Excretion into Gastrointestinal Tract of Irinotecan Lactone and Carboxylate Forms and Their Pharmacodynamics in Rodents

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Purpose. To investigate the excretion of irinotecan hydrochloride (CPT-11) and its active metabolite, SN-38, into the gastrointestinal lumen via the biliary and/or intestinal membrane route after dosing with lactone and carboxylate forms of CPT-11, and to evaluate the toxic and antitumor effects of the two forms.

Methods. The excretions of CPT-11 and SN-38 were investigated by the *in situ* perfusion technique using rats. The incidence of delayed diarrhea was evaluated after i.v. dosing (60 mg/kg) with CPT-11 lactone and carboxylate forms for 4 days. Antitumor activity and changes in body weight were investigated in mice with Meth A tumors.

Results. The excretion of CPT-11 into bile was greater in dosing with CPT-11 carboxylate than that with its lactone form, whereas the exsorption across intestinal membrane was greater in dosing with CPT-11 lactone than that with its carboxylate form. Dosing with CPT-11 lactone dose-dependently inhibited the increase in tumor weights in Meth A tumor mice, whereas the dosing with its carboxylate form reduced the antitumor effect.

Conclusions. The decreased antitumor effect caused by dosing with the CPT-11 carboxylate form could be due to less accumulation in the tissue including tumor cells resulting from the rapid elimination of the form in the body.

KEY WORDS: irinotecan; pharmacokinetics; lactone; carboxylate; antitumor activity.

INTRODUCTION

Irinotecan hydrochloride (CPT-11) is a semisynthetic water-soluble analogue of camptothecin (CPT) that has a potent antitumor activity by inhibiting topoisomerase I. CPT-11 exerts its antitumor activity after transformation *in vivo* to its more active metabolite, 7-ethyl-10-hydroxycamptothecin (SN-38), which has 100- to 1000-fold more potent antitumor activity than CPT-11 *in vitro* (1,2). Both CPT-11 and SN-38 have an α-hydroxy-δ-lactone ring, which undergoes reversible and pH-dependent hydrolysis and lactonization reactions of each compound in aqueous solution. The closed lactone form predominates under acidic conditions, and the open carboxylate form predominates under alkaline conditions (3,4). Only the closed lactone forms of CPT-11 and SN-38 are effective topoisomerase I inhibitors, and the carboxylate forms are inactive *in vitro* (5).

Scott *et al.* (6,7) reported that there are significant differences in the pharmacokinetic behavior of the two forms after separate i.v. administration of both lactone and carboxylate forms of CPT in rats. They found that the plasma concentrations and area under the curve (AUC) values for the lactone were significantly higher after dosing with CPT than after dosing with sodium salt of CPT. In the case of CPT-11, no comprehensive pharmacokinetic studies combined with pharmacodynamic studies on both CPT-11 lactone and carboxylate forms have been reported. The pharmacokinetic and pharmacodynamic studies have been performed only by administration of the active lactone form (8,9). It is also an important factor for performing chemotherapy whether CPT-11 is stable or not for a long time when admixed with commercial injections in i.v. fluids. We previously reported on the compatibility of CPT-11 following admixing with 44 kinds of commercial injections, which would have an occasion of the admixture with CPT-11 in i.v. fluids in the case of chemotherapy (10,11). We reported that admixing CPT-11 with alkaline injections such as 0.5% adenosine 5′-triphosphate disodium injection, 2% inosine injection, and 7% sodium bicarbonate injection revealed marked reduction in the lactone form within 0.5 h after mixing.

In the present study, we investigated the pharmacokinetics of CPT-11 following i.v. administration of each of the lactone or carboxylate forms of CPT-11, and furthermore evaluated the toxic and antitumor effects of the two forms in rats and mice.

MATERIALS AND METHODS

Materials

CPT, CPT-11, and SN-38 were obtained from Daiichi Pharmaceutical Co. Ltd.(Tokyo, Japan). The lactone form of CPT-11 for injection (10 and 20 mg/ml) was dissolved in pyrogen-free distilled water (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) just prior to use and when necessary adjusted with 0.01 N hydrochloric acid at pH 4.0. The carboxylate form of CPT-11 for injection (10 and 20 mg/ml) was dissolved in Hartmann's solution (pH 8; Yoshitomi Pharmaceutical Co. Ltd., Osaka, Japan) by heating and after cooling was adjusted with 0.01 N sodium hydroxide at pH 8.0. The conversion of the carboxylate into lactone form was virtually complete at pH 4.0 (>99%) and that of the lactone into the carboxylate was 92.7% at pH 8.0, as determined by high-performance liquid chromatography (HPLC). All other chemicals were commercial products and analytical grade.

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Animals

The studies were approved by the Animal Research and Use Committee of Kumamoto University. Male Wistar rats were purchased from Kyudou Co. Ltd. (Kumamoto, Japan). Male 6-week-old BALB/c mice were purchased from Japan SLC, Inc. (Shizuoka, Japan). They were housed under specific pathogen-free conditions at least for 1 week at 22 ± 2°C and 60 ± 10% relative humidity. During the acclimatization they were allowed free access to a standard diet (CE-2 food pellets for rats: Clea Japan, Tokyo, Japan; F-2 food pellets for mice: Funabashi Farms, Chiba, Japan) and tap water. The body weights of rats and mice were 150–300 g and 20–25 g, respectively. For the pharmacokinetic study, the rats were fasted overnight with free access to water before experiments.

