Altered Irinotecan and SN-38 Disposition after Intravenous and Oral Administration of Irinotecan in Mice Bearing Human Neuroblastoma Xenografts

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ABSTRACT

The antitumor activity of irinotecan in vitro primarily results from its hydrolysis by carboxylesterase to the active metabolite SN-38. The present study was conducted to evaluate the effect of human neuroblastoma xenografts on irinotecan and SN-38 disposition after i.v. and oral irinotecan administration. Non-tumor-bearing mice and mice bearing three different human neuroblastoma xenograft lines (NB1691, NB1643, and NBE) were given irinotecan (10 mg/kg) by short i.v. injection into the tail vein or oral gavage. Serial plasma samples were obtained, processed to isolate irinotecan and SN-38 lactone, and assayed with a sensitive and specific high-performance liquid chromatography assay. Noncompartmental and compartmental pharmacokinetic analyses were performed. A four-compartment model was used for analysis of irinotecan and SN-38 concentration-time data after i.v. administration. The presence of tumor increased irinotecan systemic exposure (1.2–3.8-fold; \( P < 0.05 \)) after i.v. and oral administration in mice bearing neuroblastoma xenografts compared to non-tumor-bearing mice. Moreover, SN-38 systemic exposures were higher (1.3–3.8-fold; \( P < 0.05 \)) in mice bearing human neuroblastoma xenografts as compared to non-tumor-bearing mice, with the greatest effect observed after oral administration of irinotecan. A schematic model is presented to provide a mechanistic basis for our observations. These results emphasize the need to perform preclinical pharmacokinetic studies to evaluate the influence of tumor on drug disposition.

INTRODUCTION

Camptothecin, a plant alkaloid extract from *Camptotheca acuminata*, is a potent inhibitor of the enzyme DNA topoisomerase I (1). Unlike other camptothecin analogues, irinotecan is a prodrug with very little inherent antitumor activity (1,2). Irinotecan undergoes hydrolysis by a CE enzyme (EC 3.1.1.1) to form the active metabolite, SN-38 (Refs. 3–6; Fig. 1). SN-38 undergoes further metabolism by the enzyme uridine glucuronyltransferase (UGT1) to form SN-38 glucuronide. In vitro studies suggest that SN-38 glucuronide has less than 1% of the antitumor activity of the nonglucuronidated form (7). In addition, several oxidative metabolites of irinotecan have been isolated from human bile and urine (8). Structure activity studies of the camptothecin analogues have shown that an intact lactone ring is essential for *in vitro* cytotoxicity, inhibition of topoisomerase I activity, and *in vivo* antitumor activity (9).

Neuroblastoma is a common malignancy of childhood, surpassed in incidence only by acute leukemia and brain tumors (10). Chemotherapy is the cornerstone of management in patients with unresectable localized tumors or disseminated disease (10). Neuroblastoma is a malignancy that consistently responds to chemotherapy, but these responses have not translated into durable remissions or long-term survival.

Thus, there is an urgent need to identify new agents and optimize strategies in the treatment of neuroblastoma. Studies indicate irinotecan is highly effective against human neuroblastoma tumor xenografts (11,12). We initially studied the disposition of irinotecan and SN-38 after oral and i.v. irinotecan in non-tumor-bearing mice (13). However, preliminary data suggest that the presence of human pancreatic ductal adenocarcinoma xenograft tumors in mice markedly alters irinotecan disposition and increases conversion to SN-38 compared to non-tumor-bearing mice (14). In addition, CE has been shown to be expressed in human neuroblastoma cell lines (15–17), with cytotoxic sensitivity related to intracellular CE activity (18). Thus, in the present study, we evaluated the effect of human neuroblastoma xenografts on irinotecan and SN-38 disposition after i.v. and oral administration of irinotecan in non-tumor-bearing mice and mice bearing NB1691, NB1643, and NBE xenografts.

The abbreviations used are: CE, carboxylesterase; CL, clearance; AUC, area under the plasma concentration-time curve.

