Cancer Chemother Pharmacol (1998) 41: 464–468

ORIGINAL ARTICLE

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Plasma and cerebrospinal fluid pharmacokinetics of 9-aminocamptothecin (9-AC), irinotecan (CPT-11), and SN-38 in nonhuman primates

Received: 15 July 1997 / Accepted: 8 October 1997

Abstract *Purpose*: The plasma and cerebrospinal fluid (CSF) pharmacokinetics of the camptothecin analogs, 9-aminocamptothecin (9-AC) and irinotecan, were studied in a nonhuman primate model to determine their CSF penetration. *Methods*: 9-AC, 0.2 mg/kg (4 mg/m^2) or 0.5 mg/kg (10 mg/m^2), was infused intravenously over 15 min and irinotecan, 4.8 mg/kg (96 mg/m^2) or 11.6 mg/kg (225 mg/m^2), was infused over 30 min. Plasma and CSF samples were obtained at frequent intervals over 24 h. Lactone and total drug forms of 9-AC, irinotecan, and the active metabolite of irinotecan, SN-38, were quantified by reverse-phase HPLC. *Results*: 9-AC lactone had a clearance (CL) of 2.1 ± 0.9 l/kg per h, a volume of distribution at steady state (Vd_{ss}) of 1.6 ± 0.7 l/kg and a half-life ($t_{1/2}$) of 3.2 ± 0.8 h. The lactone form of 9-AC accounted for 26 ± 7% of the total drug in plasma. The CSF penetration of 9-AC lactone was limited. CSF 9-AC lactone concentration peaked 30 to 45 min after the dose at 11 to 21 n*M* (0.5 mg/kg dose), and the ratio of the areas under the CSF and plasma concentration-time curves (AUC^{CSF}: AUC^{P}) was only 3.5 ± 2.1%. For irinotecan, the CL was 3.4 ± 0.4 l/kg per h, the Vd_{ss} was 7.1 ± 1.3 l/kg, and the $t_{1/2}$ was 4.9 ± 2.2 h. Plasma AUCs of the lactone form of SN-38 were only 2.0% to 2.4% of the AUCs of irinotecan lactone. The lactone form of irinotecan accounted for 26 ± 5% of the total drug in plasma, and the lactone form of SN-38 accounted for 55 ± 6% of the total SN-38 in plasma. The AUC^{CSF}: AUC^{P} ratio for irinotecan lactone was 14 ± 3%. SN-38 lactone and carboxylate could not be measured (< 1.0 n*M*) in CSF. The AUC^{CSF}: AUC^{P} ratio for SN-38 lactone was estimated to be ≤ 8%. *Conclusion*: Despite their structural similarity, the CSF penetration of 9-AC and SN-38 is substantially less than that of topotecan which we previously found to have an AUC^{CSF}: AUC^{P} ratio of 32%.

Key words Irinotecan · 9-Aminocamptothecin · CSF penetration · Topotecan

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Introduction

The camptothecin analogs are novel anticancer drugs that selectively inhibit the nuclear enzyme, topoisomerase I. This enzyme produces transient DNA single-strand breaks which release the torsional strain that occurs during DNA replication and transcription. In the presence of camptothecin or one of its semisynthetic analogs [topotecan, the active metabolite of irinotecan, and 9-aminocamptothecin (9AC)] , the covalent complex between topoisomerase I and the ligated DNA strand is stabilized and religation of the DNA is blocked [6, 11]. This new class of agents has significant antitumor activity, and topotecan and irinotecan have recently received FDA approval for the treatment of refractory ovarian carcinoma and refractory metastatic colorectal cancer, respectively.

Camptothecin analogs are pentacyclic compounds (Fig. 1) that require an intact lactone ring (E ring) in order to exert a cytotoxic effect. In solution, these drugs undergo pH-dependent, reversible spontaneous hydrolysis of the lactone ring to yield the hydroxy acid form which is the predominant form at physiologic pH. Irinotecan is a water-soluble prodrug that is converted to the active metabolite, SN-38, by carboxylesterase [8, 9, 18].

