturation (suckling > weanling > adult). We are currently analyzing the effect of ontogeny on lucifrease activity in the jejunum of mice expressing the *SLC19A3* promoter. Conclusions: Our results show that the intestinal thiamin uptake process undergoes ontogenic regulation and that this regulation appears to be mediated via a down regulation of the mTHTR-1 and mTHTR-2 mRNA levels. In addition, our studies with transgenic mice carrying the human *SLC19A2* promoter suggest the possible involvement of a transcriptional regulatory mechanism in the down regulation event. Supported by grants form the DVA and the NIH (DK58057 and DK56061). Jack Reidling is the recipient of a NIH NRSA award 1 F32 DK062629.

W1747

Characterization of Selenoprotein Levels in Normal and Neoplastic Colon Tissue.

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Purpose: Selenium may play a role in reducing one's risk for developing colorectal cancer (CRC), however we have previously shown that U.S. patients with CRC are not selenium deficient based on plasma selenoprotein levels. Selenium may impact CRC risk by affecting selenoprotein levels in colon tissue. We characterized selenoprotein expression in normal and neoplastic colon tissue. Methods: Patients undergoing screening colonoscopy participated in the study. Our institutional review board approved the study and informed consent was obtained. Eight biopsies were taken from normal appearing rectosigmoid mucosa and 30 ml of blood was taken from each subject. RNA was prepared from fresh biopsy specimens. One microgram of RNA was converted into single stranded cDNA, and five percent of each resulting cDNA was then used for duplicate PCR reactions for selenoprotein P (SEPP), glutathione peroxidase (GPX) 3, and beta-actin on a SDS7000 instrument (Applied Biosystems). Gene expression was calculated using the relative standard curve method, where serial dilutions of a reference pool of stage III CRC RNAs were used as a reference. Plasma selenium was determined using a fluorometric method. Plasma GPX activity with hydrogen peroxide as the substrate was determined using a method optimized for plasma. Plasma SEPP concentration was determined by radioimmunoassay using stored plasma as a reference. Results: Plasma SEPP, GPX activity and selenium were measured in the first 30 subjects and tissue SEPP and GPX3 expression was determined in the first 43 subjects. The mean plasma SEPP was 0.98 +/-0.15 units/ml plasma. The mean plasma GPX activity was 153 +/-33 nmol NADPH oxidized/ml plasma/min. The mean selenium concentration was 14 +/-3 ug selenium/dl plasma. The average relative expression of SEPP and GPX3 in normal tissue was not different than that of cancer tissue (mean 1.97, std 1.3 and mean 1.37, std 0.9, respectively). Plasma SEPP and GPX activity did not corelate with tissue expression of SEPP or GPX3 respectively. Conclusion: SEPP and GPX3 expression was not different in the normal tissue of our subjects compared to cancer tissue. Additionally, plasma SEPP and GPX activity did not correlate with tissue expression of SEPP or GPX3 in normal tissue. Therefore, it is possible that selenium affects development of CRC at the tissue level, independent of selenium status as assessed by measuring plasma selenoproteins. Further studies are needed to clarify tissue selenoprotein expression, including the effect of selenium supplementation.

W1748

Absence of a Chemoprotective Effect of Folate in an AOM Mouse Model

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Background: Folate supplementation has been linked to reduced colon cancer risk and is a potential chemopreventive agent. To evaluate the protective effects of folate we studied folate deplete (0 mg/kg), replete (2 mg/kg), and supplemented (8 mg/kg) diets in 8wk male CF-1 mice in an AOM model of colon cancer with respect to crypt proliferation, ACF and tumor formation Methods: 232 mice (160 treated + 72 controls) divided between each of the 3 dietary group received proscribed diets for 4 wks followed by injection with 10mg/kg AOM sc or saline in 6 divided doses. Treated (24) and control animals (12) on each folate diet were sacrificed at 5 wk intervals up to 30 wks post initiation of AOM. Mice were sacrificed every five weeks and proliferation was asses sed by metaphase arrest and flow cytometry. ACF and tumor number were enumerated by gross inspection and methylene blue stereomicroscopy. Ras activation and downstream phospo-proteins were measured by immunoprecipitation and western blotting. Results: ACF number were increased in folate depleted animals at 10 wks (18+/-4 vs 9+/- 3) and were persistently elevated (15-25 wks) compared to the other two diets but the increase did not reach significance or predict subsequent tumor development. Cancer occurr ed earlier (15 wks) and were more prevalent (70% vs 39%) and larger overall in the 8 mg/kg diet compared to 0mg/kg of folate (40 +/-7 mg vs20+/-4 mg). The 2 mg/kg diet results were similar to the 8 mg/kg diet in overall tumor size and number. The tumor r ed uction in the folate depleted animals correlated with significantlyy decreased crypt proliferation in the distal colon from 10 wks onward though the initial generalized proliferative response at 5 wks did not differ among the AOM treated animals. Cell cy cle arrest by folate could not be detected at early or late timepoints. The increased proli feration in folate supplemented animals was associated with early (5 wks) increased ras activation (3X) but without significant changes in pAKT or pERK. Conclu sions Folate supplementation had no demonstrated chemopreventive or antiproliferative effects in this colon carcinogen model. r