Metabolism and Pharmacokinetics of the Camptothecin Analogue CPT-11 in the Mouse

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ABSTRACT

A new water-soluble derivative of camptothecin, 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carboxyloxygenycamptothecin (CPT-11), did not exhibit potent antitumor activity in vitro against experimental tumor cells. The 50% effective doses of CPT-11 against KB and L1210 cells were 1100 and 5500 ng/ml, respectively. These values were markedly higher than those of camptothecin (CPT, 0.98 and 3.7 ng/ml) or 7-ethyl-10-hydroxycamptothecin (SN-38, 0.37 and 3.6 ng/ml).

CPT-11 was found to be converted into SN-38 in mouse serum. In vitro incubation of CPT-11 in mouse serum or tissue homogenate enhanced the growth-inhibitory activity much more than that expected from the concentration of CPT-11. This enhancement of the activity coincided with that expected from the SN-38 concentration in incubated serum or homogenate, though the contribution of CPT-11 could not be refuted. SN-38 is considered to play a major role in the antitumor activity when CPT-11 is incubated in serum or homogenate.

The plasma CPT-11 concentration decreased biexponentially after i.v. administration of CPT-11 into mice with a biological half-life of 0.8 to 1.1 h. The area under the plasma CPT-11 concentration-time curve showed dose dependency. The SN-38 concentration decreased for the first 30 min after administration and was then maintained for a few hours at about 0.1 μg/ml after i.v. administration of 20 and 40 mg/kg of CPT-11 followed by the log-linear terminal phase with a half-life of about 2 h which was independent of the dose. It is suggested that the maintenance of plasma SN-38 concentration might be necessary for it to exhibit antitumor activity in vivo.

INTRODUCTION

CPT2 (Fig. 1) is an alkaloid extracted from Camptotheca acuminata (1). Although CPT shows strong antitumor activity in vitro (2, 3) and in experimental animal tumor systems (1, 4), it has not demonstrated encouraging clinical results because of the therapeutic responses and severe toxicity (5–9). Currently, CPT is not used clinically.

The other important reason that makes the clinical use of CPT impractical is that it is only slightly soluble in water. For clinical use, CPT is dissolved in NaOH and administered as the sodium salt which has an open lactone ring. CPT-11 has been reported to have higher activity and less toxicity than CPT, ECPT, and 7-ethyl-10-hydroxycamptothecin (SN-38; Fig. 1) against murine tumors (16, 17) and higher activity against pleiotropic drug-resistant tumors in vitro and in vivo (18). While studying the conditions for determining CPT-11, we found that CPT-11 is metabolized to SN-38 in mouse serum. In this paper we present the in vitro activation of CPT-11 in mouse serum and tissue homogenates, its plasma pharmacokinetics in mice, and a discussion of the antitumor activity in vitro and in vivo.

MATERIALS AND METHODS

Chemicals. All the chemicals used were of analytical grade. CPT-11 (hydrochloride trihydrate, M, 677), SN-38 (monohydrate, M, 410), and CPT (M, 348) were semisynthesized at our institute. CPT-11 was dissolved in saline solution by sonication and warming. SN-38 and CPT were used as sodium salts for animal administration.

Preparation of Tissue Homogenate. Mice were killed by exsanguination after overnight fasting. The liver was perfused with ice-cold 0.15 m KCl, and the small intestinal mucosa was scraped with a glass slide to collect epithelium. Tissue (liver or scraped epithelium) was homogenized with 4 volumes of ice-cold 0.15 m KCl with a Teflon homogenizer. The supernatant, after centrifugation at 9000 x g for 20 min at 2°C, was used as tissue homogenate.

Incubation of CPT-11 in Serum and Tissue Homogenate. CPT-11 was added to mouse serum or tissue homogenate at 100 μg/ml, and it was incubated for various periods at 37°C. Then the concentrations of CPT-11 and SN-38 were determined by HPLC as described later. For bioassay, the incubated samples were fixed with methanol hydrochloride (100/1, v/v) and then centrifuged. The supernatant was dried in air, resuspended, and diluted with culture medium to the appropriate concentrations.

Bioassay System. KB and L1210 cells were cultured in Eagle's minimal essential medium and RPMI 1640 medium, respectively, containing 10% fetal bovine serum under a humidified atmosphere containing 5% CO2 at 37°C. About 1.5 x 10⁴ cells per ml were exposed to medium containing test materials at various concentrations for 3 days. The treated cells were counted with a Coulter Counter. The growth inhibition rate and Ed50 were determined as described previously (11). Under these conditions extracts of mouse serum and tissue homogenates without drug were not toxic to cell growth.

