Urodinamica 15: 64-67, 2005

The bladder urothelium: passive permeability and intravesical drug passive diffusion

A. Giannantoni[1], S.M. Di Stasi[2], E. Mearini[1], V. Nardicchi[3], G. Goracci[3], and M. Porena[1]

[1]Department of Urology, University of Perugia, [2]Department of Urology, "Tor Vergata" University of Roma, [3]Department of Internal Medicine, Biochemistry Section, University of Perugia, Italy

ABSTRACT. The urothelium allows the urinary bladder to minimize alterations in the composition of the urine during storage. Modifications of either cellular or tight junctions permeability alter the efficacy of the barrier properties of urothelium. Changes within the physiological range for urine pH, calcium or urea concentrations do not alter the barrier function of the urothelium, as determined from measurements of the transepithelial resistance. The barrier function may be destroyed by bacterial infection, toxic chemicals, or mechanical damage, but also by non-bacterial, non-chemical inflammatory response. Direct administration of drug solutions into the bladder through a urethral catheter overcomes systemic adverse events of drugs used for bladder disease. Indeed, this treatment modality represents a complex and not completely understood process. Several factors influence the drug transport across the urothelium: the barrier properties of the urothelium itself, pressure gradients, times of exposure, molecular weight and configuration and degree of ionization of the drugs. Further experimental studies and laboratory experiences are needed to transform an empirical methodology, that is intravesical drug passive diffusion, in a really scientific treatment modality.

Urodinamica 15: 64-67, 2005

Correspondence
D.ssa Antonella Giannantoni,
Department of Urology,
University of Perugia,
Policlinico Monteluce,
Via Brunamonti 51,
06100 Perugia, Italy.

E-mail
uropg@unipg.it

INTRODUCTION

The main function of the urinary bladder is to act as a short-term storage site for urine, while maintaining the composition of the urine similar to that produced by the kidneys. Structurally, the mammalian urinary bladder is a hollow sphere, with the wall of the sphere comprising (from outside to inside) the serosa, muscularis, submucosa, muscularis mucosa, and lamina propria (1). Within the above structures there are a circulatory

Urodinamica 15: 64-67, 2005

system, sensory and motor neurons, and an immune system. A layer of epithelial cells (urothelial cells) lies on the top of the lamina propria and covers the inside surface of the sphere. The luminal surface of the urothelium is covered by an adhering glycosaminoglycan layer (GAG) (2). The urothelium allows the urinary bladder to minimize alterations of urine composition during storage. Urothelial cells should have different properties to perform this function. First of all, urothelium should expose a minimum surface to intravesical volume, to avoid large movements of urine components across the bladder wall. As bladder geometry is similar to a sphere, there is a minimum epithelial surface area to urine volume. Thus, the movement of substances between urine and blood is reduced. Furthermore, urothelial cells should be impermeable to all substances present in the urine or blood. Movement across the urothelial cells occurs via two parallel pathways: the "trans-cellular pathway" (through the cells) and the "paracellular pathway" (through the tight junctions and lateral intercellular space) (2). Thus, both tight junctions and cell membranes should be impermeable to urine or blood components, as well as to any drug contained into both the compartments. Modifications of either cellular or tight junctions permeability alter the efficacy of the barrier properties of urothelium.

PASSIVE PERMEABILITY

It is well known that the bladder has a small but finite passive permeability to most substances (electrolytes and nonelectrolytes) found in the urine and blood (3). It is possible to have a measurement of the ion permeability of an epithelium by calculating the *transepithelial electrical resistance* (4). On the basis of the magnitude of this resistance, epithelial cells are divided into two categories: *leaky* and *tight* epithelial cells. Leaky cells usually have a resistance <500 Ω cm^2, whereas tight epithelial cells show resistances >500 Ω cm^2. It has been observed that the electrical resistance of the rabbit urinary bladder ranges from 10,000 to 75,000 Ω cm^2, thus the bladder epithelium is considered a tight epithelium (5). This epithelium has the highest recorded transepithelial resistance of all epithelia measured to date. The transepithelial resistance is caused by the parallel arrangement of the cell resistance and the tight junction resistance. The cell resistance is the sum of the resistance of the apical membrane (urine-facing) and the basolateral membrane (blood-facing). The cell resistance may vary from 10,000 to >100,000 Ω cm^2.

It has been observed that the rabbit urothelium has a very low permeability to sodium and chloride. Other observations about nonelectrolyte movements across the urothelium, both in vitro and in vivo, showed very low values for urea, ammonia, water and proton permeabilities, thus suggesting that the bladder is an excellent barrier to the movement of these substances from urine to blood (6, 7).

