Metabolic Fate of Irinotecan in Humans: Correlation of Glucuronidation with Diarrhea

Elora Gupta, Timothy M. Lestingi, Rosemarie Mick, Jacqueline Ramirez, Everett E. Vokes, and Mark J. Ratain

Section of Hematology/Oncology, Department of Medicine [E. G., T. M. L., R. M., J. R., E. E. V., M. J. R.], Committee on Clinical Pharmacology [R. M., M. J. R.], Cancer Research Center [R. M., E. E. V., M. J. R.], and Department of Radiation and Cellular Oncology [E. E. V.], University of Chicago Pritzker School of Medicine, Chicago, Illinois 60637

Abstract

Irinotecan (7-ethyl-10-[[4-(1-piperidino)1-piperidinio]carboxyloxy]camptothecin; CPT-11) is hydrolyzed by the enzyme carboxyl esterase to 7-ethyl-10-hydroxycamptothecin (SN-38), which further undergoes glucuronide formation to form the corresponding SN-38 glucuronide (SN-38G). SN-38 is believed to be the cause of treatment-related diarrhea, a dose-limiting toxicity of CPT-11 observed in phase I clinical trials. This study investigated the effect of glucuronidation on the concentrations of SN-38 following CPT-11 infusion in 21 patients undergoing a phase I trial. To assess the relationship between gastrointestinal toxicity and pharmacokinetics of CPT-11 and its metabolites, we defined a "biliary index" of SN-38 which was the product of the relative area ratio of SN-38 to SN-38G and the total CPT-11 area under the plasma concentration-time curve. Nine patients with grade 3-4 diarrhea had higher biliary indexes than 12 patients with grade 0-2 diarrhea (median 2228 versus 5499, P = 0.0004). The relatively higher index values, suggestive of higher biliary concentrations of SN-38, were possibly due to low glucuronidation rates. Hence, modulation of glucuronidation may be effective in increasing the therapeutic index of CPT-11.

Introduction

CPT-11 is a water-soluble semisynthetic derivative of CPT, a plant alkaloid isolated from Camptotheca acuminata. CPT-11 acts as a prodrug in vivo and is converted to SN-38 by the enzyme carboxyl esterase (1). SN-38 has been shown to undergo glucuronic acid conjugation to form the corresponding glucuronide which is the major elimination pathway of SN-38 (2). SN-38G is reported to be deconjugated by the intestinal microflora to form SN-38 (3). The topoisomerase I inhibition and single strand breaks after treatment with CPT-11 is determined primarily by SN-38 concentration (4). Accumulation of SN-38 in the intestine was shown to be responsible for the diarrhea attributed to CPT-11 administration in nude mice (5). Thus the in vivo activity and toxicity of CPT-11 are dependent on SN-38 concentration, and characterization of the disposition of the metabolite following CPT-11 administration is important for designing optimal dosing schedules. Prior studies have shown inconsistent relationships between the dose or pharmacokinetics of CPT-11 with SN-38 pharmacokinetics and gastrointestinal toxicity (6-9). There have been no reports on the pharmacokinetics of SN-38G in humans. The goals of this study were (a) to characterize the plasma profile of SN-38G following CPT-11 administration and (b) to estimate the relationship of gastrointestinal toxicity and SN-38 glucuronidation. The complete results of the phase I clinical trial will be reported separately.

Materials and Methods

Patient Selection, Treatment Plan, and Toxicity Evaluation. Patients with solid tumors or lymphoma were eligible for treatment if they were refractory to standard treatment or if no effective standard treatment existed. All patients had either measurable or evaluable disease, were at least 18 years of age with Karnofsky’s performance status of at least 70%, and had a life expectancy of at least 3 months. All patients met the standard laboratory criteria including criteria for adequate organ function. Informed, written consent was obtained from all patients prior to their first dose of CPT-11. The drug was given in 500 ml normal saline i.v. infusion over 90 min on a weekly basis for four doses in a 6-week cycle. Weekly dosing was assigned by a standard phase I design using the following dose levels: (a) 100 mg/m2; (b) 120 mg/m2; (c) 145 mg/m2; and (d) 175 mg/m2. Following the first dose, blood and urine sampling was performed for the first 24 h after the infusion for pharmacokinetic evaluations. A second cycle was given with the same dose and schedule used during cycle 1. If dose-limiting toxicity was observed during cycle 1, patients were treated at the previous dose level for all subsequent cycles. Patients who experienced dose-limiting neutropenia were eligible to receive granulocyte-colony-stimulating factor at 5 μg/kg/day according to the criteria defined below.