Antitumor Activity in Vivo. CD2F mice were inoculated i.p. with L1210 cells (1.0 x 10⁶ cells/animal) on Day 0 and were given SN-38 or CPT i.p. on Days 1, 5, and 9 to give a total dose of 1.5 to 400 mg/kg. The relative survival rate for the animals was calculated as follows.

\[ T/C (%) = \frac{\text{median no. of survival days of drug-treated mice}}{\text{median no. of survival days of untreated mice}} \times 100. \]

Identification of SN-38. CPT-11 was incubated with serum from CD2F mice at the initial concentration of about 10 μg/ml at 37°C overnight. The fluorescence emission spectrum at the excitation wavelength of 300 nm in the samples at the beginning of and after overnight incubation in mouse serum and SN-38 in mouse serum was monitored with a Model RF-510 spectrofluorophotometer (Shimadzu Seisakusho, Kyoto, Japan).

Mouse serum was incubated with CPT-11 at the concentration of 1
A and B are intercepts, and α and β are the rate constants of drug distribution and elimination phases, respectively. The inverse square of the observed plasma concentration served as a weighing factor.

\[ t_{\text{lag}} = \frac{0.693}{\beta} \quad (B) \]

AUC was calculated by the trapezoidal rule with estimation of AUC from the last sampling time to infinity by Equation C.

\[ \int_{t_{\text{last}}}^{\infty} C dt = C_{\text{last}}/t_{\text{lag}} \text{ or last log-linear phase slope} \quad (C) \]

The last log-linear phase slope in the AUC calculation of CPT-11 equals β.

\[ \text{CL}_p = \frac{\text{dose}}{\text{AUC}} \quad (D) \]

\[ V_{\text{dss}} = \text{dose} \times \frac{\text{AUMC}}{(\text{AUC})^2} \quad (E) \]

**RESULTS**

Growth-inhibitory Activity of CPT-11 in Vitro. The growth-inhibitory activities of CPT-11, CPT, and SN-38 against L1210 leukemia cells and KB cells are shown in Fig. 2. CPT-11 showed very weak growth inhibition activity in comparison with CPT and SN-38. SN-38 showed the strongest activity. ED50 values of CPT-11, CPT, and SN-38 against L1210 were 5500, 3.6, and 3.7 ng/ml (8100, 8.7, and 11 nmol), respectively. Those against L1210 were 5500, 3.6, and 3.7 ng/ml (8100, 8.7, and 11 nmol), respectively.

Identification of SN-38. SN-38 was identified by a fluorescence emission spectrum, HPTLC, and HPLC. The fluorescence emission spectrum of CPT-11 in mouse serum was monitored at the beginning of incubation and after overnight incubation (Fig. 3). A peak around 520 nm appeared after overnight incubation, which was also observed in the serum containing SN-38.

Pharmacokinetics of CPT-11 and SN-38 in Mice after i.v. Administration. CD2F1 mice were given CPT-11 and SN-38 i.v. through the tail vein, and blood was collected by cardiac puncture under light ether anesthesia. The blood was immediately centrifuged, and plasma was immediately diluted 10-fold with 0.1 N HCl containing CPT-11 and SN-38 incubated in mouse serum overnight, and the supernatants were applied to a C18 cassette of an advanced automated sample processor (Analytichem International, Harbor City, CA) which was activated with 1.5 ml of methanol and water as described by Wallemacq and Lesne (19). The HPLC apparatus (Model LC-4A; Shimadzu Seisakusho) was linked to the advanced automated sample processor, and a C18 reversed-phase column (LiChrosorb RP-18; 25 x 0.4 cm; Merck) with an RP-18 precolumn was used for chromatography. The mobile phases consisted of acetonitrile/ethanol/0.8% ammonium carbonate (2/1/1, v/v) and CH3CN/water (1/4, v/v) for CPT-11 and SN-38, respectively. The peak area of CPT-11 and SN-38 was determined by HPLC as described above.

Plasma CPT-11 concentration-time curves were fitted to the polynomials by MULTI weighted nonlinear regression analysis (20). A biexponential equation (Equation A) gave the best fit according to Akaake's information criteria.

\[ C = A \exp(-\alpha t) + B \exp(-\beta t) \quad (A) \]

A and B are intercepts, and α and β are the rate constants of drug distribution and elimination phases, respectively. The inverse square of the observed plasma concentration served as a weighing factor.

