Plasma Pharmacokinetics of 7-Ethyl-10-hydroxycamptothecin (SN-38) after Intravenous Administration of SN-38 and Irinotecan (CPT-11) to Rats

Norimasa Kaneda,*,† Yukiko Hosokawa,* Teruo Yokokura,* and Shoji Awazu

Yakult Central Institute for Microbiological Research,* 1796 Yabo, Kunitachi, Tokyo 186, Japan and School of Pharmacy, Tokyo University of Pharmacy and Life Science, b 1432-1 Horinouchi, Hachioji, Tokyo 192-03, Japan. Received March 10, 1997; accepted May 29, 1997

We studied the plasma pharmacokinetics of the lactone form and the carboxylate form of 7-ethyl-10-hydroxycamptothecin (SN-38), the active metabolite of irinotecan (CPT-11), after intravenous bolus administrations of each form of SN-38 and of CPT-11 to rats. After the SN-38 lactone injection, SN-38 lactone was predominant at first, and then the lactone to the carboxylate concentration ratio (LC ratio) was maintained from 30 to 90 min after the injection. The carboxylate was predominant throughout the period after the carboxylate dosing. Model-dependent analyses showed that the SN-38 lactone had greater plasma clearance and a greater distribution volume than the carboxylate. The CPT-11 administration resulted in a predominant plasma SN-38 lactone concentration. The contribution of the SN-38 lactone AUC to the total SN-38 AUC (57%) was independent of the dose of CPT-11. These results suggest that it is possible to estimate the SN-38 lactone concentration and AUC from the total SN-38 concentration without separate determination of the lactone and carboxylate. Our results showed that both SN-38 lactone and CPT-11 administration gave the predominant SN-38 lactone in plasma; however, only CPT-11 could sustain the lactone concentration at a high level, which is necessary for antitumor activity.

Key words 7-ethyl-10-hydroxycamptothecin (SN-38); irinotecan (CPT-11); plasma pharmacokinetics; SN-38 lactone; SN-38 carboxylate; reversible interconversion

Camptothecin (CPT) has a lactone ring in its structure which closes and opens reversibly depending on the pH value: CPT has a lactone form in acidic pH and a carboxylate form in neutral and basic pH (Fig. 1). The kinetics and the mechanism of this ring opening and closure were identified by Fassberg and Stella. 1 The lactone form of CPT is only slightly water-soluble; thus, clinical studies of CPT have been carried out using its water soluble carboxylate form (as a sodium salt). Since these studies showed little therapeutic efficacy and severe side effects, 2,3 the clinical trials of CPT were stopped in the early 1970s. Later studies on the mechanism of the action of CPT revealed a novel mechanism by inhibition of DNA topoisomerase I. 4,5 In addition, the carboxylate form was found to be less potent in the inhibition of the enzyme. 6,7 The antitumor activities of the carboxylate of CPT and its derivatives were weaker than those of the lactone against various murine tumors in vivo. 8 Several investigators, therefore, attempted to develop new water-soluble CPT derivatives with a closed lactone ring. Sawada et al. synthesized irinotecan (CPT-11, Fig. 1), which met these requirements. 9 CPT-11 showed stronger activity than those of CPT and its derivatives against various murine tumors. 8,10 CPT-11 was later found to be a prodrug which gives an active metabolite, 7-ethyl-10-hydroxycamptothecin (SN-38, Fig. 1). 11 CPT-11 has been approved as a new drug for the treatment of lung, cervical, ovarian, gastric, colorectal, breast, skin cancer and malignant lymphoma in Japan and of colorectal cancer in France and the U.S.A. 12,13

The early pharmacokinetic studies of CPT and its derivatives, including CPT-11, were based on its total concentration, that is, the sum of the lactone and carboxylate concentrations, because (1) the importance of the lactone was not recognized, and (2) no method had been established for separately determining the lactone and the carboxylate. High performance liquid chromatography (HPLC) methods have recently been developed for the quantification of lactone- and carboxylate-forms of CPT and its derivatives in biological samples. 14-16 The clinical plasma CPT-11 and SN-38 concentrations of both forms were also reported by various investigators. 17-20 Although the plasma pharmacokinetics and excretion after CPT-11 and carboxylate administration have been studied, 21,22 those after SN-38 lactone and carboxylate administration have not. The objectives of this study were first to clarify the plasma pharmacokinetics of SN-38 lactone and carboxylate after an intravenous (i.v.) bolus administration, and second, to determine the plasma profiles of SN-38 lactone and carboxylate after an i.v. bolus administration of CPT-11 in rats.