Toxicity assessment was done according to the Cancer and Leukemia Group B expanded toxicity criteria. Patients who experienced grade 2 diarrhea at any time while on study were given loperamide 4 mg p.o. followed by 2 mg p.o. after every stool up to a total dose of 16 mg/day. If loperamide was unsuccessful in controlling diarrhea, treatment was begun with octreotide acetate, 100-600 μg for 2-3 doses/day. Stool collections were also obtained to test for any coexisting infection. CPT-11 dosages were withheld until diarrhea resolved to ≤ grade 2.

Sample Analysis. To determine drug and metabolite levels, heparinized blood samples obtained on the first cycle of therapy were centrifuged and the plasma was stored at −70°C until analysis. CPT (1 μg/ml, obtained from the National Cancer Institute, Bethesda, MD) was used as an internal standard. One hundred μl of plasma were extracted with 2 ml of methanol and centrifuged at 2500 × g for 10 min, and the supernatant was evaporated to dryness. Reconstitution was done with 200 μl of methanol containing 0.1% 10 HCl (pH ~2.0). For the estimation of SN-38G, plasma samples were extracted as described above. Prior to reconstitution, the samples were incubated with 1000 units of β-glucuronidase (Sigma Chemical Co., St. Louis, MO) for 2 h at 37°C.

The total CPT-11 and SN-38 concentrations in the plasma were estimated by modification of the high performance liquid chromatography method of Bariloro et al. (10). Analysis was done using a C18 column (μBondapak, 10 μm, 3.9 x 300 mm; Waters Associates, Milford, MA) preceded by a C18 Novapak guard column. The mobile phase was a mixture of 35% acetonitrile:65% 0.1% potassium dihydrogen phosphate containing 3 mm sodium heptane sulfonate (pH 4.0). Detection was monitored by a Hitachi F1050 fluorescence detector (Hitachi Instruments, Inc., Naperville, IL) with a λem at 375 nm and λex at 566 nm. Standard curves of CPT-11 (Yakult Honsha Co., Ltd., Tokyo, Japan) and SN-38 (Yakult Honsha Co., Ltd.) were linear within the range of 5.0–2365.3 nm/g (r = 0.99) and 9.8–116.5 nm/g (r = 0.99), respectively.

SN-38G concentrations were determined as the increase in SN-38 concentrations following incubation with β-glucuronidase.

Data Analysis. The plasma concentration-time data of CPT-11, SN-38, and SN-38G were analyzed by noncompartmental analysis using PCNONLIN (SCI, Lexington, KY). The AUC from time zero (predose) to the time of the last
quantifiable concentration (AUC,) was calculated by the trapezoidal rule. The
AUC extrapolated to time infinity (AUC,ₐₙₐₜ) was estimated by dividing the last
quantifiable concentration by the terminal rate constant obtained by the log-linear
regression of the terminal elimination phase. The AUC was the summation of
AUCₐₙₐₜ and AUCₐₙₐₜ. CL was estimated as the ratio of the dose and AUC.

Since CPT-11-induced diarrhea in nude mice was associated with intestinal
accumulation of SN-38 (5), biliary concentrations of the metabolite might be
predictive of gastrointestinal toxicity. The principle of area analysis has been
used for assessing the disposition of biotransformed drugs (11). The present
study used this principle to obtain an estimate of SN-38 excreted in the bile.
Since glucuronidation is the major pathway of elimination of SN-38, the
fraction of SN-38 not conjugated would be primarily excreted in the bile. The
net biliary concentration of SN-38 would then be a resultant of its formation
and elimination. This concentration was expressed as the "biliary ratio" which
was the ratio of AUC of SN-38 to SN-38G. To control for individual variability
in the amount of available drug, the ratio was multiplied by the AUC of
CPT-11 to obtain a "biliary index" of SN-38. This was expressed as

$$\frac{\text{AUC}_{\text{CPT-11}}}{{\text{AUC}}_{\text{SN-38}}} \times \frac{\text{AUC}^2_{\text{SN-38}}}{\text{AUC}^2_{\text{SN-38G}}}$$

A patient with a low rate of glucuronidation would have relatively higher
concentrations of SN-38 in the bile draining into the intestine and would be at
a higher risk of gastrointestinal toxicity. Also, patients receiving high doses of
CPT-11 may have saturation of the glucuronidation pathway, leading to
elevated biliary SN-38 concentrations. Overall, the higher the biliary index of a
patient, the greater would be the risk of diarrhea.