We previously studied the central nervous system pharmacology of topotecan in a nonhuman primate model [19]. Topotecan penetration into the cerebrospinal fluid (CSF) following intravenous (i.v.) administration is

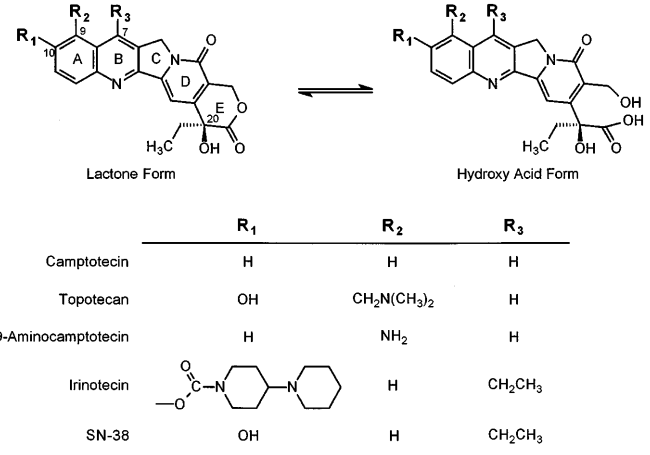

Fig. 1 Structures of camptothecin and its semisynthetic analogs in the active lactone form and the hydroxy acid form. Structure-activity relationship studies have shown that in addition to an intact lactone ring, an α-hydroxyl group at position 20 on the E ring is essential for cytotoxicity. The addition of an amino group at the 9-position (9-aminocamptothecin) results in enhanced potency relative to camptothecin, but this analog is also relatively water insoluble. Topotecan has a stable basic side chain at the 9-position of the A-ring which enhances water solubility. Irinotecan is a water-soluble prodrug that undergoes enzymatic hydrolysis in vivo by carboxylesterase to yield the active metabolite, SN-38. SN-38 also exists in a lactone and hydroxy acid form, and subsequently undergoes glucuronidation

32% (ratio of the area under the CSF concentration-time curve to the area under the plasma concentration-time curve) [2]. Baker et al. subsequently confirmed these findings in children who were receiving a continuous i.v. topotecan infusion [1]. In the present study, the CSF penetration of 9-AC, irinotecan, and SN-38 was characterized in the same nonhuman primate model.

Materials and methods

Drugs

9-AC [9-amino-20(*S*)-camptothecin] was supplied by the Investigational Drug Branch, National Cancer Institute (Bethesda, Md.) in a 1-ml ampule at a concentration of 5 mg/ml 9-AC in dimethylacetamide. This was further diluted to a final concentration of 100 μg/ml with a sterile diluent containing 50% polyethylene glycol 400 and 50% 0.01 *M* phosphoric acid, and was administered over 15 min through either a peripheral venous or a central venous catheter. Irinotecan was supplied by Yakult Honsha Co. (Tokyo, Japan) in 5-ml vials each containing 100 mg. The appropriate dose of drug was diluted with normal saline to a final total volume of 30 ml and administered over 30 min through either a peripheral venous or a central venous catheter. Camptothecin was obtained from the Investigational Drug Branch, National Cancer Institute.

Animals

Seven adult male rhesus monkeys (*Macaca mulatta*) ranging in weight from 9.1 to 11.4 kg were used in these pharmacokinetic studies. The animals were fed NIH Open Formula Extruded Non-Human Primate Diet twice daily and group housed in accordance with the Guide for the Care and Use of Laboratory Animals [13]. Blood samples were drawn through a catheter placed in either the femoral or the saphenous vein contralateral to the site of drug administration. CSF samples were obtained from a chronically indwelling Pudenz catheter attached to a subcutaneously implanted Ommaya reservoir [12]. The reservoir was pumped four times before and after each CSF sample collection to ensure adequate mixing with ventricular CSF.

