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ORIGINAL ARTICLE

Clinton F. Stewart · William C. Zamboni
William R. Crom · Peter J. Houghton

Disposition of irinotecan and SN-38 following oral and intravenous irinotecan dosing in mice

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Abstract The present study was conducted to quantitate the disposition of irinotecan lactone and its active metabolite SN-38 lactone in mice following oral and intravenous administration, and to evaluate the systemic exposure of irinotecan lactone and SN-38 lactone associated with antitumor doses of irinotecan lactone in mice bearing human tumor xenografts. Nontumor-bearing mice were given a single oral or intravenous irinotecan dose (5, 10, 40, or 75 mg/kg), and serial plasma samples were subsequently obtained. Irinotecan and SN-38 lactone plasma concentrations were measured using an isocratic HPLC assay with fluorescence detection. The disposition of intravenous irinotecan lactone was modeled using a two-compartment pharmacokinetic model, and the disposition of oral irinotecan and SN-38 lactone was modeled with noncompartmental methods. Irinotecan lactone showed biphasic plasma disposition following intravenous dosing with a terminal half-life ranging between 1.1 to 3 h. Irinotecan lactone disposition was linear at lower doses (5 and 10 mg/kg), but at 40 mg/kg irinotecan lactone clearance decreased and a nonlinear increase in irinotecan lactone AUC was observed. The steady-state volume of distribution ranged from 19.1 to 48.1 l/m^2. After oral dosing, peak irinotecan and SN-38 lactone concentrations occurred within 1 h, and the irinotecan lactone bioavailability was 0.12 at 10 mg/kg and 0.21 at 40 mg/kg. The percent unbound SN-38 lactone in murine plasma at 1000 ng/ml was 3.4 ± 0.67%, whereas at 100 ng/ml the percent unbound was 1.18 ± 0.14%. Irinotecan and SN-38 lactone AUCs in micebearing human neuroblastoma xenografts were greater than in nontumor-bearing animals. Systemic exposure to unbound SN-38 lactone in nontumor-bearing animals after a single oral irinotecan dose of 40, 10, and 5 mg/kg was 28.3, 8.6, and 2.9 ng h/ml, respectively. Data from the present study provide important information for the design of phase I studies of oral irinotecan.

Key words Irinotecan · SN-38 · Pharmacokinetics

Introduction

Camptothecin, a plant alkaloid extract from *Camptotheca acuminata*, is a potent inhibitor of the nuclear enzyme DNA topoisomerase I [27]. Irinotecan was synthesized as a water-soluble derivative of camptothecin to improve antitumor activity as well as decrease toxicity [6, 7, 27]. Irinotecan is hydrolysed by a carboxylesterase enzyme in vivo to form 7-ethyl-10-hydroxycamptothecin (SN-38) [3, 25, 29]. In humans and rodents, SN-38 further undergoes glucuronic acid conjugation to form SN-38 glucuronide. The extent of glucuronidation of SN-38 has been related to the extent and severity of diarrhea seen in humans [12]. In vitro experiments have shown that SN-38 glucuronide has less than 1% of the antitumor potency of SN-38 [9].

Unlike other camptothecin analogs, irinotecan is a prodrug with little inherent biological activity. The antitumor activity observed with irinotecan lactone (hereafter referred to as irinotecan unless otherwise stated) is primarily attributed to its active metabolite SN-38

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C.F. Stewart (✉) · W.C. Zamboni · W.R. Crom
Department of Pharmaceutical Sciences,
St Jude Children's Research Hospital, Memphis,
TN 38101-0318, USA
Tel. 901-495-3663; Fax 901-525-6869

P.J. Houghton
Department of Molecular Pharmacology,
St Jude Children's Research Hospital, Memphis, TN, USA

C.F. Stewart · P.J. Houghton
The Center for Pediatric Pharmacokinetics and Therapeutics,
University of Tennessee, Memphis, TN, USA

