Pharmacokinetics of Irinotecan and Its Metabolites SN-38 and APC in Children with Recurrent Solid Tumors after Protracted Low-Dose Irinotecan


ABSTRACT

Irinotecan (IRN), a topoisomerase I interactive agent, has significant antitumor activity in early Phase I studies in children with recurrent solid tumors. However, the disposition of IRN and its metabolites, SN-38 and APC, in children has not been reported. Children with solid tumors refractory to conventional therapy received IRN by a 1-h i.v. infusion at either 20, 24, or 29 mg/m² daily for 5 consecutive days for 2 weeks. Serial blood samples were collected after doses 1 and 10 of the first course. IRN, SN-38, and APC lactone concentrations were determined by an isocratic high-performance liquid chromatography assay. A linear four-compartment model was fit simultaneously to the IRN, SN-38, and APC plasma concentration versus time data. Systemic clearance rate for IRN was 58.7 ± 18.8 liters/h/m² (mean ± SD). The mean ± SD ng/ml/h single-day lactone SN-38 area under the concentration-time curve (AUC₀₋₆) was 90.9 ± 96.4, 103.7 ± 62.4, and 95.3 ± 63.9 at IRN doses of 20, 24, and 29 mg/m², respectively. The relative extent of IRN conversion to SN-38 and metabolism to APC measured after dose 1 were 0.49 ± 0.33 and 0.29 ± 0.17 (mean ± SD). No statistically significant intrapatient difference was noted for SN-38 area under the concentration-time curve. Large interpatient variability in IRN and metabolite disposition was observed. The relative extent of conversion and the SN-38 systemic exposure achieved with this protracted schedule of administration were much greater than reported in adults or children receiving larger intermittent doses.

INTRODUCTION

Irinotecan, a water-soluble camptothecin analogue, has antitumor activity in pediatric xenograft tumors (1–3), and more recently, we have found that it also has significant antitumor activity in children with recurrent solid tumors (4). As depicted in Fig. 1, IRN undergoes deesterification by carboxylesterase to an active metabolite, SN-38 (7-ethyl-10-hydroxycamptothecin). SN-38 subsequently undergoes conjugation to form SN-38G. Recently, two oxidative metabolites, APC (7-ethyl-10-[4-N-(5-aminoantepantoic acid)-1-piperidino]-carbonyloxycamptothecin) and NPC [7-ethyl-10-(4-amino-1-piperidino)-carbonyloxycamptothecin], have been identified in an adult clinical trial (5, 6). The biotransformation of IRN to either APC or NPC was catalyzed mainly by cytochrome P450 3A4 (6, 7).

Results of our preclinical studies have shown that the antitumor activity of IRN is schedule and exposure dependent (1–3). Houghton et al. (8) have reported that IRN given at low doses on a protracted schedule (given for 5 consecutive days, off 2 days, and repeated for 5 consecutive days) every 21 days was associated with an increased antitumor activity over schedules in which the same total dose was given but within a shorter period. Specifically, the SN-38 systemic exposure measured by the AUC is related to antitumor activity (3). This suggests that adjusting the IRN dose to attain a desired SN-38 plasma systemic exposure may enhance antitumor efficacy in children with recurrent solid tumors.

Although much was known regarding the pharmacokinetics and pharmacodynamics of IRN in adults (9–11), the disposition of IRN and metabolites in children has not been reported. Before initiating a pediatric clinical trial in which the IRN dose is adjusted to attain the desired SN-38 plasma systemic exposure, the inter- and intrapatient variability in drug disposition must be defined. Thus, we conducted this study to describe the pharmacokinetics and pharmacodynamics of IRN and its metabolites in children with recurrent solid tumors.

PATIENTS AND METHODS

Patients. Patients younger than 21 years of age with recurrent solid tumors unresponsive to conventional therapy were included in this pharmacokinetic study. Other eligibility
Criteria included an adequate renal function (serum creatinine ≤ 3 × normal for age), adequate hepatic function (bilirubin and alanine aminotransferase ≤ 3 × normal), and normal metabolic parameters (serum electrolytes, blood sugar, calcium, and phosphorous). Before study entry, written informed consent was obtained from patients, parents, or guardians according to institutional guidelines.

**Treatment Protocol.** IRN (Camptosar) was diluted with 50 ml of 5% dextrose injection (D5W), USP, and administered by 1-h i.v. infusion once daily for 5 consecutive days followed by a 2-day rest and then an additional 5 consecutive days of treatment [(d × 5 × 2)]. The dosages studied in this pharmacokinetic study included 20, 24, and 29 mg/m²/day.

