Pharmacokinetics of the 9-Amino and 10,11-Methylenedioxy Derivatives of Camptothecin in Mice

Jeffrey G. Supko and Louis Malspeis

ABSTRACT

Although 20(S)-camptothecin (CA) exhibited potent cytotoxicity against a broad spectrum of tumor models, clinical trials with the sodium salt of its opened lactone ring form were discontinued due to highly variable and severe toxicity. Recently, the 9-amino (AC) and 10,11-methylenedioxy (MC) derivatives of CA were selected for preclinical evaluation by the National Cancer Institute. In the present investigation, the pharmacokinetic behavior of CA, its sodium salt CA, AC, and MC in mice was characterized using specific liquid chromatographic assays which permitted determination of the intact lactone and opened ring carboxylic forms of these compounds. CA disposition was triexponential with a prolonged terminal phase that had a 24.6-h half-life (t1/2) that comprised only 14.6% of the area under the concentration-time profile. The relative magnitudes of the total body apparent volume of distribution (V) and terminal phase rate constant suggest that the high observed total plasma clearance (CL, 104 ml/min/kg) may be associated with extensive accumulation in peripheral tissue regions from which the drug is slowly released. In comparison, the terminal disposition phase of MC accounted for 49.7% of the area under the curve profile. It also had a shorter t1/2 (15.2 h) and appreciably greater CL (526 ml/min/kg) and V (694 liters/kg). This suggested that the degree of binding to tissues relative to plasma proteins was enhanced by the methylenedioxy moiety. In contrast, the 9-amino substituent profoundly diminished the apparent extent of tissue distribution, as indicated by the magnitude of V (7.7 liters/kg), effecting an enhanced rate of elimination (t1/2, 1.4 h). Comparison of the CL of CA and its two derivatives provided an inaccurate indication of drug elimination due to the influence of their unusually large V values. For these compounds, the relative ease of elimination from the body was best represented by mean residence times, which were 0.55, 7.24, and 11.2 h for AC, CA, and MC, respectively. Intact lactone plasma levels achieved after dosing with the lactone form of CA and its 9-amino and 10,11-methylenedioxy derivatives exceeded the far less active carboxylic at all times. In summary, these studies indicate that considerable alterations in pharmacokinetic behavior result from structural modification of the A ring of CA. The large differences in pharmacokinetic parameters of the potential candidates for clinical development, AC and MC, will play an important role in the selection of a therapeutically effective dose regimen.

INTRODUCTION

CA\(^1\) and several of its A ring substituted derivatives exhibit impressive in vivo activity against a variety of murine leukemia and solid tumor lines (1–11). Some of these compounds are highly active against a broad spectrum of human xenografts in athymic mice, including cells lines resistant to clinically available chemotherapeutic agents (2, 12–16). Comprehensive investigations on the mechanism of cytotoxic, antineoplastic, and antiviral effects of CA have conclusively identified DNA topoisomerase I as the molecular target of CA and structurally related compounds. Much of this work has been reviewed recently (17–19).

The structural features of CA required for topoisomerase I inhibition and the consequent antitumor effects have been thoroughly established (1, 19, 20). Among the various functional groups present in the molecule, the α-hydroxy-B-lactone moiety that constitutes the E ring is a primary determinant of its pharmacological and physicochemical properties (Fig. 1). In addition to an intact E ring, the tertiary alcohol and arrangement of groups about the chiral center at position 20 in the S configuration are all absolute requirements for antitumor activity. The hydroxy function renders the lactone highly reactive by enhancing the electrophilicity of the adjacent carbonyl group (1). Consequently, these compounds exist as an equilibrium mixture of their intact lactone and opened ring forms in aqueous solution and biological media (21–24). The opened ring form predominates in neutral and alkaline solution while acidic conditions favor the lactone structure (Fig. 2). The rate of equilibration also exhibits marked pH dependence. It is rapidly achieved in strongly acidic and alkaline solution and approached rather slowly in solutions closer to neutrality. These properties are extremely important when considering the design and interpretation of pharmacokinetic investigations of CA and its analogues (22, 23).

The active intact lactone form of CA is very poorly soluble in vehicles suitable for parenteral administration due to the unusually weak basicity of the quinoline nitrogen atom (1). Consequently, to facilitate the initial clinical evaluation of CA, the drug was converted to the water-soluble sodium salt of the carboxylic acid resulting from saponification of the lactone ring (2). Clinical trials with CA-Na, administered to cancer patients as a short i.v. infusion according to several different schedules, were discontinued in 1972 at the phase II stage because of a poor objective response rate coupled with the development of dose-limiting myelosuppression, severe gastrointestinal toxicities, and cystitis (2, 12). There has since been a continued effort to identify congeners with greater therapeutic effectiveness than the lead compound. Interest in new structure development was accentuated following resolution of the mechanism of action and findings of elevated topoisomerase I activity in several types of tumor cells, making the enzyme an attractive target for chemotherapeutic agents (13).