P.J. Houghton
Department of Pharmacology,
University of Tennessee, Memphis, TN, USA

lactone (also, hereafter referred to as SN-38 unless otherwise stated). In isolated nuclei from HT-29 cells, more DNA single-strand breaks are formed after exposure to SN-38 than to camptothecin, whereas irinotecan is considered to be inactive [28]. The inhibition of relaxation of SV40 DNA by topoisomerase I prepared from P388 cells is 3600-fold greater for SN-38 than irinotecan (irinotecan IC_{25} 0.2μ*M* vs SN-38 IC_{25} 720 μ*M*) [28]. The significant in vitro cytotoxic potency observed with SN-38 compared with campothecin is postulated to result from more stable cleavable complexes, possibly as a consequence of slower dissociation of SN-38 compared with camptothecin [28]. However, greater antitumor activity (measured as survival of drug-treated animals relative to controls, T/C%) against L1210 leukemia cells has been observed when irinotecan is administered as a prodrug for SN-38, compared with direct administration of SN-38 [19]. The authors speculated that in vivo antitumor activity would be greater after irinotecan administration than after direct administration of SN-38 [19].

Irinotecan and SN-38 are pentacyclic compounds (Fig. 1) with an α-hydroxy lactone system in ring E. At physiologic pH, both irinotecan and SN-38 exist in an equilibrium between the active lactone (closed form) and inactive hydroxy acid (ring-opened form), with the lactone form favored at acidic pH [2]. The presence of human serum albumin also affects the lactone–hydroxy acid equilibrium as the lactone form of SN-38 is preferentially bound to human serum albumin shifting the equilibrium in favor of the lactone form [4].

Structure–activity relationship studies of the camptothecin analogs have shown that an intact lactone ring is essential for in vitro cytotoxicity, inhibition of topoisomerase I activity, and in vivo antitumor activity [30]. However, most studies of irinotecan pharmacokinetics have determined parameters for total irinotecan (the sum of irinotecan lactone and hydroxy acid) and total SN-38 (the sum of SN-38 lactone and hydroxy acid) [13, 22, 26]. A recent clinical pharmacokinetic study has shown wide interpatient variability (7–75%) in the SN-38 lactone to total AUC ratio [24], whereas the proportion in the lactone form is relatively constant, emphasizing the importance of measuring the active lactone form [23]. Based upon the above considerations systemic exposure to nonprotein-bound nonglucuronidated SN-38 lactone may be the most appropriate pharmacokinetic measure of active drug.

Although many schedules and methods of administration for topoisomerase I inhibitors have been evaluated, the optimal schedule and method of administration for irinotecan have not been determined. Favorable results of preclinical studies of the topoisomerase I inhibitor topotecan given on an intermittent, protracted oral schedule [15, 21] has led to ongoing phase I studies of oral topotecan in children and adults. Significant antitumor activity in mice bearing human tumor xenografts has been observed after protracted oral dosing of irinotecan. In the present study, the disposition of irinotecan and SN-38 after intravenous and oral administration were evaluated to determine the oral bioavailability of irinotecan, and to determine the level of systemic exposure to irinotecan and SN-38 associated with doses of irinotecan found to be effective in mice bearing human tumor xenografts. The results of this study could be used to evaluate different dosing strategies and methods of administration for irinotecan in humans.

Materials and methods

Chemicals and animals

Irinotecan ([7-ethyl-10-(4-1-piperidino)-1-piperidino-carbonyloxy - camptothecin]) and SN-38 (7-ethyl-10-hydroxy camptothecin) powder were provided for these experiments by Upjohn Pharmaceutical Co. (Kalamazoo, Mich.). For intravenous administration, irinotecan was dissolved in a solution of 0.26 ml sorbitol (70% w/w), 0.9 mg/ml lactic acid, and sterile water (75 °C, 20 min). The pH was adjusted to 3.9 by the addition of 1 *N* HCl. The solution of 20 mg/ml was filtered, sterilized, and kept in a foil-wrapped sterile vial. This solution was used for both the intravenous and oral studies. All solvents used were analytical grade or HPLC grade.

Immune-deprived female CBA/CaJ mice (Jackson Laboratories, Bar Harbor, Me.) weighing 23 to 28 g were used in all experiments [14]. Mice were allowed free access to food and water overnight and during the study. Pharmacokinetic studies after oral and intravenous administration of irinotecan were also performed in mice bearing human neuroblastoma tumor xenografts (NB-EB).

