Plasma Pharmacokinetics of the Lactone and Carboxylate Forms of 20(*S*)-Camptothecin in Anesthetized Rats

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Received October 29, 1992; accepted April 2, 1993

20(*S*)-Camptothecin exists in equilibrium between its lactone (CPT) and its carboxylate forms (Na-CPT) under simulated physiological conditions, with the equilibrium favoring the carboxylate form. The rates of lactone hydrolysis were studied in plasma, serum albumin, and blood and were found to be faster than in aqueous buffers at equivalent pH values. From mechanistic information and *in vivo* activity data, the lactone appears to be the active form of the drug. It has been argued, therefore, that if an equilibrium existed between the lactone and the carboxylate, Na-CPT could be used to deliver the lactone effectively. In the present study, plasma pharmacokinetics were performed in sodium pentobarbital-anesthetized rats treated with both CPT (lactone) and the sodium salt of camptothecin (carboxylate, Na-CPT) and the lactone and carboxylate, as well as the total drug, concentration versus time profiles were assessed. It was found that plasma concentrations and AUC values for the lactone were significantly higher after dosing with CPT than after dosing with Na-CPT. After i.v. administration, the ratio of plasma lactone to carboxylate was skewed by the apparent rapid and extensive uptake of the lactone into tissues and the rapid clearance of both species. From our results, it appears that the lower *in vivo* activity of Na-CPT compared to that from CPT administration might be attributed to the altered conversion of carboxylate into lactone *in vivo* compared to that predicted from *in vitro* data.

KEY WORDS: camptothecin; reversible metabolism; pharmacokinetics; hydrolysis.

INTRODUCTION

20(*S*)-Camptothecin (CPT; NSC-94600) is a naturally occurring antineoplastic agent which has shown promising *in vitro* and *in vivo* activity (1–3). The antitumor activity of CPT is exhibited by inhibition of the enzyme Topoisomerase I, which has been implicated in various functions of DNA and RNA synthesis (4–7).

It has been shown that the E ring of CPT (the lactone ring) is quite labile (1) and that the opening of the ring under basic conditions produces the water-soluble carboxylate form (Na-CPT, NSC-100880; Fig. 1). Due to the poor water solubility of CPT (1), Na-CPT was introduced into clinical trials in the early 1970s (8–10), however, low activity and severe toxicity discouraged further clinical testing. Later it was discovered that the intact lactone ring is required for the biological activity of CPT (11). The approximate 10-fold greater activity of CPT versus Na-CPT (2) has been proposed to be due to a small amount of *in vivo* conversion of the carboxylate to the lactone (12), though this has not been comprehensively demonstrated.

Pharmacokinetic investigations involving camptothecin and its analogues have, in large part, looked only at the total drug due to the inability of the available analytical methods to differentiate between the two forms (10,13,14). Recently, LC methods have been reported which allow for the simultaneous determination of both the carboxylate and the lactone forms of CPT or CPT analogues in both aqueous buffers (15) and biological samples (16,17). No comprehensive pharmacokinetic study has been reported on camptothecin, however, a number of abstracts (18–20) have recently appeared describing some studies in mice for various camptothecin analogues. Therefore, the objective of this investigation was to study the pharmacokinetics of the two forms of camptothecin, namely, the lactone and the carboxylate in rats following i.v. doses of each of CPT and Na-CPT.

MATERIALS AND METHODS

Chemicals

CPT (NSC-94600) and Na-CPT (NSC-100880) were obtained from the National Cancer Institute (Bethesda, MD). Five percent human serum albumin (HSA) was obtained from Miles Inc., Ontario, Canada. All other chemicals were of reagent grade or better and were used as received.

Chromatographic Analysis

The analysis of CPT and Na-CPT was accomplished using an LC system consisting of an Altex Model 110A pump, a Rheodyne 7125 injection valve, and a RF-535 fluorescence detector (Shimadzu Scientific Instruments, Inc., Columbia, MD), which was operated at an excitation wavelength of 370 nm and an emission wavelength of 435 nm. The separation was achieved using a 5-μm (4.2 × 150-mm) ODS Hypersil column with a mobile phase consisting of 0.025 *M* phosphate buffer, pH 6.5, with 27% (v/v) acetonitrile and 5 m*M* t-butylammonium dihydrogen phosphate (TBA) as the ion pairing agent. A flow rate of 2 mL/min and an injection volume of 20 μL were used for all experiments. The resulting retention times were 2.2 min for the carboxylate and 3.5 min for the lactone. To avoid carryover between injections, the injection syringe and the injection loop were washed thoroughly with 0.1 *M* KOH and distilled water between successive injections. The KOH converted all lactone to the water-soluble carboxylate, which was easily washed from the system with distilled water. Quantification was achieved using external standards which bracketed the *in vivo* concentrations. Standard curves with concentrations ranging from 1.0 ng/mL to 1.0 μg/mL of the lactone or the carboxylate exhibited good linearity with a correlation coefficient (mean ± SD) of 0.998 ± 0.001 (n = 3).

