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Gimatecan and other camptothecin derivatives poison Leishmania DNA-topoisomerase IB leading to a strong leishmanicidal effect

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ABSTRACT

The aim of this work is the *in vitro* and *ex vivo* assessment of the leishmanicidal activity of camptothecin and three analogues used in cancer therapy: topotecan (Hycantim®), gimatecan (ST1481) and the prodrug irinotecan (Camptosar®) as well as its active metabolite SN-38 against *Leishmania infantum*. The activity of camptothecin and its derivatives was studied on extracellular *L. infantum* infrared-emitting promastigotes and on an *ex vivo* murine model of infected splenocytes with *L. infantum* fluorescent amastigotes. *In situ* formation of SDS/KCl precipitable DNA–protein complexes in *Leishmania* promastigotes indicated that these drugs are DNA topoisomerase IB poisons. The inhibitory potency of camptothecin derivatives on recombinant *L. infantum* topoisomerase IB was assessed *in vitro* showing that gimatecan is the most active compound preventing the relaxation of supercoiled DNA at submicromolar concentrations. Cleavage equilibrium assays in *Leishmania* topoisomerase IB show that gimatecan changes the equilibrium towards cleavage at much lower concentrations than the other camptothecin derivatives and that this effect persists over time. Gimatecan and camptothecin were the most powerful compounds preventing cell growth of free-living *L. infantum* promastigotes within the same concentration range. All these compounds killed *L. infantum* splenocyte-infecting amastigotes within the nanomolar range. The amastigote form showed higher sensitivity to topoisomerase IB poisons (with high therapeutic selectivity indexes) than free-living promastigotes. All the compounds assayed poisoned *L. infantum* DNA topoisomerase IB leading to a strong leishmanicidal effect. Camptothecin derivatives are suitable for reducing the parasitic burden of *ex vivo* infected splenocytes. The selectivity index of gimatecan makes it a promising drug against this neglected disease.

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1. Introduction

Visceral leishmaniasis in Europe is a disease caused by the protozoan pathogen *Leishmania infantum* that affects all countries of the Mediterranean region. The disease has a low prevalence in humans – the definitive host – but not in dogs, which act as reservoirs of the parasite. The risk of human disease significantly increased in immunosuppressed individuals mainly linked to HIV, but due to highly active antiretroviral therapies its presence has dramatically decreased [1]. Current pharmacopoeia against leishmaniasis includes old-fashioned pentavalent antimonium derivatives, as well as amphotericin B, paromomycin and alkylphospholipids [2]. Most of them have many undesirable side effects or require parental administration and long-term treatments, which can make treatment difficult to adhere to [3].

Searching for differentiable targets between the host and pathogen is a recognized strategy for designing new drugs. Type IB DNA topoisomerases (TopIB) were indicated as putative targets in proliferative processes when their mechanism of action was originally shown [4]. Since then, numerous compounds have shown antiproliferative effects: (i) by interfering with the catalytic properties of enzymes (TopIB inhibitors) or (ii) by stabilizing the

Abbreviations: Top, DNA topoisomerases; TopIB, DNA topoisomerase IB; LdTopIB, *L. donovani* TopIB; LiTopIB, *L. infantum* TopIB; IFP1.4, infrared fluorescent protein 1.4; SI_{48h}, selectivity index at 48 h; MDR-1, multidrug resistant protein 1.

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enzyme–DNA complex – a transient step of all Top activities – that can be hindered in time by many compounds (TopIB poisons). TopIB poisons prevent the religation step and produces single DNA breaks that interfere with the replication fork of the dividing cells [5]. An amazing result found in 2003 was that *L. infantum* TopIB (LiTopIB) was a heterodimeric enzyme encoded by two genes that were placed on different chromosomes [6]. This characteristic is only shared by certain phylogenetically close microorganisms such as *Trypanosoma cruzi* and *T. brucei* [7]. Despite these differences, most of the domains related to the enzymatic activity are conserved between both subunits, which are interconnected by two polypeptide extensions that play the role of a putative linker. This region is not needed for TopIB activity, but it contributes to DNA binding and camptothecin inhibition, theoretically by slowing down the religation step of the nicking-closing reaction [8–10].

Camptothecin and derivatives, known as TopIB poisons, develop their function by binding in a specific and reversible manner to the transient DNA–enzyme complexes [11]. These drugs effectively target the TopIB–DNA binary complex, while they do not bind to the enzyme alone and display a weak affinity for DNA in the absence of the enzyme [12]. The presence of cleavage complexes generates collisions with the replication fork, causing DNA breaks by converting transient complexes to permanent strand damage and consequently making these compounds powerful anticancer drugs. Camptothecin in fact shows a remarkable antiproliferative potential *in vitro* against a wide range of tumor cells in the submicromolar range [13,14] and it has been the first compound described as a specific inhibitor of eukaryotic cell TopIB with no effect on the bacterial TopIA and a well-defined mechanism of action. The development of camptothecin derivatives against several types of cancer has resulted in two water-soluble compounds currently used in clinical practice: topotecan (Hycantim®) and the pro-drug irinotecan (Camptosar®). In addition a third compound, gimatecan (ST1481) is an orphan drug that is being studied in clinical phase II against astrocytoma, glioblastoma and oligodendroglial tumors [15].