MATERIALS AND METHODS

Drugs and Reagents SN-38 and CPT-11 were provided by Yakult Honsha Co. (Tokyo, Japan). Other reagents

---

* To whom correspondence should be addressed.
were obtained from commercial sources and were of analytical or HPLC grade. They were used without further purification.

**Partition Coefficient** The apparent partition coefficients ($P_{app}$) of the lactone and carbamate forms of SN-38 were obtained as follows. SN-38 dimethylsulfoxide (DMSO) solution was added to the aqueous phase to give a final concentration of 1 μg/ml (0.1 M phosphate buffer, pH 3.0 and pH 10.0 for the lactone and the carbamate, respectively). This aqueous phase was shaken overnight at 37°C to keep all of the lactone rings closed or opened. Two ml of water-saturated n-octanol was added to the respective aqueous-phases (2 ml) in capped tubes, followed by shaking for 24h at 37°C. The tubes were then centrifuged, and the drug concentrations of the aqueous phase were determined by the HPLC method described below. $P_{app}$ was calculated by the following equation.

$$P_{app}=\frac{(C_0-C_{aq})}{C_{aq}}$$

where $C_0$ and $C_{aq}$ are the concentrations in the aqueous phase at the start of and after 24h of shaking, respectively.

**Animals and Operation** Male Wistar rats were purchased from Charles River Japan, Inc. (Atsugi, Japan). Animals were used after a 1-week acclimatization period at 25±2°C and 55±5% relative humidity. During the acclimatization, they were allowed free access to a standard diet (MF; Oriental Yeast, Tokyo, Japan) and tap water. Their body weight was 300±23 g (mean±S.D.) when used for the experiments.

Under light ether anesthesia, cannulas (Intramedic PE-50; Clay Adams, Parsippany, NJ) were implanted into the right femoral vein and the left artery for drug administration and blood sampling, respectively. The animal was kept in a restraining cage after this operation. Drugs were administered after the animal recovered from anesthesia. Only water was given during the experiments.

**Injection Sample Preparation** The SN-38 lactone injection solution was prepared by dissolving SN-38 in 10 volumes of DMSO at the concentration of 4.4 mg/ml followed by the addition of 1 volume of 10 mM phosphoric acid to give a final concentration of 4 mg/ml. The SN-38 carbamate injection solution was prepared by dissolving SN-38 in 0.1 N NaOH to give a final concentration of 4 mg/ml (lactone equivalent). CPT-11 was dissolved in saline to a concentration of 2 or 20 mg/ml by sonication and heating in boiling water. Injection samples were prepared just before administration.

**Drug Administration** SN-38 lactone and carbamate were administered at doses of 4 mg/kg and CPT-11 was given at 2 or 20 mg/kg. Drugs were administered by bolus i.v. injection over a period of about 10s. The cannula was flushed with 0.2 ml of 10 mM phosphoric acid after SN-38 lactone injection, and with 0.2 ml saline after SN-38 carbamate or CPT-11 administration.

**Sampling** Blood was sampled at 2, 5, 10, 20, 30, 45 min, 1, 1.5, 2, and 3 h after SN-38 administration, and at 5, 15, 30, 45 min, 1, 1.5, 2, and 3 h after CPT-11 injection. About 0.5 ml of blood was collected into Eppendorf centrifuge tubes containing 20 μl of heparin. The cannula was flushed with about 0.2 ml of saline containing heparin after sampling.

**Determination of SN-38 Lactone and Carbamate in Plasma** Plasma was separated immediately after blood sampling by centrifugation for 30 s (15000 rpm, -10°C) (MRX-150, Tomy Seikou, Tokyo) to prevent the lactone ring from opening or closing and to prevent the further metabolism of CPT-11 to SN-38. Separated plasma samples were immediately prepared for HPLC analysis in the following manner and injected into the HPLC system. Aliquots (100 μl) of plasma were placed in new Eppendorf centrifuge tubes followed by the addition of 100 μl of MeOH chilled in a dry ice-isopropanol bath and 4 μl of 10% zinc sulfate. The tubes were then vortexed for a few seconds and centrifuged for 1 min. The supernatant (100 μl) was directly injected into the HPLC system. The HPLC was performed with an IS-21P (Kurashiki Boseki, Osaka, Japan) using a Waters Puresil C18 5 μm column (150×4.6 mm i.d., Waters Associates, Milford, MA) and Waters Puresil C18 guard cartridge. The mobile phase consisted of 0.1 M ammonium acetate buffer (pH 5.5)/acetonitrile (70/30, v/v) containing 20 mM tetra-n-pentylammonium bromide. The flow rate was 1.0 ml/min at ambient temperature. SN-38 lactone and carbamate were detected with a Shimadzu RF-355 fluorescence spectrometer (Shimadzu Seisakusho, Kyoto, Japan) at EX 380 nm and EM 540 nm. Chromatograms were analyzed with a Maxima 820 Chromatography Workstation (Waters). The quantification limits of SN-38 lactone and carbamate were both 5 ng/ml plasma.