Sample Preparation

During *in vitro* kinetic studies, plasma samples were prepared for LC analysis by the addition of 150 μL of ice-cold acetonitrile to 50 μL of plasma or serum albumin fol-

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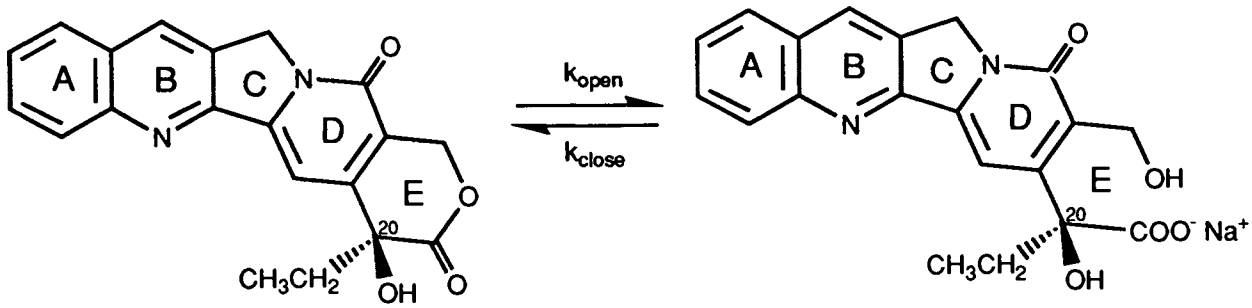

Fig. 1. Equilibrium between CPT (lactone; left) and Na-CPT (carboxylate; right). The rate constants k_{open} and k_{close} are the forward and reverse rate constants for interconversion of lactone to carboxylate.

lowed by the addition of 50 μL of ice-cold 0.1 *M* phosphate buffer, pH 6.0. The mixture was vortexed for 10 sec and centrifuged for 1 min. The supernatant (20 μL) was then injected onto the LC and analyzed simultaneously for the intact lactone and the carboxylate.

For the pharmacokinetics experiments, the intact lactone was determined by adding 150 μL of ice-cold ACN to 50 μL of plasma, followed by the addition of 50 μL of ice-cold 0.1 *M* phosphate buffer, pH 6.0. The mixture was vortexed for 10 sec and centrifuged for 1 min. The supernatant (20 μL) was then injected onto the LC and analyzed for the intact lactone. The delay between sample collection and analysis generally was less than 3 min. Total drug was determined as the lactone in the same manner except that 50 μL of 0.5 *M* HCl was added instead of phosphate buffer, pH 6.0, to cause the complete lactonization of the drug. The carboxylate form of the drug could then be determined by subtraction of the lactone concentration from the total drug concentration (16).

In Vitro Kinetic Studies

In vitro reactions were initiated by adding a small aliquot of stock solution of the drug in DMSO to the buffer solution or the physiological fluid. The initial drug concentration was kept between 7 and 20 μ*M*, with the DMSO concentration ranging between 1 and 5% (v/v) in the reaction mixtures. The reaction solutions were kept at a constant temperature of 37°C for the entire experiment, and no attempts were made to maintain the pH of the sample during the experiment. Reactions in buffers required no sample preparation, while plasma samples were prepared as described earlier. The rate of the lactone conversion to the carboxylate was followed by monitoring the disappearance of the starting material (CPT).

The pseudo-first-order rate constants, k_{obs}, for the reactions were determined from the slopes of plots of $-\ln|A_t - A_{eq}|$ versus t, where A_{eq} and A_t are the peak areas of lactone peak after the reaction has reached equilibrium and at time t, respectively. The equilibrium constant, K_{obs}, for the conversion of lactone to the carboxylate was calculated from $[\text{carboxylate}]_{eq}/[\text{lactone}]_{eq}$, where $[\text{carboxylate}]_{eq}$ and $[\text{lactone}]_{eq}$ are the equilibrium concentrations of carboxylate and lactone, respectively.