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**Table 1 Validation of CPT-11 and SN-38 determination**

<table>
<thead>
<tr>
<th>Added (ng)</th>
<th>Intraday (n = 3)</th>
<th>Interday (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>CV (%) error</td>
</tr>
<tr>
<td>CPT-11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.02</td>
<td>0.94 ± 0.06</td>
<td>6.4</td>
</tr>
<tr>
<td>2.04</td>
<td>2.14 ± 0.08</td>
<td>3.7</td>
</tr>
<tr>
<td>5.10</td>
<td>5.07 ± 0.15</td>
<td>3.0</td>
</tr>
<tr>
<td>10.20</td>
<td>10.19 ± 0.30</td>
<td>2.9</td>
</tr>
<tr>
<td>SN-38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.6</td>
<td>12.8 ± 0.5</td>
<td>4.2</td>
</tr>
<tr>
<td>25.2</td>
<td>25.1 ± 0.3</td>
<td>1.4</td>
</tr>
<tr>
<td>63.0</td>
<td>62.8 ± 1.8</td>
<td>2.8</td>
</tr>
<tr>
<td>126.0</td>
<td>126.1 ± 0.6</td>
<td>0.5</td>
</tr>
</tbody>
</table>

* CV, coefficient of variation.
Fig. 2. Growth-inhibitory activity of CPT-11, SN-38, and CPT on KB (A) and L1210 (B) cells. KB and L1210 cells were exposed to drugs for 3 days at various concentrations and counted, and growth inhibition rates were calculated. Points, mean of four experiments; bars, SD. □, CPT-11; ●, SN-38; △, CPT.

Drug concentration decreased rapidly during the first 5 min and linearly thereafter, and SN-38 increased rapidly and then linearly with time. The CPT-11 equivalent concentrations of CPT-11 and SN-38 were constant throughout the incubation (Fig. 4). Fig. 5 shows the time course of growth-inhibitory activity of the incubated mouse serum or tissue homogenate containing CPT-11 against KB cells. The activity determined by bioassay was markedly enhanced with incubation time. This activity and the activity calculated by the ratio of SN-38 concentration, determined by HPLC, to the ED50 of SN-38 showed good agreement in both serum and tissue homogenates. This result strongly suggests that this enhanced activity was mainly due to converted SN-38.

Antitumor Activity in Vivo. Fig. 6 shows the dose-response curves of the antitumor activity of SN-38 and CPT in comparison with that of CPT-11. CPT-11 gave a higher T/C value than SN-38 or CPT at the doses examined when administered i.p. on Days 1, 5, and 9. The maximum T/C values of CPT, SN-38, and CPT-11 were 288, 286, and 500% at the total doses of 100, 400, and 200 and 400 mg/kg, respectively. Forty-day survivors were observed in the CPT-11- and SN-38-treated groups. In the 200- and 400-mg/kg CPT-11 groups, all the animals survived to the end of the experiment (40 days). CPT-11 was found to be superior to SN-38 and CPT in both antitumor activity and toxicity.

Plasma Pharmacokinetics of CPT-11 and SN-38 after i.v. Administration. Plasma concentrations of CPT-11 and its metabolite SN-38 after i.v. administration at the doses of 10, 20, and 40 mg of CPT-11 per kg are shown in Fig. 7. The phar-
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Fig. 6. Antitumor activity of CPT-11, SN-38, and CPT. On Day 0, $1 \times 10^5$ L1210 cells were inoculated i.p. into mice. SN-38 or CPT was given i.p. on Days 1, 5, and 9. Median survival of drug-treated animals relative to that of control animals (T/C) was plotted against the total dose of the drugs. Numbers in parentheses, number of 40-day survivors per 6 animals. Experiments were performed as three different experiments for each drug. O, CPT-11; •, SN-38; △, CPT.

Fig. 7. Plasma concentration profiles of CPT-11 and the metabolite SN-38 in mice. CPT-11 (10, 20, and 40 mg/kg) was administered to female CD2F, mice i.v. Animals were sacrificed to collect blood samples at various time intervals by cardiac puncture. Plasma CPT-11 and SN-38 concentrations were determined by HPLC. Points, mean of all the animals in three experiments (n = 7 to 12); bars, SD. Open and closed symbols are concentrations of CPT-11 and SN-38, respectively, after i.v. administration. □, 10 mg/kg; △, 20 mg/kg; ○, 40 mg/kg.

