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ORIGINAL ARTICLE

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Pharmacokinetics and biodistribution of the camptothecin–polymer conjugate IT-101 in rats and tumor-bearing mice

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Abstract *Purpose*: IT-101 is a camptothecin–polymer conjugate prepared by linking camptothecin (CPT) to a hydrophilic, cyclodextrin-based, linear polymer through ester bonds. In previous studies, these polymer conjugates with high molecular weights (ca 90 kDa) have shown significant antitumor effects against human colon carcinoma xenografts. The pharmacokinetics of IT-101 in plasma of rats and its biodistribution in nude mice bearing human LS174T colon carcinoma tumors is reported here. *Methods*: Sprague–Dawley rats were injected intravenously with three different doses of IT-101. Serial plasma samples were analyzed for polymer-bound and unconjugated CPT by high-performance liquid chromatography (HPLC). Concentration vs time data were modeled using non-compartmentalized methods and compared to CPT alone, injected intravenously at an equivalent dose. Tumor-bearing mice were injected intravenously with IT-101 and intraperitoneally with CPT alone, and sacrificed after 24 and 48 h, and serum, heart, liver, spleen, lungs and tumor collected. Tissue samples were extracted and analyzed for polymer-bound and unconjugated CPT by HPLC. *Results*: Plasma concentrations and the area under the curve for polymer-bound CPT are approximately 100-fold higher than those of unconjugated CPT or CPT alone injected intravenously at an equivalent dose. The plasma half-life of IT-101 ranges from 17–20 h and is significantly greater than that of CPT alone (1.3 h). When CPT is conjugated to polymer, the biodistribution pattern of CPT is different from that taken alone. At 24 h post injection, the total CPT per gram of tissue is the highest in tumor tissue when compared to all other tissues tested. Tumor concentrations of active CPT released from the conjugate are more than 160-fold higher when administered as a polymer conjugate rather than as CPT alone. *Conclusions*: The studies presented here indicate that intravenous administration of IT-101, a cyclodextrin based polymer–CPT conjugate, gives prolonged plasma half-life and enhanced distribution to tumor tissue when compared to CPT alone. The data also show that active CPT is released from the conjugate within the tumor for an extended period of time. These effects likely play a significant role in the enhanced antitumor activity of IT-101 when compared to CPT alone or irinotecan.

Keywords Camptothecin · Polymer conjugate
Pharmacokinetics · Biodistribution

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Introduction

20(S)-Camptothecin (CPT) is a naturally - occurring alkaloid with a wide spectrum of antitumor activity which acts through interaction with the nuclear enzyme topoisomerase I [11, 18, 26]. However, the clinical use of CPT has been hampered by its low water solubility [26], the presence of a chemically unstable E-ring [15], low clinical activity, and severe toxicity [16, 17]. The presence of the unstable lactone ring (E-ring) is of particular concern, since it undergoes reversible spontaneous hydrolysis to yield the inactive carboxylate form, predominant at physiologic pH. Both the lactone and the carboxylate form of CPT bind to plasma proteins to a great degree, with the carboxylate showing the greater affinity [11, 15, 21]. For example, the degree of binding of the lactone form to human serum albumin (HSA) was found to be approximately 81%, whereas that of carboxylate was approximately 98% [21]. This effect forces the distribution of CPT to further disfavor the lactone

form. To address some of these issues, several water soluble small- molecule analogues have been synthesized, of which two, topotecan (Hycamptin, Glaxo-SmithKline) and irinotecan (Camptosar, Pharmacia & Upjohn), are currently approved for the treatment of certain types of human cancer [13].

Another approach to overcome the drawbacks of CPT is to conjugate it to water-soluble polymers [24]. In addition to improved solubility, polymer conjugates may also improve the biodistribution to tumor tissue through the so-called enhanced permeability and retention (EPR) effect [14]. This process relies on two factors: abnormally leaky tumor vasculature allowing macromolecular extravasation, and lack of effective tumor lymphatic drainage, preventing effective clearance of the penetrating macromolecules. Covalent attachment of CPT to a number of different polymers such as poly(ethylene glycol) (PEG) [6, 9, 10], poly-*N*-(2-hydroxypropyl)methacrylamide (HPMA) [3, 27], carboxymethyldextran [8], and poly (L-glutamic acid) (PG) [1, 22, 28] has been reported. In preclinical studies, these conjugates have shown improved solubility and efficacy when compared to CPT alone. The conjugates that have entered phase I/II of clinical trials are PG–CPT ($M_w = 17$ kDa, CT-2106, Cell Therapeutics, Seattle, WA), PEG–CPT ($M_w = 40$ kDa, Prothecan, Enzon, Piscataway, NY) [19], MAG–CPT, an HPMA conjugate ($M_w = 18$ kDa, PNU-166148, Pharmacia and Upjohn, Milan, Italy) [2, 25], and DE-310, a conjugate between the CPT analog DX-8951f and carboxymethyldextran ($M_w = 340$ kDa, Daiichi Pharmaceutical, Tokyo, Japan) [23]. Early clinical results have been published for MAG–CPT: a Phase I study showed that MAG–CPT was tolerated up to 200 mg CPT equivalents/kg and that plasma half-life was extended to >6 days [2]. However, a Phase II study showed no preferential accumulation of polymer-bound or unconjugated CPT in tumor tissue of colorectal cancer patients [20], and a subsequent study showed severe cumulative bladder toxicity when weekly doses were administered. [25]. A Phase I study of PEG–CPT showed a prolonged plasma half-life of 77 h for released CPT , the absence of dose - limiting toxicity up to 7,000 mg/m^2 (drug conjugate containing ca 1% CPT), and some preliminary antitumor activity [19]. A Phase I study of DX-310 showed prolonged plasma half-life of 13 days for the conjugate, a ratio of conjugated to free drug of 600, and some preliminary antitumor activity [23].