Pharmacokinetic Studies

Rats were anesthetized by i.p. injection of ethyl carbamate (1.2g/kg). The small intestine was exposed by a midline abdominal incision. The upper duodenum and the ileocecal junction were cannulated with a polyethylene tube and the small intestine was washed with saline maintained at 37°C and was perfused with lactated Ringer's solution at the rate of 1.3 ml/min from the duodenum through the small intestine to the ileocecal junction (12). CPT-11 lactone and carboxylate forms were intravenously administered at 10 mg/ml/kg *via* the femoral vein in about 1 min to rats. After the injection, blood samples (0.2–0.3 ml) were collected periodically every 15 min through a cannula introduced into the femoral artery. Perfusates were also collected every 15 min from the ileal outflow, and the bile was collected separately every 15 min from a cannula introduced into the common bile duct. The serum was separated immediately by centrifugation at 8000 rpm for 2 min at –10°C and stored at –80°C until assay. After completing the blood collection periods, the rats were sacrificed and tissues (heart, lung, liver, spleen, kidney, intestine) were removed. The AUCs of the lactone and carboxylate forms of both CPT-11 and SN-38 were calculated by a trapezoidal rule. The unpaired *t*-test was used to assess the pharmacokinetic parameters. A probability level of $P<0.05$ was considered significant.

Analytical Method

An HPLC equipped with a fluorescence detector was used to determine the lactone and carboxylate forms of both CPT-11 and SN-38 in the serum and tissue according to a described technique (13) with minor modifications. Briefly, to 50–100 μl of serum in polypropylene tubes (Iuchi Bio Systems Co., Osaka, Japan) were added 0.2 ml of cold methanol (–20°C) containing 0.2 μg/ml CPT as an internal standard and 100 μl of the mobile phase. The tubes were vortex-mixed for 5 s and centrifuged at 8000 rpm for 2 min at –10°C. Part of the supernatant (100 μl) was transferred to a fresh tube and 70 μl of the mobile phase buffer was added. The solution was briefly vortex-mixed and 50 μl injected onto the column. For the analysis of tissue samples, they were homogenized in 15-fold volumes (w/v) of cold methanol (–20°C) and then centrifuged at 3000 rpm for 2 min at –10°C. The supernatant (100 μl) was transferred to a fresh tube and 70 μl of mobile phase buffer was added. The solution was briefly vortex-mixed and 50 μl injected onto the column. The HPLC system was composed of a Shimadzu LC-6A pump (Kyoto, Japan). A TSK gel ODS-80TS column (4.6 × 150 mm, Tosoh Co., Tokyo, Japan) was used for separation. The mobile phases consisted of 0.075 M ammonium acetate buffer (pH 6.4)-acetonitrile (8:2, v/v). The flow rate was 1.5 ml/min at 30°C. Detection was monitored with an excitation wavelength at 355 nm and an emission wavelength at 515 nm.

Incidence of Diarrhea

CPT-11 (60 mg/kg) was administered intravenously to rats once daily for 4 consecutive days, as reported by Kase *et al.* (14). Body weight and onset of diarrhea were examined daily until the 7th day after CPT-11 treatment. CPT-11 is known to produce acute and delayed diarrhea; the former is associated with anticholinesterase activity by CPT-11 (15), whereas the latter is associated with gastrointestinal impairment by SN-38 excreted in the gut (16). Thus, diarrhea that appeared within 1 h of CPT-11 administration was regarded to be acute diarrheal symptoms and diarrhea appearing after 6–24 h was regard as delayed diarrheal symptoms. Diarrhea was scored judging by the feces as 0 for no diarrhea (normal feces), 1 for slight diarrhea (softened feces keeping shape), 2 for moderate diarrhea (diarrhea being out of shape) and 3 for severe diarrhea (watery diarrhea).

Antitumor Effects

Meth A fibrosarcoma (Meth A) was maintained by serial i.p. passage in syngeneic BALB/c mice. Ascitic fluid was collected 7 days after implantation, and tumor cells were washed twice with Hanks' balanced salt solution (HBSS, Gibco-BRL, New York, NY). Meth A cells (1×10^6 cells/0.1 ml) were inoculated subcutaneously into the right flank of syngeneic BALB/c mice (day 0). Mice with tumors were randomly divided into experimental groups (seven mice per group) on day 9 after tumor inoculation, when the mean estimated tumor weight (ETW) reached about 100 mg, and were treated intravenously using a schedule of every other day for a total of four treatments. The ETW was calculated using the formula: $ETW = L \times W^2/2$, where L and W represent the length and the width of the tumor mass, respectively. Mice were killed under anesthesia on day 23 after tumor inoculation, and the tumor masses were then excised and weighed. The growth inhibition rate (IR) was calculated on the basis of tumor weight using the formula: $IR = (1 - TWt/TWc) \times 100(\%)$, where TWt represents the mean of tumor weight for a treated group and TWc that for the control group. The significance of differences in tumor weights between test and control groups was analyzed using Dunnett's test or the Tukey-Kramer test. Furthermore, to evaluate the intensity of the side effects, the rate of body weight loss (BWL) and D/U were used as parameters of toxicity. BWL was calculated using the formula: $BWL = (1 - BWn/BWs) \times 100(\%)$, where BWn and BWs represent the mean body weights of mice on day n and the day of initial administration, respectively. The maximum value of BWL was designated as BWLmax, and BWLmax less than zero indicates no body weight loss. D/U indicates the ratio of the number of mice that died of toxic effects to the number of mice used.