Drug administration and sample collection

For intravenous studies, the following doses of irinotecan were administered as single doses by direct injection (duration of infusion < 2 min) into a lateral tail vein: 5 mg/kg (16.5 mg/m^2), 10 mg/kg (33 mg/m^2), 40 mg/kg (132 mg/m^2), or 75 mg/kg (247.5 mg/m^2). For oral studies the following doses of irinotecan were administered as single doses by oral gavage into the stomach: 5 mg/kg (16.5 mg/m^2), 10 mg/kg (33 mg/m^2), 40 mg/kg (132 mg/m^2), or 75 mg/kg (247.5 mg/m^2). Heparinized blood samples (~1 ml) were collected (four animals per point) at 0.08, 0.125, 0.25, 0.5, 1, 2, 4, and 8 h after the dose.

All blood samples were immediately centrifuged at 12 000 *g* for 2 min on a tabletop centrifuge. Plasma was separated and proteins precipitated by the addition of 200 μl plasma to 800 μl cold methanol (−30 °C), followed by vigorous agitation with a vortex mixer, and centrifuging again at 12 000 *g* for 2 min. The supernatant was decanted and stored at −70 °C until analysis.

Irinotecan and SN-38 plasma concentrations were determined using an isocratic HPLC assay with fluorescence detection as previously described in detail [22]. The initial excitation and emission wavelengths were 375 nm and 500 nm, respectively. The fluorescence detector was programmed to change the emission wavelength to 540 nm (optimum emission wavelength for SN-38) 1 min prior to elution of SN-38. The lower level of quantitation was 10 ng/ml for irinotecan and 5 ng/ml for SN-38. All calibrators and controls were prepared in murine plasma (Hill Top Laboratory Animals, Scottdale, Pa.).

Protein binding studies

The extent of protein binding of SN-38 in murine and human plasma was determined using an Amicon Micropartition System-1 (MPS-1, Amicon Corporation, Danvers, Mass.). SN-38 (1000 ng/ml and 100 ng/ml) was added to aliquots of murine and human plasma, brought to 25 °C, vortexed for 15 s, and then centrifuged using a fixed-angle rotor at 1000 *g* for 15 min. The ultrafiltrate was collected, tested for microprotein leakage, and immediately analyzed by HPLC for SN-38. Microprotein leakage was not detected through any of the filtration membranes. SN-38