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2 To whom requests for reprints should be addressed, at Department of Pharmaceutical Sciences, St. Jude Children’s Research Hospital, 332 North Lauderdale, Memphis, TN 38105.
Hydroxy acid

CH₂CH₃ O

SN-38CH₂I₃O

Ir

Glucuronyl

APC

SN-38

Glucuronide

Fig. 1 Metabolic schema for the camptothecin derivative irinotecan lactone, showing enzymatic formation of the active metabolite SN-38 and hydrolysis of both compounds to their hydroxy acid forms. Irinotecan also undergoes several steps of oxidative metabolism to form a series of metabolites. SN-38 undergoes glucuronidation to form SN-38 glucuronide.

MATERIALS AND METHODS

Immune Deprivation of Mice. Female CBA/CaJ mice (Jackson Laboratories, Bar Harbor, ME), 4 weeks of age, were immune-deprived by thymectomy, followed 3 weeks later by whole-body irradiation (1200 cGy) using a 137Cs source. Mice received 3 x 10⁶ nucleated bone marrow cells within 6–8 h of irradiation (19). Tumor pieces of approximately 3 mm³ were implanted in the space of both dorsal lateral flanks of the mice to initiate tumor growth. Tumor-bearing mice were randomized into groups of seven before initiating therapy.

Human Neuroblastoma Tumor Xenograft Lines. Each of the neuroblastoma xenografts has been described in detail elsewhere (20). All tumors demonstrated amplification of N-MYC, with the exception of xenograft NBEB. For chemotherapy studies, all tumors were used within four passages of their engraftment in mice. Each tumor grew routinely in >95% of recipient mice, and all were human as determined by karyotype. All tumors were 1–2 cm in diameter at the time of study.

Formulation and Administration. Irinotecan powder, provided by Pharmacia and The Upjohn Co. (Kalamazoo, MI), was dissolved in a solution of sorbitol (70% w/w) and lactic acid. The irinotecan solution was heated to 65°C for 20 min and then cooled at room temperature. The pH was adjusted to 3.9 with the addition of hydrochloric acid. The solution was diluted in sterile water USP, (Fujisawa, Deerfield, IL), to 20 mg/ml, filter sterilized, and stored in foil-wrapped tubes (–20°C). The drug was further diluted (0.1 ml/20 g body weight) in sterile water, USP and 0.9% sodium chloride, USP (Fujisawa) before oral and i.v. administration, respectively.

Drug Administration and Sample Collection. The disposition of irinotecan and SN-38 was evaluated after a single dose of i.v. or oral irinotecan in non-tumor-bearing mice and in mice bearing NB1691, NB1643, and NBEB human neuroblastoma tumor xenografts. For i.v. studies, a single dose of irinotecan, 10 mg/kg (33 mg/m²), was administered by direct injection (duration of infusion, <1 min) into a lateral tail vein. For oral studies, a single dose of irinotecan, 10 mg/kg (33 mg/m²), was administered by oral gavage into the stomach. Heparinized blood samples (~1 ml) were collected (three animals/time point) before and 0.25, 1, 2, 4, and 6 h after i.v. administration and before and 0.25, 0.5, 1, 2, and 4 h after oral administration.

All blood samples were immediately centrifuged at 12,000 rpm for 2 min in a centrifuge (Microcentrifuge: Denver Instru-
eluded volume of the central compartment for irinotecan and calculated using standard equations (23).

determined by an isocratic high-performance liquid chromatography assay with fluorescence detection as described previously in detail (13, 21). The excitation and emission wavelengths were 375 and 520 nm, respectively. The lower level of quantitation was 5 ng/ml for irinotecan and SN-38. All calibrators and controls were prepared in murine plasma (Hill Top Lab Animals, Inc., Scottsdale, PA).