Protein Binding Studies

Protein binding studies were performed with both the lactone and the carboxylate forms of CPT in 5% human serum albumin (HSA) and in freshly collected rat plasma. Rat plasma and HSA were brought to 4°C prior to the addition of CPT or Na-CPT. Stock solutions of CPT or Na-CPT in DMSO were added to the plasma or HSA in the amounts required to achieve the desired concentration (1 μg/mL). DMSO concentrations were kept below 1% (v/v) for all experiments. After addition of the drug to the plasma or HSA the solution was allowed to equilibrate for 30 min at 4°C to minimize the interconversion between lactone and carboxylate during the incubation period. The sample was then divided into two aliquots. One aliquot (500 μL) was transferred to an ultrafiltration tube (Amicon, Beverly, MA) with a YMT membrane having a molecular weight cutoff of 5000. The sample was centrifuged in a Beckman GH-3.7 refrigerated centrifuge (Beckman instrument, Inc., Palo Alto, CA) for 10 min at 2000*g* and 4°C. Following centrifugation the ultrafiltrate was analyzed immediately for the free fraction of the drug. The second aliquot was kept at 4°C for the duration of the centrifugation and was then frozen on dry ice until analysis. This sample was analyzed for the total (free + bound) drug. The same procedure was used for both CPT and Na-CPT.

Pharmacokinetic Studies

All pharmacokinetic experiments were conducted using nonfasted, male, anesthetized (sodium pentobarbital; 50 mg/kg i.p. doses; Nembutal, Abbott Laboratories, Chicago, IL) Sprague-Dawley rats (maintained at 37°C, heating pad; tracheotomized) weighing between 300 and 350 g. Anesthesia was maintained during the experiment by i.p. injections of pentobarbital (approx. 5 mg) given at 1-hr intervals. Blood sampling was performed through an indwelling cannula (PE-50 tubing, Clay Adams, Parsippany, NJ) which was implanted into the right external jugular vein. Drug dosing was performed through an i.v. infusion cannula (25-G needle) which was inserted into the left external jugular vein.

Animals were given doses of 1 mg/kg of either CPT or Na-CPT in all experiments. Na-CPT was delivered in 0.02 *M* phosphate buffer (pH 8.0). CPT was delivered in a solution composed of 20% DMSO, 20% PEG 400, 30% EtOH, and 30% pH 3.5 phosphoric acid (10 m*M*). The formulation was prepared by first dissolving CPT in DMSO followed by the addition of the other solvents in the order shown above. The solution was immediately administered to the animal after preparation. This formulation allowed for the delivery of CPT as a solution, as opposed to a suspension, while minimizing the DMSO and PEG 400 concentrations. Intravenous doses were administered over a 1-min period. Intravenous infusions of Na-CPT (100 μg/mL in 0.02 *M* phosphate buffer,

pH 8.0) were performed at an infusion rate of 1 mL/hr. Total doses of 1 mg/kg were given during infusion experiments. Blood samples (100 μL) were collected at 5, 15, 30, 45, 60, 90, 120, 180, and 240 min postdosing into chilled microcentrifuge tubes containing 5 μL of 0.7% EDTA. Samples were immediately centrifuged at 13,600*g* for 1 min, plasma separated and analyzed as described earlier. Except for the i.v. infusion experiment, six animals were dosed with CPT and six with Na-CPT.

Pharmacokinetic Calculations

The plasma concentration versus time profiles for both the lactone and the carboxylate following dosing by both species were fit (Sigma Plot 5.0, Jandel Scientific, San Rafael, CA), using nonlinear least squares, to Eq. (1),

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

where λ_i is the rate constant in min^{-1} and C_i describes the amplitude of each exponential term in ng/mL.

Area under the curves (AUC) values were calculated using the integrated form of Eq. (1) and by the trapezoidal rule with addition of C_f/λ_n to account for the area between the concentration of the final point at 240 min and infinity, where C_f is the concentration of the final sample (t = 240 min) and λ_n is the apparent rate constant for the terminal phase. AUC values for the first 5 min were calculated using C_0 (concentration at t = 0 min) values determined from the fitted curves. Further pharmacokinetic parameters were calculated using the equations which were previously reported (22).