Experiments

The plasma and CSF pharmacokinetics of 9-AC were studied in four animals following an i.v. dose of 0.2 mg/kg ($n = 1$) or 0.5 mg/kg ($n = 3$) infused over 15 min. Blood was collected in heparinized tubes prior to the dose, at 7.5 min during the infusion, at the completion of the infusion, and at 5, 15, and 30 min, and 1, 2, 4, 6, 8, 10, 24 h following the completion of the infusion. In two animals 48-h postinfusion samples were also obtained. Plasma was separated immediately by centrifugation at 12 000 *g* for 3 min in a rapid acceleration/deceleration centrifuge. CSF samples were collected prior to the dose and at 0.5, 1, 2, 4, 6, and 8 h after the dose.

The plasma and CSF pharmacokinetics of irinotecan were studied in three animals following an i.v. dose of 4.8 mg/kg ($n = 2$) or 11.4 mg/kg ($n = 1$) infused over 30 min. Blood was collected in heparinized tubes prior to the dose, at 15 min during the infusion, at the completion of the infusion, and at 5, 15, and 30 min, and 1, 2, 4, 6, 8, 10, 24 h following the completion of the infusion. In two animals 48-h postinfusion samples were also obtained. CSF samples were collected prior to the dose and at 0.5, 1, 2, 3, 4, and 6 h.

Sample processing and analysis

9-AC was measured in plasma and CSF following solid-phase extraction using a recently described reverse-phase HPLC assay [20]. Briefly, a Bond-Elut, 100 mg, C_{18} solid-phase extraction column was conditioned with 1 ml methanol followed by 1 ml water. A 1 ml aliquot of the heparinized plasma sample or 500 μl CSF was immediately combined with 10 μl 100 n*M* camptothecin internal standard in a 1.5 ml polypropylene tube. The sample was vortexed for 15 s and immediately loaded at a rate of 0.25 ml/min onto the solid-phase extraction cartridge. The cartridge was washed twice with 1 ml water followed by 1 ml 25% (v/v) methanol. The 9-AC lactone and the camptothecin internal standard were eluted with 0.75 ml 75% (v/v) methanol/25 m*M* KH_2PO_4, pH 2.55. The eluted fraction was combined with 0.25 ml 25 m*M* KH_2PO_4 (pH 2.55) and stored at −70 °C for subsequent HPLC analysis. A 100-μl aliquot of heparinized plasma or CSF for measurement of total drug concentration was combined with 900 μl 8.5% phosphoric acid in a 1.5-ml polypropylene tube and incubated for 15 min at room temperature to completely convert the 9-AC carboxylate to 9-AC lactone. Following the addition of a 10-μl aliquot of the camptothecin internal standard, the sample was extracted as described above. The 9-AC carboxylate plasma level was then calculated by subtracting the concentration of 9-AC lactone from the total plasma concentration. 9-AC was detected with a Waters 470 scanning fluorescence detector (Milford, Mass.) at a λ_{ex} of 365 nm and a λ_{em} of 440 nm with a bandwidth of 18 nm [20]. The lower limit of quantitation was 0.25 n*M*.

Irinotecan (lactone and carboxylate forms) and its active metabolite, SN-38 (lactone and carboxylate forms), were measured in plasma and CSF using a previously described reverse-phase HPLC method with slight modifications [17]. Total drug concentrations were determined by adding the concentration of the lactone and carboxylate forms for each particular compound. Briefly, heparinized blood samples were immediately centrifuged at 8100 *g* for 2 min in a rapid acceleration/deceleration centrifuge at 4 °C. A 200-μl aliquot of plasma was transferred to each of two microfuge tubes containing a 1:1 mixture of methanol/acetonitrile on ice. The microfuge tubes were vigorously vortexed for 8 s and then centrifuged at 8100 *g* for 2 min in a rapid acceleration/deceleration centrifuge at 4 °C. The supernatant from both microfuge tubes was

transferred to a single polypropylene screw-top vial and stored at −70 °C until further analysis. The remaining plasma was placed in a polypropylene screw-top vial and frozen for subsequent analysis of SN-38 glucuronide (SN-38G).