The design of CA analogues has largely focused on substitution of the A ring in order to achieve enhanced aqueous solubility and increased antitumor potency. The introduction of ionizable functional groups into the molecule served to enhance water solubility, thereby facilitating direct parenteral administration of the intact lactone form (7, 10, 11, 25). Two compounds of this type, the 9-(N,N-dimethylaminomethyl)-10-hydroxy (CPT-11) (topotecan) and 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carboxyloxy derivatives of CA, were recently entered into clinical trials (26–28). Moreover, numerous derivative of CA were prepared and evaluated to determine if anti tumor potency could be significantly improved through appropriate structural modifications (5–8, 25). These efforts culminated in iden-
facilitate an unambiguous comparison of the disposition of the derivatives with the intact lactone and opened ring forms of the parent compound.

MATERIALS AND METHODS

Chemicals. 9-Amino-20(S)-camptothecin (NSC 603071), 20(S)-camptothecin (NSC 94600), camptothecin sodium (NSC 100880), and 10,11-methylenedioxy-20(RS)-camptothecin (NSC 606174) were provided by the Developmental Therapeutics Program of the National Cancer Institute. The purity of these compounds was verified by the supplier using methods that included elemental, chromatographic, and spectroscopic analyses. 9-Amino-[10,12-3H]-20(RS)-camptothecin (NSC 629971) was obtained from the Research Triangle Institute (Research Triangle Park, NC). It had an indicated specific activity of 5.06 mCi/mg (1.84 Ci/mmol). All other chemicals were purchased from commercial sources and used as received.

Drug Disposition Studies. Unfasted male Harlan BALB/c × DBA/2 F1 mice were treated, without anesthesia, by 1-min tail vein injection using a 27-gauge, 0.5-inch Yale hypodermic needle (Becton-Dickinson, Lincoln Park, NJ). The animals received 10-mg/kg doses of each compound, with the exception of AC, for which the dose was 5 mg/kg. Injectable solutions were formulated shortly before administration such that the desired dosage was delivered in a volume of 100 μl. Dimethyl sulfoxide solutions of AC, MC, and CA were diluted 1:4 (v/v) with propylene glycol 400-sterile water for injection, with an apparent pH of 3.0–3.5. The resulting solution was sufficiently acidic to prevent the lactone ring from opening prior to injection. CA-Na was dissolved directly in sterile water for injection, USP. An electric minute timer (Precision Scientific, Chicago, IL) was used to monitor the time of drug administration and blood withdrawal.

It was necessary to use different sampling schedules for each compound in order to accurately define their respective plasma concentration-time profiles. Thus, blood specimens were acquired at 15–19 time intervals, ranging from 5 min to 72 h postinjection, with a minimum of 12 points obtained during the initial 8-h period. Animals were bled by puncture of the retroorbital plexus under ether anesthesia using heparinized microhematocrit capillary tubes (Scientific Products, McGaw Park, IL). The whole blood was collected in 1.5-ml polypropylene tubes (VWR Scientific, Media, PA), rapidly cooled without freezing in a dry ice-propanol bath for approximately 5 s and, immediately thereafter, centrifuged at 12,000 × g for 1 min using an Eppendorf 5412 microcentrifuge (Brinkmann Instruments, Westbury, NY). Three aliquots (50 μl) of the separated plasma were promptly transferred to individual microcen-
trifuge tubes and prepared for analysis of the intact lactone form of the administered compound. The remaining plasma was separated from blood cells, flash frozen, and stored at -20°C until assayed for total drug.

Drug levels in plasma specimens acquired from treated animals were monitored using analytical methods with specificity to permit quantitation of the reversibly associated intact lactone and carboxylate forms of each compound. These procedures have been reported in considerable detail (23, 39). Briefly, the intact lactone form of the drug was separated from the carboxylate component and endogenous compounds by reversed-phase high performance liquid chromatography after removing macromolecules from the sample matrix by precipitation with a methanolic solution of a structurally related internal standard maintained in a dry ice-propanol bath. The intrinsic intense fluorescence of CA and MC provided a highly sensitive and selective direct means of detection (23). Although nonfluorescent in the presence of the chromatographic mobile phase, AC was readily converted to a fluorescent species by inline postcolumn acification prior to detection (39). The concentration of drug present as the intact lactone was specifically determined by direct analysis of deproteinized plasma. Under the chromatographic conditions used, the opened ring form of the drug eluted in the presence of poorly retained endogenous components and was not amenable to direct quantitation. Therefore, the total drug concentration, defined as the sum of concentrations of the lactone and carboxylate species, was also determined in each sample. This was achieved by acidifying the sample to pH 3 before deproteinization, effecting the rapid and quantitative lactonization of drug present in the carboxylate form. The difference between total drug and intact lactone levels provided the concentration of the carboxylate species.