The nonparametric Mann-Whitney test was used to test for differences in
pharmacokinetic outcomes between two patient groups, defined by the worst
severity of diarrhea experienced in the first two cycles of CPT-11 treatment.
Statistical tests were performed in the Number Cruncher Statistical System
(Dr. Jerry Hintz, Kaysville, UT). A two-sided significance level of \( \leq 0.05 \) was
considered statistically significant.

Results and Discussion

Metabolism of CPT-11. Following i.v. infusion of CPT-11, two
metabolites could be detected in the plasma, SN-38 and SN-38G. The
glucuronide was the major metabolite, with peak plasma concentra-
cions occurring 0.5 to 3 h after the SN-38 peak and plasma levels
generally exceeding that of SN-38 (Fig. 1). In addition, a prominent
secondary peak was observed in the SN-38 profile (Fig. 1b). These
observations were in agreement with preclinical studies in rats that
reported that 55, 22, and 9% of the biliary radioactivity excreted over
24 h was unchanged CPT-11, SN-38G, and SN-38 and approximately
18% of the biliary radioactivity was reabsorbed from the intestine (2).
Pharmacokinetic estimations of the drug and metabolites in the four
dose levels are listed in Table 1. There was no effect of pretreatment
with G-CSF on the pharmacokinetics of CPT-11 and its metabolites
(data not shown). A nonlinear 2.6-fold increase of AUC of CPT-11
from the 100-mg/m² to the 175-mg/m² dose level correlated to the
decrease in CL estimates and was in accordance with previous reports
of nonlinear pharmacokinetics of CPT-11 (3, 6, 12). However, there
was also a 3.7- and 2.7-fold increase in the AUC estimations of SN-38
and SN-38G, respectively, over the 1.75-fold dose range. Interest-
ingly, there appeared to be no increase in the SN-38G AUC between
the 145-mg/m² and the 175-mg/m² dose levels. The nonlinear increase
in CPT-11 AUC seen in the present study could be due to progressive
saturation of both the nonmetabolic and metabolic pathways of elim-
nation of CPT-11. The plateau concentrations of SN-38G at the two
highest dose levels indicate saturation of glucuronidation of SN-38
and SN-38G. The increase in the SN-38 AUC irrespective of decreasing
CL of CPT-11 could be due to the capacity limitation of the glucu-
ronidation pathway of SN-38. The second peak in the plasma
profile contributing to about a 12% increase in the AUCₐₙₐₜ is
suggestive of hydrolysis of SN-38G by β-glucuronidase resulting in
enterohepatic circulation of SN-38.

Interpatient Variability in Disposition. Across dose levels there
was a 17–57% variability in the AUCₐₙₐₜ and 2–44% variability in
the AUCₐₙₐₜ estimates as measured by the percentage coefficient of
variation. It has been suggested that variability in CPT-11 disposition
was due to interpatient differences in carboxyl esterase levels (6–9).
However, estimation of carboxyl esterase activity in predose plasma
samples of patients in this study showed poor correlation to dose-
normalized AUC of SN-38 or summation of SN-38 and SN-38G (13).
This indicated that formation from CPT-11 was not the rate-determi-
ning step in the disposition of SN-38. Moreover, on the average 0.25
and 3% of the dose were excreted in the urine as SN-38 and SN-38G,
respectively (data not shown). Hence, renal clearance is a minor route
of elimination with the major fraction of SN-38 undergoing conjuga-
tion and elimination in the bile, an observation consistent with pre-