**Pharmacokinetic Parameters** Plasma concentration profiles of the lactone and the carbamate after SN-38 lactone and carbamate injection were also analyzed by the method of Cheng and Jusko based on the model by Scott et al. shown in Fig. 2. The plasma lactone and carbamate concentrations were simulated by the

![Fig. 2. The Pharmacokinetic Model Used for the SN-38 Lactone and Carbamate in Vivo Interconversion](image-url)
following differential equations for the model in Fig. 2 with the parameters obtained.

\[
\begin{align*}
\frac{dC(1)}{dt} &= \left( (\text{CL}_{10} - \text{CL}_{12} - \text{CL}_{42}) \times C(1) + \text{CL}_{41} \times C(2) \\
+ \text{CL}_{31} \times C(3) \right) / V_{sl} \\
\frac{dC(2)}{dt} &= \left( \text{CL}_{41} \times C(1) - \text{CL}_{41} \times C(2) \right) / V_{sl} \\
\frac{dC(3)}{dt} &= \left( (\text{CL}_{20} - \text{CL}_{21} - \text{CL}_{42}) \times C(3) + \text{CL}_{41} \times C(4) \\
+ \text{CL}_{12} \times C(1) \right) / V_{sc} \\
\frac{dC(4)}{dt} &= \left( \text{CL}_{42} \times C(3) - \text{CL}_{42} \times C(4) \right) / V_{sc}
\end{align*}
\]

Eq. 1

where \( C(1) \) and \( C(2) \) are lactone concentrations in central and peripheral compartments, and \( C(3) \) and \( C(4) \) are carboxylate concentrations in central and peripheral compartments, respectively. \( V_{sl} \) and \( V_{sl} \) are the distribution volumes of the lactone form in the central and peripheral compartments, respectively. \( V_{sc} \) and \( V_{sc} \) are those of the carboxylate form in the central and the peripheral compartments, respectively.

The total SN-38 concentration after CPT-11 injection was calculated as the sum of the lactone and carboxylate concentration. The pharmacokinetic parameters of plasma SN-38 (lactone, carboxylate and total) after CPT-11 administration were obtained by moment analysis.\(^{23}\)

The slope of the terminal linear phase of the plasma concentration (logarithm–time curve, \( \lambda_2 \)), was estimated by fitting the terminal linear phase of the curve to the monoequation by the nonlinear least squares method. The area under the plasma concentration–time curve (AUC) from 0 to infinity was calculated by the trapezoidal method with the estimation that the AUC from the last point to infinity is given as \( C_{last} / \lambda_2 \), where \( C_{last} \) is the concentration of the last sampling point within the determinable range.

Statistics Statistical significance was estimated at the level of \( p < 0.05 \) by Student’s \( t \)-test.

RESULTS AND DISCUSSION

Partition Coefficients between \( n \)-Octanol and Buffer The \( P_{app} \) of SN-38 lactone and carboxylate were 29.60 and 0.06 (means of 6 experiments), respectively.

Plasma Concentrations of SN-38 Lactone and Carboxylate after i.v. Administration to Rats Figure 3 shows the concentration time courses after the i.v. administrations of SN-38 lactone and carboxylate. The doses of both compounds were 4 mg/kg, equivalent to that of SN-38 lactone. SN-38 lactone was hydrolyzed very rapidly, and the maximum concentration of its carboxylate form was observed even at 2 min after the administration of the lactone form, then both compounds were eliminated with similar time courses. In contrast, SN-38 carboxylate barely formed a lactone ring.

The plasma concentration data were analyzed by the method of Cheng and Jusko based on the model in Fig. 2, and the resultant pharmacokinetic parameters are listed in Table 1. The simulated lines derived from the parameters are shown in Fig. 3. Taking into account that the parameters were not obtained by direct curve fitting, the simulated values are considered to coincide well with the observed ones.