Quantitation was performed by similarly assaying series of 8-9 plasma standards with known concentrations of the analyte and a drug-free sample. Standard curves were constructed by plotting the peak height ratio of each analyte to the internal standard against its concentration. Linear least squares regression was performed with a weighting factor of 1/σ², without inclusion of the origin, to determine the slope, y-intercept, and correlation coefficient of the best fit line. Analyte concentrations in unknown samples were calculated using the results of the regression analysis. Specimens with an analyte level exceeding the upper range of the standard curve were reassayed upon appropriate dilution with drug-free plasma. All samples were initially assayed in duplicate, and if the replicate determinations deviated from their average by more than 10%, additional analyses were performed.

Pharmacokinetic Data Analysis. Time points were determined as the difference between the blood collection interval midpoint and starting time of dose administration. Geometric mean plasma concentrations at each time point were calculated from the average determinations of at least three mice. The resulting plasma concentration-time profiles were pharmacokinetically analyzed by model-independent methods (40). The conventions recommended by Rowland and Tucker (41) for the symbols of pharmacokinetic terms have been adopted. Nonlinear least squares regression was performed using the PCNON-LIN software package (Statistical Consultants, Lexington, KY) and initial parameter estimates determined by preliminary data analysis with the stripping routine RSTRIP (MicroMath, Salt Lake City, UT).

Since drug administration was not strictly instantaneous, but rather a short duration continuous rate i.v. infusion, the appropriate equation of the general form:

\[ C = \sum_{i=1}^{n} \left( C_i / A_i R \right) e^{-k_i \tau} \]

was fit to observed time courses for the plasma concentration of drug by weighted nonlinear regression. The equation was derived according to the principles developed by Benet (42) with subsequent simplification to eliminate compartmental attributes. Thus, the value of \( t' \) is zero until the infusion of duration \( \tau \) has terminated and subsequently becomes defined as:

\[ t' = t - \tau \]

where \( \tau \) denotes time from the initiation of treatment. The coefficients \( C_i \) are the intercept values, corresponding to i.v. bolus administration of the dosage, of each log-linear phase with slope \(-k_i\), such that \( \lambda_1 > \lambda_2 > \cdots > \lambda_n \). Parameters associated with the terminal decay phase are designated with a subscript \( z \) by convention.

Excretion Studies. The urinary and fecal excretion of each compound was evaluated by treating three groups of five mice in the same manner as the corresponding disposition study. The animals were housed in stainless steel metabolic cages and given food and water ad libitum during the course of the experiment. Pooled urine and feces from each group was collected in silanized glass test tubes at 12 to 24-h intervals for 2-5 days following drug administration. The specimens were stored at -20°C until assayed after measuring the urine volume and feces dry weight for each collection period. Excreta from a group of 5 untreated mice was similarly obtained during a 12-h period.

The concentration of unchanged compound in urine specimens was determined directly by the total drug method of analysis as described above. Fecal specimens (0.1 g) were initially homogenized in 0.1 N HCl (1 ml) in a silanized glass test tube for 5 min at 0-5°C using an SDT Tissumizer equipped with a model SHT080EN stainless steel microprobe (Tekmar, Cincinnati, OH). Dimethylformamide (4.0 ml) was added to the mixture and homogenization was continued for 10 min before aliquots (50 μl) were removed for total drug analysis. Specimens of urine and feces collected from the untreated mice were similarly processed for blank determinations.

Renal clearance was calculated as:

\[ CL_r = \frac{\int_0^t C(t) dt}{\int_0^t AUC(t) dt} \]

where \( A(0 \rightarrow t) \) is the cumulative amount of total drug excreted unchanged from the time of dosing to the end of the least void interval and \( AUC(0 \rightarrow t) \) represents the area under the total drug plasma concentration-time profile during this period (43). Values of \( AUC(0 \rightarrow t) \) were calculated using nonlinear regression estimates of the total drug macroscopic disposition parameters \( C, A, \) and \( λ \).

Excretion of Radiolabeled AC. The 9-amino-[10,12-3H]-20(RS)-camptothecin was formulated in the same vehicle used for the disposition study and diluted with the nonlabeled compound to deliver a total dosage of 5.0 mg/kg in 100 μl of solution. Specific activity of the dose, 0.200 ± 0.011 (SD, n = 5) mcI/mg, was ascertained by liquid scintillation counting upon mixing aliquots (2 μl) of the injectable with 10 ml of Formua-963 scintillation cocktail (NEN Products/Dupont, Boston, MA). A single group of 5 mice (26.0 ± 0.1 g) was treated, and their excreta were collected according to the protocol described in the preceding section. At the end of each collection period, the trap and funnel of the metabolic cages was washed with a methanol-soaked filter paper to recover drug present in urine that failed to reach the collection tube.