IT-101 is a conjugate between CPT and a beta-cyclodextrin based polymer (Fig. 1). The molecular weight of IT-101 is 85 ± 23 kDa, significantly higher than MAG–CPT, PG–CPT, or PEG–CPT but lower than DX-310. CPT is attached to the polymer at its 20-OH position via a single glycine linker. This conjugate, previously also named HG6 (high MW, single glycine linker, 6% CPT loading) shows dramatically increased solubility while maintaining CPT in its active lactone form [5]. Free CPT is released from this polymer prodrug by esterolytic cleavage. Compared to conjugates with lower molecular weight (35 kDa) and triglycine linker, IT-101 gave increased antitumor effect and reduced toxicity, respectively. IT-101 also showed improved antitumor activity when compared to CPT alone or irinotecan in preclinical studies [4]. The aim of the present studies is to define the pharmacokinetics of IT-101 in the plasma of rats and its biodistribution in nude mice bearing human LS174T colon carcinoma tumors.

Materials and methods

Reagents

CPT was purchased from Boehringer Ingelheim (Ingelheim, Germany). IT-101 was synthesized as previously described [5]. Properties of the polymer–CPT conjugate IT-101 are described in Table 1. IT-101 is similar to compounds previously denoted as HG6, representing a conjugate with high M_w (H, 85 ± 23 kDa), a single glycine linker (G) and approximately 6 wt% (5.0%) drug loading [4].

Plasma sample pretreatment

To determine the level of unconjugated CPT in each plasma sample, 15 µl of plasma (thawed on ice) was mixed with 15 µl of 0.1 N HCl. The resulting mixture was allowed to stand in ice for 5 min. Proteins were precipitated by the addition of acetonitrile (120 µl) to the plasma mixture and incubation at 4°C for 3 h. The suspension was centrifuged at 14,000 rpm for 10 min at 10°C. The supernatant (120 µl) was transferred to a 300 µl HPLC vial, 0.1 N HCl (120 µl) was added, and the sample analyzed by reverse phase HPLC.

To determine the level of total CPT in each plasma sample, 15 µl of plasma (thawed in ice) was mixed with 10 µl of 0.1 N NaOH and stored at room temperature for 1 h, releasing the CPT from the polymer. Fifteen microliters of 0.1 N HCl was then added, followed by 110 µl of methanol. Proteins were precipitated by incubation at room temperature for 3 h. The suspension was centrifuged at 14,000 rpm for 10 min at 10°C. The supernatant was further diluted with 0.1 N HCl and analyzed by reverse phase HPLC.

Solid tissue sample pretreatment

Tissue samples were weighed and placed in bead-containing 2 ml homogenizer tubes (Matrix D for mammalian tissue sampling, Qbiogen). HCl (0.1 N, 100 µl) and 400 µl of lysis reagent (Cellytic-MT Mammalian Tissue Lysis Reagent, Sigma) were added to each tube. The tissue sample was homogenized using a homogenizer (Qbiogene FastPrep F12) at a speed of 5 m/s for