Pharmacokinetic Analysis. A four-compartment model (Fig. 2) using maximum likelihood estimation was fit simultaneously to the irinotecan and SN-38 plasma concentration data after i.v. administration (ADAPT II). Model parameters included volume of the central compartment for irinotecan and SN-38 (Vc), irinotecan elimination rate constant (k30), irinotecan intercompartment rate constants (k12 and k21), SN-38 elimination rate constant (k30), CE conversion of irinotecan to SN-38 (k13), and SN-38 intercompartment rate constants (k34 and k43). Irinotecan and SN-38 undergo linear disposition at doses <20 mg/kg i.v. in mice, thus a linear rate constant (k1) was used to represent hydrolysis by CE. Systemic CL of irinotecan and SN-38 was calculated from these model estimates. Area under the irinotecan and SN-38 plasma concentration-time curve from zero to infinity (AUC0) was calculated using the log-linear trapezoidal rule to the last measurable data point. The elimination rate constant (k1) was determined by log-linear regression analysis of the terminal phase of the plasma concentration-time curve and was used to extrapolate the AUC to infinity. The molar ratio of SN-38 formation was calculated by dividing SN-38 AUC by irinotecan AUC.

Schematic Model of SN-38 Formation after Oral and i.v. Irinotecan Administration in Mice Bearing Human Neuroblastoma Xenografts. The schematic model of SN-38 formation has three assumptions: (a) systemic baseline formation of SN-38, defined as formation of SN-38 by liver and plasma, was calculated after irinotecan i.v. administration in non-tumor-bearing mice; (b) systemic baseline formation of SN-38 is equal to 48% of irinotecan plasma systemic exposure, up to a maximum of 532 ng·h/ml; and (c) intestinal formation of SN-38 was calculated after oral irinotecan in non-tumor-bearing mice and is equal to 149 ng·h/ml. After i.v. irinotecan, tumor formation of SN-38 was calculated as the difference between measured SN-38 AUC and SN-38 systemic baseline formation. After oral irinotecan, tumor formation was calculated as the difference between measured SN-38 AUC and the sum of the systemic baseline and intestinal formation.

Statistical Analysis. Compartmental and noncompartmental parameters were determined from the average concentration of three mice at each time point. The concentration-time profile was modeled through a shape curve, and model parameters were examined by applying linear regression to log concentration (24).

RESULTS

Plasma Irinotecan and SN-38 Pharmacokinetics after i.v. Administration. Irinotecan and SN-38 lactone plasma concentration-time profiles after i.v. administration in non-tumor-bearing and NB1691-bearing mice are presented in Fig. 3. The pharmacokinetic parameters for irinotecan are presented in Table 1. Irinotecan and SN-38 AUC0-∞ values after i.v. administration, estimated from compartmental and noncompartmental methods, were similar (data not shown). Thus, to be consistent with the noncompartmental estimations of AUC0-∞ for irinotecan and SN-38 after oral administration, noncompartmental estimation of irinotecan and SN-38 AUC0-∞ after i.v. administration was used for further analysis. Irinotecan CL was decreased 20, 40, and 33% in mice bearing NBEB, NB1691, and NB1643 human neuroblastoma xenografts compared to non-tumor-bearing mice, respectively. Moreover, irinotecan AUC0-∞ was increased 26, 58, and 42% in mice bearing NBEB, NB1691, and NB1643 human neuroblastoma xenografts compared to non-tumor-bearing mice, respectively (P < 0.05; Table 1).

The pharmacokinetic parameters for SN-38 are presented in Table 1. After i.v. administration, peak SN-38 concentrations occurred within 0.25 h (Fig. 3). SN-38 lactone CL was 44–59% lower in tumor-bearing mice compared to non-tumor-bearing mice. SN-38 lactone AUC0-∞ after i.v. irinotecan administration increased 72, 93, and 49% in mice bearing NBEB, NB1691, and NB1643 human neuroblastoma xenografts compared to non-tumor-bearing mice, respectively (P < 0.05; Table 1). The molar ratio of SN-38 formation was similar between non-tumor-bearing and tumor-bearing mice.
Neuroblastoma Alters Irinotecan and SN-38 Disposition

SN-38 concentrations peaked 1 h after oral administration of irinotecan (Fig. 4). The increase in SN-38 lactone AUC after oral administration of irinotecan was 266, 241, and 209% in mice bearing NBEB, NB1691, and NB1643 tumor xenografts compared to non-tumor-bearing mice, respectively (*P < 0.05; Table 2). The molar ratio of SN-38 formation was similar between non-tumor-bearing mice and mice bearing NBEB, whereas it was 2-fold higher in mice bearing NB1691 and NB1643 xenografts.