Urine specimens (2-10 μl) were counted in triplicate after thoroughly mixing with Formula-963 scintillation cocktail (10 ml). Radioactivity present on the cage filters was similarly determined. The feces was permitted to dry and then ground to a fine powder. Two 50 μg samples were combined with distilled water (300 μl) and 4.7 ml of Beckman Tissue Solubilizer 450 (Beckman Instruments, Fullerton, CA) in separate scintillation vials. Most of the feces was solubilized after allowing the mixture to digest at ambient temperature, with intermittent shaking, for 2 days. Aliquots of the digest (200 μl) were separated from undissolved particles and added to 10 ml of Cocktail Neutralizer (Research Products International, Elk Grove Village, IL). The samples were thoroughly mixed and kept in the dark for 12 h prior to liquid scintillation counting.

A Beckman Instruments model LS-6800IC liquid scintillation counter was utilized for measuring radioactivity. All samples were counted for 10 min, which generally provided a counting error of less than 1%, except for those from the later collection periods that contained very low levels of radioactivity. Counting efficiency in each matrix, verified by the internal standard method using Lipophilic Oxi-test 'H-O standards (Radiomatic Instruments & Chemical, Meriden, CT), was approximately 43%.

RESULTS

Plasma Pharmacokinetics

Plasma concentration-time profiles depicting the observed intact lactone and total drug geometric mean plasma levels and best-fit curves for each compound following administration to mice by 1 min i.v. injection are shown in Fig. 3. Time courses for the corresponding carboxylate component were generated from the difference between
nonlinear regression fits of the total drug and intact lactone plasma profiles. This served to diminish variability introduced by the propagation of error in the experimental data. Nonlinear least squares estimated pharmacokinetic parameters and derived values for the administered form of drug are summarized in Table 1. Thus, with the exception of CA-Na, for which parameters associated with carboxylate disposition are shown, the parameters pertain to the intact lactone structure of CA and the two derivatives. Table 2 contains similarly calculated parameters for the total drug plasma profile of each compound.

**Camptothecin Sodium.** An initial carboxylate concentration of 26.9 µM was achieved in the plasma of mice treated by 1 min i.v. injection with 25.9 µmol/kg of CA-Na (Fig. 3A). During the subsequent 90 min, CA-Na plasma levels decayed 100-fold in an apparent first-order manner with a half-life of 5.3 min. This was followed by a considerably slower terminal disposition phase with a 2.2-h half-life. However, the initial rapid phase was the primary determinant of carboxylate disposition, inasmuch as it accounted for 97% of the AUC. Consequently, the MRT of the carboxylate in the body was only

Table 1 Pharmacokinetic parameters for the administered form of camptothecin and its 9-amino and 10,11-methyleneoxy analogues

<table>
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<tr>
<th>Compound</th>
<th>Parameter</th>
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<th>CA</th>
<th>AC</th>
<th>MC</th>
<th>Units</th>
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<td>0.62</td>
<td>0.68</td>
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Table 2 Pharmacokinetic parameters for the total drug plasma profiles of camptothecin sodium, camptothecin, and its 9-amino and 10,11-methyleneoxy analogues

<table>
<thead>
<tr>
<th>Compound</th>
<th>Parameter</th>
<th>CA-Na</th>
<th>CA</th>
<th>AC</th>
<th>MC</th>
<th>Units</th>
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<tr>
<td>Dose</td>
<td>25.9</td>
<td>28.7</td>
<td>13.8</td>
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<td>C1</td>
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**See Table 1 for definitions of parameter abbreviations.**
DISPOSITION OF CAMPTOTHECIN AND DERIVATIVES

13 min and its CL, 114 ml/min/kg, was similarly high. The magnitudes of $V_f$ (0.9 liter/kg) and $V_e$ (1.5 liters/kg) were both relatively low, indicative of limited distribution into peripheral regions of the body, consistent with the hydrophilic nature of CA-Na.

Lactonization of the carboxylate species was a significant component of CA-Na elimination. A peak lactone concentration of 0.63 μM was observed in plasma specimens acquired at the first sampling interval (5.5 min). Plasma levels of the lactone exceeded the carboxylate component within 45 min. Approximately 3 h after treatment, an apparent distribution equilibrium between the two components was achieved, characterized by similar terminal rates of elimination from plasma, with biological half-lives of 2.2 h. The ratio of the lactone to carboxylate plasma concentration was $1.45 \pm 0.02$ ($n = 6$) during this time. However, the AUC of the lactone was only 12.5% of the total drug AUC (3.87 μM·h). This was very similar to the absolute bioavailability of the generated lactone (11.5%) determined from the ratio of intact lactone AUC values observed in animals treated with CA-Na in comparison to CA, corrected for differences in the actual amount of each compound administered. Therefore, while in vitro lactonization appears to occur very rapidly, the extent of conversion to the active form is relatively minor.

