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ORIGINAL ARTICLE

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Alleviation of side effects induced by irinotecan hydrochloride (CPT-11) in rats by intravenous infusion

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Abstract *Purpose*: Irinotecan hydrochloride (CPT-11) is a potent topoisomerase I inhibitor and is established and used widely as an antitumor agent. However, it sometimes causes severe side effects such as myelosuppression and diarrhea. These dose-limiting toxicities prevent the adoption of CPT-11 in aggressive chemotherapy. Thus we sought to determine in a rat model whether extending the period of infusion of CPT-11 would ameliorate the adverse reactions. *Methods*: CPT-11 was administered intravenously (i.v.) to rats at a dose of 60 mg/kg per day for four consecutive days as a bolus injection or as 3-, 8- or 24-h infusions, and then blood cell counts and the incidence of acute and delayed-onset diarrhea were monitored. *Results*: Serious acute and delayed-onset diarrhea and marked decreases in the number of neutrophils and lymphocytes were observed in the bolus injection group. These symptoms were alleviated in the infusion groups with the degree of alleviation dependent on infusion time. In the bolus injection group, mucosal impairment of the cecal epithelium including wall thickening, edema, a decrease in the number and size of crypts, and the formation of a pseudomembrane-like substance was observed, whereas these changes were less severe in the infusion groups. Areas under the plasma concentration-time curves (AUC_{pla}) of CPT-11 and its metabolite, 7-ethyl-10-hydroxycamptothecin (SN-38), differed little among the bolus injection group, and the 3-h and 8-h infusion groups. However, the AUC_{pla} values of CPT-11 and SN-38 were significantly decreased and increased, respectively, in the 24-h infusion group. The maximum plasma concentrations (C_{max}) of CPT-11 decreased with increasing infusion time, but those of SN-38 did not. *Conclusions*: It was confirmed that the side effects of CPT-11 were alleviated by extending the infusion time. The pharmacokinetic parameters suggested that the C_{max} of CPT-11 is closely related to the incidence and severity of adverse reactions such as myelosuppression and acute and delayed-onset diarrhea.

Keywords Irinotecan hydrochloride · CPT-11 · SN-38 · Myelosuppression · Acute diarrhea · Delayed-onset diarrhea

Abbreviations AUC_{cec} Area under the cecal tissue concentration-time curve · AUC_{mar} Area under the bone marrow tissue concentration-time curve · AUC_{pla} Area under the plasma concentration-time curve · C_{max} Maximum concentration · CL_{tot} Total clearance · *CPT* Camptothecin · *CPT-11* Irinotecan hydrochloride [7-ethyl-10-(4-(piperidino)-1-piperidino) carbonyloxycamptothecin] · *G-CSF* Granulocyte colony-stimulating factor · *HPLC* High-performance liquid chromatography · i.v. Intravenous(ly) · *MRT* Mean residence time · *SN-38* 7-Ethyl-10-hydroxy-camptothecin · *SN-38G* SN-38 glucuronide · $T_{1/2}$ Half-life · *UGT* UDP-glucuronosyltransferase

Introduction

Irinotecan hydrochloride (CPT-11), a water-soluble derivative of camptothecin (CPT) [14, 28], is used clinically to treat colorectal, gastric, lung, uterine cervical and ovarian cancers, malignant lymphoma and other malignancies [12, 38, 46, 47, 52]. However, at high dosages, CPT-11 sometimes causes severe side effects such as diarrhea and myelosuppression. These dose-limiting toxicities prevent the adoption of CPT-11 in aggressive chemotherapy [1, 12, 34, 37, 40, 46]. Myelosuppression, especially neutropenia, is frequently observed in patients who have received CPT-11 chemotherapy. Granulocyte colony-stimulating factor

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(G-CSF) is usually used to prevent and treat life-threatening neutropenia and makes the dose-escalation of CPT-11 possible [2, 9, 11, 35].