RESULTS

Serum Concentration-Time Profiles

Figure 1 shows the serum concentration-time curves of the lactone and carboxylate of CPT-11 and SN-38 after i.v. dosing with CPT-11 lactone and carboxylate forms at a dose of 10 mg/kg in rats. It was observed that a part of CPT-11 administered in the both forms was rapidly converted to its lactone or carboxylate form in the serum and further metabolized to that of SN-38. Dosing CPT-11 with the lactone form showed higher serum CPT-11 lactone levels than that after dosing with the carboxylate form. In contrast, although dosing CPT-11 with the carboxylate form showed high serum CPT-11 carboxylate levels at the initial time, it was very rapidly eliminated from the serum. The serum levels of SN-38 after dosing with both forms of CPT-11 were much lower compared with those of CPT-11.

Table I lists the AUC values of CPT-11 and SN-38 from 0 to 4 h after i.v. dosing with CPT-11 lactone and carboxylate forms. The AUC value of CPT-11 lactone was markedly larger in dosing with the lactone form than with the carboxylate form. The AUC ratio for dosing with the CPT-11 lactone form to the carboxylate form was 9.32. Thus, there was a marked difference in AUC ratios of CPT-11 between the doses with the lactone and carboxylate forms of CPT-11. On the other hand, there was no definitive difference in AUC values of SN-38 between the doses with both forms of CPT-11.

Table I. Comparison of AUC Values of CPT-11 Lactone and Carboxylate and SN-38 Lactone and Carboxylate After i.v. Doses of CPT-11 Lactone and Carboxylate Forms at a Dose of 10 mg/kg in Rats

AUC (μg · h/ml) (0–4 h)	i.v. CPT-11 lactone	i.v. CPT-11 carboxylate	AUC ratio[a]
CPT-11			
Lactone	7.18 ± 0.56	0.77 ± 0.27[b]	9.32
Carboxylate	3.35 ± 0.38	3.41 ± 0.85	0.98
Total	10.5 ± 0.93	4.18 ± 1.12	2.51
SN-38			
Lactone	0.07 ± 0.02	0.09 ± 0.02	0.78
Carboxylate	0.07 ± 0.02	0.15 ± 0.06	0.47
Total	0.15 ± 0.04	0.25 ± 0.07	0.60

[a] AUC (i.v. lactone form/i.v. carboxylate form). Each value represents the mean ± SEM of four rats.
[b] $P < 0.01$ (i.v. lactone forms vs. i.v. carboxylate form).

Excretion into the Bile and Intestinal Lumen

We examined the excretion of CPT-11 and SN-38 into gastrointestinal lumen via the biliary duct and/or intesti-

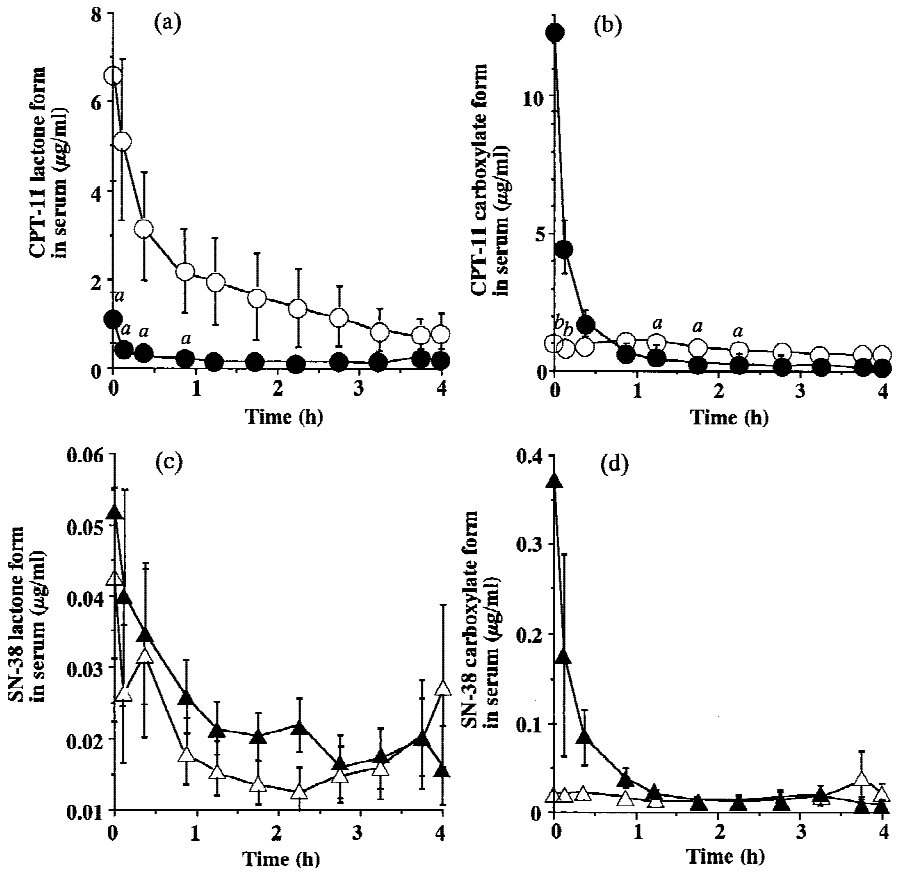

Fig. 1. Serum concentration-time profiles for CPT-11 lactone (a), CPT-11 carboxylate (b), SN-38 lactone (c), and SN-38 carboxylate (d) after i.v. doses of CPT-11 lactone form (open circles and open triangles) and CPT-11 carboxylate form (filled circles and filled triangles) at a dose of 10 mg/kg in rats. Each value represents the mean ±SEM of four rats. (a) $P<0.05$; (b) $P<0.01$ (i.v. lactone form vs. i.v. carboxylate form).