TopIB is essential for DNA replication, recombination and repair mechanisms. Most organisms are unable to survive in the absence of this enzyme [16]. Previous works have unsuccessfully tried to create *Leishmania* [17] or *T. brucei* [18] strains lacking the small monomer or both protomers, respectively. For these reasons and because of distinct structural differences between human and leishmanial TopIB, this protein is considered a valuable target for chemotherapy [19].

This paper explains the effect of these camptothecin derivatives on an *ex vivo* murine model of infected splenocytes with *L. infantum*. Furthermore, the *in vitro* effect of these compounds reveals for the first time their ability to trap TopIB–DNA covalent complexes on *Leishmania* parasites, thus preventing the religation step at micromolar concentrations.

2. Materials and methods

2.1. Reagents and culture media

Pyrococcus furiosus (*Pfu*), klenow polymerases and restriction enzymes were acquired from Roche (Roche Farma SA, Spain) and GE Healthcare (Spain). T4 DNA ligase was obtained from Stratagene (La Jolla, CA, USA). Cell culture media, camptothecin, irinotecan and SN-38 were purchased from Sigma (Sigma–Aldrich, Spain). Topotecan (Hycantim®) was obtained from GlaxoSmithKline (UK). Gimatecan and camptothecin-N-oxide were kind gifts of Sigma Tau to Alessandro Desideri. Primers for PCR amplification were from Sigma Genosys (UK).

2.2. Biological material

L. infantum-IFP1.4 promastigotes and infecting amastigotes were obtained by stable transfection of *L. infantum* (BCN-150 strain) with the pLEXSY-IFP1.4 vector [20]. This vector contains the 987 bp ORF region of the infrared fluorescent protein 1.4 (IFP1.4) derived from *Discosoma* sp., [21] kindly provided by Dr. Roger Y. Tsien (Department of Pharmacology; Department of Chemistry and Biochemistry, University of California, San Diego, USA).

To obtain naturally amastigote-infected splenocytes cultures, BALB/c mice were inoculated intraperitoneally with 10^8 *L. infantum*-IFP1.4 purified metacyclic promastigotes [22]. Five weeks post-infection spleens were aseptically dissected and infected splenocytes were cultured as described previously [20]. Different concentrations of miltefosine (included as positive control) and camptothecin derivatives were administered to the explants for 48 h. The viability of infecting amastigotes was assessed registering the fluorescence emission at 708 nm in an Odyssey (Li-Cor) infrared imaging system. To determine the cytotoxicity of the drugs in mammalian cells, the Alamar Blue staining method was used on drug-exposed splenocytes derived from uninfected mice, according to manufacturer's recommendations. The animal research described in this manuscript complied with Spanish (Ley 32/2007) and European Union Legislation (2010/63/UE). The used protocols were approved by the Animal Care Committee of Universidad de León (Spain).

2.3. DNA relaxation assays

Cloning of *LiTopIB* ORFs (encoding large and small subunits), expression and purification of the enzyme were carried out as previously described [9]. TopIB activity was assayed by the relaxation of negatively supercoiled plasmid DNA. The reaction mixture, in a total volume of 20 μL, contained 0.5 μg of supercoiled DNA from the pBluescript SK(−) phagemid (pSK), 10 mM Tris–HCl buffer pH 7.5, 5 mM $MgCl_2$, 0.1 mM EDTA, 15 μg/mL bovine serum albumin, 50 mM KCl, 1 unit of the leishmanial enzyme and 1 μL of the tested drugs at different concentrations (dose dependent) or at a fixed drug concentration (75 μM) at different time points ranging from 0.2 to 15 min (time-course). Reaction mixtures were incubated at 37 °C. Enzyme reactions were stopped by the addition of up to 1% SDS (w/v) (final concentration) and digested with 1 mg/mL proteinase K at 37 °C during one extra hour to remove protein that remained attached to the DNA fragments. The extent of plasmid DNA relaxation was assessed in 1% agarose gels by electrophoresis in 0.1 M Tris borate EDTA buffer (pH 8.0) at 2 V/cm for 16 h. Gels were visualized with UV illumination after staining with ethidium bromide (0.5 μg/mL). A further electrophoresis was run in the presence of 0.1 μg/mL ethidium bromide, in order to separate the nicked DNA from the relaxed topoisomers.