Table 1  Pharmacokinetic estimates of CPT-11, SN-38, and SN-38G by dose level

<table>
<thead>
<tr>
<th>Dose level</th>
<th>AUCₐₙₐₜ (ng h/ml)</th>
<th>AUCₐₙₐₜ (ng h/ml)</th>
<th>AUCₐₙₐₜ (ng h/ml)</th>
<th>CPT-11-CL (l/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 mg/m²</td>
<td>5,603 ± 967</td>
<td>102.4 ± 28</td>
<td>399.4 ± 344</td>
<td>20.3 ± 4.37</td>
</tr>
<tr>
<td>(n=3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120 mg/m²</td>
<td>5,031 ± 1,111</td>
<td>127.4 ± 45</td>
<td>268.9 ± 233</td>
<td>24.93 ± 5.98</td>
</tr>
<tr>
<td>(n=6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>145 mg/m²</td>
<td>11,972 ± 6,790</td>
<td>271.2 ± 119</td>
<td>1,152 ± 1,199</td>
<td>13.91 ± 5.98</td>
</tr>
<tr>
<td>(n=10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>175 mg/m²</td>
<td>14,543 ± 5,220</td>
<td>376.1 ± 6.29</td>
<td>1,058 ± 622</td>
<td>12.86 ± 4.62</td>
</tr>
<tr>
<td>(n=2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± SD.
variable was the biliary index with median values of 2276 and 4747 which ranged from 0.15 to 2.53. The interpatient variability (coefficient estimates above 4000. In 4 of 5 patients with grade 3–4 diarrhea in the intestine. Since glucuronidation represents the major detoxification pathway of SN-38, patients deficient in this enzyme activity should have a greater susceptibility to diarrhea. Interindividual differences coupled with intraracial differences in glucuronidation have been reported (17). Deficient as well as capacity-limited glucuronosyltransferase activity has been shown to be responsible for the toxicity of drugs such as acetaminophen (18, 19). Therefore, one approach to increasing the therapeutic index of CPT-11 would be to induce glucuronosyltransferase activity.

### References

18. De Moraes, S. M. F., and Wells, P. G. Enhanced acetaminophen toxicity in rats with relatively low glucuronidation rates had progressive accumulation of SN-38 leading to toxicity. The hypothesis was supported by the fact that urinary estimates of the SN-38G were on the average 2.5-fold lower in patients with grade 3–4 diarrhea (data not shown).

Pharmacogenetic variations in drug metabolism have contributed to treatment-related toxicities of several anticancer drugs (14–16). In the case of CPT-11, variability in glucuronidation, which may be genetic, was primarily responsible for differential accumulation of SN-38 in the intestine. Since glucuronidation represents the major detoxification pathway of SN-38, patients deficient in this enzyme activity should have a greater susceptibility to diarrhea. Interindividual differences coupled with intraracial differences in glucuronidation have been reported (17). Deficient as well as capacity-limited glucuronosyltransferase activity has been shown to be responsible for the toxicity of drugs such as acetaminophen (18, 19). Therefore, one approach to increasing the therapeutic index of CPT-11 would be to induce glucuronosyltransferase activity.

### Table 2 Correlation of pharmacokinetic estimates to CPT-11 induced diarrhea

<table>
<thead>
<tr>
<th>Pharmacokinetic estimate</th>
<th>Grade 0–2 (n=5)</th>
<th>Grade 3–4 (n=5)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC-CPT-11 (ng/ml/h)</td>
<td>9.160</td>
<td>14.879</td>
<td>0.75</td>
</tr>
<tr>
<td>AUCSN-38 (ng/ml)</td>
<td>211.5</td>
<td>269.1</td>
<td>0.35</td>
</tr>
<tr>
<td>AUCSN-38G (ng/ml)</td>
<td>(170.0–282.5)</td>
<td>(161.8–544.5)</td>
<td>0.46</td>
</tr>
<tr>
<td>&quot;Biliary ratio&quot;</td>
<td>389.9</td>
<td>762.3</td>
<td>0.27</td>
</tr>
<tr>
<td>&quot;Biliary index&quot;</td>
<td>(413.1–2135)</td>
<td>(242.4–4206)</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>(0.12–0.41)</td>
<td>(0.13–0.87)</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>(1.812–3.812)</td>
<td>(3.028–7.856)</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

Fig. 2. Correlation of pharmacokinetic estimates to CPT-11 induced diarrhea. Patients receiving a dose of 145 mg/m² were classified according to the worst grade of diarrhea in treatment cycle 1 or 2. Values are represented as median values with the range in parentheses.
Metabolic Fate of Irinotecan in Humans: Correlation of Glucuronidation with Diarrhea

Elora Gupta, Timothy M. Lestingi, Rosemarie Mick, et al.


Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/54/14/3723

Sign up to receive free email-alerts related to this article or journal.

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.