CPT-11 induces both acute and delayed-onset diarrhea [42]. In acute diarrhea, it is assumed that the cholinergic activity of CPT-11 stimulates the intestinal contractility and disturbs normal intestinal mucosal absorptive and secretory functions [15, 23, 48]. This diarrhea is short-lasting and can be prevented and rapidly suppressed with atropine [15, 42]. In contrast, delayed-onset diarrhea is so severe that it can be life-threatening. Moreover, it remains unpredictable despite many pharmacokinetic studies [18, 27, 37, 43]. The great interpatient variability in both the severity of the diarrhea and the effect of antidiarrheal agents such as loperamide makes it difficult to elucidate the mechanism of this diarrhea [1, 12, 17, 34, 42, 46]. A possible explanation for delayed-onset diarrhea is that the mitotic activity of the active metabolite of CPT-11, 7-ethyl-10-hydroxycamptothecin (SN-38), which is much more cytotoxic than CPT-11, damages the gastrointestinal epithelium both structurally and functionally [18, 19, 24, 26, 48, 49, 50]. The pharmacokinetics of SN-38 are therefore considered to be mainly responsible for the induction of the diarrhea.

In our previous study using a rat model [30], acute and delayed-onset diarrhea induced with CPT-11 were alleviated by modification of the administration schedule from an injection of 60 mg/kg once daily for four consecutive days to 30 mg/kg twice daily. The areas under the plasma and cecal tissue concentration-time curves (AUC_{pla} and AUC_{cec}) of CPT-11, AUC_{pla} of SN-38, the maximum plasma concentrations (C_{max}) of SN-38 and the biliary excretions of CPT-11 and SN-38 were similar between the two schedules. Moreover, the C_{max} of CPT-11 was significantly lower and AUC_{cec} of SN-38 was a little larger in the latter schedule than the former. These results suggest that the C_{max} of CPT-11 is closely related to the incidence and severity of delayed-onset diarrhea induced by CPT-11. In order to investigate this possibility, in this study, we sought to determine in a rat model whether extending the period of infusion of CPT-11 would ameliorate the adverse reactions, acute and delayed-onset diarrhea and myelosuppression.

Materials and methods

Materials

CPT-11 (lot 115126), SN-38 (lot 30091R) and SN-38 glucuronide (SN-38G; lot 970326) were provided by Yakult Honsha Company (Tokyo, Japan). CPT was purchased from Sigma Chemical Co. (St. Louis, Mo.). Sodium 1-decanesulfonate was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). The water used was of Milli-Q grade (Millipore Company, Bedford, Mass.), and all other chemicals were of analytical or HPLC grade and were obtained from commercial sources.

Animals

Male Sprague-Dawley rats were purchased from Japan SLC (Hamamatsu, Japan) and used for experiments after a 1-week acclimatization with free access to water and commercial animal chow (F-2; Funabashi Farm, Funabashi, Japan). Rats weighing 185–250 g were used in all experiments.

Monitoring of CPT-11-induced adverse reactions

Animals were cannulated in the right cervical vein (Intramedic PE-50; Clay Adams, Parsippany, N.J.) under light anesthesia, and then placed in free-movement cannulation cages (Tsumura, Tokyo, Japan) with free access to an ordinary diet and water. CPT-11 was administered 3 days later via the cervical vein cannula at a dose of 60 mg/kg per day for four consecutive days (days 1–4) as a bolus injection, or as a 3-h, 8-h or 24-h infusion via a syringe pump (Harvard Apparatus, Holliston, Mass.). Each infusion was started at 10:00 a.m. Body weight changes and the severity of diarrhea were monitored throughout the experimental period (11 days from the first administration). Diarrhea observed during and for 3 h after infusion was defined as acute diarrhea. That observed more than 3 h from the end of infusion was defined as delayed-onset diarrhea. The severity of the diarrhea was scored as follows: 0 (normal—normal stools or absent), 1 (slight—wet and soft stools), 2 (moderate—wet and unformed stools with moderate perianal staining of the coat), and 3 (severe—watery stools with severe perianal staining of the coat). Blood (0.5 ml) was collected via the left cervical vein before cannulation and on days 5, 7, 9 and 11, and each type of blood cell was counted with a Technicon HE1 hematological diagnostic device (Bayer Corporation, N.Y.).