2.4. Oligonucleotide assays

A 202-bp PvuII/HindIII fragment of pSK DNA substrate [^{32}P]-labeled at a single 3′-end, was prepared as described elsewhere [23]. Equal concentrations of LiTopIB were incubated with at least 1.5 μL of DNA containing a minimum of 100,000 cpm in 10 mM Tris–HCl buffer pH 7.5, 5 mM $MgCl_2$, 5 mM DTT, 0.1 mM EDTA, 15 μg/mL bovine serum albumin, 50 mM KCl, and different concentrations of camptothecin, topotecan, SN-38, gimatecan and camptothecin-N-oxide in 1% DMSO. Following incubation for 2 min at room temperature, reactions were either stopped directly with 1% SDS or treated with 0.5 M NaCl for 1 h at 25 °C to force LiTopIB religation and then stopped with 0.5% SDS at indicated times. The samples were heated at 75 °C for an extra

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15-min period, treated with proteinase K and subjected to double-ethanol precipitation prior to electrophoresis in a 16% polyacrylamide/7 M urea gel. Stabilized cleavage products were visualized with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

2.5. *In situ sensitivity to camptothecin derivatives*

For drug-induced protein–DNA complex determination, *L. infantum* promastigotes, previously labeled for 24 h with 0.5 μCi/mL [2-^{14}C] thymidine, were exposed to different concentrations of camptothecin and its derivatives for 30 min, followed SDS/KCl method as previously described [20]. All assays included solvent controls. DNA fragments formation, as a percentage of total labeled DNA, was calculated as follows: [(dpm in SDS/KCl drug − dpm in SDS/KCl solvent)/(dpm total incorporation)] × 100. Each experiment was run at least in triplicate. For each drug the concentration causing 50% of DNA to be cleaved (CC_{50}) was calculated from dose/response curves.

3. Results

3.1. *Leishmanicidal effect of camptothecin and analogues*

We have analyzed the effect of the specific TopIB inhibitors outlined in Fig. 1 on the proliferation rate of *L. infantum*-IFR1.4 promastigotes and amastigotes. Table 1 shows the IC_{50} values obtained from dose–response plots, based on the percentage of infrared fluorescence signal at 708 nm, obtained for free-living promastigotes and amastigote-infected splenocytes, exposed for 48 h to several concentrations of the tested compounds in comparison to untreated controls. The IC_{50} value obtained with miltefosine (a leishmanicidal alkylphospholipid) is also reported in Table as positive control. No differences in drug sensitivities are found between the *IFR1.4*-transfected strain and the wild-type (data not shown). Gimatecan and camptothecin are the most powerful compounds in killing promastigotes with IC_{50} values near 1 μM. The water-soluble derivative topotecan and SN-38 are less effective, but both have IC_{50} values lower than the positive-control miltefosine, that is more effective than camptothecin-N-oxide and irinotecan. The table also shows the IC_{50} value obtained for the amastigote-infected splenocytes. Gimatecan is the most effective one, having IC_{50} value on the nanomolar range, followed by camptothecin, SN-38 and topotecan. Camptothecin-N-oxide and miltefosine are the weakest compounds in killing amastigotes working in the lower micromolar range. Irinotecan does not show a significative leishmanicidal effect likely due to its pro-drug nature.

The leishmanicidal effect of these compounds has been validated checking their potential toxicity on mammalian cells. For this purpose, freshly isolated splenocytes, from uninfected BALB/c mice, have been exposed to different drug concentrations for 48 h and the percentage of living cells has been fluorimetrically determined with the Alamar Blue staining technique. The selective