RESULTS AND DISCUSSIONS

Sample Preparation

By using a mobile phase buffered at a pH of 6.5, the rate of on-column interconversion between the lactone and the carboxylate during analysis was negligible (15) and the errors introduced in the measurement of lactone, because of the delay of 2 to 3 min during sample preparation, were also minimized (<1%) by adjusting the pH of the deproteinized plasma to 6.0 and maintaining the sample at low temperature. However, these errors were significant when the deproteinizing solution did not contain the pH 6.0 buffer. Without the pH control, ring opening continued at a much faster rate during sample preparation since the apparent pH of the deproteinized plasma was between 8.5 and 9.0.

Relative recoveries of the lactone and carboxylate forms of the drug from plasma were 100 ± 5% (n = 5) and 103 ± 4% (n = 5), respectively, over the concentration range observed in the pharmacokinetics experiments (1 ng/mL–1 μg/mL). The recoveries shown are presented as the average recovery ± standard deviation for five different concentrations within the above concentration range.

Rate and Equilibrium Constants

The equilibrium between the lactone and the carboxylate forms of camptothecin was rapidly established at physiological pH (Table I) and was consistent with previously reported values (15). The rate of lactone hydrolysis was found to be slightly faster in plasma, whole blood, and HSA than in buffers at similar pH values; however, the equilibrium was shifted in favor of the carboxylate form in all cases. Although this rate enhancement and shift in equilibrium can partially be attributed to the difference in apparent pH between the plasma and the buffer, the differences cannot be totally explained by the pH difference. It was confirmed that the pH of plasma can increase by 0.5 to 1 pH unit over the course of several hours due to changes in the partial pressure of carbon dioxide. For example, the pH of the plasma slowly increased from 7.8 to a pH of 8.3 over a period of 6 hr, while the pH of the HSA remained relatively constant. Since no attempt was made to maintain the pH of the samples, the upward drift in the pH may account for the lower equilibrium values of the lactone observed in plasma and in blood. However, the kinetics of hydrolysis were unaffected by this shift in pH since there is relatively no change in the pH of the sample during the first hour, which is the time period which was generally used for the kinetic determinations. Since there is no observable change in the pH of HSA during incubation at 37°C, the slight increase in the rate of hydrolysis relative to pH 7.2 buffer is probably not due to pH differences.

An explanation for the rate and equilibrium changes in physiological media might be differences in protein binding between the two forms of camptothecin. Since the lactone

Table I. Observed Rate (k_{obs}) and Equilibrium Constants (K_{obs}) for the Hydrolysis of Camptothecin in Various Aqueous Buffers (μ = 0.15 *M*) and Physiological Media at 37°C

Medium	Apparent pH	k_{obs} (min^{-1})	$t_{1/2}$ (min)[a]	K_{obs}	% closed E ring at equilibrium
0.02 *M* PBS[b]	7.2	0.021	33	4.0	20
0.02 *M* PBS	7.4	0.032	22	7.5	12
0.02 *M* PBS	8.0	0.13	5.3	28	3.5
Human plasma	7.8	0.16	4.3	>99	<1
5% HSA (w/v)	7.2	0.029	24	52	1.9
Rat plasma	7.7	0.15	4.6	61	1.6
Rat blood	7.4	0.073	9.5	39	2.5

[a] Half-life for the approach to equilibrium.
[b] Phosphate-buffered saline.

form of the drug is rapidly and almost completely converted to the carboxylate form in plasma at 37°C, it was necessary for the lactone binding experiments to be carried out at 4°C in order to keep the majority of the drug in the closed form (>80%) during incubation and centrifugation. Other complications, caused by nonspecific adsorption of the lactone form to the ultrafiltration membrane and the small amount of conversion of the lactone to the carboxylate even at 4°C, complicated the assessment of the binding of the lactone. It was found that both forms of the drug were highly protein bound, with the carboxylate being more highly bound than the lactone in both plasma and in HSA. For example, the binding of the lactone was found to be approximately 89% in rat plasma and 81% in HSA, whereas the carboxylate was found to be approximately 98% bound in both plasma and in HSA a value similar to that previously reported at 37°C (10,21). The higher binding of the carboxylate may partially account for the enhancement in hydrolysis rate and the shift in equilibrium, although catalysis of the rate process by plasma proteins cannot be ruled out. Erythrocyte binding by both species was found to be insignificant.

Plasma Pharmacokinetics After i.v Bolus Dosing

Shown in Figs. 2a and b are the concentration–time profiles for the lactone and carboxylate forms of camptothecin, respectively, after dosing with CPT and Na-CPT. As expected, the initial plasma concentrations of the lactone are significantly higher following dosing with CPT than after dosing with Na-CPT (Fig. 2a). However, after 90 min the concentration of the lactone appears relatively independent of the form administered. The same appears to be true for the carboxylate (Fig. 2b). When a control experiment was performed by administration of Na-CPT in the same formulation as that used for the lactone, no effect was observed on the pharmacokinetic behavior of either form of the drug or on the formation of the metabolite (see later discussion).