Urine components and the "blood-urine barrier"

An essential requirement for normal bladder function is that urine components should not compromise the barrier properties of the bladder (6). Changes within the physiological range for urine pH, calcium or urea concentrations do not alter the barrier function of the urothelium, as determined from measurements of the transepithelial resistance. Thus, acid pH, low Ca^{+2} or high urea increase the ion permeability of the urothelium (8). In experimental studies, urine seem to be able to influence the volume-pressure response of the bladder. It has been observed that bladder capacity can be reduced by administering intravesical solutions of isotonic KCl, hypertonic NaCl and ph 5 (9). On the contrary, it can be increased by hypotonic NaCl, isotonic mannitol and

Urodinamica 15: 64-67, 2005

ph 8. Furthermore, extracellular K^+ and hyperosmolality directly depolarize smooth muscle cells and generate increased activity of the detrusor, while hypo-osmolality produces opposite changes (9).

A number of non-physiological factors causes alterations of the urothelial barrier function. Bacterial products such as amphotericin B, nystatin, polymyxin B, and perhaps α-hemolysin, as well as positively charged proteins released from eosinophils and found in sperm (histones and protamine), increase the ion permeability of the urothelium by interacting with the apical membrane and causing a non-selective increase in membrane ion permeability. If the increase in membrane permeability persists, cell swelling and lysis will occur (6). The loss of cells from the epithelial layer results in a loss of barrier function. Acetate, propionate, butyrate, or succinate at pH 4.4 also alter the transepithelial permeability of the rabbit urothelium, but not at pH 5.0 (9, 10). The increase in transepithelial permeability due to volatile fatty acids is rapid (minutes) and is due in part to an increase in the apical membrane permeability to sodium and chloride (3). However, neither the mechanism by which these volatile fatty acids increase the apical membrane ion permeability at low pH nor the long-term effect of these agents on the barrier function of the urothelium are known.

The barrier function may be destroyed by bacterial infection, toxic chemicals (e.g. cyclophosphamide), or mechanical damage, but also by nonbacterial, non-chemical inflammatory response. Infection, radiation and toxic chemicals can lead to loss of urothelial barrier function either by a direct effect on the urothelial cells, or by secondary effect of inflammation. The loss of barrier properties and inflammation leads to the movement of urine constituents into the underlying connective and muscle tissues, exacerbating the cystitis.

DRUG TRANSPORT ACROSS THE BLADDER WALL: A PHENOMENON NOT COMPLETELY UNDERSTOOD

Direct administration of drug solutions into the bladder through a urethral catheter overcomes systemic adverse events of drugs used for bladder disease. Indeed, transport of drugs across the bladder urothelium represents a complex and not completely understood process. Commonly used for the treatment of superficial bladder cancer, intravesical drug administration has been introduced in the treatment of bladder dysfunction since the first report of Brendler et al., in 1989, which used intravesical oxybutynin to treat neurogenic detrusor overactivity (11). The variability of the results obtained after treatment with intravesical oxybutynin passive diffusion should be due to different factors, related both to the properties of the urothelium and to the characteristics of therapeutic agents. These factors may result in: barrier properties of the urothelium, pressure gradients, times of exposure, molecular weight and configuration and degree of ionization of the drugs. The recent observations about the presence of several types of receptors for different neurotransmitters (cholinergic, adrenergic, vanilloid receptors) also at the level of urothelial cells suggest that target sites for pharmacological modulation of bladder dysfunction should be easily available; anyway the ideal condition for each instilled drug solution needs yet to be investigated and standardized. The risk is that we continue to treat patients without knowing exactly all the physical and chemical mechanisms involved in intravesical drug penetration. Increased permeability, as seen in experimental cystitis, after dimethyl sulfoxide (DMSO) exposure and after overdistension, suggests favored access of urine to detrusor nerves and muscle cells. Urine is frequently hypertonic and differs markedly with respect to K^+ and pH from blood. Changes in intravesical ions, osmolality and pH can alter urothelial permeability and increase (or decrease) drug pene-