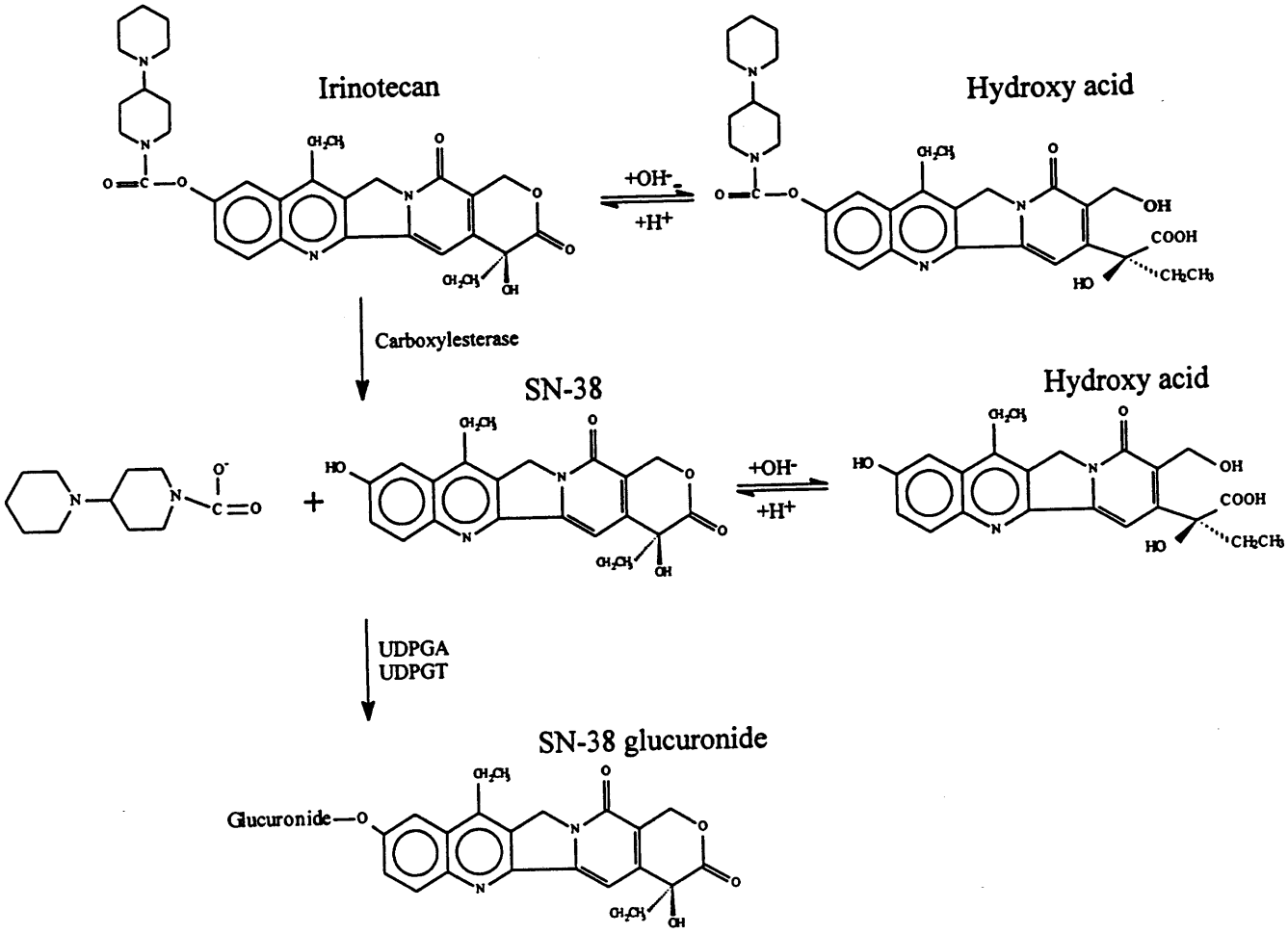

Fig. 1 Metabolic schema for camptothecin derivative irinotecan lactone, showing enzymatic formation of active metabolite SN-38, and hydrolysis of both compounds to their hydroxy acid forms. SN-38 further undergoes glucuronidation to form SN-38 glucuronide

bound concentrations were determined using a curve constructed from murine plasma, and SN-38 unbound concentrations were determined using a curve constructed from murine plasma ultrafiltrate. The percent SN-38 unbound (%unbound) was calculated from the concentration of SN-38 in the ultrafiltrate in relation to the SN-38 total plasma concentration determined in a separate aliquot of the initial SN-38 plasma solution.

Pharmacokinetic analysis

Irinotecan plasma concentration versus time data from intravenous administration were modeled using maximum likelihood estimation as implemented in ADAPT II [8]. One- and two-compartment pharmacokinetic models were evaluated, and model fits were compared by examination of residuals between predicted versus observed concentrations, correlation coefficients, and relative precision of parameter estimates. In each case the irinotecan plasma concentration data were best fit by a two-compartment model. Parameters of the two-compartment model estimated included the volume of the central compartment (V_c), elimination rate constant (K_{10}), and the intercompartment rate constants (k_{12}, k_{21}). Pharmacokinetic parameters calculated from these estimates included sytemic clearance (CL), volume of distribution at steady-state (Vd_{ss}), and area under the plasma concentration versus time curve (AUC).

Irinotecan plasma concentration versus time data from oral administration were analyzed using noncompartmental methods. The AUC and the area under the first moment curve (AUMC) for irinotecan were calculated by the trapezoidal rule to the last measurable data point. The elimination rate constant (k_e) determined by log-linear regression analysis of the terminal phase of the plasma concentration-time curve was used to extrapolate the AUC to infinity. The apparent oral clearance (CL) and bioavailability (F) of irinotecan were calculated using standard equations [10].

SN-38 AUC after oral and intravenous administration of irinotecan was determined by noncompartmental methods as described above. The maximum observed SN-38 plasma concentration (C_{max}) and time to maximum plasma concentration (T_{max}) for each dose of irinotecan were determined.

Statistical analysis

Pharmacokinetic parameters were summarized using descriptive statistics including mean, median, and range. The r^2 value reported for the pharmacokinetic modeling was calculated as the regression sum of squares/total sum of squares, and was used as a measure of goodness of fit for the modeling of irinotecan.