Shown in Fig. 3 is the ratio of lactone to carboxylate concentration as a function of time following dosing with CPT and Na-CPT. After dosing with CPT, the ratio is initially greater than unity (as expected), drops below unity, and approaches a ratio which is approximately equal to that found *in vitro*. However, at longer times, the ratio again appears to increase. The etiology of this increase is unknown. A similar observation was made following Na-CPT dosing.

The concentration–time profiles of lactone and carboxylate camptothecin were empirically fit to multiexponential equation, Eq. (1). Equations adequately defining both species over the 4-hr collection period from the two delivery forms are given below:

$$C_L^L(t) = 281e^{-0.28t} + 137e^{-0.054t} + 4.54e^{-0.0026t} \quad (2)$$

$$C_C^L(t) = -224e^{-0.35t} + 208e^{-0.034t} + 26.2e^{-0.010t} \quad (3)$$

$$C_L^C(t) = 23.5e^{-0.066t} + 4.53e^{-0.0016t} \quad (4)$$

$$C_C^C(t) = 2302e^{-0.19t} + 428e^{-0.051t} + 16.4e^{-0.0070t} \quad (5)$$

Equation (2) describes the lactone concentration (ng/mL) after dosing with CPT (C_L^L, Eq. (3) describes the carboxylate concentration (ng/mL) after dosing with CPT (C_C^L), Eq. (4) describes the lactone concentration (ng/mL) after dosing with Na-CPT (C_L^C), and Eq. (5) describes the carboxylate concentration (ng/mL) after dosing with Na-CPT (C_C^C). It can be seen that the administered form of the drug displays