tration into the bladder wall. Furthermore, one of the crucial point to obtain successful responses seems to be the vehicle of the drug solution used to increase urothelial permeability. For capsaicin and resiniferatoxin, ethanol and water alone, ethanol at different concentration rates, glucidic solvents or liposomes with hydrogel have been used in different experimental and/or clinical studies with different success rates (12-15). Actually, we really do not know neither the best vehicle for each intravesical drug, nor the ideal bladder conditions to perform a useful intravesical treatment. Recently, a scientific debate started about the variability of clinical results concerning RTX treatments, someone attributing this fact to a possible reduced RTX stability or to a phenomenon of drug absorption by the plastic material of drug containers (16). A recent laboratory study made light to these hypotheses by demonstrating that the stability of RTX solutions can be maintained if ampoules containing the drug are stored at low temperature (4°C) and in dark conditions, and that glass or plastic materials of drug containers do not cause any drug absorption nor alter the stability of the drug (17). The same authors conducted a laboratory study to determine the best conditions for intravesical administration of other drugs, such as lidocaine and epinephrine (18). We retain that such experimental studies and other laboratory experiences should be performed before administering any drug by intravesical passive diffusion, with the aim of transforming an empirical methodology in a really scientific treatment modality.

REFERENCES

1. Hossler F.E., Monson F.C.: Microvasculature of the rabbit urinary bladder. Anat. Rec. 243: 438-448, 1995.
2. Parsons C.L., Lilly J.D., Stein P.: Epithelial dysfunction in nonbacterial cystitis (interstitial cystitis). J. Urol. 145: 732-735, 1991.
3. Mann F.C., Magoun J.A.H.: Absorption from the urinary bladder. Am. J. Med. Sci. 166: 96-106, 1923.
4. Lewis S.A.: Epithelial electrophysiology. In: Wills N.K., Reuss L., Lewis S.A. (Eds.), Epithelial transport: a guide to methods and experimental analysis. Chapman & Hall, London, 1996, pp. 93-117.
5. Lewis S.A., Diamond J.M.: Na+ transport by rabbit urinary bladder, a tight epithelium. J. Membr. Biol. 28: 1-40, 1976.
6. Lewis S.A.: Everything you wanted to know about the bladder epithelium but were afraid to ask. Am. J. Physiol. Renal Physiol. 278: 867-874, 2000.
7. Lewis S.A., Berg J.R., Kleine T.J.: Modulation of epithelial permeability by extracellular macromolecules. Physiol. Rev. 75: 561-589, 1995.
8. Lewis S.A., Clausen C.: Urinary protease degrade epithelial sodium channels. J. Membr. Biol. 122: 77-88, 1991.
9. Hohlbrugger G., Lentsch P.: Intravesical ions, osmolality and pH influence the volume pressure response in the normal rat bladder and this is more pronounced after DMSO. Eur. Urol. 11: 127-130, 1985.
10. Ifshin M.S., Johnson K.E., Eaton D.C.: Acid pH and weak acid induce Na-Cl cotransport in the rabbit urinary bladder. J. Membr. Biol. 76: 151-164, 1983.
11. Brendler T.B., Appell R.A., Lopez M.A., et al.: Pharmacokinetic evaluation of intravesical oxybutynin: bolus and continuous delivery. J. Urol. 165: 252, 2001 (Abstract 1039).
12. Lazzeri M., Spinelli M., Zanollo L., et al.: Intravesical vanilloids and neurogenic incontinence: ten years experience. Urol. Int. 72: 145-149, 2004.
13. Giannantoni A., Di Stasi S.M., Stephen R.L., et al.: Intravesical capsaicin versus resiniferatoxin in patients with detrusor hyperreflexia: a prospective randomized study. J. Urol. 167: 1710-1714, 2002.
14. deSeze M., Wiart L., de Seze M.P., et al.: Intravesical capsaicin versus resiniferatoxin for the treatment of detrusor hyperreflexia in spinal cord injured patients: a double-blind, randomized, controlled study. J. Urol. 171: 251-255, 2004.
15. Tyagi P., Chancellor M.B., Li Z., et al.: Urodynamic and immunohistochemical evaluation of intravesical capsaicin delivery using thermosensitive hydrogel and liposomes. J. Urol. 171: 483-489, 2004.
16. Brady C.M., Harper M., Fowler C.J.: RTX trials experience. Proceedings of the International Continence Society, 32nd Annual Meeting, 28-30 August 2002, pp. 35-36.
17. Di Stasi S.M., Giannantoni A., Massoud R., et al.: Stability of resiniferatoxin stock solutions. Eur. Urol. Suppl. 3: 106, 2004 (Abstract 414).
18. Di Stasi S.M., Giannantoni A., Navarra P., et al.: The stability of lidocaine and epinephrine solutions exposed to electric current and comparative administrations rates of the two drugs into pig bladder wall. Urol. Res. 31: 169-176, 2003.