nal membrane by using an *in situ* single-pass perfusion technique as reported previously (12). Figure 2 shows the cumulative biliary excretion curves of the lactone and carboxylate of both CPT-11 and SN-38 after i.v. dosing with CPT-11 lactone and carboxylate forms at a dose of 10 mg/kg in rats. The biliary excretion rates of CPT-11 lactone and carboxylate after dosing with the CPT-11 carboxylate form were significantly greater than after dosing with the CPT-11 lactone form. The amounts of cumulative excretion of CPT-11 lactone and carboxylate were 8.63% and 56.3% of the dose in 4 h after dosing with the carboxylate form, respectively, whereas they were 4.39% and 40.0% after dosing with the lactone form, respectively. The biliary excretion profiles of SN-38 were similar to those of CPT-11, and the excretion rates of SN-38 lactone and carboxylate tended to be greater in dosing with the CPT-11 carboxylate form.

Figure 3 shows the cumulative exsorption (secretion) curves of CPT-11 and SN-38 from blood into intestinal lumen across the intestinal membrane. The exsorption rates of CPT-11 lactone and carboxylate were both significantly greater in dosing with the CPT-11 lactone form than in dosing with the carboxylate form. In particular, administration of the lactone form showed marked exsorption as compared with that of the carboxylate form. The amount of exsorption of the CPT-11 lactone form was approximately 11% in 4 h. The exsorption of SN-38 lactone form across the intestinal membrane also tended to be greater in dosing with the CPT-11 lactone form than in dosing with the CPT-11 carboxylate form, but there was no significant difference in the exsorption of SN-38 between the doses with the two forms of CPT-11. The amounts of exsorption of SN-38 lactone and carboxylate were less than 1% of the dose in 4 h. These results indicate that the excretion of CPT-11 into the gastrointestinal lumen occurs not only via the biliary route but also appreciably via the intestinal membrane route.

Tissue Distribution

Figure 4 shows the concentrations of CPT-11 and SN-38 in the tissue at 4 h after i.v. dosing with CPT-11 lactone and carboxylate forms in rats. Significant amounts of CPT-11 lactone and carboxylate were distributed to the tissues after dosing with the CPT-11 lactone form compared with after dosing with the carboxylate form. On the other hand, SN-38 lactone and carboxylate forms in the tissue showed approximately one-hundredth and one-tenth lower levels than those of the CPT-11 lactone and carboxylate forms, respectively. There was no significant difference in the tissue levels of SN-38 between dosing with the CPT-11 lactone and carboxylate forms. Thus, it was found that the tissue distributions of CPT-11 were markedly reduced by dosing with the carboxylate form.

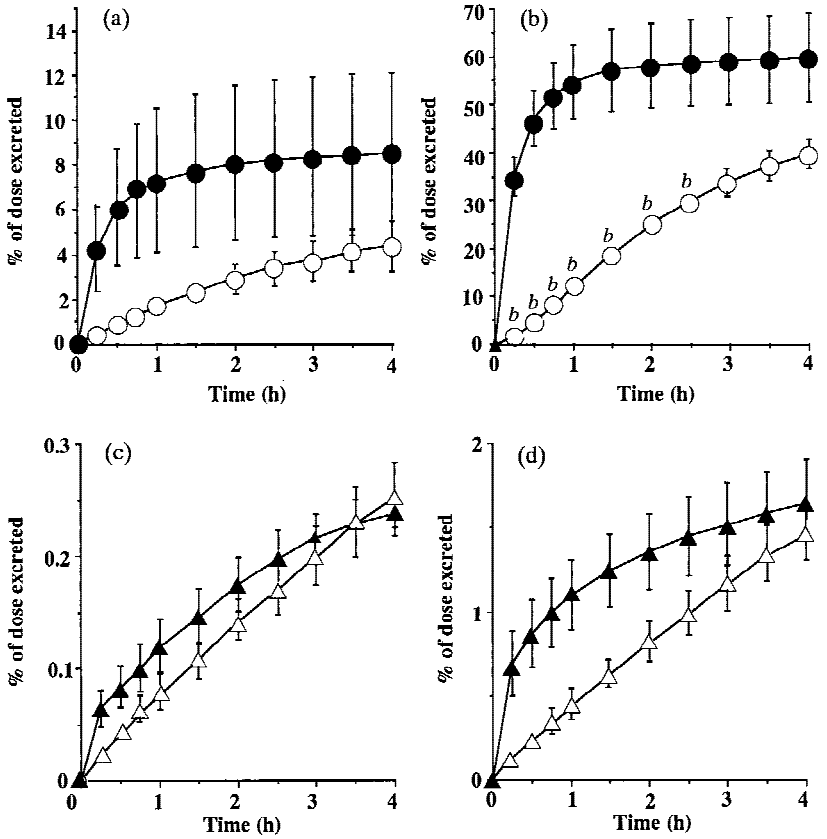

Fig. 2. Cumulative biliary excretion curves for CPT-11 lactone (a), CPT-11 carboxylate (b), SN-38 lactone (c), and SN-38 carboxylate (d) after i.v. doses of CPT-11 lactone form (open circles and open triangles) and CPT-11 carboxylate form (filled circles and filled triangles) at a dose of 10 mg/kg in rats. Each value represents the mean ±SEM of four rats. (b) $P<0.01$ (i.v. lactone form vs. i.v. carboxylate form).