Results

Plasma irinotecan and SN-38 pharmacokinetics

Single intravenous irinotecan doses of 5, 10, and 40 mg/kg were tolerated without acute toxicities,

whereas all mice receiving 75 mg/kg intravenous irinotecan died within 30 min of the infusion, presumably related to the rapidity of infusion (< 2 min). The irinotecan and SN-38 concentration-time data after 5, 10, and 40 mg/kg dose are presented in Fig. 2. After intravenous administration irinotecan and SN-38 plasma concentrations were above the lower limit of quantitation for at least 4, 6, and 8 h after 5, 10, and 40 mg/kg doses, respectively. The pharmacokinetic parameters for irinotecan and SN-38 are summarized in Tables 1 and 2, respectively. At each intravenous dose irinotecan showed biphasic plasma disposition, with a terminal half-life ranging between 1.1 to 3 h. The irinotecan AUC increased linearly with dose from 5 to 10 mg/kg, but from 10 to 40 mg/kg the irinotecan AUC increased nonlinearly (i.e. fourfold increase in dose, sixfold increase in AUC). The less than proportional increase in SN-38 AUC from 10 to 40 mg/kg is consistent with saturation of carboxylesterase. The steady-state volume of distribution was 10 to 20 times greater than estimates of mouse total body water (~0.7 l/kg), suggesting extensive tissue distribution and binding.

Irinotecan was absorbed rapidly after oral administration and the observed time to peak irinotecan and SN-38 concentration was within 1 h of administration. After an initial absorption phase, plasma irinotecan concentrations declined monoexponentially. After oral administration of 5 mg/kg irinotecan, concentrations were below the limit of quantitation of our assay. Whereas, after administration of 40 and 75 mg/kg, irinotecan and SN-38 concentrations were measurable for at least 8 h, and for at least 4 h for the 10 mg/kg dose. Although irinotecan concentrations were below the limit of quantitation of our assay, SN-38 was measurable for at least 4 h. Irinotecan bioavailability varied with dose: at 10 mg/kg the bioavailability was 0.12 and at 40 mg/kg it was 0.21. The irinotecan and SN-38 AUCs after the 75 mg/kg oral dose were 4483 and 1341 ng h/ml, respectively. Shown in Fig. 3 are the SN-38 concentration-time data after oral irinotecan administration at 5 and 40 mg/kg.

As shown in Fig. 4, after intravenous irinotecan administration the molar ratio of SN-38 AUC to irinotecan AUC was 0.7, 0.7, and 0.2 for the 5, 10, and 40 mg/kg doses, respectively. The ratio of SN-38 AUC to irinotecan AUC decreased with increasing irinotecan oral dose: 3.1, 2.3, and 0.6 for the 5, 10, and 40 mg/kg doses, respectively.

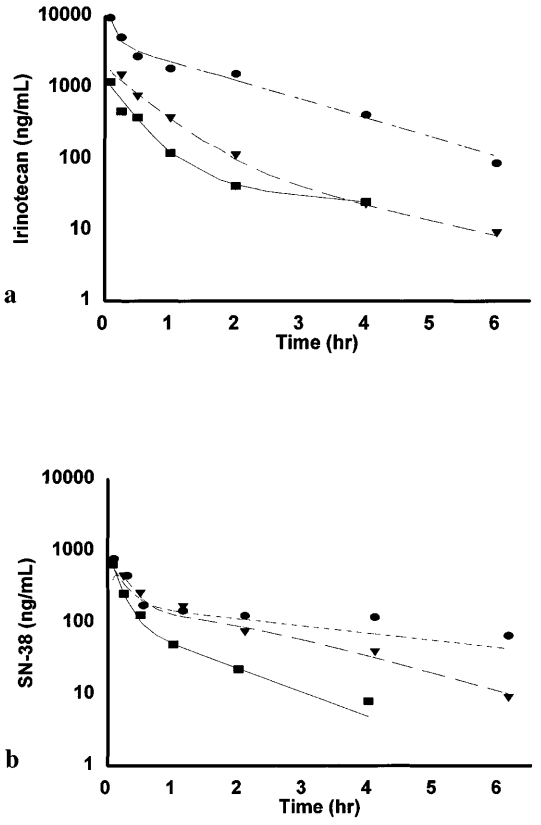

Fig. 2 **a** Mean plasma irinotecan concentration-time plot for 5 (■), 10 (▼), and 40 (●) mg/kg irinotecan administered intravenously. The lines represent the best-fit line of the average of three data points at each observation time (range CV% of the average of three data points 3.3 to 25%). **b** Plasma SN-38 concentration-time plot for 5 (■), 10 (▼), and 40 (●) mg/kg irinotecan administered, intravenously. The solid line represents the best-fit line of the average of three data points at each observation time (range CV% of the average of three data points 2.1 to 44%)