**Pharmacokinetic Studies.** Pharmacokinetic studies were performed after doses 1 and 10 of the first course. Blood samples (3 ml) were collected in heparinized tubes immediately before IRN infusion and at 0.25, 0.5, 1, 2, 4, and 6 h after the end of the infusion. All blood samples were obtained from a site contralateral to the infusion site. All blood samples were immediately centrifuged at 10,000 rpm for 2 min on a table-top centrifuge. Plasma was separated, and proteins were precipitated by the addition of 200 ml of plasma to 800 ml of cold methanol (−30°C), followed by vigorous agitation with a vortex mixer and repeat centrifugation at 10,000 rpm for 2 min. The supernatant was decanted and stored at −70°C until analysis (3, 4).

**Quantification of IRN, SN-38, and APC.** IRN, SN-38, and APC lactone plasma concentrations were determined using an isocratic high-performance liquid chromatography assay with fluorescence detection as described previously in detail (12).
parameters for each set of data were initially fit by ML estimation as implemented in ADAPT II, and the mean and SD for each parameter were calculated (13). These mean parameter estimates were then used as the revised initial estimates for another ML estimation. The mean and SD for each parameter were updated until the parameter estimates for all parameters were stable (defined as no net change in the third significant digit). The refined mean and SD from the final ML run were used to construct a diagonal covariance matrix for use in a Bayesian algorithm. All sets of data were modeled using a MAP-Bayesian approach to further refine estimates and update the covariance matrix. This was repeated until stable estimates of the model parameters were obtained. Each observation was assessed for the goodness of fit by an estimate of the variance for the predicted values.

AUCs for IRN, SN-38, and APC from the beginning of the infusion to 6 h (the last sample time point after the end of the infusion) were calculated by integration of the simulated concentration-time data from model estimates.

Although the ratio of metabolite:parent compound is not a direct measure of conversion or metabolism, the ratio is useful to characterize the relative conversion from the parent compound to metabolites among patients (14–16). The calculation of the REC is defined as the ratio of SN-38 AUC:IRN AUC (14). The same principle was applied to the REM, defined as the ratio of APC AUC:IRN AUC.

**SN-38 Protein Binding.** In patients with adequate plasma samples, SN-38 lactone protein binding studies were conducted as described previously (17). Briefly, a plasma sample was obtained from a patient before IRN administration and spiked with SN-38 lactone to attain a final concentration of 1.2

### Table 2 Summary of pharmacokinetic model parameters (n = 43)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_c ) (liters/m²)</td>
<td>36.9</td>
<td>14.2</td>
<td>35.4</td>
<td>15.2–77.0</td>
</tr>
<tr>
<td>( k_{10} ) (h⁻¹)</td>
<td>1.40</td>
<td>0.63</td>
<td>1.28</td>
<td>0.42–3.58</td>
</tr>
<tr>
<td>( k_{12} ) (h⁻¹)</td>
<td>2.62</td>
<td>0.78</td>
<td>2.60</td>
<td>1.33–4.9</td>
</tr>
<tr>
<td>( k_{21} ) (h⁻¹)</td>
<td>0.79</td>
<td>0.19</td>
<td>0.75</td>
<td>0.45–1.41</td>
</tr>
<tr>
<td>( k_{13} ) (h⁻¹)</td>
<td>0.17</td>
<td>0.10</td>
<td>0.16</td>
<td>0.02–0.47</td>
</tr>
<tr>
<td>( k_{34} ) (h⁻¹)</td>
<td>0.17</td>
<td>0.10</td>
<td>0.15</td>
<td>0.03–0.51</td>
</tr>
<tr>
<td>( k_{36} ) (h⁻¹)</td>
<td>0.64</td>
<td>0.42</td>
<td>0.54</td>
<td>0.08–2.41</td>
</tr>
<tr>
<td>( k_{40} ) (h⁻¹)</td>
<td>0.45</td>
<td>0.13</td>
<td>0.46</td>
<td>0.20–0.83</td>
</tr>
<tr>
<td>IRN clearance (liters/h/m²)</td>
<td>58.8</td>
<td>18.8</td>
<td>56.6</td>
<td>26.7–95.2</td>
</tr>
<tr>
<td>IRN ( t_{1/2} ) (h)</td>
<td>2.66</td>
<td>0.57</td>
<td>2.55</td>
<td>1.82–4.47</td>
</tr>
<tr>
<td>SN-38 ( t_{1/2} ) (h)</td>
<td>1.58</td>
<td>1.34</td>
<td>1.29</td>
<td>0.29–8.28</td>
</tr>
<tr>
<td>APC ( t_{1/2} ) (h)</td>
<td>1.68</td>
<td>0.53</td>
<td>1.52</td>
<td>0.84–3.44</td>
</tr>
</tbody>
</table>