Camptothecin. A comparison of Fig. 3, A and B, illustrates that the disposition of CA and CA-Na differ considerably. The initial concentration of total camptothecin (5.3 μM) in mice treated with 28.7 μmol/kg of CA was approximately 5-fold lower than observed for a similar dose of CA-Na (Fig. 3B). However, the systemic duration of the drug was dramatically enhanced by direct administration of the lactone. Whereas the total drug could only be monitored for 8 h following treatment with CA-Na, plasma levels were amenable to quantitation for 72 h in animals receiving CA by bolus i.v. injection. The time course of CA in plasma was distinctly triexponential. The two initial disposition phases of the administered lactone were characterized by half-lives of 19.2 min and 3.6 h, while the biological half-life was 24.6 h. Since the contribution of the prolonged terminal phase to AUC was only 14.6%, it represented a relatively minor component of CA disposition. The MRT of CA lactone was 7.2 h.

In contrast to the slow apparent terminal phase elimination, the CL of CA (104 ml/min/kg) was relatively high, exceeding both murine hepatic blood flow (63.0 ml/min/kg) and creatinine clearance (7 ml/min/kg) (44). However, the magnitudes of $V_f$ and $V_e$, respectively, 45.2 and 222 liters/kg, were also extremely large. Therefore, to the extent that the pharmacokinetics of CA is linear, the relationship:

$$\text{CL} = \frac{V_e \cdot \lambda}{V_f}$$

suggests that the high apparent CL is more likely a consequence of extensive drug depletion from systemic circulation due to accumulation in peripheral tissue regions than actual elimination from the body. It follows that the slow terminal disposition phase is a result of rate limiting release of drug from peripheral compartments. Since the terminal phase was much slower than the other disposition phases and also associated with a comparatively minor fraction of the dose, which are factors that amplify the magnitude of $V_e$, $V_f$ represents a more realistic estimate of the apparent distribution space (45). In addition, $V_f$ (5.4 liters/kg) was found to be more than 5 times body weight, suggesting that CA lactone is very highly bound to erythrocytes or other rapidly equilibrated tissues.

The total drug plasma profile was comparatively similar to the intact lactone time course. Plasma levels of the generated carboxylate increased rapidly to a maximum of 0.30 μM at 21 min after dosing with CA. However, the carboxylate concentration remained below the intact lactone form of the drug at all times. The plasma profile of the carboxylate was most adequately described by an empirical model incorporating a first-order input function and three exponential terms for decay. The CL determined in the study of CA-Na disposition permitted calculation of the actual fraction of the CA dose converted to inactive carboxylate as 39.9%. This represents a significantly greater amount than predicted by direct comparison of the lactone and total drug AUC values (ratio, 0.80) of CA.

9-Aminocamptothecin. Acute toxicities resulting in rapid death were observed in mice upon i.v. injection with 10 mg/kg of AC, the dose used for the other compounds evaluated in this study. Inasmuch as 5 mg/kg (13.8 μmol/kg) appeared to be well tolerated, the plasma pharmacokinetics of AC was characterized at this dose level. Despite the approximately 50% lower dose, the initial plasma concentration of AC, 11.8 μM, was more than 2-fold greater than achieved following administration of CA (Fig. 3C). However, plasma levels remained above 13.3 nm, the lower limit of quantitation, for only 6 h. The plasma profile of the intact lactone form of AC was biexponential, characterized by a relatively rapid initial phase with a half-life of 10.5 min that accounted for 82.9% of the AUC. The biological half-life and MRT were 1.4 and 0.55 h, respectively. The CL was 64.4 ml/min/kg and respective values for $V_f$ and $V_e$ were 1.1 and 2.1 liters/kg. Therefore, the presence of the amino substituent appeared to profoundly diminish the extent of tissue distribution, thereby enhancing overall elimination of the derivative, relative to CA.

Conversion of the lactone to carboxylate proceeded at a much faster rate than was evident in the case of the parent compound. The maximum concentration of AC carboxylate (5.4 μM) occurred at the first time point (5.6 min). During the subsequent 2 h, the relative amount of carboxylate in plasma increased, until an apparent equilibrium between the two components was established. Plasma levels of the carboxylate were 94.6 ± 3.1% ($n = 5$) of the lactone concentration during the terminal phase. Furthermore, the ratio of the lactone to total drug AUC values (0.62) suggested that the extent of conversion to carboxylate was greater for AC than the parent compound.