Schematic Model of SN-38 Formation after Oral and i.v. Irinotecan Administration in Mice Bearing Human Neuroblastoma Xenografts. A schematic model describing SN-38 formation in mice bearing NBEB, NB1691, and NB1643 human neuroblastoma tumor xenografts after oral and i.v. administration of irinotecan is presented in Fig. 5. Estimates of tumor SN-38 formation after oral and i.v. administration of irinotecan are presented in Table 3. Ratios of SN-38 tumor formation after oral as compared to i.v. irinotecan administration were 1.0, 0.7, and 1.5 in mice bearing NBEB, NB1691, and NB1643 human neuroblastoma tumor xenografts, respectively.

DISCUSSION

Although other investigators have suggested that tumor xenografts may influence irinotecan and SN-38 disposition, this is the first report of altered irinotecan and SN-38 disposition after oral and i.v. administration of irinotecan in mice bearing human neuroblastoma xenografts. The importance of these data is underscored by the use of xenograft models to evaluate potential chemotherapeutic agents, especially when comparing systemic exposures associated with antitumor response in the xenograft models with systemic exposures tolerable in humans (20). The results of this study emphasize the need to perform preclinical xenograft studies in non-tumor-bearing and tumor-bearing mice to evaluate the influence of tumor-related factors on drug disposition.

Several possible explanations can account for the increase in irinotecan systemic exposures and the decrease in CL after i.v. administration in tumor-bearing mice. Several studies have reported altered drug disposition in tumor-bearing mice and rats, secondary to inhibition of hepatic oxidative metabolism (25–27). In one study, surgical removal of the tumor abolished the impairment of drug metabolism (27). Moreover, data suggested that the serum of tumor-bearing animals contained substances that were able to inhibit the activity of microsomal liver oxidative enzymes. Interestingly, hepatic oxidation has been shown to be a pathway of irinotecan metabolism (8). Due to limited sample volume, the oxidative metabolites were not measured in this study (8). Similar molar ratios of SN-38 formation and an increase in irinotecan systemic exposure in tumor-bearing mice compared to non-tumor-bearing mice suggest that inhibition of irinotecan CL is a potential factor responsible for altered irinotecan and SN-38 disposition.

The 2-fold higher SN-38 lactone AUC after i.v. administration of irinotecan in mice bearing neuroblastoma xenografts compared to non-tumor-bearing mice may be explained by increased conversion of irinotecan to SN-38 by neuroblastoma...
Table 1  Irinotecan and SN-38 pharmacokinetic parameters after i.v. administration

Irinotecan and SN-38 pharmacokinetic parameters after irinotecan (10 mg/kg) was administered i.v. in non-tumor-bearing mice and in mice bearing human neuroblastoma (NBEB, NB1691, and NB1643) tumor xenografts.