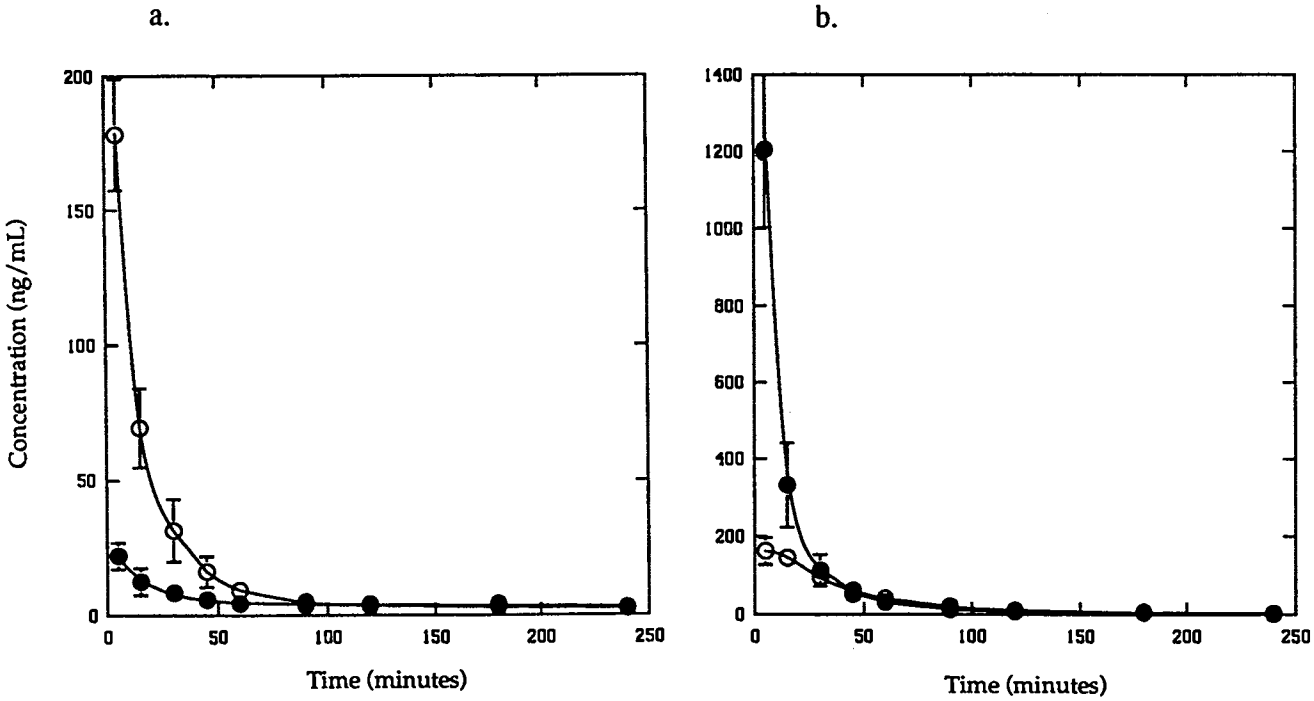

Fig. 2. (a) Concentration–time profiles for camptothecin lactone following a 1 mg/kg i.v. dose of CPT (open circles) and a 1 mg/kg (CPT equivalent) i.v. dose of Na-CPT (filled circles). (b) Concentration–time profiles for camptothecin carboxylate following a 1 mg/kg i.v. dose of CPT (open circles) and a 1 mg/kg i.v. dose of Na-CPT (filled circles). The individual points represent the average concentration for all animals studied and the error bars represent standard deviations.

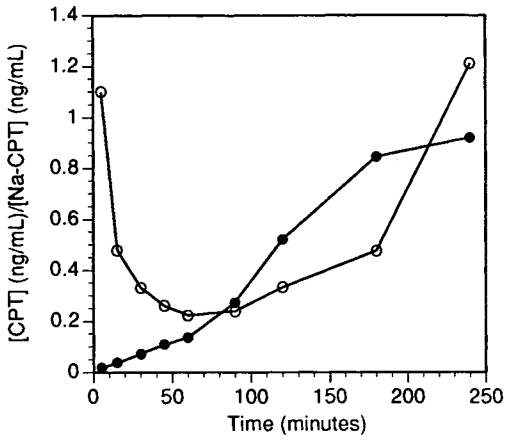

Fig. 3. Ratio of lactone-to-carboxylate plasma concentration as a function of time following dosing with CPT (open circles) and Na-CPT (filled circles).

a triexponential decay curve. However, the form of the drug which is produced after administration displays a biexponential decay curve. Although the initial behavior of the lactone and the carboxylate is dependent on the form in which it was administered, the terminal phase of each form of the drug appears to be much less dependent on the form administered.

A first approximation of a model that might be adequate to describe these pharmacokinetic observations is given in Fig. 4. This model was first developed by Cheng and Jusko (22) to describe the pharmacokinetics of methylprednisolone, which undergoes reversible metabolism to form methylprednisone. The main features of the model are the clearances associated with the conversion of lactone to carboxylate (CL_{12}), carboxylate to lactone (CL_{21}), clearance of the lactone (CL_{10}) and the carboxylate (CL_{20}) by other pathways, total elimination clearances for the lactone (CL_{11}) and the carboxylate (CL_{22}), and distribution clearances (CL_{D1} and CL_{D2}). Assuming this model, these parameters can be calculated from AUC values of the lactone and the carboxylate after dosing with CPT and Na-CPT using equations described by Cheng and Jusko (22). These clearance parameters, as well as other parameters, are shown in Fig. 4 and Table II. Although the clearance values for the lactone are

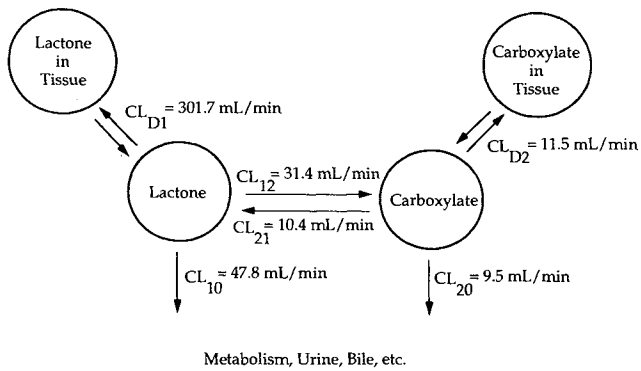

Fig. 4. Pharmacokinetic model used to describe the behavior of camptothecin lactone and carboxylate. Included in this figure are the estimated values for various clearances as defined by the model of Cheng and Jusko (22).

larger than the corresponding values for the carboxylate, the volume of distribution for the lactone is also much larger than that of the carboxylate. It can be seen that the lactone has a larger apparent distribution volume compared to the carboxylate, suggesting higher tissue accessibility and binding. For models with multiple extravascular compartments, the distribution clearances (CL_D) represent the total clearance into all peripheral compartments. The values of CL_D for the lactone and the carboxylate indicate that the lactone is quickly distributed into the tissue compartment relative to the carboxylate.