Table 1 Summary of irinotecan pharmacokinetic parameters (C_{max} maximum plasma concentration, t_{max} time of maximum plasma concentration, $t_{½\beta}$ elimination half-life, $AUC_{0\rightarrow\infty}$ area under the concentration time curve from 0 to infinity)

Pharmacokinetic parameter	5 mg/kg[a]	10 mg/kg		40 mg/kg		75 mg/kg
	Intravenous	Intravenous	Oral	Intravenous	Oral	Oral
V_c (l/m^2)	13	14.7	–	9.3	–	–
k_e (h^{-1})	1.8	1.7	1.1	1.8	1.5	2.3
$t_{½\beta}$ (h)	3.3	2.1	1.1	1.1	1.3	0.31
Vd_{ss}(l/m^2)	48.1	21.6	–	23.8	–	–
CL (l/h/m^2)	23.4	24.8	–	16.5	–	–
$AUC_{0\rightarrow\infty}$ (ng h/ml)	686	1301	154	7749	1588	4366
C_{max} (ng/ml)	1067	1796	74	9544	758	960
t_{max} (h)	–	n/a	1	n/a	1	1

[a]Concentrations for oral dose were below the limit of quantification

Table 2 Summary of SN-38 pharmacokinetic parameters (See Table 1 for listing of abbreviations)

Pharmacokinetic parameter	5 mg/kg		10 mg/kg		40 mg/kg		75 mg/kg
	Intravenous	Oral	Intravenous	Oral	Intravenous	Oral	Oral
C_{max} (ng/ml)	647	30	443	130	783	236	296
t_{max} (h)	–	1	–	1	–	1	0.25
k_e (h^{-1})	0.52	0.43	1.02	0.90	0.40	0.32	0.21
AUC $_{0\rightarrow\infty}$ (ng h/ml)	279	86	534	253	1012	831	1341
% extrapolated	5.6	21	1.8	6.1	6.4	8.9	24

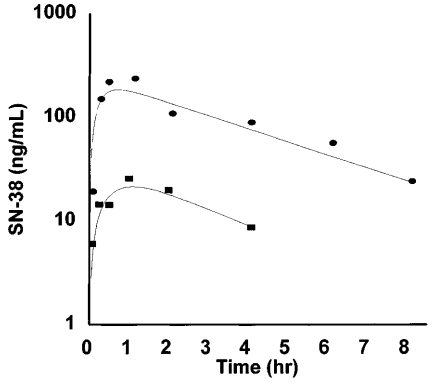

Fig. 3 Plasma SN-38 concentration-time plot after oral administration of 5 (■) and 40 (●) mg/kg irinotecan. The solid lines represent the best-fit line of the average of three data points at each observation time (range CV% of the average of three data points 7.6 to 58.1%)

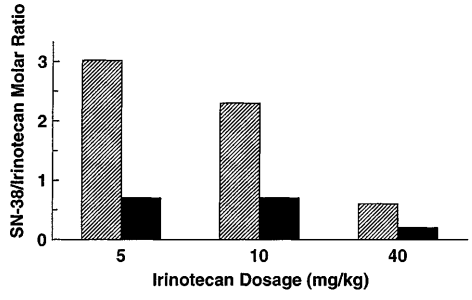

Fig. 4 The molar ratio of SN-38 AUC to irinotecan AUC for the 5, 10, and 40 mg/kg oral (▨) and intravenous (■) doses. (The irinotecan AUC from the 5 mg/kg oral dose was not measurable, so the AUC used in this table was estimated from model parameters.)

