Antitumor Activity of 7-Ethyl-10-[4-(1-piperidino)-1-piperidinocarbonyloxy-camptothecin, a Novel Water-soluble Derivative of Camptothecin, against Murine Tumors

Takehiko Kunimoto, Kazuo Nitta, Tomiko Tanaka, Nobuaki Uehara, Hiroyasu Baba, Mieko Takeuchi, Teruo Yokokura, Siego Sawada, Tadashi Miyasaka, and Masahiko Mutai

Chemotherapy Division [T. K., K. N., N. U., H. B., M. T.] and Pharmacology Division [T. T.], National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104; Yakult Central Institute, Kanisachi, Tokyo 186 [T. Y., S. S., M. M.]; and Showa University School of Pharmaceutical Sciences, Hatanodai, Shinagawa-ku, Tokyo 142 [T. M.], Japan

ABSTRACT

The search for new water-soluble analogues of camptothecin (CPT) with higher activity and less toxicity has led to the development of a novel compound, 7-ethyl-10-[4-(1-piperidino)-1-piperidinocarbonyloxy-camptothecin (CPT-11), which showed significant antitumor activity against a broad spectrum of experimental tumor models by i.p., i.v., or oral administration. When its activity against L1210 was compared with that of CPT and known derivatives, CPT-11 was most effective, giving the highest maximum increase in life span (ILS) and showing good activity over a wide dose range. The antitumor activity of CPT-11 was shown against tumors not only in the ascites form but also in the solid form. Included among the more susceptible murine tumors are S180, Meth A fibrosarcoma, Lewis lung carcinoma, Ehrlich carcinoma, M1H134 hepatoma, mammary carcinoma of C3H/HeN mice, L1210, and P388 leukemia. Probable cures of these tumors were induced frequently by CPT-11. The antitumor activity of CPT-11 against i.p.-implanted L1210 was superior to that of Adriamycin in maximum ILS, the number of cured mice, and the therapeutic ratio. CPT-11 at a dose of 100 mg/kg produced an ILS in excess of 300% with five of six mice surviving tumor free, and effected 100% tumor regression at 200 mg/kg, whereas the optimum dose of Adriamycin, 12.5-25 mg/kg, brought about 114-129% ILS with one of six mice surviving. The acute toxicity of CPT-11 was extremely low, particularly in the case of oral administration. CPT-11 is expected to be clinically useful.

INTRODUCTION

CPT (Fig. 1) is a plant antitumor agent which was isolated from Camptotheca acuminata, a tree native to south China by Wall et al. (1). It has a very good spectrum of activity against experimental animal tumor models in ascites as well as solid form but its application as a therapeutic has been hampered by severe toxicity. Clinical and preclinical studies revealed reversible bone marrow depression and hemorrhagic cystitis as the major dose-limiting toxicity (2, 3).

Efforts have been directed at finding new derivatives of CPT with higher antitumor activity and less toxicity (for review, see Ref. 4). For example, 10-hydroxy-CPT has been reported to have almost the same antitumor activity as CPT with less toxicity (4). We prepared many partially synthesized CPT derivatives including 7-ethyl-CPT (Fig. 1, SN-22) and 7-ethyl-10-hydroxy-CPT (Fig. 1, SN-38) having strong activity against various murine tumors (5). However, SN-22 and most of the derivatives synthesized previously by us or other investigators as well as CPT itself are not soluble in water. Poor aqueous solubility posed a number of problems in their clinical application. Alkaline treatment of the compounds resulted in water-soluble sodium salt forms with the β-lactone ring opened. Since it was pointed out that the hydroxy lactone ring in CPT is absolutely required for its antitumor activity (4), water-soluble derivatives having an intact β-lactone ring were investigated. Among them, 7-ethyl-10-[4-(1-piperidino)-1-piperidinocarbonyloxy-camptothecin (CPT-11) showed marked activity against mouse leukemia L1210, with little toxicity.