The areas under the curves (AUC) for both camptothecin species are shown in Table III following dosing with CPT and Na-CPT. All AUC values presented in Table III were calculated using the trapezoidal rule in order to maintain consistency for comparison purposes. For the zero-to-infinity calculations, the AUCs from integration of Eqs. (2)–(5) are also shown. Following a 1 mg/kg dose of CPT, the AUC_0^∞ of the lactone is about two times larger, and the AUC_0^∞ of the carboxylate is approximately two times smaller, than the corresponding AUC values following 1 mg/kg dose of Na-CPT. This observation can be explained by differences in the elimination clearances of the two forms of the drug compared to interconversion clearances between the two species, as well as tissue uptake clearances. That is, the elimination clearance of the carboxylate (CL_{20}) compared to its clearance to the lactone (CL_{21}) suggests that much of the carboxylate is eliminated prior to its conversion. Similarly comparing CL_{10} to CL_{12} suggests that some of the lactone is eliminated, probably as a metabolite (see discussion which follows) before its conversion to the carboxylate.

The model of Cheng and Jusko (22) must be considered an approximation for camptothecin since it is likely that the interconversion of the two species occurs in all tissues to some extent. Therefore, the conclusion based on this model should be tempered. For example, this model does not adequately predict the upswing in the ratio of lactone to carboxylate at later time points seen in Fig. 3.

Table III also shows the AUC values for the first 240 min and the first 60 min after dosing. For the carboxylate, it can be shown (compare the ratio C_1/λ_1 to C_2/λ_2, etc.) that a major contribution to the AUC_C (AUC of the carboxylate) is from the initial portion of the curve, consistent with this portion of the curve representing both elimination as well as interconversion to the lactone and distribution. The AUC_L (AUC of the lactone) ratio from early time points to infinity, decreased with increasing time. The same trend is followed

Table II. Pharmacokinetic Parameters Based on the Reversible Metabolism Model of Cheng and Jusko (22) as Illustrated in Fig. 4

Lactone		Carboxylate	
CL_{10} (mL/min)	47.8 ± 5.52	CL_{20} (mL/min)	9.5 ± 3.3
CL_{12} (mL/min)	31.4 ± 4.18	CL_{21} (mL/min)	10.4 ± 0.86
CL_{11} (mL/min)	79.2 ± 1.36	CL_{22} (mL/min)	19.9 ± 4.17
CL_D (mL/min)	301.7 ± 159.3	CL_D (mL/min)	11.5 ± 4.42
V_{ss} (mL)	4727 ± 732.7	V_{ss} (mL)	232 ± 93.5
V_c (mL)	1246 ± 175.2	V_c (mL)	152 ± 5.17
V_t (mL)	3481 ± 907.0	V_t (mL)	80 ± 98

Table III. AUC Values, AUC Ratios, and Concentrations for Camptothecin Lactone and Carboxylate After i.v. Administration of 1 mg/kg CPT and 1 mg/kg Na-CPT

AUC and concentration values	1 mg/kg CPT[a]	1 mg/kg Na-CPT[a]	AUC ratio[b]
AUC_0^{∞} (ng · min/mL)[c]			
Carboxylate	8,803 ± 1,343	22,225 ± 5,502	0.39
Lactone	5,573 ± 466	2,911 ± 449	1.9
Total drug	12,786 ± 2,024	24,395 ± 4,873	0.52
Carboxylate[d]	7,994 ± 2,983	22,420 ± 3,092	0.36
Lactone[d]	5,265 ± 105	3,279 ± 919	1.6
Total drug[d]	13,259 ± 3,088	25,699 ± 4,011	0.52
AUC_0^{240} (ng · min/mL)[c]			
Carboxylate	8,278 ± 1,004	21,750 ± 5,227	0.38
Lactone	4,260 ± 294	1,410 ± 751	3.0
Total drug	11,813 ± 1,712	23,588 ± 4,808	0.50
AUC_0^{60} (ng · min/mL)[c]			
Carboxylate	8,268 ± 992	21,693 ± 3,337	0.38
Lactone	3,741 ± 311	866 ± 489	4.31
Total drug	10,001 ± 1,726	21,875 ± 3,014	0.46
Concentration at t = 5 min (ng/mL)			
Carboxylate	180.7 ± 33.1	1,256.0 ± 163.1	0.14
Lactone	190.0 ± 22.8	21.2 ± 5.6	8.9
Total drug	329.7 ± 55.6	1,286.0 ± 163.0	0.26

[a] Mean ± SD (n = 6).
[b] AUC [CPT dosing]/AUC [Na-CPT dosing].
[c] AUCs determined from trapezoidal method.
[d] AUCs determined from integration of Eqs. (2)–(5).

when the concentrations at 5 min (Table III) are compared. Since the lactone appears to be significantly more active than the carboxylate, AUC and plasma concentration values may indicate that the differences in *in vivo* activity of CPT relative to Na-CPT may be due to the higher initial concentrations of the lactone after dosing with CPT allowing effective drug (lactone) uptake into tissues.