- \( V_c \), volume of the central compartment; \( k_{10} \), IRN elimination constant; \( k_{12} \) and \( k_{34} \), IRN intercompartment rate constants; \( k_{13} \), linear conversion of IRN to SN-38; \( k_{40} \), oxidation of IRN to form APC; \( k_{36} \), SN-38 elimination rate constant; \( k_{40} \), APC elimination rate constant.

Excitation and emission wavelengths were 370 and 520 nm. The lower level of quantitation for this assay was 1 ng/ml for CPT-11, SN-38, and APC (2). All calibrators and controls were prepared using single-donor plasma.

**Pharmacokinetic Analysis.** The disposition of IRN and metabolites was evaluated using a four-compartment model with linear distribution and elimination. As depicted in Fig. 2, the four-compartment pharmacokinetic model consisted of an IRN central compartment, an IRN peripheral tissue compartment, and SN-38 and APC plasma compartments. A simplifying assumption was made that the apparent volumes of distribution for IRN, SN-38, and APC were identical. Pharmacokinetic
RESULTS

Patient-specific characteristics are summarized in Table 1. Of the 24 patients evaluated, 19 had pharmacokinetic studies after doses 1 and 10. Four patients had studies completed only after the first dose, and one patient was studied at dose 10 only.

The pharmacokinetic parameters for IRN, SN-38, and APC are summarized in Table 2. IRN, SN-38, and APC lactone plasma concentration-time data from a representative patient given IRN at a dose of 20 mg/m² are depicted in Fig. 3. IRN exhibited biphasic plasma elimination with a rapid decrease after the end of the 60-min infusion. SN-38 plasma concentrations displayed monoeXponential first-order elimination. In two patients, SN-38 plasma concentrations revealed a bimodal distribution, suggesting enterohepatic recycling. APC terminal plasma concentrations displayed first-order decay.

The summary AUC values for IRN, SN-38, and APC are listed in Table 3. The mean ± SD ng/ml h single-day lactone SN-38 AUC₀→∞ was 90.9 ± 96.4, 103.7 ± 62.4, and 95.3 ± 63.9 at IRN doses of 20, 24, and 29 mg/m², respectively. The IRN AUC₀→∞ accounted for greater than 70% of the IRN AUC₀→∞ in all of the 43 pharmacokinetic studies. Likewise, the SN-38 AUC₀→∞ accounted for greater than 70% of the SN-38 AUC₀→∞ in 38 of 43 pharmacokinetic studies. Thus, we chose AUC₀→∞ to report the systemic exposure. As shown in Table 3, large interpatient variability was observed in IRN, SN-38, and APC systemic exposure or AUC₀→∞ after the first IRN dose. A 5-fold variation in IRN systemic exposures among patients was observed during course 1 over three dosage levels, and more than 30- and 10-fold variations in SN-38 and APC systemic exposures were observed, respectively. In the 19 patients who had pharmacokinetic studies after doses 1 and 10, we observed no statistically significant difference in SN-38 AUC (P = 0.10, exact Wilcoxon test).

The relationship between the parent compound and its metabolites can be expressed and evaluated further in terms of ratios. The median REC measured was 0.37 (range, 0.10–1.44), 0.51 (range, 0.07–0.86), and 0.21 (range, 0.08–0.51) at IRN doses of 20, 24, and 29 mg/m², respectively. The median REM was 0.29 (range, 0.10–0.57), 0.33 (range, 0.13–0.76), and 0.25 (range, 0.08–0.52) at IRN doses of 20, 24, and 29 mg/m², respectively.

Adequate sample volume was available to determine the extent of SN-38 protein binding in 13 patients. The serum albumin levels were normal in these patients, ranging from 3.0–4.4 g/dl. Median SN-38 percentage unbound was 3.3 (range, 0.7–6.5) over all dose levels. No relation was noted between serum albumin and SN-38 percentage unbound or BR.

Myelosuppression was minimal and was not dose limiting in any child during the first course of therapy, except for one patient that developed a grade 3 neutropenia lasting for 11 days after starting an IRN dose of 24 mg/m². We were unable to identify any pharmacodynamic relationship between SN-38 systemic exposure and myelosuppression.