SN-38 plasma protein binding

The plasma protein binding for SN-38 in mice was evaluated using ultrafiltration methodology. Initially, the recovery of SN-38 from the MPS-1 device was less than 50%, suggesting nonspecific binding to the ultrafiltration apparatus. After pretreatment of the ultrafiltration device with blank murine plasma, percent recoveries improved (101 ± 17%). At 1000 ng/ml the mean (±SD) SN-38 %unbound was 3.4% ± 0.7% ($n = 10$), whereas at 100 ng/ml the %unbound was 1.18 ± 0.14%. For comparison, similar experiments were performed using human plasma, and the mean (±SD) SN-38 %unbound was 1.6% ± 0.4% ($n = 11$). To approximate systemic exposure to unbound drug in mouse plasma, SN-38 AUC (bound and unbound) was multiplied by 1.18% (%unbound) to estimate $AUC_{unbound}$. Systemic exposure to unbound SN-38 was 1.01, 2.98, 9.81 ng h/ml for oral doses of 5, 10, and 40 mg/kg, respectively.

Irinotecan and SN-38 disposition in mice bearing human neuroblastoma xenografts

After an oral irinotecan dose of 10 mg/kg the irinotecan AUC in mice bearing NB-EB xenografts was greater than in nontumor-bearing mice (522.2 vs 139.9 ng h/ml, respectively). Similarly the SN-38 AUC in mice bearing NB-EB xenografts was greater than in nontumor-bearing mice (789.9 vs 216.0 ng h/ml, respectively).

Discussion

This is the first report of the pharmacokinetics of irinotecan and SN-38 in mice after oral administration of irinotecan. Irinotecan has shown significant activity against both murine tumors and human xenograft models after many different schedules, routes of administration, and dosages. Kunimoto et al. evaluated parenteral (intraperitoneal and intravenous) and oral irinotecan given as a single dose once weekly for three consecutive weeks against murine tumors in ascites and solid forms [20]. Oral irinotecan at total dosages of 200 to 800 mg/kg (i.e. 67 to 267 mg/kg per dose) produced cures and was associated with very low acute toxicity. However, they did not report irinotecan lactone or SN-38 plasma concentration data for comparison with the present study.

We evaluated oral irinotecan in two regimens: 25 and 50 mg/kg per day for 5 days per week for 12 consecutive weeks (or [(dx5)12]), and 50 and 75 mg/kg per day for 5 days per week for 2 consecutive weeks repeated at 21 days for four cycles (or [(dx5)2]4). When irinotecan was administered [(dx5)12], there was significant weight loss at the highest dose level (e.g. 50 mg/kg per dose), with mice weighing 81 ± 4% of their pretreatment weight at the end of drug administration. Intestinal toxicity was manifest by lose feces, but not diarrhea, with an onset at week 8 of treatment. Thus, we do not feel the mouse model reflects the intestinal toxicity seen in patients. Both schedules were associated with

significant antitumor activity against a panel of human colon adenocarcinomas, whereas the intermittent schedule [(dx5)2]4 was less toxic than the protracted 12-week schedule. The antitumor activity of oral irinotecan will be presented in detail elsewhere [28a]. However, given by oral gavage on the schedules tested, this drug was as efficacious as the optimal schedules of intravenous irinotecan in each of the tumor models examined.

In the current study the disposition of irinotecan and SN-38 was studied after intravenous as well as oral administration. However, only the lactone forms were measured. Previously published studies of irinotecan in animals have reported pharmacokinetic parameters for total irinotecan and SN-38 after intravenous administration which make comparison between the results of this study and those reported from previous studies impossible [5, 19]. Measurement of the lactone form of camptothecin analogs such as irinotecan and SN-38 requires careful and immediate plasma sample processing. However, to correlate drug exposure and activity, measurement of the active form is essential. This is especially true with the camptothecin analogs (e.g. SN-38) since the lactone structure is required for binding to topoisomerase I [11, 13, 17]. Several investigators have suggested measurement of total irinotecan or total SN-38 is an adequate intermediate, or surrogate, measure of irinotecan (or SN-38) systemic exposure [26]. However, until a well-designed study shows total SN-38 (sum of lactone and hydroxy acid) to be predictive of outcome (e.g. toxicity or efficacy), the measurement of systemic exposure to the lactone form is basic to understanding the pharmacology of irinotecan.