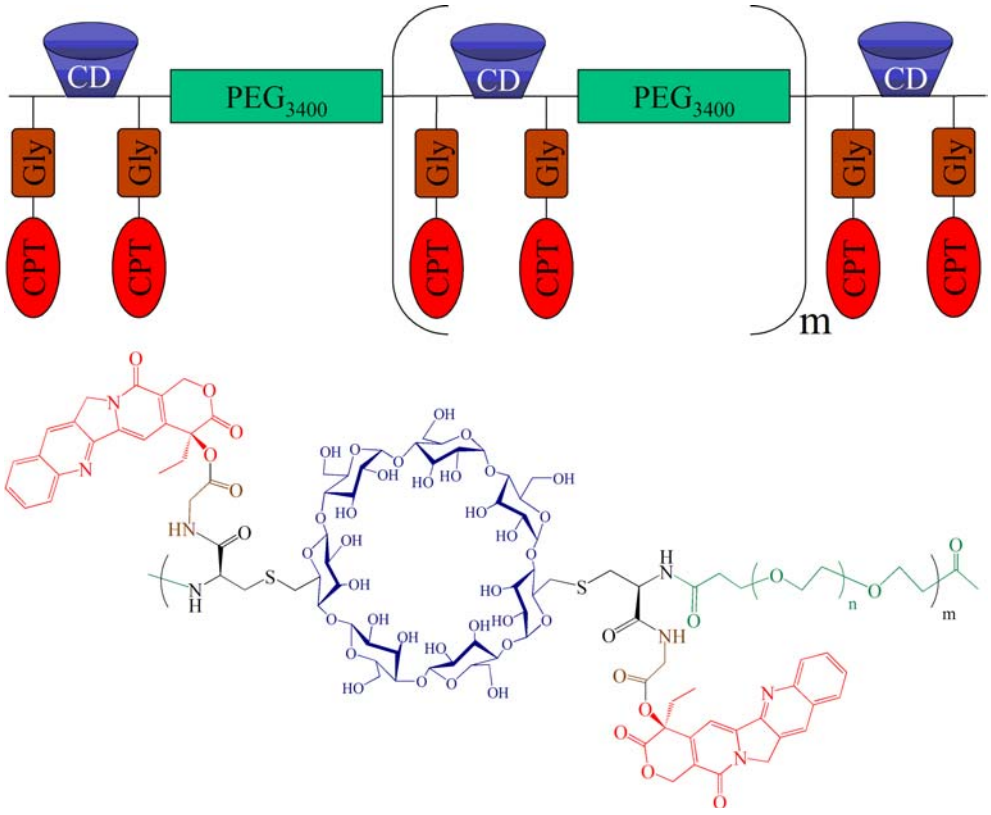

Fig. 1 Schematic representation of the structure of IT-101, a conjugate of 20(S)-camptothecin (CPT) and a linear, cyclodextrin-based polymer (CDP). The components of the parent polymer are β-cyclodextrin (indicated in *blue*) and poly-ethylene glycol (PEG, *green*), both of which are widely used in pharmaceutical formulations, and the natural amino acid L-cystein (*black*). CPT (*red*) is attached to the polymer via a single glycine amino acid linker (*brown*). *n* Number of ethylene glycol repeating units (average $n = 77$ for PEG with M_w 3,400); *m* number of repeating units of (CDP–CPT) in the polymer–CPT conjugate (average $m = 18 \pm 5$ for parent polymer M_w of 85 ± 23 kDa)

40 s. This process was repeated 15 times at 5-min intervals for each sample. Tissue samples were cooled on ice after every other homogenization procedure. Following completion of the 15-cycle homogenation process, the homogenate was centrifuged at 14,000 rpm for 30 min at 10°C. Eight hundred microliters of cold methanol (stored at 4°C) was added to 200 µl of supernatant to precipitate tissue proteins. The mixed solution was centrifuged at 14,000 rpm for 15 min at 10°C.

To determine the level of unconjugated CPT in each tissue sample, 100 µl of the supernatant solution from the centrifuged solution referred to previously was mixed with 0.1 N HCl (100 µl). The resulting solution was analyzed by reverse phase HPLC.

To determine the level of total CPT in each tissue sample, 150 µl of supernatant was mixed with 150 µl of 0.1 N NaOH. The mixture was allowed to stand for 3 h at room temperature. HCl (0.1 N, 1200 µl) was added to this solution. The solution was analyzed by reverse phase HPLC.

Determination of polymer-bound and unconjugated CPT by reverse phase HPLC

Chromatography was performed on a Beckman Coulter HPLC System Gold equipped with a 126P solvent module, a 508 autosampler, a fluorescence detector (GTI/Spectrovision FD-500), and a column heater

Table 1 Properties of IT-101

M_w of the parent polymer	M_w/M_n[a]	CPT loading (wt%)	Unconjugated CPT (%)[b]	Particle size[c]
85 kDa	1.48	5.03	1.3	78 nm

[a]Polymer poly-dispersity determined by light scattering techniques
[b]Measured by HPLC methods
[c]Mean particle size of the conjugate determined in water (pH 3.0) by dynamic light scattering using a ZetaPals instrument (Brookhaven Instruments, Holtsville, NY)