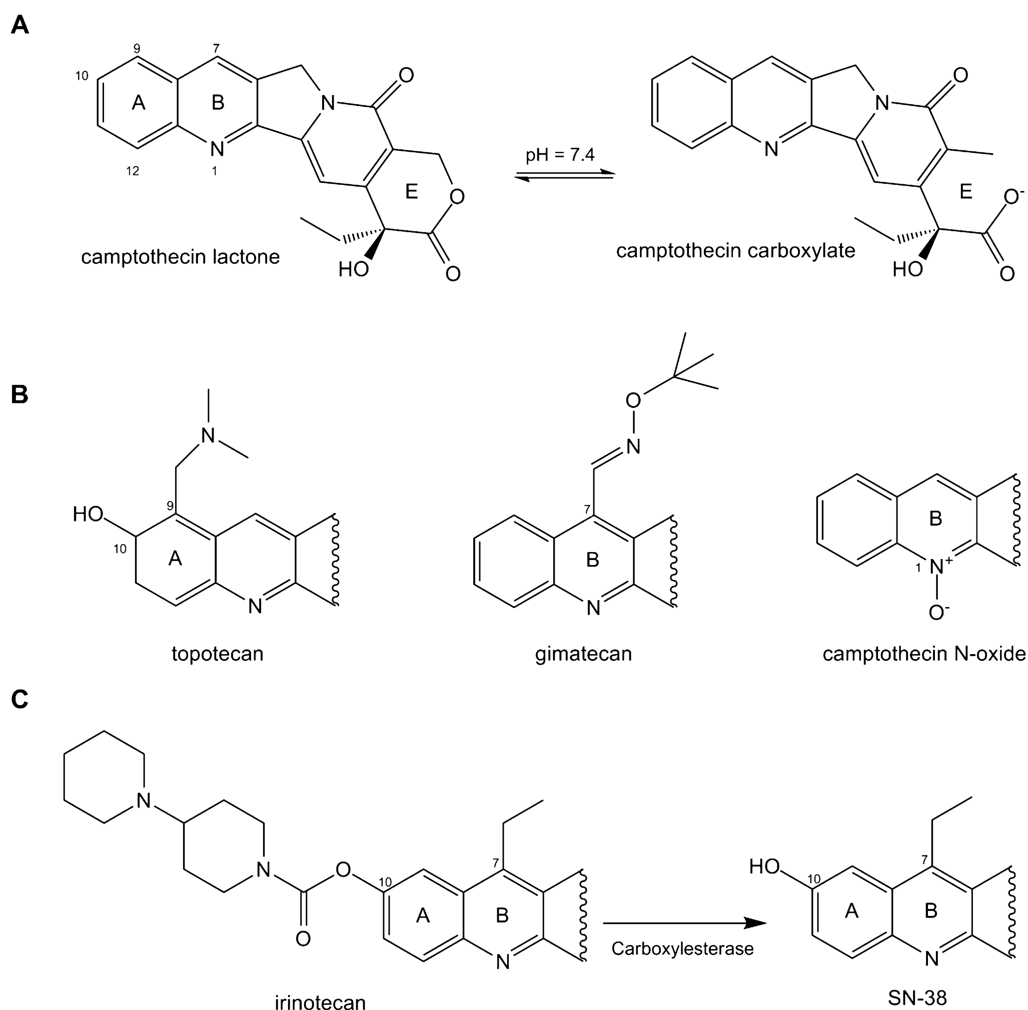

Fig. 1. Chemical structures of the camptothecin derivatives used in the study. (A) Lactone/carboxylate equilibrium of the camptothecin E-ring; (B) different moieties substituting the A and B rings of topotecan, gimatecan and camptothecin-N-oxide; (C) enzymatic hydrolysis of the prodrug irinotecan to the active compound SN-38.

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Table 1
IC_{50} calculation after a 48-h period of exposure to the selected compounds of *L. infantum* promastigotes, *ex vivo* infected splenocytes and uninfected splenocytes.[a]

Drug	IC_{50} for[b]:			
	L. infantum promastigotes (μM)[c]	Infecting amastigotes (μM)[c]	Uninfected splenocyte culture (μM)[d]	SI_{48}[e]
Miltefosine	25.15 ± 2.35	8.7 ± 0.07	504.1 ± 7.0	57.9
Camptothecin	1.12 ± 0.13	0.03 ± 0.01	0.62 ± 0.13	20.7
Topotecan	10.86 ± 1.64	0.16 ± 0.05	4.96 ± 0.95	31.0
Gimatecan	1.73 ± 0.10	0.001 ± 0.000	0.21 ± 0.00	175
Camptothecin-N-oxide	90.02 ± 1.20	1.48 ± 0.54	3.95 ± 1.55	2.7
SN-38	12.20 ± 2.11	0.05 ± 0.03	0.54 ± 0.08	9.8
Irinotecan	>200	>100	>200	ND

ND = no determined.
[a] IC_{50} at 48 h of the compounds in freshly uninfected splenocyte culture and SI_{48h} values between this cell line and infected splenocytes with *L. infantum* amastigotes *ex vivo* were calculated from the dose–response curves determined in triplicate in separate experiments after performing a nonlinear fitting with the SigmaPlot software program.
[b] Mean ± SD.
[c] The viability of both *L. infantum* IFP1.4-promastigotes and infecting IFP1.4-amastigotes was assessed registering their infrared-fluorescence emission at 708 nm in an Odyssey (Li-Cor).
[d] The viability of uninfected splenocyte culture was assessed by using the Alamar Blue staining method on drug-exposed splenocytes derived from uninfected mice.
[e] Selectivity index; SI_{48h}, IC_{50} for uninfected splenocyte culture/IC_{50} for amastigotes.

index (SI_{48h}), calculated as the IC_{50} ratio of the uninfected and *L. infantum*-IFR1.4 infected splenocytes, reported in Table 1, shows that gimatecan is the safest compound with a SI_{48h} value three fold higher than miltefosine and significantly higher than the other tested compounds.

3.2. In situ *formation of cleavable complexes*

The formation of enzyme–DNA adducts, in promastigotes exposed to camptothecin analogues, has been evaluated by protein precipitation with SDS. *L. infantum* promastigotes, grown in the presence of [2-^{14}C]-thymidine, have been exposed to increasing drugs concentrations over a period of 30 min. The percentage of labeled SDS/KCl precipitable complexes with respect to total labeled DNA has been determined using camptothecin as reference drug (Fig. 2A). Gimatecan displays the maximum DNA-cleaving potency (CC_{50} = 0.02 μM) since a gimatecan concentration of 0.3 μM is enough to obtain 80% of cleaved DNA (Fig. 2C). A camptothecin or topotecan concentration of 1 and 10 μM respectively is needed to obtain a comparable DNA breakage percentage (Fig. 2A and B). Camptothecin-N-oxide reaches the same percentage at a concentration of 90 μM (Fig. 2D), whilst in the case of SN-38 the maximum concentration of cleaved DNA is 50% even at a concentration of 90 μM (Fig. 2E).