10,11-Methylenedioxy camptothecin. The A ring methylenedioxy substituent modified the pharmacokinetic behavior of the parent compound in a very different manner than the 9-amino group. In mice treated i.v. with 25.7 μmol/kg of the derivative, MC plasma levels decayed triexponentially from an initial concentration of 1.85 μM, which was lower than achieved with a similar dose of CA (Fig. 3D). The compound was rapidly eliminated from plasma during the first 3 h after administration. With half-lives of 5.6 and 49.4 min, the influence of the two early exponential phases became insignificant within 5 h. Consequently, the terminal phase contribution to the AUC, 49.7%, was greater than either of the preceding disposition phases. Although the biological half-life of MC (15.2 h) was shorter than CA, it had a greater MRT (11.2 h). However, the total body plasma clearance and apparent volumes of distribution were the most significantly altered pharmacokinetic parameters. The extremely high values realized for CL (526 ml/min/kg) and $V_e$ (353 liters/kg) strongly suggest that the methylenedioxy substituent greatly enhances affinity of the compound for tissues relative to plasma proteins. A considerable fraction of the drug was apparently tightly bound in peripheral regions of the body.

The fraction of the dose converted to MC carboxylate (0.68), estimated from the lactone to total drug AUC ratio, appeared to be intermediate between CA and AC. As with the 9-amino analogue, the lactone ring of MC was subject to rapid opening in vivo. The maximum carboxylate concentration (0.57 μM) was observed in the first plasma specimens, collected 5.2 min after dosing, in which the corresponding mean concentration of MC lactone was 1.15 μM. However, the lactone concentration remained greater than the carboxylate component throughout the 24-h plasma profile.
Drug Excretion

The equilibrium position between the lactone and carboxylate components of CA and related compounds is very sensitive to pH. The pH differential between whole blood and the excretory tracts precludes determination of the amount of drug excreted as the intact lactone or opened ring form. Accordingly, excretion of the camptothecins can be reported only as total drug. Excretion profiles of unchanged total drug in mice treated i.v. with CA-Na, CA, MC, and AC, at doses similar to the corresponding disposition study, are summarized in Table 3. Values for the fraction of the dose excreted and renal clearance are expressed as the mean ± SD of three independent experiments. Due to its limited supply, the excretion of radiolabeled AC was determined in a single group of animals and results are reported without expressing variability.

The cumulative amount of unchanged drug detected in the urine and feces of mice for 5 days after treatment with CA-Na (23.8 ± 6.8%) was considerably greater than that found in animals that received a similar dose of CA (3.6 ± 0.7%). During this time, 19.6 ± 5.3% of the CA-Na dose was found in the urine, primarily in the first 24-h void (19.4 ± 4.3%), which greatly exceeded the amount present in feces (4.0 ± 4.3%). The pattern of excretion for the 10,11-methylenedioxy and 9-amino derivatives differed considerably. Urinary excretion was a minor pathway of MC elimination, with only 0.67 ± 0.49% of the dose recovered during 5 days after treatment. A similar amount of the compound was detected in the feces (0.84 ± 0.14%). However, 15.0 ± 1.9% and 28.9 ± 5.2% of the AC given to mice were eliminated, respectively, in the urine and feces collected over a 48-h period. Following the administration of 9-amino-[10,12-3H]-20(RS)-camptothecin to a separate group of animals, urinary radioactivity accounted for 22.5% of the dose excreted during 44 h, most of which (93%) was excreted during the first 12 h. Fecal excretion was found to be the primary route of radiolabel elimination, accounting for 54.7% of the dose. As with elimination by the urinary pathway, fecal excretion of the radiolabel was largely complete within 24 h.

DISCUSSION

The i.v. route of administration is traditionally used in the initial clinical evaluation of investigational antitumor agents to achieve control over the rate and amount of the xenobiotic introduced systemically. However, as with many promising drug candidates, CA was found to be inadequately soluble in parenteral vehicles. This formulation problem had been resolved by converting the compound into a water-soluble sodium salt (CA-Na) through hydrolysis of the lactone moiety. Unfortunately, CA-Na was introduced into phase I trials (35-37). More recently, studies on the disposition of CA-Na (9) and its p.o. absorption in mice were conducted (38). In each of these investigations, plasma concentrations of the drug were monitored using analytical methods in which the sample was acidified to quantitatively lactonize the analyte prior to its isolation. However, these assays are inherently nonspecific because total drug levels were measured rather than individual concentrations of the intact and opened lactone forms of camptothecin. The need to characterize the pharmacokinetic behavior of both components is indicated by the requirement of the intact lactone for antitumor activity (20, 47) and the possible association of dose-limiting toxicities with excretion of the carboxylate (37, 48). Furthermore, in vitro studies have demonstrated that equilibrium between the intact lactone and opened E ring forms of the camptothecins is slowly achieved (21, 23, 24), suggesting that the in vivo behavior of CA and CA-Na may differ significantly. Specific assays for the lactone and carboxylate forms of CA and several of its structural analogues in biological fluids were recently developed to facilitate monitoring the individual species (23, 39).