Diarrhea was seen at all three dose levels. Among six patients who developed grade 3 diarrhea, two patients had positive cultures for Clostridium difficile. Acute onset of diarrhea within 8 h of the initial IRN dose occurred in two patients. The median onset of diarrhea occurred on day 9 (range, 1–16

\[
\text{% Change in ANC} = \left(\frac{\text{pre-nadir}}{\text{pre}}\right) \times 100
\]
days) after the first dose of the first course of IRN. All patients took loperamide at the first onset of loose stools. Diarrhea was resolved after a median of 4 days (range, 0–16 days) after the last dose of IRN. We observed no significant correlation between the duration or severity of diarrhea and pharmacokinetic measures of drug exposure, including IRN, SN-38, and APC AUC0–36.

DISCUSSION

Although the disposition of IRN and metabolites has been widely reported in adults (9, 10, 18–21), this is the first report of the disposition of IRN and its metabolites in pediatric cancer patients. A four-compartment pharmacokinetic model adequately fit the IRN, SN-38, and APC plasma lactone concentration-time data simultaneously after a single i.v. IRN dose in children with recurrent solid tumors.

IRN displayed a biexponential elimination, whereas SN-38 exhibited a monophasic decline, perhaps because of the low measured SN-38 plasma concentrations. In addition, we measured APC plasma concentrations as an indicator of the extent of oxidative metabolism of IRN. We incorporated APC plasma concentration data into the model and reported the first APC pharmacokinetic parameters in children. The mean terminal half-lives of IRN and SN-38 were shorter than those reported in adults (22). The mean IRN systemic clearance in our population was comparable to that observed in adults; however, a greater interpatient variability was observed in this pediatric population than in adults (22). IRN systemic clearance appeared to be dose independent, although the dosage range evaluated was narrow (20–29 mg/m²), and the sample size, especially at the 29 mg/m² dosage level, was small.

Table 3  Summary of IRN, SN-38, and APC AUC0–36 at each dosage level

<table>
<thead>
<tr>
<th>IRN dosage</th>
<th>20 mg/m²/day (n = 17)</th>
<th>24 mg/m²/day (n = 22)</th>
<th>29 mg/m²/day (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRN (ng/mlh)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>326.8 (109.9)</td>
<td>368.6 (91.9)</td>
<td>645.0 (273.9)</td>
</tr>
<tr>
<td>Median</td>
<td>309.9</td>
<td>358.1</td>
<td>613.7</td>
</tr>
<tr>
<td>Range</td>
<td>199.7–514.1</td>
<td>218.8–521.2</td>
<td>355.1–998.3</td>
</tr>
<tr>
<td>SN-38 (ng/mlh)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>90.9 (96.4)</td>
<td>103.7 (62.4)</td>
<td>95.3 (63.9)</td>
</tr>
<tr>
<td>Median</td>
<td>71.1</td>
<td>90.4</td>
<td>103.8</td>
</tr>
<tr>
<td>Range</td>
<td>14.0–427.9</td>
<td>19.4–242.3</td>
<td>16.5–156.9</td>
</tr>
<tr>
<td>APC (ng/mlh)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>83.7 (43.9)</td>
<td>119.6 (61.9)</td>
<td>128.5 (45.9)</td>
</tr>
<tr>
<td>Median</td>
<td>80.1</td>
<td>113.6</td>
<td>133.4</td>
</tr>
<tr>
<td>Range</td>
<td>24.5–194.3</td>
<td>45.2–309.0</td>
<td>76.9–170.3</td>
</tr>
</tbody>
</table>

Table 4  Potential interaction of dexamethasone and anticonvulsant agents with IRN