Histological experiments

Intestinal tissues (ileum, cecum and colon) were extirpated after exsanguination on day 5 and fixed in 10% neutral buffered formaldehyde. Tissue samples were processed for histology by preparing slides from segments embedded in paraffin wax. The slides were stained with hematoxylin-eosin for light microscopy.

Pharmacokinetic experiments

Animals were cannulated in the right femoral vein and the left femoral artery (Intramedic PE-50) or the bile duct (Intramedic PE-10) under light ether anesthesia. They were kept in Bollman cages after cannulation and had free access to an ordinary diet and water. After the animals had completely recovered from the anesthesia, CPT-11 was administered via the right femoral vein cannula at a dose of 60 mg/kg as a bolus injection or as 3-, 8- or 24-h infusions via a syringe pump, and the treatment was followed by flushing with physiological saline. Blood (200 μl) was collected from the left femoral artery cannula at 2, 5, 10 and 30 min, and at 1, 2, 3, 4, 6, 9, 12, 15, 24 h (3-h infusion), at 1, 3, 6, 7, 8, 9, 12, 15, 24 h (8-h infusion) or at 1, 3, 6, 9, 12, 15, 24, 27, 30 h (24-h infusion) after the start of infusion. Bile was collected for 24 h (30 h for the 24-h infusion) after the start of infusion and kept on ice. We confirmed that CPT-11 and the two metabolites were stable under these conditions.

In different experiments, animals were cannulated in the right cervical vein (Intramedic PE-50) under light anesthesia, and then placed in free-movement cannulation cages with free access to an ordinary diet and water. The next day, CPT-11 was administered via the cervical vein cannula at a dose of 60 mg/kg as a bolus injection or as 3-, 8- or 24-h infusions via a syringe pump, and the animals were killed by exsanguination at 1, 3, 8, 12 and 24 h (as

well as 30 and 36 h in the 24-h infusion group) after the start of infusion, and their bone marrow and cecum extirpated.

Sample preparation

The plasma was separated immediately after sampling, diluted fivefold with 0.146 *M* H_3PO_4, and then added to an equal volume of the internal standard (IS) solution (0.146 *M* H_3PO_4 containing 1 μg/ml of CPT as IS). The bile was diluted 500-fold with Milli-Q water and then added to an equal volume of IS solution. The cecal tissue was rinsed until clear with cold physiological saline, blotted and weighed. It was then homogenized with a tenfold volume of cold 10% IS solution/methanol on ice with a Teflon homogenizer. The bone marrow was collected from the femur by centrifugation and weighed. It was then homogenized with a tenfold volume of cold 10% IS solution/methanol on ice with a sonicator (frequency 20 KHz, amplitude 250 μm, output 10 W, 1 min; Model 450 Sonifier, Branson Ultrasonics Company, Danbury, Ct.). The homogenates were centrifuged at 15,000 rpm for 5 min, and the supernatants were diluted fivefold with 0.146 *M* H_3PO_4 and analyzed.

Determination of CPT-11, SN-38 and SN-38G

A previously reported HPLC method with a fully automated online solid-phase extraction system (PROSPEKT; Spark Holland, Emmen, The Netherlands) [29] was used. Briefly, 100, 100, 500 and 500 μl of the plasma, bile, bone marrow tissue and cecal tissue samples, respectively, were used for the solid-phase extraction with a Bond Elut C18 cartridge (Spark Holland). A Symmetry C18 reversed-phase column (150×4.6 mm ID, 5 μm; Waters, Milford, Mass.) was used at 50°C for chromatography. The fluorescence detector (470 scanning fluorescence detector; Waters) was set at 373 and 428 nm (excitation and emission, respectively) for 0–2.7 min, at 380 and 540 nm for 2.7–3.8 min, and at 373 and 428 nm for 3.8–8.5 min. The mobile phase consisted of 0.05 *M* KH_2PO_4/acetonitrile (70:30, v/v) containing 4 m*M* sodium 1-decanesulfonate (pH 3.5 with H_3PO_4) and the flow rate was 1.5 ml/min. The quantification limits of CPT-11, SN-38 and SN-38G were 5, 2.5 and 2.5 ng/ml for plasma, 0.5, 0.25 and 0.25 μg/ml for bile, 10, 2 and 2 ng/g for bone marrow, and 100, 2 and 2 ng/g for cecal tissue, respectively.