The present work was undertaken to evaluate the effect of CPT-11 administered through diverse routes on various kinds of murine tumors both in ascites and in solid forms. The activity was compared with that of CPT and its known derivatives, or current clinically useful anticancer agents including Adriamycin. The acute toxicity of CPT-11 for experimental animals was also quantitated. Portions of this work have been presented in preliminary form (6).

MATERIALS AND METHODS

Animals and Tumors. An appropriate strain of SPF adult mice for each system was obtained commercially (Table 1). All tumors used were maintained in our laboratory by serial transplantation.

Antitumor Testing. The route and size of the tumor inoculum and host mouse used in each experiment are summarized in Table 1. Mice were inoculated with the appropriate number of viable tumor cells i.p. or s.c. on day 0. CPT-11 was dissolved in sterile physiological saline immediately before use and administered to mice i.p., i.v., or p.o. on days 1, 5, and 9, and also on days 13 and 17 for B16 melanoma. When the activity of CPT-11 against L1210 was evaluated concomitantly with that of Adriamycin, 5 × 10⁴ cells were inoculated i.p. into CDF1 mice on day 0 and the drugs were administered i.p. on days 1–9. The activity of CPT-11 was determined in parallel with that of 5FU against Sarcoma 180 (S180), 2 × 10⁶ cells being inoculated s.c. on day 0 and drugs being administered p.o. on days 1, 5, and 9. The activity of CPT-11 was compared with that of CPT and derivatives against L1210, 5 × 10⁶ cells being implanted i.p. into CDF1; on day 0 and the compounds being injected i.p. on days 1–5.

Evaluation of Antitumor Activity. For the ascites type of tumor the percentage of ILS was calculated by the following formula for comparison:

\[ \text{ILS} \% = \left( \frac{T}{C} - 1 \right) \times 100 \]  

where \( T \) and \( C \) are the survival periods for treated and control mice, respectively. The survival times were compared by employing the generalized Wilcoxon test. ILS 30% or \( T/C \) 130% was adopted for the criteria of therapeutic efficacy against ascites type of tumor. Longer survivors than 40 days in L1210 and P388 and 60 days in the other ascites tumors with no retention of ascites were considered to be cured. Solid tumors were extirpated and weighed on day 21, and the tumor IR was calculated by the following formula:

\[ \text{IR} \% = \left( 1 - \frac{T}{C} \right) \times 100 \]  

where \( T \) and \( C \) are the tumor weight in treated and control mice, respectively. Student’s \( t \) test was used to assess the statistical signifi-
canee of the observed differences between mean tumor weights. IR, was calculated by the following formula:

\[ IR = \frac{dose \ for \ ILS \ maximum}{dose \ for \ ILS \ 30\%} \]

Toxicity Testing. Male 7-week-old ICR mice were used for the toxicity evaluation. Mice in groups of 10/dose were treated by single i.p. injection of CPT-11. I\textsubscript{L}D\textsubscript{50} values were calculated by the method of Litchfield-Wilcoxon. Similarly, the toxicity test was performed using male and female 6-week-old Fisher rats.

Materials. CPT-11 was synthesized by chemical modification of camptothecin, introducing an ethyl group at the 7-position (SN-22), then a hydroxyl group at the 10-position (SN-38) via [N\textsubscript{14}]oxide of SN-22 (8, 9) and then binding a piperadinopiperadinocarbonyl group to the hydroxyl group (9, 10).