The present investigation has shown that following systemic introduction of CA and CA-Na conversion to the other species, in addition to distribution and elimination by alternate pathways, affects the subsequent reduction in plasma levels of the administered form of the drug. The two dosage forms provided very different plasma concentration-time courses for the closed and opened E ring structures of camptothecin. Initially after treatment with CA-Na, the inactive carboxylate was the predominant form of the drug in plasma. Subsequently, the relative concentration of the generated lactone increased until an apparent state of equilibrium between the two species was achieved at approximately 3 h, after which plasma levels of the lactone exceeded the carboxylate by a factor of 1.45. Following the administration of CA, the lactone concentration was greater than the

### Table 3 Excretion of unchanged total drug in mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Compound</th>
<th>CA-Na</th>
<th>CA</th>
<th>MC</th>
<th>AC</th>
<th>[10,12-3H]-AC</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>f&lt;sub&gt;e, urine&lt;/sub&gt; (Δt)</td>
<td>19.4 ± 5.3 (24)</td>
<td>1.8 ± 0.7 (24)</td>
<td>0.48 ± 0.49 (12)</td>
<td>12.8 ± 1.9 (12)</td>
<td>21.0 (12)</td>
<td>% dose (h)</td>
<td></td>
</tr>
<tr>
<td>f&lt;sub&gt;e, stool&lt;/sub&gt; (Δt)</td>
<td>19.6 ± 5.3 (120)</td>
<td>2.4 ± 0.7 (120)</td>
<td>0.67 ± 0.49 (120)</td>
<td>15.0 ± 1.9 (48)</td>
<td>22.9±44</td>
<td>% dose (h)</td>
<td></td>
</tr>
<tr>
<td>f&lt;sub&gt;e, total&lt;/sub&gt; (Δt)</td>
<td>4.0 ± 4.3 (24)</td>
<td>0.67 ± 0.22 (24)</td>
<td>0.37 ± 0.003 (12)</td>
<td>16.1 ± 3.6 (12)</td>
<td>57.8±14</td>
<td>% dose (h)</td>
<td></td>
</tr>
<tr>
<td>CL&lt;sub&gt;e&lt;/sub&gt; (μL/min/kg)</td>
<td>52 ± 4.8</td>
<td>3.85 ± 1.33</td>
<td>2.38 ± 2.43</td>
<td>7.22 ± 1.52</td>
<td>77.2±44</td>
<td>% dose (h)</td>
<td></td>
</tr>
<tr>
<td>CL&lt;sub&gt;e&lt;/sub&gt; (LIT)</td>
<td>21.6</td>
<td>43</td>
<td>0.7</td>
<td>18.3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Values for amount excreted and renal clearance are represented as the mean ± SD of 3 experiments.

<sup>b</sup> Abbreviations: f<sub>e</sub>, fraction of the dose excreted unchanged; Δt, time interval for excretion collection relative to drug administration; CL<sub>e</sub> (LIT), clearance determined from the total drug plasma profile; CL<sub>e</sub> (μL/min/kg), renal plasma clearance; [10,12-3H]-AC, 9-amino-[10,12-3H]-20(RS) camptothecin.
derived carboxylate throughout the plasma profile. However, the ratio of the lactone to carboxylate plasma concentrations decreased over time to a constant value of 1.90 ± 0.11 (n = 5) at 12 h postinjection. Therefore, the disposition of CA and CA-Na are significantly influenced by the relatively slow rate of in vivo lactone-carboxylate interconversion. Elimination of the hydrophilic carboxylate species from the body is considerably more facile than the nonpolar lactone form of camptothecin, which is reflected by the mean residence times, 13.2 min and 7.24 h, respectively. These differences were not indicated by the total plasma clearance values of the two species due to offsetting magnitudes of their total body apparent volumes of distribution and terminal disposition phase rate constants.

The total drug plasma profile of CA-Na determined in this study was in good agreement with the only previous investigation of its disposition in mice (9). In animals treated with 10 mg/kg i.v., the biological half-life and total plasma clearance were reported to be 1.06 h and 119 ml/min/kg, respectively. The plasma profile was described as triexponential with half-lives of 1.23 and 3.99 min for the initial two phases of drug decay; however, the number of early time points was inadequate to definitively establish the presence of two very rapid disposition phases. In the present study, although the loss of total drug from plasma was distinctly biexponential following i.v. injection of 10 mg/kg to mice, the existence of an undetected slower disposition phase for CA-Na must be considered. Its apparent terminal phase half-life was similar to the intermediate disposition phase in animals treated with CA (Table 2). However, assay sensitivity did not permit drug level monitoring beyond 8 h after treatment with CA-Na, while the terminal phase was not achieved until more than 24 h after CA was administered. The presence of a third exponential phase is also supported by the total drug plasma pharmacokinetics determined in 15 cancer patients treated with 1–10 mg/kg of CA-Na by 5–10 min i.v. infusion (35). The terminal phase with an apparent half-life of 19.9 ± 7.3 h became evident approximately 24 h after dosing.