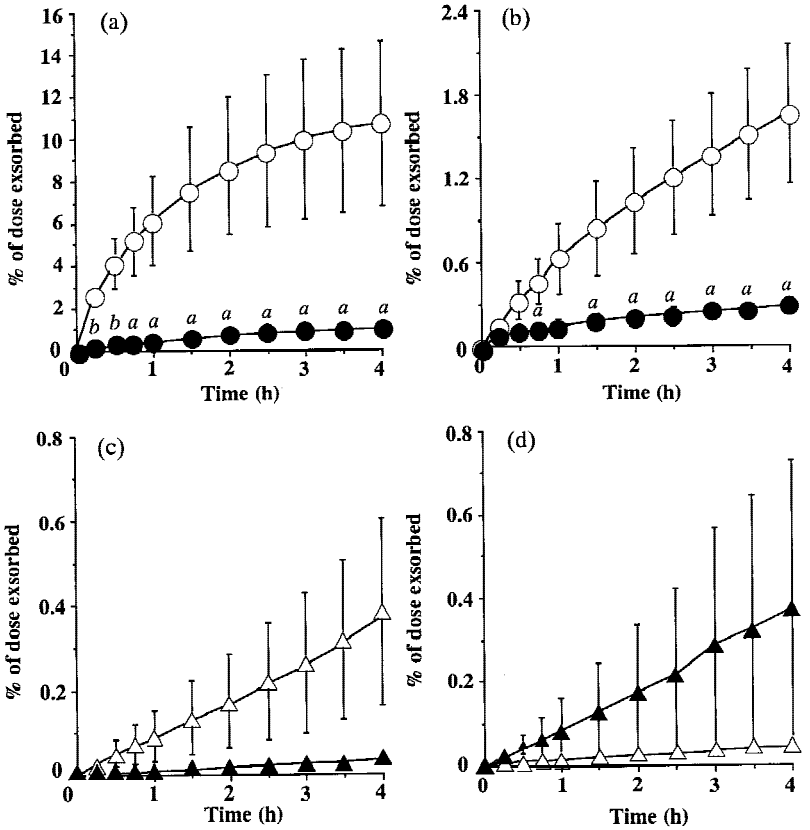

Fig. 3. Cumulative intestinal exsorption curves for CPT-11 lactone (a), CPT-11 carboxylate (b), SN-38 lactone (c), and SN-38 carboxylate (d) after i.v. doses of CPT-11 lactone form (open circles and open triangles) and CPT-11 carboxylate form (filled circles and filled triangles) at a dose of 10 mg/kg in rats. Each value represents the mean ±SEM of four rats. (a) $P<0.05$ (i.v. lactone form vs. i.v. carboxylate form).

Antitumor Effect and Toxicity

The antitumor activity and the change in the body weight after i.v. administration of CPT-11 were investigated in mice with Meth A tumors. Figure 5 shows the time profiles of the tumor weights after i.v. dosing with the CPT-11 lactone and carboxylate forms on alternate days for a total of four treatments from day 9 after Meth A tumor inoculation in mice. Dosing with the CPT-11 lactone form at each dose of 50 or 100 mg/kg inhibited dose-dependently the increase in the tumor weights as compared with the control group. On the other hand, dosing with the CPT-11 carboxylate form greatly reduced the antitumor activity although it showed a tendency to inhibit the increase in the tumor weights as compared with the control.

To evaluate the toxicity of CPT-11, the body weight loss and mortality were used as an index. Figure 6 shows the time profiles of the body weights. It was observed that dosing at 100 mg/kg of the CPT-11 lactone form markedly decreased the body weight, peaking on day 9 after the dose. However, there was no definitive difference in the increase in the body weight between the treatments with CPT-11 carboxylate and the control.

Table II lists the results of the antitumor effect and the toxicity of CPT-11 lactone and carboxylate forms against Meth A tumors in mice. Dosing with the CPT-11 lactone form significantly reduced the tumor weight after Meth A tumor inoculation as compared with the control, and the growth IRs at 50 and 100 mg/kg doses were 48 and 76%, respectively. On the other hand, the administration of the carboxylate form pronouncedly decreased the inhibitory effect of the tumor growth as compared with dosing with the lactone form. No mice died from CPT-11 toxicity in either group.

Incidence of Diarrhea

We investigated the incidence of diarrhea, which is one of severe adverse reactions to CPT-11. Table III lists the incidence of delayed diarrhea after i.v. dosing with CPT-11 lactone and carboxylate forms at 60 mg/kg once a day for 4 days in rats. It was observed that acute diarrheal symptoms with watery diarrhea occurred within 1 h on the second or third day of CPT-11 administration with the lactone form in all rats (data not shown) and that delayed diarrhea symptoms occurred on the fourth to seventh day, and particularly the severe diarrhea was at its peak on the fifth day. On the other hand, dosing with the CPT-11 carboxylate form scarcely showed acute diarrhea symptoms (data not shown) and showed delayed diarrhea symptoms on the fourth or fifth day after the dose with only a slight diarrhea.