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Non-tumor-bearing mice</th>
<th>NBEB</th>
<th>NB1691</th>
<th>NB1643</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Irinotecan</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_c$ (liter/m$^2$)</td>
<td>12.8</td>
<td>11.2</td>
<td>5.8</td>
<td>4.6</td>
</tr>
<tr>
<td>$k_{o1}$ (h$^{-1}$)</td>
<td>0.01</td>
<td>0.07</td>
<td>0.51</td>
<td>0.66</td>
</tr>
<tr>
<td>$k_{i1}$ (h$^{-1}$)</td>
<td>0.51</td>
<td>3.9</td>
<td>1.5</td>
<td>5.0</td>
</tr>
<tr>
<td>$k_{i2}$ (h$^{-1}$)</td>
<td>0.85</td>
<td>6.5</td>
<td>1.7</td>
<td>2.7</td>
</tr>
<tr>
<td>$k_{i3}$ (h$^{-1}$)</td>
<td>1.9</td>
<td>1.6</td>
<td>2.0</td>
<td>2.8</td>
</tr>
<tr>
<td>CL (liter/h/m$^2$)</td>
<td>23.9</td>
<td>19.1</td>
<td>14.3</td>
<td>31.4</td>
</tr>
<tr>
<td>$AUC_{irino}$ (ng-h/ml)</td>
<td>1116.9$^{a,b,c}$</td>
<td>1403.0$^c$</td>
<td>1762.0$^d$</td>
<td>1583.1$^e$</td>
</tr>
<tr>
<td>$C_{max}$ (ng/ml)</td>
<td>2079.0</td>
<td>2601.3</td>
<td>3432.0</td>
<td>2500.0</td>
</tr>
</tbody>
</table>

| **SN-38**                 |                        |      |        |        |
| $V_c$ (liter/m$^2$)       | 12.8                   | 11.2 | 5.8    | 4.6    |
| $k_{o2}$ (h$^{-1}$)       | 4.4                    | 2.6  | 3.9    | 6.9    |
| $k_{i3}$ (h$^{-1}$)       | 3.0                    | 1.3  | 5.4    | 8.6    |
| $k_{i2}$ (h$^{-1}$)       | 1.2                    | 1.3  | 1.9    | 1.3    |
| CL (liter/h/m$^2$)        | 55.9                   | 29.1 | 22.4   | 31.4   |
| $AUC_{SN-38}$ (ng-h/ml)   | 532.7$^{a,c,e}$        | 914.0$^d$ | 1029.1$^e$ | 794.2$^e$ |
| $C_{max}$ (ng/ml)         | 443.3                  | 485.3 | 673.3  | 508.5  |
| Molar SN-38 formation     | 0.48                   | 0.65 | 0.59   | 0.50   |

$a,b,c P < 0.05.$
$d,e,f P < 0.05.$

xenograft cells. CE has been shown to be endogenous to a wide range of organ tissues in humans, with high, intermediate, and low activity in liver, colon, and brain tissue, respectively (28, 29). CE expression has shown interindividual variation in human liver microsomes (30). In addition, CE has been isolated from rat serum (5). CE is highly expressed in human neuroblastoma cell lines (15–17), with cytotoxic sensitivity related to intracellular CE activity (18). In addition, intracellular CE activity has been shown to correlate with irinotecan cytotoxicity in a wide range of human tumor cell lines (31–33). Thus, CE expression in neuroblastoma tumor xenografts may be responsible for the increased SN-38 systemic exposure in mice bearing neuroblastoma xenografts. Decreased SN-38 hepatic glucuronidation, and thus an increased nonglucuronidated SN-38, may account for the apparent decrease in SN-38 systemic CL. Due to limited sample volume, SN-38 glucuronide was not measured in this study.

Pharmacokinetic model estimations of CE conversion of irinotecan to SN-38 ($k_{13}$) were similar between non-tumor-bearing and tumor-bearing mice, whereas the SN-38 CL was 44–59% lower in tumor-bearing mice compared to non-tumor-bearing mice. Thus, the model parameter estimations suggest that the increase in SN-38 systemic exposure may be due to a decrease in SN-38 CL rather than an increase in SN-38 formation.

The higher molar ratio of SN-38 formation in non-tumor-bearing mice after oral as compared to i.v. irinotecan administration suggests the presence of intestinal factors that increase irinotecan bioavailability with subsequent systemic conversion to SN-38 and/or increased conversion to SN-38 during absorption. CE has been shown to be highly expressed in the proximal and distal small intestine and colon (28, 29, 34). In addition, CE is expressed in colon adenocarcinoma cell lines (28). Thus, intestinal epithelium CE may be responsible for increased conversion of irinotecan to SN-38 during absorption.