The antitumor activity of CA and CA-Na against i.p. implanted L1210 leukemia in mice is consistent with their pharmacokinetic behavior. To achieve efficacy similar to that of CA, a 12-fold higher dose of CA-Na was required, when given i.p. on an every fourth day schedule (2). When administered i.v. with the same dosing regimen, activity with CA-Na was not observed (25). Repeated daily treatment afforded only moderate antitumor effects (2). These observations are consistent with the view that an intact E ring is essential for antitumor activity (25). The present finding that the ionized species predominates following administration of CA-Na further supports this hypothesis. Systemically formed camptothecin lactone in mice treated with i.v. CA-Na was only 11.5% of the dose whereas the lactone constituted 60% of total drug when CA was given. Furthermore, the plasma concentration of lactone decayed below 10 nm within 8 h after i.v. treatment with 10 mg/kg of CA-Na whereas a similar dose of CA provided concentrations sustained above this level for more than 2 days. Therefore, the concentration and duration of camptothecin lactone in plasma were both significantly greater when CA was given.

We have also shown that the presence of neutral substituent groups on the A ring of CA can significantly affect the pharmacokinetics. The 9-amino analogue provides a significantly higher initial plasma concentration of the intact lactone species following a 13.8 μmol/kg dose than observed after 28.7 μmol/kg of CA. However, AC levels decayed below 10 nm within 8 h after treatment, whereas CA remained above this concentration for almost 48 h. In contrast, the initial lactone concentration achieved with MC was almost 3-fold lower than evident after an approximately equivalent dose of CA; however, plasma levels of the derivative exceeded 10 nm for 12 h. The 9-amino and 10,11-methylenedioxy substituents appear to influence disposition primarily through effects on tissue distribution. The extremely large apparent volumes of distribution Vₐ and Vₐ determined for CA are indicative of extensive accumulation into peripheral tissues. Rate-limiting release of CA back into the blood stream could account for its prolonged systemic duration. Within this context, the comparatively rapid elimination of the 9-amino analogue originates from a diminished affinity for tissue sites, as shown by its profoundly lower apparent volumes of distribution relative to the parent compound. Analogous considerations suggested that the extent of tissue distribution and duration of the drug were significantly enhanced by the 10,11-methylenedioxy group.

As noted earlier, when comparing the elimination of camptothecin lactone and its sodium salt from the body, the relative ease of eliminating these A ring substituted derivatives are best represented by their mean residence times. These were 0.55, 7.24, and 11.2 h for AC, CA, and MC, respectively. The trend was not shown by the observed CL values due to the influence of the unusually large Vₐ on the magnitude of CL for each of these compounds. In comparison to CA, the more facile elimination of AC and greater duration of MC in the body are consistent with the changes in polarity imparted to the molecule by the A ring substituents. However, since the lactone form of both analogues is uncharged at physiological pH, it is possible that the substituents influence disposition by affecting binding characteristics with plasma proteins and tissues. The only previous studies of plasma protein binding have been with CA-Na, which was shown to be very highly bound to plasma proteins (34–36). The marked propensity for adsorption onto dialysis and ultrafiltration membranes has precluded experimental determination of plasma protein binding for CA, AC, and MC.

In summary, these studies have shown that rapid i.v. administration of camptothecin in its lactone form will provide sustained levels of the therapeutically effective form of the drug, whereas treatment with the compound as the carboxylate salt failed to achieve comparable plasma levels due to its rapid elimination from the body. Should the clinical evaluation of the lead compound be considered again, a trial with CA appears more likely to afford therapeutic responses with a lower degree of toxicity than was found with CA-Na. Our findings further suggest that the c.i.v. infusion of CA-Na should provide plasma levels of the active lactone species that exceed the inactive opened E ring form after the achievement of steady state. However, due to its more rapid elimination, considerably higher doses of CA-Na would be required than for CA, together with an increased potential for toxicity.

The pharmacokinetics of MC was generally similar to that of CA; however, its elimination from the body was significantly slower. Although this would permit intermittent dosing of the compound, there is also the potential that accumulation with consequent toxicity may occur in patients who were slow eliminators. AC, a compound reported to be particularly effective against MDR-1-expressed cell lines (32), exhibited pharmacokinetics that differed significantly from that of CA. Its more rapid elimination, considerably higher doses of CA-Na would be required than for CA, together with an increased potential for toxicity.

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REFERENCES


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