DISCUSSION

The present study showed that dosing with the CPT-11 carboxylate form markedly reduced the antitumor activity

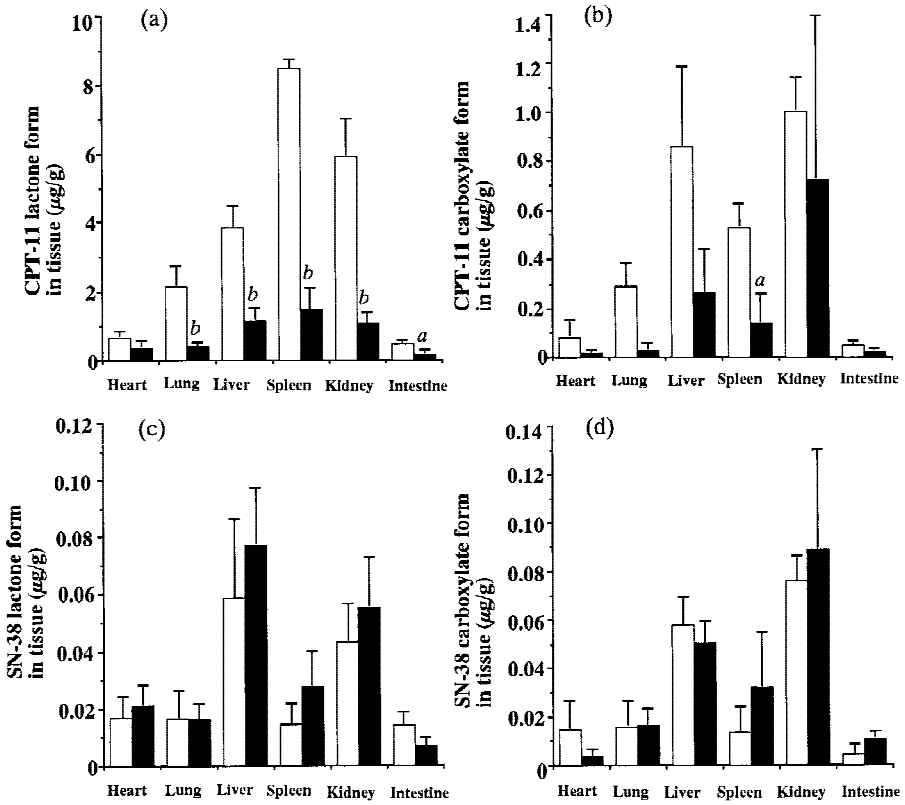

Fig. 4. Distribution of CPT-11 lactone (a), CPT-11 carboxylate (b), SN-38 lactone (c), and SN-38 carboxylate (d) at 4 h after i.v. doses of CPT-11 lactone form (open columns) and CPT-11 carboxylate form (filled columns) at a dose of 10 mg/kg in rats. Each value represents the mean ±SEM of 4 rats. (a) $P<0.05$, (b) $P<0.01$ (i.v. lactone form vs. i.v. carboxylate form).

and the toxicity expected by dosing CPT-11. The antitumor activities of the CPT-11 carboxylate form are found to be weaker than those of the lactone form *in vitro* (5). Our present study suggests that the reduced pharmacological activities may be due to not only the difference in the antitumor activity between the lactone and carboxylate forms but also the difference in the pharmacokinetic behaviors of the forms. CPT-11 lactone, after dosing with the CPT-11 carboxylate

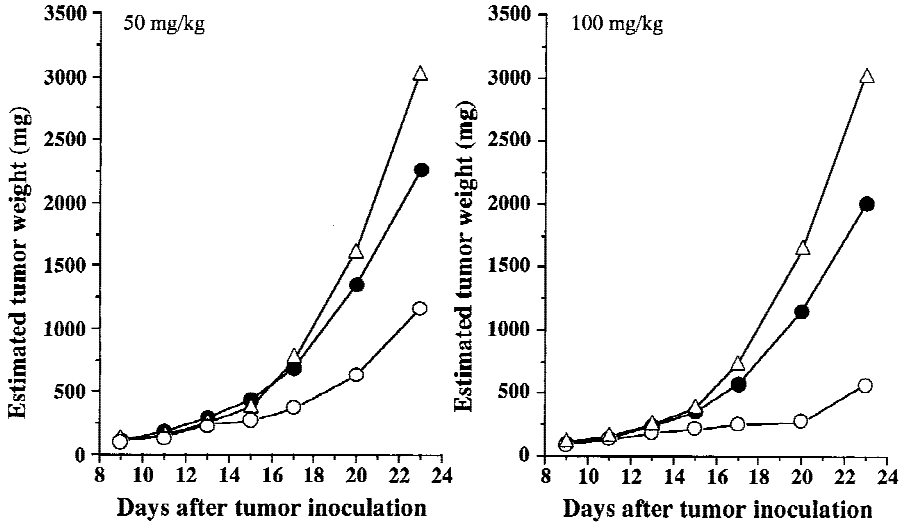

Fig. 5. Change in tumor weight after i.v. doses of CPT-11 lactone form (open circles) and CPT-11 carboxylate form (filled circles) with a schedule of every other day for a total of four treatments (q2d×4) from the ninth day after Meth A tumor inoculation in mice. Open triangles: control (no dose of CPT-11); open circles: i.v. dose of CPT-11 lactone form (50 or 100 mg/kg, q2d×4); closed circles: i.v. dose of CPT-11 carboxylate form (50 or 100 mg/kg, q2d×4). Each point represents the mean of seven mice.