3.3. *LiTopIB inhibition by camptothecin and derivatives*

The effect of the different compounds on the relaxation activity of LiTopIB has been monitored over time incubating 1 U of LiTopIB with 75 μM of each derivative in the presence of 0.5 μg of 37 °C pre-warmed supercoiled pSK DNA. Aliquots have been then taken at different time points, ranging from 0.2 to 15 min. As shown in Fig. 3A, in absence of the drug, the supercoiled substrate is fully processed after 0.2 min. Camptothecin and SN-38 inhibit the

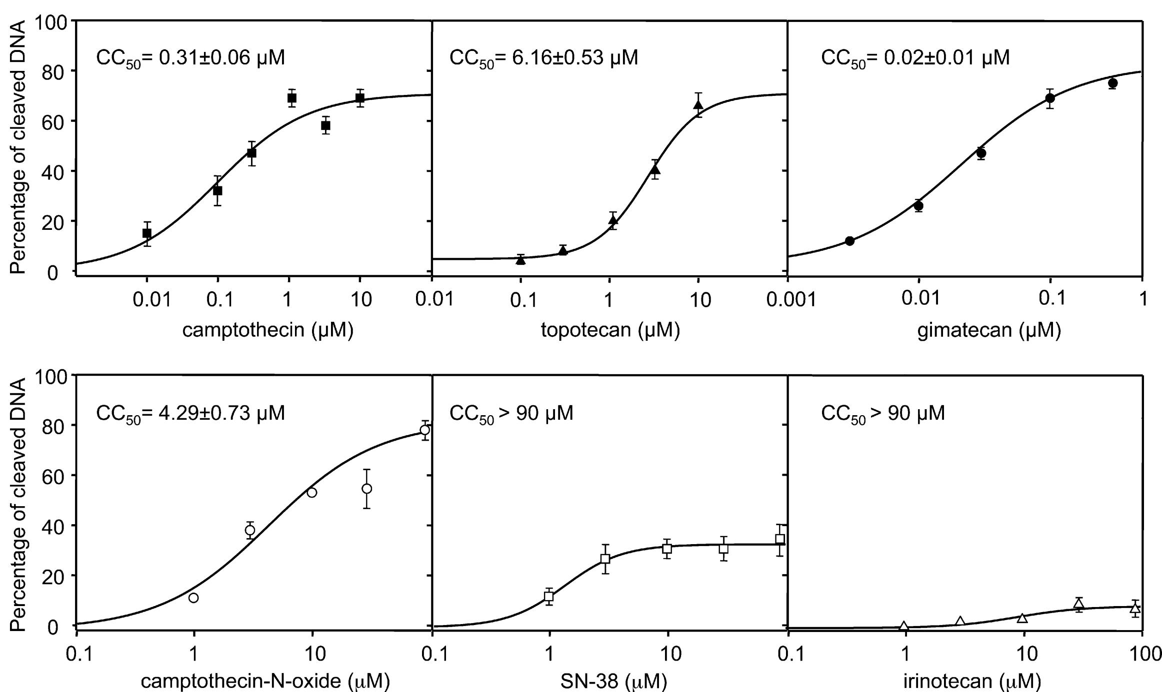

Fig. 2. *In vivo* determination of SDS/KCl-precipitable enzyme–DNA complexes at increasing concentrations of the drugs under study: (A) camptothecin; (B) topotecan; (C) gimatecan; (D) camptothecin-N-oxide; (E) SN-38 and (D) irinotecan in promastigotes of *L. infantum* after 48 h growth in presence of [2-^{14}C] thymidine. Results are expressed as mean ± SE of at least three different experiments by duplicate.