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Median</th>
<th>Patient 1</th>
<th>Patient 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>REC</td>
<td>0.42</td>
<td>0.16</td>
<td>0.37</td>
</tr>
<tr>
<td>REM</td>
<td>0.28</td>
<td>0.58</td>
<td>0.76</td>
</tr>
<tr>
<td>IRN AUC0–36 (ng/mlh)</td>
<td>389.5</td>
<td>317.0</td>
<td>273.6</td>
</tr>
<tr>
<td>SN-38 AUC0–36 (ng/mlh)</td>
<td>82.3</td>
<td>29.5</td>
<td>59.4</td>
</tr>
<tr>
<td>APC AUC0–36 (ng/mlh)</td>
<td>83.5</td>
<td>269.3</td>
<td>307.6</td>
</tr>
<tr>
<td>Concomitant medications</td>
<td>Valproic acid, gabapentin, dexamethasone, omeprazole, oxycodone, and acetaminophen</td>
<td>Carbamazepine, phenobarbital, dexamethasone, and morphine</td>
<td></td>
</tr>
</tbody>
</table>
pediatric study in which a 10-fold higher IRN dose of 200–420 mg/m² every 3 weeks was administered (15). Vassal et al. (15) reported a median REM of 0.12 (range, 0.04–0.25) in a group of children whose age ranged from 10 months to 17.5 years. Estimated REM measurements in both groups of children were much lower than that reported previously in adults (median, 2.2; range, 0.65–3.9) receiving IRN at 115 mg/m² over a 90-min period every 3 weeks (14). This illustrates the differences in metabolite formation between children and adults. The exact mechanisms by which APC metabolism is mediated in children and adults are still unknown.

In a recent study of adult glioma patients, Friedman and colleagues (26, 27) suggested that the concurrent administration of anticonvulsants and corticosteroids with IRN altered the disposition of IRN and metabolites. Their results showed that the mean IRN and SN-38 AUC was approximately 39% and 22%, respectively, of that measured in a group of gastrointestinal cancer patients treated with IRN on a similar schedule and dose, but without concurrent anticonvulsants and corticosteroids. They did not report the APC data from their patients. Although the present study was not designed to evaluate the interaction of corticosteroids and anticonvulsants with IRN, two patients were treated at 24 mg/m² and received concomitant dexamethasone and anticonvulsants (e.g., valproic acid, phenobarbital, and carbamazepine). The REC value for each patient was below the median determined for all patients after dose 1 (see Table 4). Similarly, these two patients receiving dexamethasone and anticonvulsants had REM values 2-fold that of the median value determined for all patients after dose 1 (see Table 4). These preliminary observations warrant further investigation into the mechanisms for possible drug interactions.

The primary toxicity in this study was diarrhea, consistent with that observed in adults treated with conventional dosages (19, 20, 22). IRN-induced diarrhea occurred at all dosage levels in children with recurrent solid tumors. The systemic exposures of IRN and SN-38 in children who developed grade 3 or 4 diarrhea overlapped with those of patients who did not report diarrhea. In contrast to our finding, Sasaki et al. (28) reported that there was a strong correlation between total SN-38 AUC and diarrhea after administering IRN at 100 mg/m² weekly in adults.

We were unable to identify a pharmacodynamic relationship between SN-38 or IRN systemic exposure and gastrointestinal toxicity. Although we used the NCI Common Toxicity Criteria for classifying the severity of diarrhea, these results remain subjective. Most end point measurements of diarrhea are based largely on patient or family self-reports. We conclude that a more systematic quantification of diarrhea in this patient population will be essential to additional studies of the mechanism of this toxicity. In single-dose studies of IRN in adults, delayed onset of diarrhea occurred at least 2 days after dosing (29). However, in our pediatric patients who received this protracted schedule, it was difficult to distinguish whether diarrhea occurring late in the schedule was attributed to cumulative systemic exposure or to a delayed onset.

The major elimination pathway of SN-38 is conjugation to glucuronic acid. SN-38G has been shown to be secreted in bile and deconjugated by intestinal flora to form SN-38 (30). This local accumulation of SN-38 is believed to contribute to the delayed gastrointestinal toxicity observed in patients receiving IRN. Gupta et al. (31) described a linear relationship between biliary index and grade of gastrointestinal toxicity in adults. Our plasma sampling schedule was not designed to identify enterohepatic recycling in these pediatric patients; however, the SN-38 plasma profiles displayed a secondary peak suggestive of enterohepatic recycling in two patients. We were unable to measure plasma SN-38G concentrations in the current study because of a limited sample volume. A follow-up study in children that will include measurements of SN-38G is ongoing.

In summary, the description of the disposition of IRN, SN-38, and APC with one pharmacokinetic model may have important clinical implications. Specifically, this model could be used to prospectively adjust the IRN dose in clinical trials to attain putative cytotoxic systemic exposures as defined in the xenograft model. However, because of the wide interpatient variability noted in IRN and metabolite disposition, additional pharmacokinetic studies are needed to refine population priors for use in a Bayesian approach to target cytotoxic systemic exposures. Moreover, additional studies of the genetic polymorphisms in IRN-metabolizing enzymes (e.g., UGT1A1, CYP3A4) or plasma protein binding may explain some of the interpatient variability of IRN and metabolite disposition in children.

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