11. Plasma SN-38 decayed triexponentially with $t_{1/2}$, $t_1$, and $t_2$ of 1.6 min, 7 min, and 1.7 h, respectively. The plasma concentration decreased $1/1000$ within 1 h. The AUC was $1.35 \mu g \cdot h/ml$ and was mainly attributed to the high concentration just after administration.

macokinetic parameters calculated are listed in Table 2.

CPT-11 decayed biphasically at the doses examined with a $t_{1/2}$ of 0.8 to 1.1 h. AUCs were 3 to 23.5 $\mu g \cdot h/ml$ and increased 8 times, while the dose increased 4 times from 10 to 40 mg/kg. In contrast, CLp decreased from 3.4 to 1.8 liters/kg-h. Dose dependency of CPT-11 was detected by the normalization of AUC and CLp to the dose. $V_{a/d}$ values were 3.3 to 2.6 liters/kg and were relatively constant, though a tendency to decrease with an increase in dose was observed.

The AUC of SN-38 was 0.41, 0.71, and 1.08 $\mu g \cdot h/ml$ after the administration of 10, 20, and 40 mg of CPT-11 per kg, respectively. The half-times of the terminal log-linear phase of SN-38 concentration were 2.2 to 3.4 h.

Plasma SN-38 Concentration Profile after i.v. Administration of SN-38. Fig. 8 shows the plasma concentration profile after i.v. administration of SN-38 (10 mg/kg), which was quite different from that of SN-38 after the administration of CPT.
DISCUSSION

A new water-soluble CPT derivative, CPT-11, showed very strong antitumor activity against mouse tumors when administered by various routes (Fig. 6) (16, 18). In spite of the activity in vivo, its in vitro activity was very poor (Fig. 2), if based on the assumption that the antitumor activity of the drug in vivo should directly reflect in vitro activity. We found that SN-38 was formed from CPT-11 in mouse serum and tissue homogenate and that the growth-inhibitory activity determined by bioassay and that calculated from the SN-38 concentration showed good agreement (Fig. 5). Therefore SN-38 was thought to play an important role in the in vitro antitumor activity of CPT-11 when it was incubated in serum or liver and intestine homogenates. It was expected that SN-38 would result in higher antitumor activity in vivo than CPT-11, but this was not the case. SN-38 had lower antitumor activity than CPT-11 (Fig. 6). The effective concentration was calculated from the plasma concentrations of CPT-11 and SN-38 after the i.v. administration of CPT-11 and SN-38. Fig. 9 shows the effective concentrations of CPT-11 and SN-38. When SN-38 was administered, an effective concentration higher than the ED₅₀ was maintained for 2.5 h. That of SN-38 was higher than the ED₅₀ for at least 5 h after injection of CPT-11. The effective concentration of CPT-11 was below the ED₅₀ just after administration. The contribution of CPT-11 to the in vivo antitumor activity was very small, though it was not negligible.

Concerning the mechanism of the antitumor activity of CPT and its derivatives, it has been reported that the antitumor activity of CPT is time dependent rather than dose dependent, except at extremely high concentrations of the drug (22). This was also observed with SN-38. Maintenance of the effective concentration of SN-38 by i.v. administration of CPT-11 is a preferable explanation for the mechanism of action.

The plasma SN-38 concentration profile after the i.v. administration of CPT-11 was quite different from that after i.v. administration of SN-38 (Figs. 7 and 8). The rapid decrease in SN-38 concentration after CPT-11 administration was due to the formation of SN-38 in the blood (serum) within a few minutes as shown in Fig. 4. Then at higher doses of CPT-11 (20 and 40 mg/kg), the SN-38 level was maintained for the next 1 to 4 h. Maintenance of this concentration is presumed to be caused by two mechanisms. One is that the concentration is the sum of the linear formation of SN-38 from CPT-11 in the blood (Fig. 4) and in the liver (Fig. 5). The other alternative is that SN-38 elimination from the plasma is saturated or inhibited by coexisting CPT-11. The details of the mechanism that maintains plasma SN-38 concentration for several hours and of the contribution of the liver in the production of SN-38 are not yet clear and require further investigation.