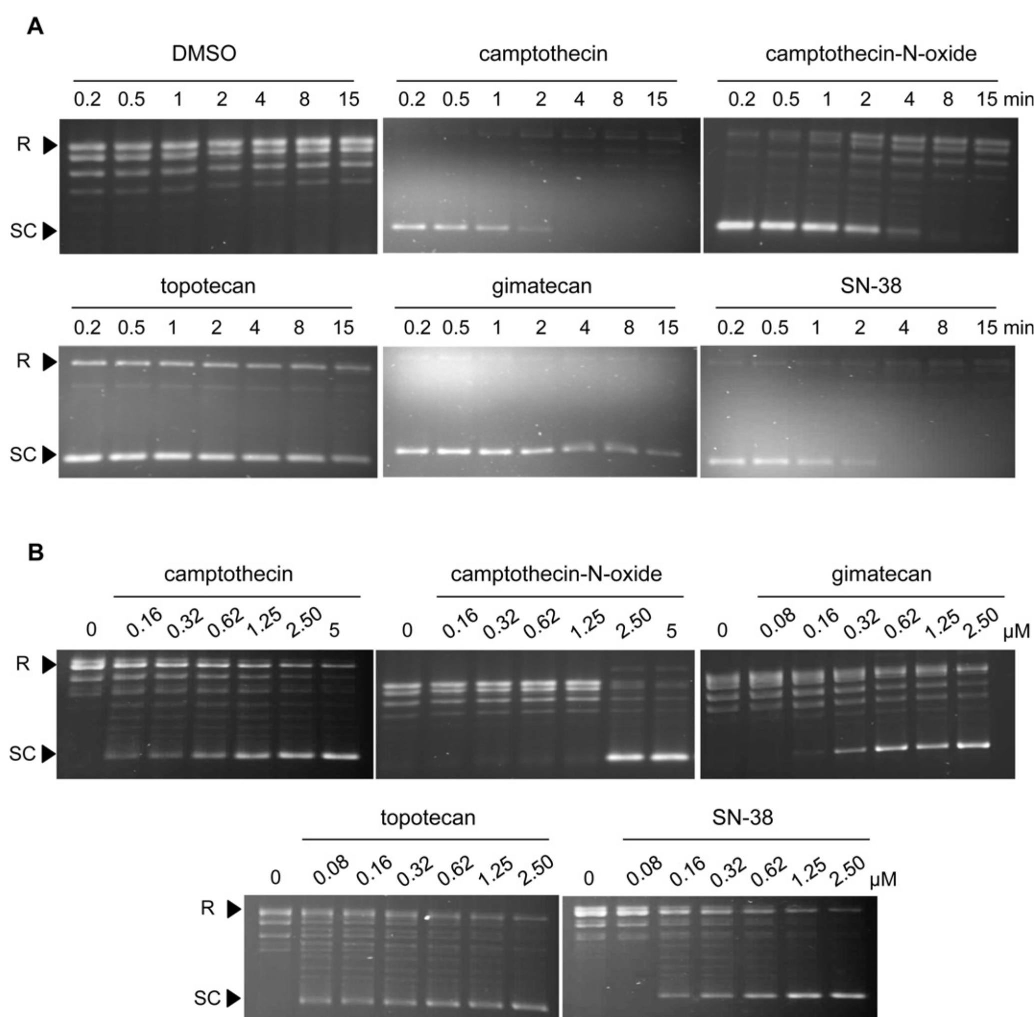

Fig. 3. Inhibition of LiTopIB-mediated DNA relaxation by camptothecin analogues (A) time-course inhibition of LiTopIB relaxation activity mediated by camptothecin analogues at a fixed 75 μM concentration; (B) dose-dependent LiTopIB relaxation activity inhibition at the optimum time-point determined in A panel. Key: R = relaxed DNA; SC = supercoiled DNA. Reactions were incubated at 37 °C in a final concentration of 150 mM KCl and then stopped with SDS up to a final concentration of 1% of reaction volume. Products were resolved in a 1% agarose gel and visualized by ethidium bromide staining. The results are representative of three independent trials.

relaxation of supercoiled DNA up to 2 min, camptothecin-N-oxide up to 4 min, whilst gimatecan or topotecan are the most efficient ones preventing the relaxation activity of the enzyme up to 15 min.

The inhibitory potency of the camptothecin derivatives on the relaxation activity of recombinant LiTopIB was also assessed after 30 sec as a function of drug concentrations (Fig. 3B). Topotecan, camptothecin, gimatecan and SN38 have a comparable efficacy whilst larger camptothecin-N-oxide concentrations are required to have the same level of inhibition (Fig. 3B).

3.4. Camptothecin derivatives poison LiTopIB

A cleavage–religation equilibrium assay has been carried out in order to assess the cleavage stabilization nature of these drugs as TopIB poisons. These compounds are expected to act stabilizing the cleavable complexes generated by the nicking action of the enzyme. Once the enzyme is attached to DNA and cuts one of its strands, camptothecin stabilizes the LiTopIB–DNA complex and hinders the religation of the nicked DNA [10]. A *Pvu*II/*Hind*III fragment of pSK DNA has been incubated with 100 U of LiTopIB in presence of different drugs concentrations (0.1, 1, 10 and 100 μM). The first lane of Fig. 4 shows that in absence of the drugs no signals of the cleaved oligo are observed, indicating that the religated/uncleaved (R/U) equilibrium is shifted towards religation. In presence of SN-38, topotecan and gimatecan the equilibrium is shifted towards cleavage, since several cleaved bands (CL1, CL2 and CL3) are appearing due to the stabilization of the cleavable complexes. Gimatecan is the most powerful compound, since it stabilizes the enzyme–DNA complexes at concentrations of 0.1 μM. Camptothecin and SN-38 require a concentration 10-fold higher, whereas topotecan a 100-fold higher concentration to reproduce comparable effects. It is interesting to notice that topotecan is not able to trap the CL2 cleavable complex, likely due to a low residence time of the drug in this site or to a different cleavage pattern for topotecan in which CL2 is not a preferred site [24].

Fig. 5 shows a cleavage complex reversal assay comparing the stability of both LiTopIB–camptothecin–DNA and LiTopIB–gimatecan–DNA ternary complexes. The reversal of the LiTopIB cleavable complexes is much slower for gimatecan than for camptothecin. The effect of camptothecin persists for two minutes, whilst the gimatecan-stabilized complex can be detected up to 15 min. The long persistence time of gimatecan on the DNA cleaved site provides an explanation to its greater potency in inducing cell death.