Irinotecan lactone and carboxylate and SN-38 lactone and carboxylate were detected with a Waters 474 scanning fluorescence detector (Milford) at a λ_{ex} of 355 nm and a λ_{em} of 515 nm (cutoff filter). The lower limits of detection were 0.4 n*M* and 0.7 n*M* and the lower limits of quantitation were 0.7 n*M* and 1.0 n*M* for irinotecan and SN-38, respectively. Total drug concentrations for irinotecan and SN-38 were calculated by adding the lactone and carboxylate forms of the respective compounds. SN-38G concentrations were determined as the increase in SN-38 concentrations following incubation with β-glucuronidase using a previously described assay [16].

Pharmacokinetic analysis

The areas under the plasma and CSF concentration-time curves (AUC) were calculated using the linear trapezoidal method [4], and extrapolated to infinity by adding the quotient of the final plasma concentration divided by the terminal rate constant. The rate constants were derived by nonlinear, least squares regression analysis using MLAB, a nonlinear curve-fitting program [10]. Total body clearance (Cl_{TB}) was determined by dividing the dose by the AUC. The volume of distribution at steady state (Vd_{ss}) was calculated by using the area under the moment curve [14]. The fraction of 9-AC (lactone and total drug), irinotecan (lactone and total drug), and SN-38 (total drug) penetrating into the CSF was derived from the ratio of the AUCs in CSF and plasma. For SN-38 lactone which was below the limit of quantification in CSF, a maximum AUC in CSF was estimated using the trapezoidal rule with a concentration of 0 n*M* at time 0, a peak concentration equal to the lower limit of detection (if no drug was detected) or the lower limit of quantitation (if drug was detected) at 0.5 h, and a terminal rate constant from the plasma concentration-time curve. The half-lives were calculated by dividing 0.693 by the rate constant.

Results

9-AC

Data from one animal that received 0.5 mg/kg of 9-AC are not included in this analysis because of technical problems with sample processing. 9-AC was quantifiable in CSF after the 0.2 mg/kg and 0.5 mg/kg doses. The CSF concentration of the lactone form of 9-AC peaked 30 to 45 min after the dose and was 2.4 n*M* in the animal that received 0.2 mg/kg and 10.9 and 20.8 n*M* in the two animals treated with 0.5 mg/kg. The plasma and CSF AUCs for the 9-AC lactone and total drug are shown in Table 1. The lactone form of 9-AC accounted for 26 ± 7% of the total drug in plasma. The penetration of 9-AC lactone into the CSF, as measured by the ratio of the AUC^{CSF} to the AUC^{P} was only 1.9 to 5.9%. A representative concentration-time profile of 9-AC in plasma and CSF is shown in Fig. 2, and other pharmacokinetic parameters are shown in Table 1.

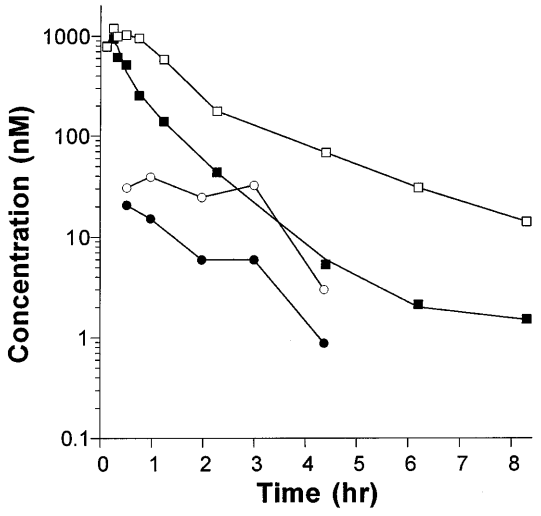

Fig. 2 Representative concentration-time profile of 9-AC lactone (*closed symbols*) and total drug (*open symbols*) in plasma (*squares*) and CSF (*circles*) following a 15-min intravenous infusion of 0.5 mg/kg

Irinotecan

The plasma disappearance curves of irinotecan, SN-38, and SN-38 glucuronide after a 30-min i.v. infusion of 11.4 mg/kg (225 mg/m^2) of irinotecan are shown in Fig. 3A. Irinotecan was rapidly converted to SN-38, but plasma AUC's of the lactone form of the active metabolite were only 2.0% to 2.4% of the AUCs of the parent drug. The AUCs of SN-38 lactone were 66 n*M* h and 45 n*M* h at the 4.8 mg/kg dose and 151 n*M* h at the 11.4 mg/kg dose. The lactone form of irinotecan accounted for 26 ± 5% of the total drug in plasma, and