(Phenomenex, Thermasphere TS-130). Separation of the compounds was performed on a Phenomenex Synergi 4 μ Hydro-RP 80A 250 mm×4.60 mm column. A guard column (4 mm×3 mm) packed with the same resin was used to protect the column. CPT was detected using an excitation wavelength of 370 nm and an emission wavelength of 440 nm. The mobile phase was a 50% potassium phosphate buffer (10 mM, pH 4.0)—50% acetonitrile, the flow rate was 0.5 ml/min, and the column temperature was 30°C. Injection volumes between 5 and 50 μl were applied. CPT eluted at a retention time of 8.5 min whereas the polymer–CPT conjugate eluted at a retention time of 4.5 min. CPT was quantitated using a CPT standard (50 and 200 ng/ml in 0.1 N HCl) injected to generate a linear standard curve between 0.25 and 10 ng CPT. Levels of bound CPT (i.e. CPT still attached to the polymer backbone) were calculated by subtracting unconjugated CPT concentration from the total CPT concentration. All samples were tested in triplicate.

Plasma pharmacokinetics study of IT-101 in Sprague–Dawley rats

Female Charles River Sprague–Dawley cannulated rats (200–225 g) were randomized into three groups of four animals each. Animals were injected intravenously with three different doses of IT-101 corresponding to an estimated 90% maximum tolerated dose (MTD, 8.8 mg/kg CPT equivalent), one-half log of MTD (2.77 mg/kg CPT equivalent), and one log below MTD (0.88 mg/kg equivalent). Dosing volume was based upon a ratio of 10 ml/kg and scaled appropriately according to actual body weight of the rats. At scheduled time-points (0, 5, 15, and 30 min, 1, 2, 4, 8, 12, 24, 48, 72, 96, and 120 h), 300 μl of blood was withdrawn from each animal via the cannula. The blood was processed for plasma, which was immediately frozen and stored at −80°C prior to analysis.

Plasma pharmacokinetics of CPT in Sprague–Dawley rats

Plasma concentrations of IT-101 are compared to previously published plasma concentration data of total CPT after a bolus intravenous injection of 1 mg/kg of CPT in male Sprague–Dawley rats [21]. Total CPT (lactone and carboxylate forms) concentration vs time data were provided by V. Stella and analyzed in the same manner as IT-101 data. Briefly, due to limited solubility, CPT was administered in a solution composed of 20% DMSO, 20% PEG 400, 30% EtOH, and 30% pH 3.5 phosphoric acid (10 mM). Blood samples were collected at 5, 15, 30, 45, 60, 90, 120, 180, and 240 min post dosing, processed for plasma and analyzed for total drug concentration by HPLC using fluorescence detection. Sample preparation was performed using a method almost identical to the one described, giving results for direct comparision.

Analysis and determination of pharmacokinetic parameters

Pharmacokinetic analysis of the HPLC results was carried out using a non-compartmental modeling software (PK Solutions 2.0, Summit Research Services, Montrose, CO). Calculations of pharmacokinetic parameters, including distribution and elimination half-life, area under the concentration–time curve, volume of distribution (V_d), and clearance rates (Cl) are based on resolving the concentration–time curve into a series of exponential terms according to Eq. 1

$$C(t) = \sum_{i=1}^{n} C_i e^{-\lambda_i t} \quad (1)$$

The number of exponential terms resulting in minimal residuals was found to be three for polymer-bound CPT and two for both unconjugated CPT and CPT alone. Since in most cases, these "model-independent" exponential terms correspond to the elimination (E) and the initial (A) and late (D) distribution phases of a drug in the blood, these terms are used throughout this paper.

Biodistribution study of IT-101 in LS174T colon carcinoma xenograft mice

The LS174T colon tumor line used for this study was maintained in athymic nude mice. A tumor fragment (1 mm^3) was implanted subcutaneously into the right flank of Charles River female athymic nude mice. Tumors were monitored until their average size was 400–500 mm^3 at which point animals were randomized into two groups of eight animals each. Animals were administered either a single dose of CPT (3 mg/kg, MTD for CPT) intraperitoneally or a single dose of IT-101 (24 mg/kg CPT equivalent, approximately 90% MTD) intravenously. IT-101 was dissolved in 5% dextrose in water (D5W) while CPT was prepared in D5W containing 3% DMSO and 3% Tween 80. Dosing volume was determined based on a ratio of 200 μl for a 20 g mouse, and was scaled appropriately according to actual body weight. Mice were euthanized at either 24 h or 48 h post administration. All the blood was collected from each animal by cardiac puncture and processed for plasma. Liver, spleen, lung, tumor, and heart were harvested from each animal, and all tissues sectioned into two pieces of approximately equivalent sizes. The weight of each tissue sample was determined. Plasma and tissue samples were immediately stored at −80°C prior to analysis.