RESULTS

Antitumor Activity of CPT-11 against Ascites Tumors. The i.p. administration of CPT-11 prolonged the survival time of the L1210-implanted leukemia L1210 (Table 2) or P388-bearing mice at total doses of 6.3 mg/kg or more and many mice were cured of the ascites tumors at total doses of 100–400 mg/kg. Against the ascites form of S180 (Table 3), Meth A and Ehrlich carcinoma, the i.p. administration was effective at total doses of less than 3.13 mg/kg in the case of Ehrlich, when administered i.p. or i.v. and at 50-100 mg/kg in the case of Ehrlich, when administered i.p. or i.v. and at 400 mg/kg or above by the p.o. route. It showed moderate significant therapeutic response in curing mice of the following tumors in ascites form: P388 and MH134, cure in two mice out of six at a total dose of 800 mg of CPT-11/kg; Ehrlich and S180, more than five out of six cured at 200–800 mg/kg. The i.v. treatment was effective against LI210, P388, and B16; however, CPT-11 administered by the i.v. route was two to four times less active than by the i.p. route. High doses of CPT-11 resulted in tumor-free mice (complete inhibition of the tumor growth) at total doses of 200 mg/kg or above, or at 50–100 mg/kg in the case of Ehrlich, when administered i.p. or i.v. and at 400 mg/kg or above by the p.o. route. The TR \textsuperscript{*} was calculated by the following formula:

\[ TR = \frac{dose \ for \ ILS \ maximum}{dose \ for \ ILS \ 30\%} \]

\textsuperscript{*} Tumor-free survivors were excluded from calculations of the mean, SD, and T/C (%).


d, day.

\textsuperscript{w}, weeks.

\textsuperscript{d}, day.
ANTITUMOR ACTIVITY OF CAMPTOTHECIN DERIVATIVE CPT-11

Activity against mammary carcinoma of C3H mice. CPT-11 was not effective against adenocarcinoma 755 under the present conditions.

Comparison of the Activity of CPT-11 and CPT and Derivatives. The antitumor activity of CPT-11 against i.p.-implanted L1210 was evaluated in parallel with that of CPT and the known derivatives SN-22 and SN-38 as shown in Fig. 3. Among those compounds, CPT-11 gave the highest T/C (%) and the widest dose range where the effect was significant. Fig. 3 also includes the activity of the corresponding sodium salt. It is clearly indicated that the sodium salt forms have greatly decreased antileukemic activity compared to the original compounds.

Comparison of the Activity of CPT-11 with That of Current Clinically Useful Anticancer Agents. Table 6 compares the efficacy of CPT-11 and Adriamycin in the treatment of i.p.-implanted L1210. CPT-11 at a total dose of 100 mg/kg produced an ILS in excess of 300% (T/C, 414%) with five of six mice surviving tumor free and effected 100% tumor regression at an ILS in excess of 300% (T/C, 414%) with five of six mice of 10 mice, and at 800 mg/kg, CPT-11 induced a cure in six out of 10 mice whereas the mice treated with 5FU exhibited 100% mortality by day 21.

Acute Toxicity of CPT-11. Acute toxicity of CPT-11 was evaluated after single administration by the i.p. and p.o. routes and compared with that of CPT. As shown in Table 8, the LD50 values of 117.5 and 765.3 mg/kg by the i.p. and p.o. routes, respectively, are much higher than those of CPT. A similar acute toxicity test showed that LD50 values of CPT-11 in rats were 83.6 (male) and 85.1 (female) mg/kg by i.v. injection and 866.9 (male) and 1026.5 (female) mg/kg by p.o. administration.

DISCUSSION

Camptothecin (CPT) and most of its known derivatives such as 7-ethyl-CPT (SN-22), 10-hydroxy-CPT, and 7-ethyl-10-hydroxy-CPT (SN-38) are not sufficiently soluble in water for clinical use. Alkaline treatment of these compounds yielded water-soluble sodium salt forms with the δ-lactone ring opened and diminished activity. The results are in general agreement with the proposal of Wall et al. that the hydroxy lactone ring in CPT is absolutely required for its antitumor activity (4).

The search for new water-soluble derivatives having an intact δ-lactone ring had led to the development of a novel compound, CPT-11, which demonstrated significant antitumor activity against a broad spectrum of experimental tumor models. Comparing the activity of CPT-11 against L1210 with that of CPT and its derivatives SN-22, SN-38, and 10-hydroxy-CPT, revealed that CPT-11 was the most effective, giving the highest maximum ILS and showing good activity over a wide dose range. The antitumor activity of CPT-11 was shown against the tumors not only in the ascites form but also in the solid form. Among the more susceptible murine tumors are S180, Meth A fibrosarcoma, Lewis lung carcinoma, Ehrlich carcinoma, MH134 hepatoma, mammary carcinoma of C3H/HeN mice, L1210 leukemia, and P388 leukemia. Probable cures of these tumors were observed frequently after CPT-11 treatment. Al-
and weighed on day 21.