Table 1 Plasma and CSF pharmacokinetic parameters for 9-AC lactone and total drug after a 15-min infusion of 9-AC (CL_{TB} total body clearance, Vd_{ss} volume of distribution at steady state, *AUC* area under the concentration-time curve, *P* plasma, *CSF* cerebrospinal fluid, $t_{1/2}$ terminal half-life, *CSF:P* ratio of the AUC^{CSF} to AUC^{P}, *NA* no data available because of analytical problems)

Monkey	Dose (mg/kg)	Lactone						Total drug		
		CL_{TB} (l/h/kg)	Vd_{ss} (l/kg)	AUC^{P} (n*M* h)	AUC^{CSF} (n*M* h)	CSF:P	$t_{1/2}$ (h)	AUC^{P} (n*M* h)	AUC^{CSF} (n*M* h)	CSF:P
85Z	0.2	3.0	2.2	183	5.0	0.027	3.2	652	NA	NA
X854	0.5	2.1	1.8	674	40	0.059	3.9	2180	114	0.052
CH957	0.5	1.3	0.90	1030	20	0.019	2.4	5840	25	0.0043
Mean		2.1	1.6			0.035	3.2			0.028
SD		0.9	0.7			0.021	0.8			0.033

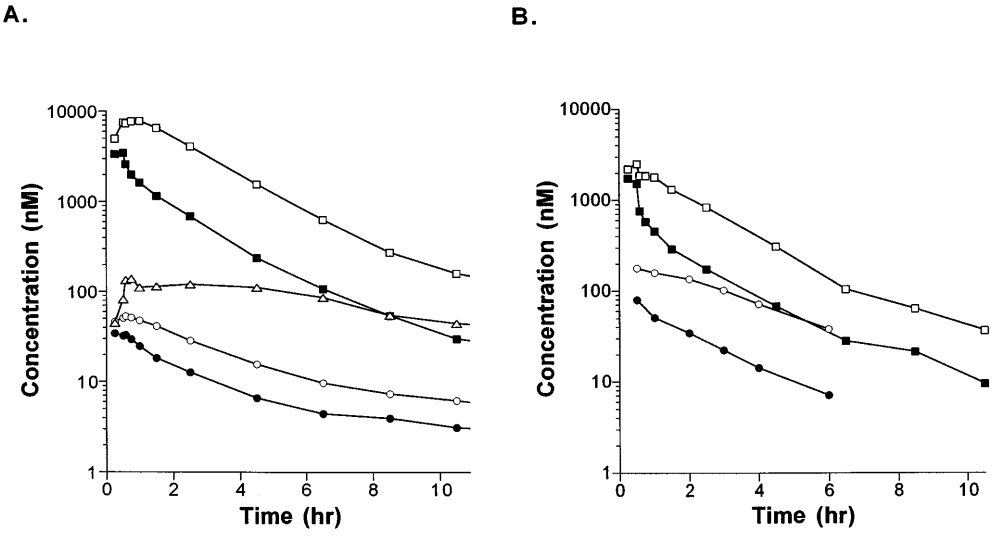

Fig. 3A Representative plasma concentration-time profile of irinotecan lactone (■) and total drug (□), its active metabolite, SN-38 lactone (●) and total drug (○), and total SN-38G (△) after a 30-min intravenous infusion of 11.4 mg/kg of irinotecan. **B** Representative concentration-time profiles of irinotecan lactone (■) and total drug (□) in plasma, and irinotecan lactone (●) and total drug (○) in CSF after a 30-min intravenous infusion of 4.8 mg/kg

Table 2 Plasma and CSF pharmacokinetic parameters for irinotecan lactone and total drug after a 30-min infusion of irinotecan. CL_{TB} total body clearance, Vd_{ss} volume of distribution at steady state, *AUC* area under the concentration-time curve, *P* plasma, *CSF* cerebrospinal fluid, $t_{1/2}$ terminal half-life, *CSF:P* ratio of the AUC^{CSF} to AUC^{P}