Results

Plasma pharmacokinetics

Plasma concentrations of polymer-bound and unconjugated CPT were analyzed by HPLC after a single bolus intravenous injection of IT-101 in Sprague–Dawley rats. Spiking studies into whole blood gave recoveries of 87.0% for IT-101. Spiking IT-101 and CPT alone into plasma resulted in recoveries of 89.4% and 103.8%, respectively. These results indicate that binding to erythrocytes is minimal for polymer-bound CPT. This was shown to be true for both the lactone and carboxylate form of CPT alone [21]. Additionally, these data demonstrate that the extensive serum protein binding expected for CPT [21] did not interfere with the analysis.

Animals were dosed at three different levels corresponding to approximately 90% MTD (8.8 mg/kg CPT equivalent), one-half log of MTD (2.77 mg/kg CPT equivalent), and one log below MTD (0.88 mg/kg equivalent). Figure 2 shows the time-course of plasma concentrations averaged over all animals for both polymer-bound and unconjugated CPT. Plasma levels of unconjugated CPT were approximately 100-fold lower than polymer-bound CPT at all time-points tested.

For comparative purposes, Fig. 2b also shows previously published [21] plasma concentration data of CPT in Sprague–Dawley rats administered intravenously with a dose of 1 mg/kg of CPT. This dose corresponds well with the lowest dose of IT-101 administered (0.88 mg/kg CPT equivalent). Direct comparison of intravenous PK data at the higher dose levels was not possible due to limited solubility and dose limiting toxicity of CPT alone. In fact, even at the 1 mg/kg dose level, CPT needed to be administered in a solution containing of 20% DMSO, 20% PEG 400, and 30% EtOH in order to completely dissolve the drug.

Compared to polymer-bound CPT, administration of CPT alone resulted in significantly lower plasma concentrations. The CPT concentration was 30-fold lower at 5 min and over 500-fold lower 4 h post administration. Interestingly, the concentration profile for CPT alone was similar to the one for CPT released from the polymer (Fig. 2b, inset).

Pharmacokinetic parameters for polymer-bound and unconjugated CPT as well as for the CPT alone control are shown in Table 2. When comparing equivalent doses, mean elimination half-life ($t_{½}$) for polymer-bound CPT was significantly greater than for CPT alone (17.2 vs 1.3 h). The $t_{½}$ for polymer-bound CPT was nearly concentration independent, increasing slightly from 17.2 to 19.8 h with increasing dose.

The area under the curve (AUC) for polymer-bound CPT was at least 100-fold higher than the corresponding AUC for unconjugated CPT. AUC scaled linearly with the dose administered for both the polymer-bound and unconjugated CPT. However, the ratio of polymer-bound to unconjugated AUC increased from 110 to 335 with decreasing dose. While the reason for this effect is unknown, one may speculate that higher exposure to unconjugated CPT may negatively affect the ability to clear the drug from the circulation. Another possible explanation is that the tail-end of the concentration–time curve was not as well- defined for the lower dose levels because the limit of quantitation of the HPLC method was reached earlier. The AUC for polymer-bound CPT also compares favorably to AUC for CPT alone injected at an equivalent dose. In fact the AUC for CPT injected alone was identical to the AUC for released CPT (0.2 μg h/ml). This result agrees well with the value of 0.2 μg h/ml reported by Scott et al. [21].