Table 2 Pharmacokinetic parameters of CPT-11 and SN-38 after i.v. administration of CPT-11 to mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Dose (mg/kg)</th>
<th>10</th>
<th>20</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>t₁/₂ (h)</td>
<td></td>
<td>0.95 ± 0.22</td>
<td>0.83 ± 0.04</td>
<td>1.07 ± 0.13</td>
</tr>
<tr>
<td>AUC (µg·h/ml)</td>
<td></td>
<td>2.96 ± 0.12</td>
<td>7.65 ± 1.09</td>
<td>23.45 ± 7.75</td>
</tr>
<tr>
<td>CLp (liter/kg·h)</td>
<td></td>
<td>3.38 ± 0.15</td>
<td>2.65 ± 0.37</td>
<td>1.82 ± 0.55</td>
</tr>
<tr>
<td>Vₘ (liter/kg)</td>
<td></td>
<td>3.34 ± 0.24</td>
<td>2.84 ± 0.17</td>
<td>2.59 ± 0.94</td>
</tr>
<tr>
<td>SN-38</td>
<td></td>
<td>2.16 ± 0.66</td>
<td>3.01 ± 1.55</td>
<td>3.40 ± 0.56</td>
</tr>
<tr>
<td>t₁/₂ (h)</td>
<td></td>
<td>0.41 ± 0.06</td>
<td>0.71 ± 0.24</td>
<td>1.08 ± 0.11</td>
</tr>
</tbody>
</table>

* Mean ± SD of three experiments.

** AUC from Time 0 to infinity.

** Unpublished data.

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**Fig. 7**. Points, mean of all the animals in three different experiments (n = 12 to 15); bars, SD.

**Fig. 8**. Plasma concentration profile of SN-38 after i.v. administration of SN-38. SN-38 (10 mg/kg) was injected i.v. into female CD2F₁ mice. The SN-38 concentration was determined by the same method as described in the legend for Fig. 7. Points, mean of all the animals in three different experiments (n = 12 to 15); bars, SD.

**Fig. 9**. Effective concentration of the drug in plasma of drug-treated mice. The CPT-11 and SN-38 concentrations after 10 mg/kg of CPT-11 administration in Fig. 7 and the SN-38 concentration after 10-mg/kg administration in Fig. 8 were divided by the ED₅₀ values of CPT-11 or SN-38 against Li210. The ED₅₀ value is 1 for the effective concentration. ▲, SN-38 administration; ○, CPT-11 after CPT-11 administration; ●, SN-38 after CPT-11 administration.

**Unpublished data.**
Plasma CPT-11 concentration-time curves after i.v. administration are quite different from those of CPT (23), ECPT (11), and SN-38 (Fig. 8). No significant decrease in concentration was observed just after the injection of CPT-11. The decrease in concentration after 0.5 to 1 h is a general characteristic of sodium salts of CPT and its derivatives, because the half-times of SN-38 and ECPT are very similar (11). The dose dependency of the AUC of CPT-11 exhibited a concave curve, and that of SN-38 after the i.v. administration of CPT-11 showed a convex curve (Table 2). This suggests that there is a saturable process during the formation of SN-38. An increase in the dose of CPT-11 from 20 to 40 mg/kg did not raise the SN-38 concentration level, while the SN-38 concentration was maintained, but lengthened the period of maintenance from 1 to 3 h. This is presumed to be favorable from the standpoint of the mechanism of action for SN-38 (Fig. 7).

The phenolic compounds phenol and naphthol have been reported to decay very fast and to be metabolized into conjugates in the lung (24, 25). SN-38 has a phenolic hydroxyl group (Fig. 1), and it has been shown in rats to be metabolized into glucuronide, which has only 1/100 of the antitumor activity in vitro. On the assumption that SN-38 metabolism in mice is the same as in rats, the following explanation for the superior antitumor activity in vivo and weaker toxicity of CPT-11 than SN-38 is possible. Some CPT-11 is metabolized to SN-38 after its administration, and the concentration is maintained for hours at the level necessary to exhibit antitumor effects; maintenance of this level is not realized after the administration of SN-38 because of its rapid disappearance from the plasma. SN-38, which is active against tumor cells but toxic to normal cells, is detoxified by conjugation and then excreted. The ratio of SN-38 glucuronide and free SN-38 after CPT-11 administration is higher than that of SN-38. Weaker CPT-11 toxicity, compared with that of SN-38, which is active against tumor cells but toxic to normal cells, is presumed to be due to the levels of SN-38 glucuronide and free SN-38. CPT-11, which has high antitumor activity and low toxicity, is thought to be suitable for clinical use.

REFERENCES

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