DrugControl
male ICR mice.

cacy of CPT-11 against s.c.-implanted S180 surpassed that of
Adriamycin, 12.5-25 mg/kg, brought about 114-129% ILS
with five of six mice surviving tumor free and effected 100%
ILS, the number of cured mice, and the therapeutic ratio. CPT-
LI210 was superior to that of Adriamycin in the maximum
most all mice bearing S180 or Lewis lung carcinoma were cured
by the p.o. route. However, since the toxicity of
CPT-11 by the oral route was 10-20 times less
safely administered p.o. at doses as large as 30 times the
effective doses by the i.p. route and induced significant per
sions of cure of S180 and Lewis lung carcinoma.

CPT-11 was shown when it was administered i.v. or p.o. against various tumors. In general,
CPT-11 administered by the oral route was 10-20 times less
active than by the i.p. route. However, since the toxicity of
CPT-11 by the p.o. route was very low, as stated above, it was

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by the oral route. However, since the toxicity of
CPT-11 by the p.o. route was very low, as stated above, it was

Table 6 Comparison of the antitumor activity of CPT-11 and Adriamycin against i.p.-implanted LI210

<table>
<thead>
<tr>
<th>Compound</th>
<th>LI210 (mg/kg)</th>
<th>LI210 (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPT</td>
<td>56.2 (50.6-62.4)*</td>
<td>50.1 (35.5-70.6)</td>
</tr>
<tr>
<td>CPT-11</td>
<td>177.5 (131.5-239.6)*</td>
<td>765.3 (358.9-1086.7)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, 95% confidence limits.

Table 7 Comparison of the antitumor activity of CPT-11 and 5FU against s.c.-implanted S180

<table>
<thead>
<tr>
<th>Drug</th>
<th>Total dose (mg/kg)</th>
<th>Tumor weight (g, mean ± SD)*</th>
<th>Inhibition ratio (%)</th>
<th>Cured mice</th>
<th>Mortality (on day 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>2.03 ± 0.88</td>
<td>0/10</td>
<td>0/10</td>
<td></td>
</tr>
<tr>
<td>CPT-11</td>
<td>200</td>
<td>0.31 ± 0.20</td>
<td>94.1</td>
<td>1/10</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>0.12 ± 0.19</td>
<td>94.1</td>
<td>3/10</td>
<td>0/10</td>
<td></td>
</tr>
<tr>
<td>800</td>
<td>0.03 ± 0.05</td>
<td>98.5</td>
<td>6/10</td>
<td>0/10</td>
<td></td>
</tr>
<tr>
<td>5FU</td>
<td>100</td>
<td>1.27 ± 0.69</td>
<td>37.4</td>
<td>0/10</td>
<td></td>
</tr>
<tr>
<td>800</td>
<td>0.38 ± 0.21</td>
<td>81.3</td>
<td>5/10</td>
<td>0/10</td>
<td></td>
</tr>
</tbody>
</table>

* Tumor-free survivors were excluded from the calculations of the mean and SD.

Table 8 Acute toxicity of CPT-11 and camptothecin

LDso was determined by the method of Litchfield-Wilcoxon with 7-week-old male ICR mice.

<table>
<thead>
<tr>
<th>Compound</th>
<th>I.p.</th>
<th>p.o.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPT</td>
<td>56.2 (50.6-62.4)*</td>
<td>50.1 (35.5-70.6)</td>
</tr>
<tr>
<td>CPT-11</td>
<td>177.5 (131.5-239.6)*</td>
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* Numbers in parentheses, 95% confidence limits.

REFERENCES

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