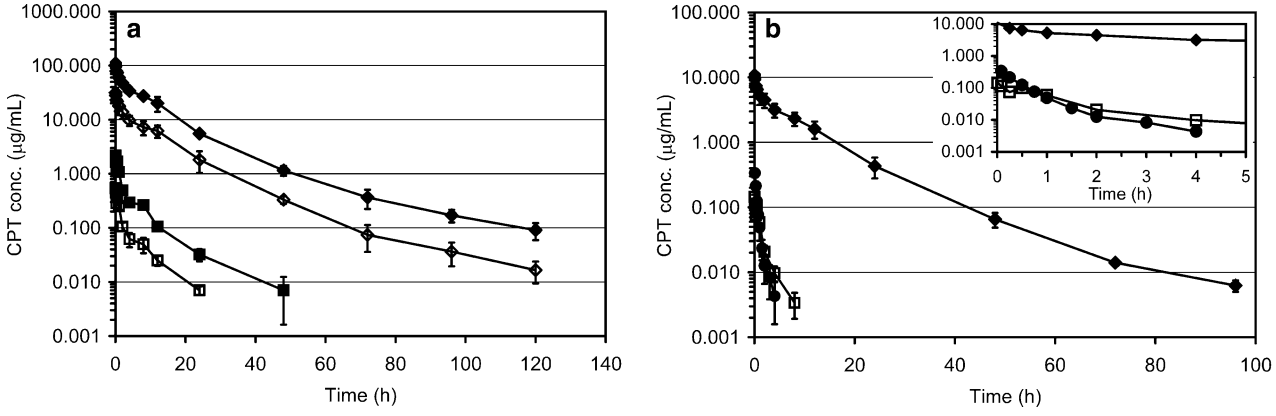

Fig. 2 *Panel A:* plasma concentration of polymer-bound (*diamonds*) and unconjugated CPT (*squares*) as a function of time after a single bolus i.v. injection of IT-101. Doses administered were 8.8 mg/kg CPT equivalent (*filled symbols*) or 2.77 mg/kg CPT equivalent (*open symbols*). *Panel B:* plasma concentration of polymer-bound (*closed diamonds*) and unconjugated CPT (*open squares*) after a single bolus i.v. injection of IT-101 (0.88 mg/kg CPT equivalent). The previously published concentration–time profile for CPT following an equivalent i.v. dose of CPT (1 mg/kg) is also depicted (*closed circles*) [21]. The inset shows a magnification of the graph between 0 and 4 h. *Error bars* indicate standard deviation

Table 2 Pharmacokinetic parameters for IT-101 and camptothecin after a single bolus i.v. injection in Sprague–Dawley rats

Parameters[a]	Units	Polymer-bound CPT			Unconjugated CPT			CPT
IT-101 dose	mg/kg	8.8	2.77	0.88	8.8	2.77	0.88	n/a
Dosage	μg/kg	8761.9	2774.6	876.2	n/a	n/a	n/a	1000
E half-life	h	19.81	17.16	17.16	n/a	n/a	n/a	1.291
D half-life	h	6.039	6.890	8.029	n/a	n/a	n/a	0.258
A half-life	h	0.392	0.529	0.815	n/a	n/a	n/a	∼
C initial (i.v.)	μg/ml	100.6	29.4	9.1	2.0	0.5	0.1	0.4
AUC∞	μg h/ml	692.6	203.8	66.9	6.3	1.3	0.2	0.2
MRT	h	12.5	12.1	17.2	7.6	6.3	2.3	0.9
V_c	ml	18.0	19.4	20.1	n/a	n/a	n/a	797.5
V_d	ml	74.8	69.3	68.0	n/a	n/a	n/a	2857.8
V_d/kg	ml/kg	363.2	338.6	325.8	n/a	n/a	n/a	8793.3
V_{ss}	ml	32.8	33.8	47.2	n/a	n/a	n/a	1305.5
CL	ml/h	2.620	2.796	3.372	n/a	n/a	n/a	1534.2
CL/kg	ml/h kg	12.71	13.67	13.16	n/a	n/a	n/a	4722.2

[a] *E*, elimination; *D*, distribution; *A*, initial distribution; AUC∞, total area under curve; MRT, mean resident time (time for 63.2% of the administered dose to be eliminated; V_c, apparent volume of the central compartment; V_d, volume of distribution; V_d/kg, V_d normalized by animal weight; V_{ss}, volume of distribution at steady state; CL, systemic clearance; CL/kg, CL normalized by animal weight. Animals were injected with three different doses of IT-101 corresponding to an estimated 90% MTD (8.8 mg/kg CPT equivalent), one-half log of MTD (2.77 mg/kg CPT equivalent), and one log below MTD (0.88 mg/kg equivalent). Camptothecin treated animals were administered a single dose of 1 mg/kg of CPT (for study details see Ref. [21], data kindly provided by V. Stella)

The volume of distribution (V_d) for CPT alone was significantly greater (42-fold) than the V_d for polymer-bound CPT. This result is an indication that IT-101, a nanoparticle drug with a hydrodynamic diameter of 78 nm (Table 1), is retained in the vasculature to a higher degree than CPT alone, a very hydrophobic, low molecular weight compound.

Biodistribution of IT-101 in mice bearing LS174T xenografts

Average tissue concentrations of total and unconjugated CPT are listed in Table 3. At 24 h post administration, IT-101 was distributed to all major organs tested, including tumor. The average 24-h total CPT concen-

Table 3 Concentration of total and unconjugated camptothecin in plasma and various tissues of nude mice bearing subcutaneous LS174T tumor xenografts