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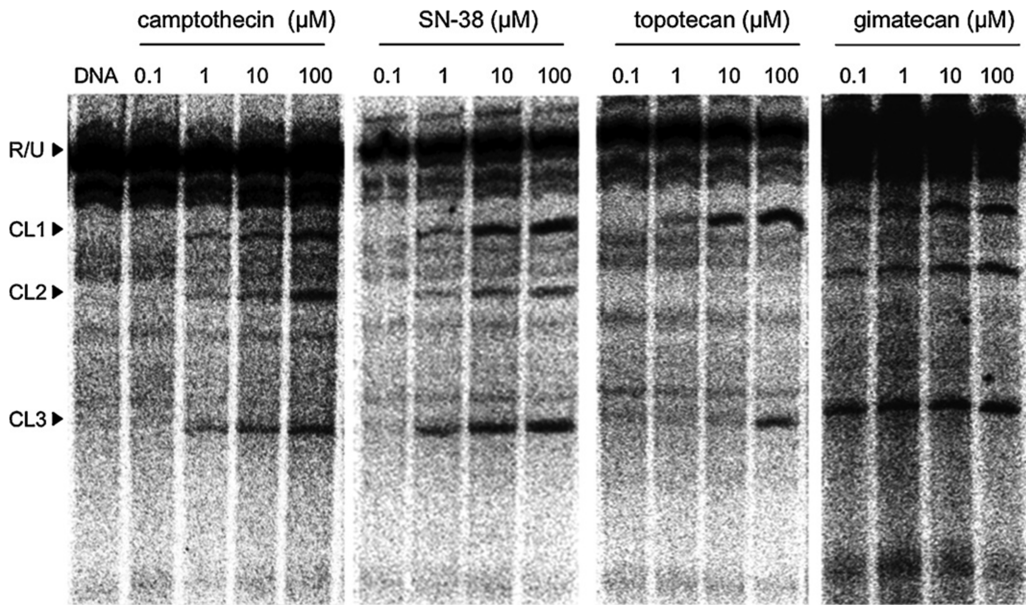

Fig. 4. SDS-mediated cleavage/religation equilibrium of a DNA substrate in presence of different concentrations of camptothecin derivatives. 75 units of purified LiTopIB enzyme were incubated with 100,000 cpm of the [α-^{32}P] end-labelled pSK DNA fragment at a final concentration of 150 mM KCl. Denaturing electrophoresis gels show the presence or absence of cleavage stabilization when reactions are carried out with solvent alone (first lane) or increasing concentrations 0.1, 1, 10 and 100 μM of camptothecin, SN-38, topotecan and gimatecan. Arrows indicate DNA preferred cleavage sites (CL1 to 3) by LiTopIB. Irinotecan and camptothecin-N-oxide did not show a significant cleaving pattern at any concentration tested. The results are representative of three independent trials.

4. Discussion

One of the most problematic aspects of leishmaniases is the fast emergence of resistant strains against conventional drugs, which hinders the treatment and consequently, constant development of new therapeutic resources is required. In the present study, we have shown that gimatecan is a powerful growth inhibitor of *L. infantum* promastigotes that is even more lethal for its