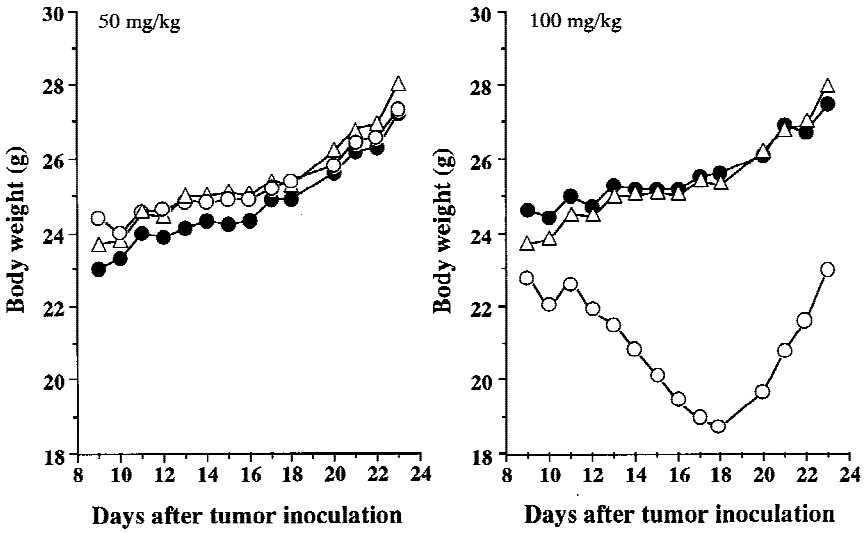

Fig. 6. Change in body weight after i.v. doses of CPT-11 lactone form (open circles) and CPT-11 carboxylate form (filled circles) with a schedule of every other day for a total of four treatments (q2d×4) from the ninth day after Meth A tumor inoculation in mice. Open triangles: control (no dose of CPT-11); open circles: i.v. dose of CPT-11 lactone form (50 or 100 mg/kg, q2d×4); closed circles: i.v. dose of CPT-11 carboxylate form (50 or 100 mg/kg, q2d×4). Each point represents the mean of seven mice.

form, maintained lower serum levels than those after dosing with the lactone form (Fig. 1, Table I).

The lower serum levels of CPT-11 lactone after the dose of the carboxylate form probably would be due to the slower conversion rate of CPT-11 carboxylate to the lactone compared with that of CPT-11 lactone to the carboxylate in the physiological conditions. At physiological pH, the equilibrium favors hydrolysis to open the lactone ring and yield the carboxylate form (3,4). Furthermore, the lactonization reaction of CPT-11 carboxylate is more than 10-fold slower than that of the hydrolysis reaction of the lactone at physiological pH (4). Therefore, when CPT-11 is administered as the carboxylate form *in vivo,* it is in an unfavorable environment to convert from the carboxylate to the lactone form.

Another explanation for the lowered serum levels of both CPT-11 lactone and carboxylate after dosing with the CPT-11 carboxylate form may be due to the increased biliary excretion of CPT-11 (Fig. 2). The excretion of CPT-11 lactone and carboxylate into bile after dosing with the CPT-11 carboxylate form were significantly greater than those after dosing with CPT-11 lactone. Chu *et al.* (17,18) reported that the carboxylate forms of both CPT-11 and SN-38 had a high affinity for the canalicular multispecific organic anion transporter (cMOAT) and that cMOAT is responsible for the biliary excretion of their carboxylate forms in rats. Our results also indicated that the carboxylate forms were appreciably excreted into bile. Thus, the administration of the CPT-11 carboxylate form may accelerate the active excretion of the carboxylate forms into bile by cMOAT before converting to the lactone.

In contrast to the biliary excretion, exsorption of CPT-11 lactone and carboxylate from blood via intestinal membrane was greater in dosing with the lactone form than in dosing with the carboxylate form (Fig. 3). Most drugs are exsorbed into the gastrointestinal lumen according to concentration

Table II. Antitumor Effect of CPT-11 Lactone and Carboxylate Forms Against Meth A Tumors in Mice

Compound	Total dose (mg/kg)	Tumor weight[a] Mean ± SEM (g)	IR (%)	BWLmax[b] (%) [day]	D/U[c]
Control	—	2.85 ± 0.25	0	<0	0/7
i.v. CPT-11 lactone	50 × 4	1.47 ± 0.26[d,e]	48	1.5 [10]	0/7
	100 × 4	0.687 ± 0.18[f,g]	76	18.1 [18]	0/7
i.v. CPT-11 carboxylate	50 × 4	2.61 ± 0.27	8	<0	0/7
	100 × 4	1.96 ± 0.22[h]	31	0.9 [10]	0/7

[a] Meth A cells were inoculated subcutaneously into BALB/s mice (day 0). Mice received i.v. injections with a schedule of every other day for a total of four treatments (q2dx4) beginning from day 9. Tumor weight was assessed on day 23.

[b] Maximum rate of body weight loss, numbers in parentheses denote the day. <0 indicates no body weight loss.