		CPT		IT-101	
		24 h	48 h	24 h	48 h
Plasma	Total CPT (ng/ml)[a]	0 ± 0	0 ± 0	21,640.5 ± 1,985.9	5,862.3 ± 1,314.4
	Unconjugated CPT (ng/ml)			72.1 ± 9.2	2.6 ± 3.3
	Percent of unconjugated CPT (%)			0.3%	0.04%
Heart	Total CPT (ng/g)[b]	0 ± 0	14.3 ± 0	6,419.6 ± 880.9	2,548.6 ± 565.6
	Unconjugated CPT (ng/g)			23.4 ± 21.3	4.5 ± 5.2
	Percent of unconjugated CPT (%)[c]			0.4%	0.2%
Liver	Total CPT (ng/g)[b]	106.4 ± 39.5	107.8 ± 74.3	12,396.1 ± 2,197.1	6,899.2 ± 1,975.4
	Unconjugated CPT (ng/g)			241.3 ± 131.9	121.4 ± 56.7
	Percent of free CPT (%)[c]			1.9%	1.8%
Spleen	Total CPT (ng/g)[b]	48.5 ± 15.2	0 ± 0	9,647.9 ± 1,799.8	7,321.2 ± 2,854.3
	Unconjugated CPT (ng/g)			45.4 ± 42.1	12.9 ± 16.7
	Percent of unconjugated CPT (%)[c]			0.5%	0.2%
Lung	Total CPT (ng/g)[b]	7.7 ± 15.1	0 ± 0	8,683.8 ± 1,072.8	3,587.4 ± 901.3
	Unconjugated CPT (ng/g)			56.8 ± 43.2	10.5 ± 8.8
	Percent of unconjugated CPT (%)[c]			0.7%	0.3%
Tumor	Total CPT (ng/g)[b]	1.1 ± 0	0 ± 0	13,901.3 ± 833.4	4,175.3 ± 337.6
	Unconjugated CPT (ng/g)			183.3 ± 115.3	55.2 ± 22.1
	Percent of unconjugated CPT (%)[c]			1.3%	1.3%

[a] ng/ml = ng of CPT/ml plasma
[b] ng/g = ng of CPT/g of corresponding solid tissue
[c] Percent of unconjugated CPT expressed as a percent of average free CPT to average total CPT recovered in each tissue. Animals received a single bolus of camptothecin (3 mg/kg) intraperitoneally or IT-101 (24 mg/kg CPT equivalent) intravenously

tration detected in animals treated with IT-101 was greatest in tumor (13,901 ng/g), followed by the liver (12,396 ng/g), spleen (9,648 ng/g), lung (8,684 ng/g), and heart (6,420 ng/g). Average 24-h total CPT level in plasma was 21,640.5 ng/ml. Average total CPT concentrations decreased in all tissues at 48 h when compared to those detected at the 24-h time-point. Average unconjugated CPT levels accounted for less than 2% of the average total CPT in all tissues at both 24 and 48 h time-points.

For reference purposes, CPT was intraperitoneally administered at 3 mg/kg. This dose and route were chosen because it represented the maximum dose that could be administered without causing unacceptable animal toxicity, and the best case scenario for treatment with CPT. Intravenous administration of CPT alone was not tolerated at that dose level. At 24 h following a single intraperitoneal injection of CPT alone, the greatest concentration of total CPT was detected in the liver of all animals (Table 3). Average total CPT concentration was greatest in the liver (106.4 ng/g), followed by the spleen (48.5 ng/g), lung (7.7 ng/g), and tumor (1.1 ng/g). Average total CPT concentration in heart and plasma at 24 h were below levels of detection. Average total CPT concentration in the liver at 48 h (107.8 ng/mg) was similar to levels detected at the 24-h time-point (106.4 ng/g). Average total CPT levels in the spleen, lung, and tumor at 48 h were decreased relative to the levels detected at the 24-h time-point, whereas average total CPT concentration in the heart increased to 14.3 ng/mg at 48 h when compared to levels below detectable limits at 24 h. Average total CPT levels in plasma at 48 h remained below detectable limits.

Discussion

The present study shows that IT-101, a polymer–CPT conjugate, gives a significantly improved pharmacokinetics and biodistribution profile for CPT when compared to CPT alone. The mean the plasma elimination half-life ($t_{½}$) for IT-101 (17–19 h) is significantly longer than that of CPT (1.3 h). A long $t_{½}$ for the polymer–conjugate when compared to the CPT molecule is most likely due to its ability to evade glomerular elimination. In terms of polymer size, glomerular filtration has been shown to be virtually eliminated for dextran and PEG polymers with molecular weights M_w above 40 kDa [12]. This corresponds to particles with a hydrodynamic (Stokes) diameter greater than approximately 10 nm. The M_w of the IT-101 parent polymer used in this study was 85 kDa which resulted in a hydrophilic drug conjugate with a particle size of 78 nm (Table 1).

A long plasma half-life may be of potential benefit because CPT is known to have a cell cycle dependent mechanism and the prolonged exposure afforded by long plasma $t_{½}$ could result in an increased antitumor effect.

The plasma levels of CPT alone, injected at a dose equivalent to the lowest dose of IT-101 administered, were significantly below those for polymer-bound CPT and resulted in a significantly higher volume of distribution for CPT alone (Fig. 2 and Table 2). This result indicates that CPT shows significant extravascular binding while IT-101 is maintained within the intravascular compartment.