Monkey	Dose (mg/kg)	Irinotecan lactone						Irinotecan total drug		
		CL_{TB} (l/h/kg)	Vd_{ss} (l/kg)	AUC^{P} (n*M* h)	AUC^{CSF} (n*M* h)	CSF:P	$t_{1/2}$ (h)	AUC^{P} (n*M* h)	AUC^{CSF} (n*M* h)	CSF:P
88033	4.8	3.1	5.6	2390	358	0.15	6.2	9930	1696	0.17
B9078	4.8	3.9	8.2	1890	181	0.096	2.3	5820	717	0.12
RQ244	11.4	3.3	7.5	5770	948	0.16	6.1	25400	2660	0.10
Mean		3.4	7.1			0.14	4.9			0.13
SD		0.4	1.3			0.03	2.2			0.04

the lactone form of SN-38 accounted for 55 ± 6% of the total SN-38 in plasma. SN-38G was also formed rapidly (Fig. 3A) and the AUCs of the glucuronide were sevenfold higher than the AUCs of SN-38 total drug.

Irinotecan was detected in CSF (Fig. 3B). The CSF concentrations of the irinotecan lactone and total drug peaked between 30 and 60 min following drug administration, and peak CSF concentrations of irinotecan lactone were 57 n*M* and 80 n*M* at the 4.8 mg/kg dose and 240 n*M* at the 11.4 mg/kg dose. The AUC^{CSF}: AUC^{P} ratio for irinotecan lactone ranged from 9.6% to 16%. SN-38 CSF concentrations were below the level of quantification at all time points. We estimate that the AUC^{CSF}: AUC^{P} ratio for SN-38 lactone was $<8\%$ and for total SN-38 was $<3\%$. Pharmacokinetic parameters for plasma and CSF irinotecan are shown in Table 2.

Toxicity

One of the two animals that received 0.5 mg/kg 9-AC required an antiemetic for nausea and vomiting. No hematological or other organ toxicity was observed following i.v. administration of 9-AC at doses 0.2 or 0.5 mg/kg or of irinotecan at doses of 4.8 or 11.6 mg/kg.

Discussion

Despite the similarities in chemical structure and clinical pharmacology of the camptothecin analogs, the CSF penetration of the lactone forms of 9-AC (3.5%), irinotecan (14%), and SN-38 ($<8\%$) were substantially lower than the previously reported CSF penetration of topotecan (32%) in the same model. These differences may be explained in part by differences in protein binding of these agents. Topotecan is minimally protein-bound ($<20\%$) [7], whereas 9-AC [3, 21], irinotecan [15], and SN-38 [22] are highly protein-bound. Because the protein content of the CSF is very low relative to the amount of protein in blood, CSF is similar to a dialysate which equilibrates with free drug in plasma across a relatively impermeable membrane (the brain capillary endothelial lining).

The AUC of SN-38 relative to irinotecan appeared to be lower in our nonhuman primate model than in humans. This hampered our ability to accurately quantify the degree of CSF penetration of the active metabolite. The average ratios of the AUCs for SN-38 to irinotecan in humans were 6.9% and 5.3% for the lactone and total drug, respectively. In contrast, in nonhuman primates,

the ratio of the AUCs for SN-38 to irinotecan were 2.2% and 1.1% for the lactone and total drug, respectively. Likewise, the ratio of AUCs of the SN-38G to SN-38 lactone was 7 in nonhuman primates compared with 2–4 in humans [5], suggesting that the glucuronidation of SN-38 is more rapid in the nonhuman primates. More rapid elimination (glucuronidation) of SN-38 could, in turn, account for the lower SN-38:irinotecan AUC ratio.

The ability of drugs to penetrate into the CSF has been used as a surrogate for penetration across the blood-brain barrier into the central nervous system. The results presented here suggest that 9-AC and SN-38 may be inferior to topotecan for the treatment of leptomeningeal tumors because of their more limited penetration into the CSF.

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