SN-38 was detected in plasma as early as 5 min after oral irinotecan administration, was maximal at 1 h and then declined monoexponentially. Hydrolysis of irinotecan by a carboxylesterase enzyme has been reported to be responsible for the conversion of irinotecan to SN-38 [29]. The detection of SN-38 at early time points may be a consequence of the presence of carboxylesterase in murine plasma [19], first pass metabolism of irinotecan, or metabolism of irinotecan by intestinal carboxylesterase, and subsequent absorption of SN-38. Regardless of the reason, the molar ratio of SN-38 AUC to irinotecan AUC was threefold higher after oral than after, intravenous administration of irinotecan at the same dose (see Fig. 4)

The SN-38 systemic exposure (AUC) was greater in mice bearing the NB-EB human tumor xenograft than in nontumor-bearing mice. Chabot et al. have reported a similar observation after administration of an intravenous dose of irinotecan (57.5 mg/kg) to mice bearing PO3 pancreatic adenocarcinoma [5]. Our preliminary observation of altered irinotecan and SN-38 disposition in mice bearing human neuroblastoma xenografts has led to additional studies with other neuroblastoma and colon carcinoma xenografts. If the presence of tumor alters irinotecan and SN-38 disposition in the xenograft model this may have potential clinical consequences as the xenograft data is extrapolated to patients. The present study provides important data to use as a baseline for comparison with studies of irinotecan and SN-38 disposition in tumor-bearing animals.

After conversion of irinotecan to SN-38 by carboxylesterase, SN-38 is further metabolized by glucuronyltransferase to an inactive glucuronide. Although Gupta et al. have reported a correlation between the ''biliary index'' and grade of gastrointestinal toxicity [12], we chose not to measure SN-38 glucuronide because of its low antitumor potency ($< 1\%$ of SN-38) and limited plasma sample volume.

Irinotecan has been reported to undergo metabolic saturation (nonlinear clearance) at the higher doses (e.g. 40 mg/kg) evaluated in this study [18, 19]. The irinotecan clearance (16.5 l/h per m^2) for the 40 mg/kg dose was less than the clearance determined from the 5 mg/kg (23.4 l/h per m^2) and 10 mg/kg (24.8 l/h per m^2) doses. Likewise, a disproportionate increase in AUC was observed as a fourfold increase in the irinotecan dose resulted in a sixfold increase in irinotecan AUC (see Table 1). An eightfold increase in the irinotecan intravenous dose resulted in only a fourfold increase in SN-38 AUC. The molar ratio of SN-38 AUC to irinotecan AUC after oral and intravenous irinotecan decreased with increasing irinotecan dose, possibly reflecting carboxylesterase saturation. Similar results have been reported for irinotecan total AUC and SN-38 total AUC in mice [18] and in humans [1]. In view of these pharmacokinetic differences, it is interesting that Houghton et al. reported similar responses in a range of human tumor xenografts for irinotecan whether treated at a dose of 20 or 40 mg/kg [16]. The disproportionate increases in irinotecan and SN-38 concentrations, and apparent plateau of antitumor effect in the xenograft model may reflect saturation of carboxylesterase.

Prior to extrapolating results of preclinical pharmacology studies of irinotecan in nontumor-bearing animals to patients with tumors receiving irinotecan, several potentially confounding factors must be considered. As our data show, SN-38 formation after oral dosing is greater than after intravenous dosing (e.g. SN-38/irinotecan molar ratio was 0.7 and 3.1 after 10 mg/kg intravenous and oral administration, respectively). Thus, the route of administration (e.g. oral, intravenous) must be considered. Data from this study suggest that the presence of tumor may alter irinotecan and SN-38 disposition in animals bearing human tumor xenografts. Thus, the presence of tumor should be systematically evaluated in the animal model if the results are to be extrapolated to patients. Further studies of irinotecan and SN-38 disposition in mice bearing human neuroblastoma and colon carcinoma xenografts are ongoing. The difference between SN-38 protein binding in murine and human plasma presents another potentially confounding factor to extrapolating results from the xenograft model to patients.

In conclusion, the overall bioavailability of oral irinotecan was low ($F = 0.12$ to 0.21). However, the molar ratio of SN-38 AUC to irinotecan AUC was threefold

higher following oral than following intravenous administration at equivalent doses. Extensive first-pass metabolism (intestinal and hepatic) could explain the higher SN-38 exposure after oral than after intravenous administration. In vitro studies showed SN-38 highly bound to murine plasma protein. Irinotecan may undergo nonlinear clearance at higher doses, which may reflect saturation of carboxylesterase conversion of irinotecan to SN-38. Data from this study will be useful to the design of clinical studies to determine the optimal dose, schedule, and route of administration of irinotecan.

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