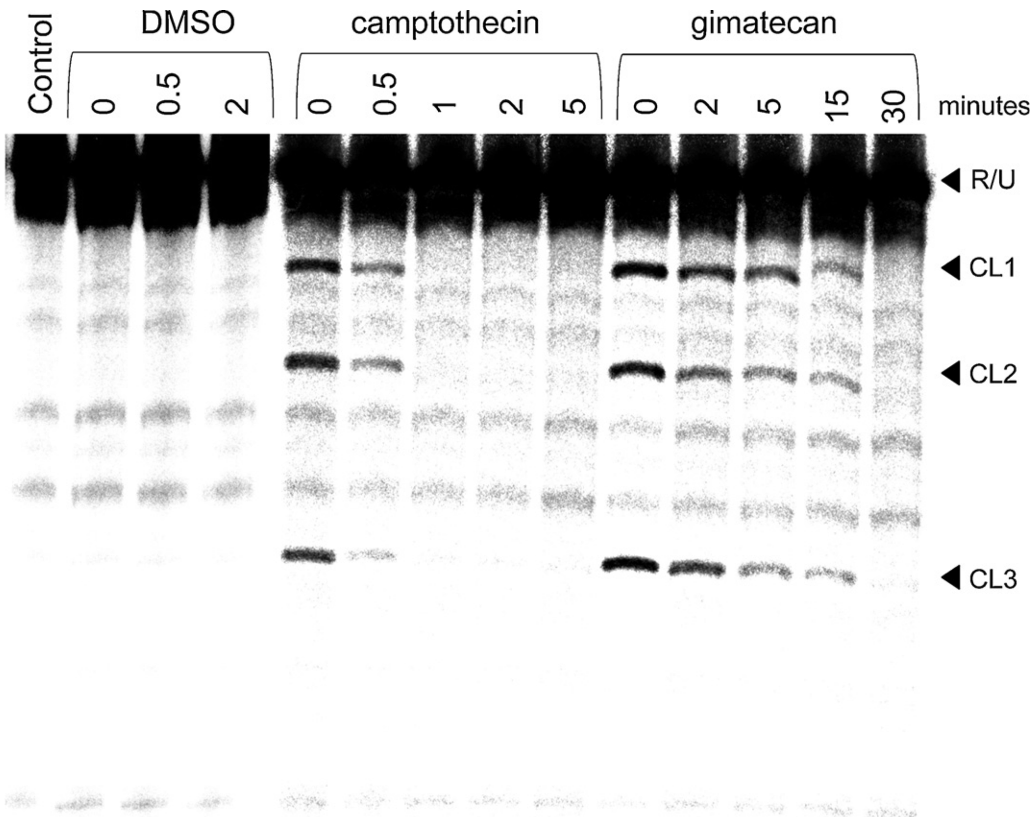

Fig. 5. Differential time stability of TopIB cleavable complexes induced by camptothecin and gimatecan. Reactions were performed at 25 °C for 2 min, after which a control was taken (time 0) and 0.35 M NaCl was added. Times above each lane, aliquots were taken after NaCl addition and stopped by adding 0.5% SDS.

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intracellular form (amastigote) (Table 1). In addition, gimatecan is also shown to be a potent LiTopIB-poison.

In vitro results show that the relaxation activity of *L. infantum* DNA topoisomerase IB is inhibited by all the camptothecin derivatives, with the exception of camptothecin-N-oxide and irinotecan, within the nanomolar range (Fig. 2B), suggesting that this enzyme is the potential target in camptothecin-treated *Leishmania* parasites. Gimatecan stabilizes the cleavable complexes at a concentration 10-fold lower than camptothecin, and of any other camptothecin derivatives (Fig. 4). Moreover the gimatecan-stabilized complexes are slowly reverted in comparison with the ones stabilized by camptothecin (Fig. 5). This fact may explain the enhanced leishmanicidal effect of gimatecan, since drug-induced DNA damage is directly proportional to the lifespan of the ternary complex. The slow reversal of the gimatecan-induced cleavable complexes is likely due to its 7-t-butoxyiminomethyl substitution, since this position has been shown to be important in other 7-modified camptothecin derivatives [25]. The long residence time of the gimatecan stabilized ternary complexes, is also in agreement with the long time that the LiTopIB relaxation activity remains hindered by this drug (Fig. 3A) and the greater potency of gimatecan in inducing SDS/KCl-precipitable DNA–topoisomerase complexes *in vivo* (Fig. 2).

The leishmanicidal activity of the camptothecin derivatives has been validated on a model of mouse splenocytes infected with a *L. infantum* strain expressing IFP1.4, which permits to study the compounds under conditions that resemble those found in the animal, including the presence of the complete range of immune host cells, infected macrophages and fibroblasts [20]. For all the studied derivatives the sensitivity of the amastigote form is much higher than that of free-living promastigotes (Table 1). This is not an unexpected result since many drugs with different intracellular targets have similar behavior [26]. However, irinotecan does not have leishmanicidal effect (up to 100 μM) in the splenocytes, suggesting that this compound is not metabolically hydrolyzed to SN-38 within the splenic explants.

A remarkable finding is the extraordinary power of gimatecan in all trials conducted both *in vitro* and *ex vivo*. This drug has a therapeutic selectivity index above 170, three times higher than miltefosine that is clinically used for the treatment of leishmaniasis (Table 1). Furthermore, gimatecan presents a series of added values, which must be taken into consideration, as they substantially improve the therapeutic index obtained for this drug and make it interesting for the treatment of visceral leishmaniasis: (i) after oral administration the highest tissue levels are found in liver and spleen [27]; (ii) the percentage of active lactone form in plasma is 80 to 100%, much higher than those of its analogues, topotecan, irinotecan and SN-38 estimated at *ca.* 20% for each [28,29]; (iii) a half-life of over 76.5 h, maintaining significant concentrations in plasma 7 days after the dose was given [28]; (iv) Zhu et al. [28] have shown in cancer patients that after weekly administration of oral gimatecan at 1.2 mg/kg concentration for 3 weeks the gimatecan is tolerated and has favorable pharmacokinetic properties. Miltefosine, despite its drug safety, has a less desirable dosage regimen that involves the administration of 1.76 mg/kg/d for 28 days for an effective oral treatment of this cutaneous disease, resulting in difficulty of adherence to treatment of infected people in developing countries [30]; (v) unlike other camptothecins the pharmacokinetics of the compound appears to be more favorable in humans than in mice [29]; (vi) it has recently been shown that gimatecan is not affected by multidrug resistant protein 1 (MDR-1) efflux in tumor cell lines (responsible for resistance to camptothecin), which have an orthologous protein-coding gene in *Leishmania* [15].

In conclusion, the present study indicates that all the here studied camptothecins with the exception of camptothecin-N-oxide, are *Leishmania* topoisomerase IB poisons in the low micromolar range, although gimatecan is the most efficient one and its excellent selectivity index suggest that this compound may be exploited as a potential drug candidate against visceral leishmaniasis.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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