The plasma levels of unconjugated CPT were approximately 100-fold lower than polymer-bound CPT at all doses and time-points tested (Fig. 2). This translated into significantly lower AUCs for unconjugated CPT when compared to polymer-bound (Table 2). Low plasma levels for unconjugated CPT may provide a potential safety benefit in that high plasma AUCs for free CPT were correlated to increased side effects in early clinical trials with sodium CPT [7, 17]. On the other hand, we have previously shown that while the polymer–CPT conjugate is readily taken up by cells through the endosomal–lysosomal pathway, it does not appear to travel to the nucleus [5], therefore topoisomerase I related activity or side effects are expected to be caused by CPT once it is released from the polymer and diffuses to the nucleus. Release of the active drug from the polymer is through cleavage of the ester linkage between the 20-OH group of CPT and a single glycine amino-acid linker (see Fig. 1).

Administration of IT-101 to tumor-bearing mice resulted in a biodistribution pattern significantly different from mice dosed with CPT alone. When average, total CPT levels are expressed in terms of percent of total injected dose per gram tissue as shown in Fig. 3, it becomes apparent that IT-101 resulted in significantly higher delivery of the drug to all major organs tested, including tumor, when compared to CPT alone. This is true for both 24 and 48 h time-points and supports the notion that a long plasma half-life of the conjugate can lead to significantly increased tissue concentrations.

More importantly, a single intravenous dose of IT-101 resulted in greater total CPT levels in the tumor at 24 h than in any other tissue tested, while a single dose of CPT alone showed negligible tumor distribution. This observation supports the hypothesis that IT-101 can accumulate in the tumor tissue by the so-called EPR effect [14]. The EPR effect can be attributed to two factors with regard to the long-circulating nanoparticulate drugs which (a) escape the vasculature through abnormally leaky tumor blood vessels (b) are subsequently retained in the tumor tissue due to lack of effective tumor lymphatic drainage.

Another interesting observation was that in animals dosed with IT-101, unconjugated CPT tumor to plasma ratios increased from approximately 2.5:1 at 24 h to 21:1 at 48 h. The increase in unconjugated CPT in tumor relative to plasma indicates that active drug is released from the polymeric prodrug within the tumor rather than distributed from plasma to tumor. Previous in vitro studies have shown that IT-101 is effectively

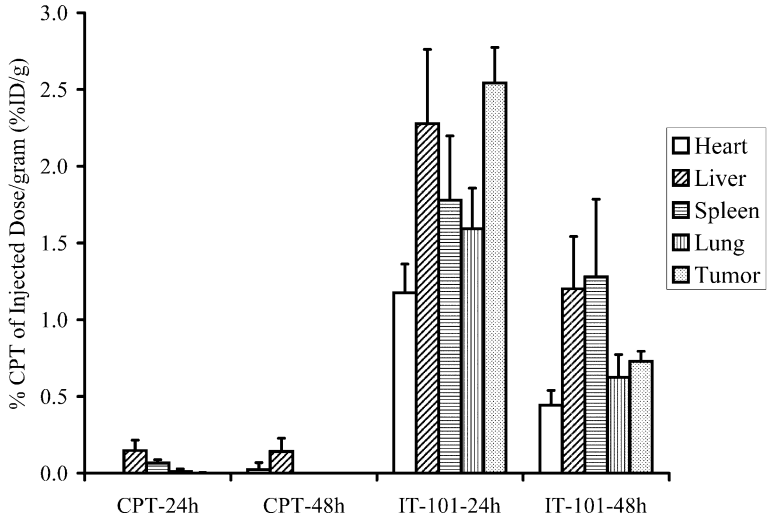

Fig. 3 Total CPT concentration expressed as percent of injected dose in tissues of nude mice bearing subcutaneous LS174T colon cancer xenografts. Mice received a bolus i.v. injection of IT-101 (24 mg CPT/kg) or i.p. injection of CPT (3.0 mg/kg, MTD for CPT). *Error bars* indicate standard deviation

taken up by cells by what appears to be endosomes–lysosomes [4]. Acidification of endosomes may stabilize the ester linkage between polymer and CPT and therefore provide a slow release mechanism for the active CPT drug. These results also indicate that tissue half-life of IT-101 may be significantly longer than plasma half-life, a hypothesis supported by the detection of both polymer-bound and unconjugated CPT in xenograft tumors for a significant time after injection [4].

In summary, the studies presented here indicate that administration of IT-101, a cyclodextrin based polymer–CPT conjugate, leads to prolonged plasma half-life and enhanced distribution to tumor tissue when compared to CPT alone. They also show that CPT is released from the conjugate within the tumor for an extended period. The combination of these observed effects likely contributes to the enhanced antitumor activity of IT-101 when compared to CPT alone or irinotecan observed in preclinical studies.

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