The ratios of the interconversion to the elimination of...
SN-38 lactone and carboxylate, $CL_{12}/CL_{10}$ and $CL_{21}/CL_{20}$ were calculated as 0.186 and 0.039 using the values in Table 1. This means that the carboxylate form of SN-38 scarcely closed into the lactone form. The lactone has larger distribution volumes and higher lipophilicity than the carboxylate. These results suggest that the lactone is more likely to be distributed to peripheral compartments, including tumor cells. This is probably one of the reasons why the lactone exhibits stronger antitumor activity than the carboxylate in vivo.5)

**Plasma Concentrations of SN-38 Lactone and Carboxylate after CPT-11 Administration**

The SN-38 lactone and carboxylate concentration profiles in plasma after CPT-11 (2 and 20 mg/kg) i.v. bolus administration are shown in Fig. 4. CPT-11 was rapidly hydrolyzed, and the maximum concentration of SN-38 appeared both in the lactone and carboxylate form at 5 min after CPT-11 administration. The $AUC$ values in Table 2 show that CPT-11 was hydrolyzed to give SN-38 non-linearly. This result coincides with the report that CPT-11 is hydrolyzed enzymatically in rat plasma, liver and intestine.26,27)

The Plasma SN-38 Lactone and Carboxylate Concentration Ratio after SN-38 and CPT-11 i.v. Administration

According to a linear pharmacokinetic theory, the concentration ratio between any two compartments is expressed by the same equation as in a Laplace transformation, independent of the input rate function, which is not necessarily linear. The concentration ratios of the lactone/carboxylate (LC ratios) reached an asymptote at about 30 min after the i.v. administration of SN-38 lactone (Fig. 5). Similar results were also observed after CPT-11 administration, irrespective of doses (Fig. 5). The simulated LC ratio derived from Eq. 1 is also shown in Fig. 5. The observed ratios are not significantly different from the simulation curve. These findings suggest that the pharmacokinetic behavior of SN-38 is linear in rat, although the behavior of CPT-11 (a prodrug of SN-38) was nonlinear, as shown in Table 2. The constant LC ratio permits us to calculate the SN-38 lactone concentration which has antitumor activity by the following equation, based on the value of the SN-38 total concentration, which

---

**Table 2. $AUC$ of SN-38 Lactone and Carboxylate after CPT-11 i.v. Bolus Administration to Rats**

<table>
<thead>
<tr>
<th>$AUC$ ($\mu g$ min/ml)</th>
<th>CPT-11 dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 mg/kg</td>
</tr>
<tr>
<td>Lactone</td>
<td>3.86 ± 0.84</td>
</tr>
<tr>
<td>Carboxylate</td>
<td>3.03 ± 1.29</td>
</tr>
<tr>
<td>Total(^b)</td>
<td>6.86 ± 0.66</td>
</tr>
<tr>
<td>Lactone/carboxylate</td>
<td>1.64 ± 1.89</td>
</tr>
<tr>
<td>Lactone/total</td>
<td>0.567 ± 0.132</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) CPT-11 was administered i.v. as a single bolus at doses of 2 and 20 mg/kg. Values are expressed as mean ± S.D. of 5 animals. \(^b\) $AUC$ is from time 0 to infinity. \(^c\) Significantly different from the low-dose group (Student's t-test, p < 0.05). \(^d\) Total SN-38 concentration was calculated as the sum of the lactone and carboxylate concentration. \(^e\) Mean ± S.D. of both dose groups combined.
Fig. 6. Plasma SN-38 Lactone Concentration after i.v. Administration of 20 mg/kg of CPT-11

Closed symbols and bars represent the means and 95% confidence intervals of the values observed. Open symbols are the estimated values from the total SN-38 plasma concentration in a separate experiment. Estimated SN-38 lactone concentration = 0.57 \times \text{total SN-38 concentration}

is easier to determine, after CPT-11 administration.

Since the asymptotic value of the LC ratio estimated by Eq. 1 is 1.32, the conversion coefficient to calculate the SN-38 lactone concentration from the total SN-38 concentration is calculated as 0.57 (\approx 1.32/2.32).

SN-38 lactone concentration = 0.57 \times \text{total SN-38 concentration}

Figure 6 shows the plasma SN-38 lactone concentration profile obtained in this study and the profile estimated from the separate experimental data of a CPT-11 (20 mg/kg) administration. Except for at 30 min, all calculated values were within 95% confidence intervals, and the value of 0.57 is fairly coincident with the value listed in Table 2.

After the CPT-11 injection, more than half of the SN-38 existed as a lactone, and it maintained its concentration longer than after the SN-38 lactone administration (Figs. 3 and 4, Table 2). Therefore, CPT-11 administration is superior to that of SN-38 lactone, because the antitumor activity depends on the exposure time to SN-38 rather than the concentration.\(^{24}\)

In conclusion, the pharmacokinetics of SN-38 were well explained by the linear model shown in Fig. 2, with a reversible conversion between the lactone and carboxylate form, and a simple equation was proposed to calculate the SN-38 lactone concentration after the administration of CPT-11, which is an aqueous soluble prodrug of SN-38.

REFERENCES