Earlier reported data from Na-CPT administration indicated that most of the drug appears in the urine and feces. Ongoing studies in our laboratory confirm these findings. Also, no metabolites of camptothecin have been observed previously. During our study, a peak, which eluted near the solvent front, was observed in the HPLC plasma chromograms. This peak was formed at much higher apparent concentrations when CPT was administered than when Na-CPT was administered. The area of the peak(s) which eluted in the solvent front increased after dosing with CPT and after dosing with Na-CPT, however, after dosing with CPT the area reached a maximum at approximately 20 min, whereas after dosing with Na-CPT the maximum is reached after 50 to 60 min (Fig. 5). The identity of this apparent metabolite has not yet been established, however, based on its temporal pattern and the fact that the AUC of the peak area versus time curve corresponds very closely to that for the relative AUCs of the lactone, it appears to be a metabolite formed from the lactone and not from the carboxylate. A study using a more reliable analytical method for peak area quantitation and homogeneity is being explored and the identity of this metabolite will be reported later.

Pharmacokinetics of Na-CPT Following i.v. Infusion

Because of the difficulties involved in delivery of CPT, it may be desirable to administer Na-CPT by infusion. To see if significant levels of the lactone could be generated, animals were infused with Na-CPT (to a total dose of 1 mg/kg) as described under Materials and Methods. After the start of infusion, concentrations of the carboxylate reached apparent steady state at about 100 min, whereas the lactone appears to reach steady state earlier (data not shown). The C_{ss} value for the carboxylate and the lactone were 210.6 ± 23.7 and 7.8 ± 1.7 ng/mL, respectively. The values for $AUC^{\infty}_{0\,\text{Carboxylate}}$ and $AUC^{\infty}_{0\,\text{Lactone}}$ were 31,894 ± 252 and 3171 ± 934 ng · min/mL, respectively. These values are nearly identical to the AUC values obtained following i.v. bolus of Na-CPT at the same total dose. Therefore, under the conditions used in these experiments, there is no apparent advantage (based on AUC values alone) of dosing by i.v. infusion over dosing by

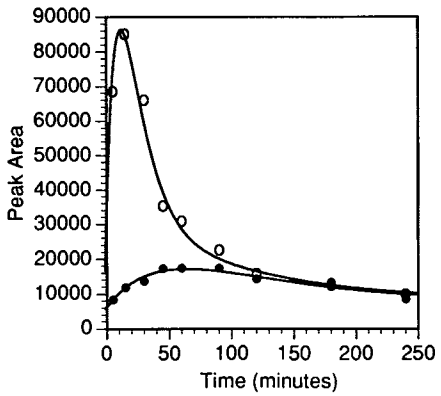

Fig. 5. Apparent metabolite plasma peak area versus time plot after dosing with CPT (open circles) and after dosing with Na-CPT (filled circles).

i.v. bolus injection. However, long-term exposure to the low levels of the lactone produced during infusion may be therapeutically effective.

In conclusion, the lactone and carboxylate forms of CPT exist in equilibrium at physiological pH with the equilibrium favoring the carboxylate form. The rates of lactone hydrolysis were found to be faster in plasma and blood than in aqueous buffers of physiological pH. This finding may be attributed to the small differences in apparent pH and to the higher protein binding of the carboxylate relative to the lactone in the biological fluids. It was found that the AUC of the lactone was significantly higher after dosing with CPT than with Na-CPT, possibly accounting for the higher potency of CPT relative to Na-CPT. The *in vivo* activity of Na-CPT might be reasonably attributed to the *in vivo* conversion of carboxylate into lactone.

ACKNOWLEDGMENTS

This work was supported by the National Cancer Institute (N01-CM-97576 and N01-CM-27755) and the Kansas Health Foundation (formerly the Wesley Foundation) of Wichita, Kansas. The authors would like to thank Steven C. Sutton for his helpful suggestions and Thea Herron for technical assistance.

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