Fig. 4  Upper panel, the irinotecan lactone plasma concentration-time profile in non-tumor-bearing mice (○) and mice bearing NBEB human tumor xenografts (●) after oral administration of irinotecan. Lower panel, the SN-38 lactone plasma concentration-time profile in non-tumor-bearing mice (○) and mice bearing NBEB human tumor xenografts (●) after oral administration of irinotecan. Points and bars represent the mean and SD, respectively, of plasma concentrations from three mice at each time point.
Irinotecan and SN-38 lactone pharmacokinetic parameters after oral irinotecan administration

Irinotecan and SN-38 pharmacokinetic parameters after irinotecan (10 mg/kg) was administered orally in non-tumor-bearing mice and in mice bearing human neuroblastoma (NBEB, NB1691, and NB1643) tumor xenografts.

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Non-tumor-bearing mice</th>
<th>NBEB</th>
<th>NB1691</th>
<th>NB1643</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioavailability (ng/ml)</td>
<td>0.13</td>
<td>0.37</td>
<td>0.13</td>
<td>0.15</td>
</tr>
<tr>
<td>Cmax (ng/ml)</td>
<td>102.3</td>
<td>448.3</td>
<td>97.0</td>
<td>127.5</td>
</tr>
<tr>
<td>AUC0→∞ (ng·h/ml)</td>
<td>139.9b,c</td>
<td>522.2b</td>
<td>233.0b</td>
<td>243.9b</td>
</tr>
<tr>
<td>k1 (h⁻¹)</td>
<td>1.6</td>
<td>3.8</td>
<td>1.6</td>
<td>1.7</td>
</tr>
<tr>
<td>SN-38</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cmax (ng/ml)</td>
<td>129.0</td>
<td>409.0</td>
<td>119.3</td>
<td>332.0</td>
</tr>
<tr>
<td>AUC0→∞ (ng·h/ml)</td>
<td>216.0e, f</td>
<td>789.9d</td>
<td>737.4d</td>
<td>668.1d</td>
</tr>
<tr>
<td>k1 (h⁻¹)</td>
<td>0.9</td>
<td>3.6</td>
<td>3.4</td>
<td>3.0</td>
</tr>
<tr>
<td>Molar SN-38 formation</td>
<td>1.5</td>
<td>1.5</td>
<td>3.2</td>
<td>2.8</td>
</tr>
</tbody>
</table>

Note: b, c, d, e, f, p < 0.05.

gastrointestinal absorption and the subsequent increased SN-38 systemic exposure. Higher SN-38 systemic exposures in neuroblastoma-bearing mice after oral as compared to i.v. irinotecan administration also suggest that the presence ofintestinal factors in addition to tumor-related factors may alter irinotecan and SN-38 disposition.

In our schematic model (see Fig. 5), we report similar estimates of tumor production of SN-38 (AUC) between oral and i.v. administration of irinotecan in the three neuroblastoma xenograft lines (see Table 3). This provides indirect evidence that our schematic model may reflect systemic disposition and tumor formation of SN-38. Future studies in human neuroblastoma xenograft lines are required to evaluate the validity of this model.

At the time of study, the neuroblastoma xenograft tumors weighed 0.8 ± 0.4 g. The xenograft tumors represented 2.6 ± 1.3% of the total body weight of the mice. Depending on the extent of disease, the percentage of total body weight represented by neuroblastoma tumors in children may be equal to or greater than that of the murine xenograft models. Thus, the effect of neuroblastoma tumors in children may be of even greater significance and clinical importance. Moreover, if the extent of neuroblastoma affects irinotecan and SN-38 disposition, the degree of influence may be diminished as the tumor responds to therapy, as compared to initial treatment. This demonstrates the need to evaluate irinotecan and SN-38 disposition at the initiation of treatment (i.e., large tumor burden) and throughout irinotecan therapy to characterize the degree of alteration.

In summary, the presence of NB1691, NB1643, and
REFERENCES


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