[c] Number of mice that died of toxicity/number of mice used. Significantly different from control (h $P < 0.05$, d $P < 0.01$, f $P < 0.001$; Dunnett's test). Significantly different from pH 8.0 group (e $P < 0.05$, g $P < 0.01$; Tukey-Kramer test).

Table III. Incidence of Diarrheal Symptoms Caused by CPT-11 (60 mg/kg) in Rats

	Diarrheal score[a]																			
	Day 4					Day 5					Day 6					Day 7				
Group	0	1	2	3	(mean)	0	1	2	3	(mean)	0	1	2	3	(mean)	0	1	2	3	(mean)
Control	4	0	0	0	(0.0)	4	0	0	0	(0.0)	4	0	0	0	(0.0)	4	0	0	0	(0.0)
i.v. CPT-11																				
lactone	3	4	0	0	(0.6)[b]*	0	1	3	3	(2.3)[†,¶]	0	3	3	1	(1.7)[‡]	4	3	0	0	(0.4)[b‡]
carboxylate	6	1	0	0	(0.1)	5	2	0	0	(0.3)	7	0	0	0	(0.0)	7	0	0	0	(0.0)

[a] Diarrheal score was defined as follows: 0, no diarrhea; 1, slight diarrhea; 2, moderate diarrhea; 3, severe diarrhea.
[b] Significantly different between control and i.v. lactone groups *$P < 0.05$; †$P < 0.005$; Fisher's PLSD test). Significantly different between i.v. lactone and carboxylate groups ‡$P < 0.05$, ¶$P < 0.005$; Fisher's PLSD test).

gradients between the blood and gastrointestinal lumen by passive diffusion (19). Therefore, the high serum concentrations of CPT-11 lactone after dosing with the CPT-11 lactone form could result in the great exsorption compared with the CPT-11 carboxylate form. CPT-11 carboxylate was also more exsorbed in dosing with CPT-11 lactone form than in dosing with the carboxylate form, although CPT-11 carboxylate in the serum maintained its low serum levels after dosing with the lactone form. This may be explained due to a favorable interconversion of the CPT-11 lactone form to its carboxylate form during the exsorption across the intestinal membrane. That is, there is a dynamic and pH-dependent equilibrium between the lactone and carboxylate forms *in vivo*. It is considered that CPT-11 lactone exsorbing across the membrane would be probably interconverted to its carboxylate in physiological pH. The exsorption of SN-38 lactone, which has a potent antitumor activity, tended to be greater in dosing with the CPT-11 lactone form than in dosing with the carboxylate form.

Although passive diffusion is generally an important mechanism of exsorption for most compounds, some are subject to specialized transport mechanisms. Recent studies indicate that P-glycoprotein is involved in the active secretion of anticancer drugs such as etoposide (20) and vinblastine (21). CPT-11 and SN-38 also have been considered to be substrates of P-glycoprotein and to have moderate affinity to P-glycoprotein (22,23). Accordingly, P-glycoprotein may contribute in part to the exsorption of both CPT-11 and SN-38. Further studies are required to confirm the contribution of P-glycoprotein to the exsorption via intestinal membrane.

Thus, in the case of dosing with the CPT-11 lactone form, both CPT-11 and SN-38 were found to be transported into gastrointestinal lumen not only via the biliary route but also via intestinal membrane. On the other hand, in dosing with the CPT-11 carboxylate form, the transport of both CPT-11 and SN-38 into the gastrointestinal lumen was exclusively responsible for via biliary route but little via intestinal membrane. These results seem to have a close relationship with gastrointestinal toxicity including the severe diarrhea induced by CPT-11. The mechanism of the delayed diarrhea is considered to be as follows. CPT-11 is metabolized in the liver by carboxylesterase to SN-38, which is further conjugated to SN-38-glucuronide and subsequently excreted in the intestine via bile. The SN-38-glucuronide excreted in the gut is hydrolyzed to SN-38 by enterobacterial β-glucuronidase and consequently impairs the gut (16). The present study suggests that the gastrointestinal impairment induced by CPT-11 may also occur as a consequence of the significant exsorption of CPT-11 via the intestinal membrane and subsequently of the high intracellular concentrations in the gut in addition to the previously mentioned mechanism. This may be supported by the fact that dosing with the CPT-11 carboxylate form poorly distributes to the intestine resulting only in slight diarrhea (Fig. 4, Table III).

The present study confirmed that the administration of CPT-11 in the form of carboxylate markedly reduced the antitumor activity and the toxicity in Meth A tumor mice. It has been conjectured that the strength of the antitumor activity between the lactone and carboxylate forms occurs because the antitumor activities of the carboxylate of CPT derivatives are more decreased than those of the lactone (5). The present study suggests that the reduced antitumor and toxic effects accomplished by dosing with the CPT-11 carboxylate form could be attributed to lower accumulation in the tissues, including tumor cells, caused by the rapid elimination of the carboxylate in the body. In contrast, dosing with the CPT-11 lactone form is expected to keep up higher CPT-11 levels in the serum and tissue for a longer time than after dosing with the carboxylate form. The retained CPT-11 could be gradually converted to the active metabolite, SN-38, in the tumor tissues by carboxylesterase (24) and then exert its antitumor activity. This is probably one of the reasons why the lactone exhibits stronger antitumor effect than the carboxylate *in vivo*. Our results are considered to be important data to call for the reasonable use of CPT-11 in clinical practice.

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