Pharmacokinetic analysis

Plasma and tissue concentration-time curves were analyzed using non-compartmental models. The areas under the plasma and the cecal tissue concentration-time curves (AUC_{pla} and AUC_{cec}, respectively) were calculated by the trapezoidal rule with estimations of the AUC from the last sampling time to infinity using Eq. 1:

$$\int_{last}^{\infty} C\,dt = C_{last}/\text{last log linear phase slope} \quad (1)$$

where C_{last} is the concentration at the last sampling time. Total clearance (CL_{tot}) was calculated as dose/AUC_{pla}, and mean resident time (MRT) as [(AUMC/AUC)−(infusion time/2)], where AUMC (area under the moment curve) = $\int_0^{\infty} tCdt$.

Statistical analysis

The results were analyzed by ANOVA. Differences were considered significant based on Dunnett's multiple comparison test at $P < 0.05$, except for diarrhea scores, which were analyzed using Wilcoxon's rank sum test.

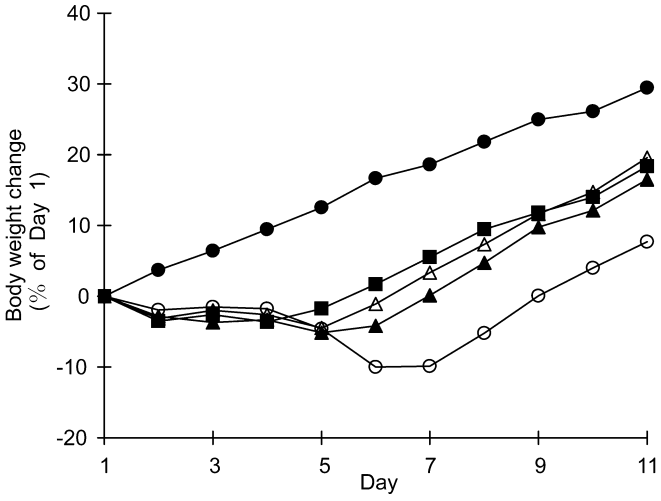

Fig. 1 Body weight changes after i.v. administration of CPT-11 at a daily dose of 60 mg/kg for four consecutive days (days 1–4) to rats (● control, physiological saline 3 ml/kg, bolus injection; ○ bolus injection; ▲ 3-h infusion; △ 8-h infusion; ■ 24-h infusion). The values are the means from six (control), seven (3-h infusion) and eight (bolus injection, 8-h infusion and 24-h infusion) rats

Results

Influence of CPT-11 infusion on body weight, incidence of myelosuppression and diarrhea

CPT-11 was administered i.v. to rats at a daily dose of 60 mg/kg as either a bolus injection or 3-, 8- or 24-h infusions for four consecutive days. In the bolus injection group, body weights decreased and reached a nadir on day 6 or 7. On the other hand, although the decreases in body weight in the infusion groups were similar to those in the bolus injection group during the periods of administration, recovery was more rapid than in the infusion groups (Fig. 1).

As myelosuppression was induced by the treatment, erythrocyte, lymphocyte and neutrophil numbers transiently decreased (Fig. 2). The decrease in the neutrophil count was serious, but in the infusion groups, it was ameliorated, the degree of amelioration being dependent on infusion time, to levels similar to the erythrocyte and lymphocyte counts. A transient decrease in the number of platelets was observed only in the bolus injection group. Serious acute and delayed-onset diarrhea were induced in the bolus injection group, but in the infusion groups the symptoms were alleviated, the degree of alleviation being dependent on infusion time such that symptoms were hardly observed in the 24-h infusion group (Tables 1 and 2).

Marked pathological differences were induced in cecal tissues by CPT-11 administration (Fig. 3 and Table 3). In the bolus injection group, macroscopic wall thickening and the formation of pseudomembrane-like substances consisting of fibrin, cell debris and enterobacteria on the mucosa occurred in some rats, and

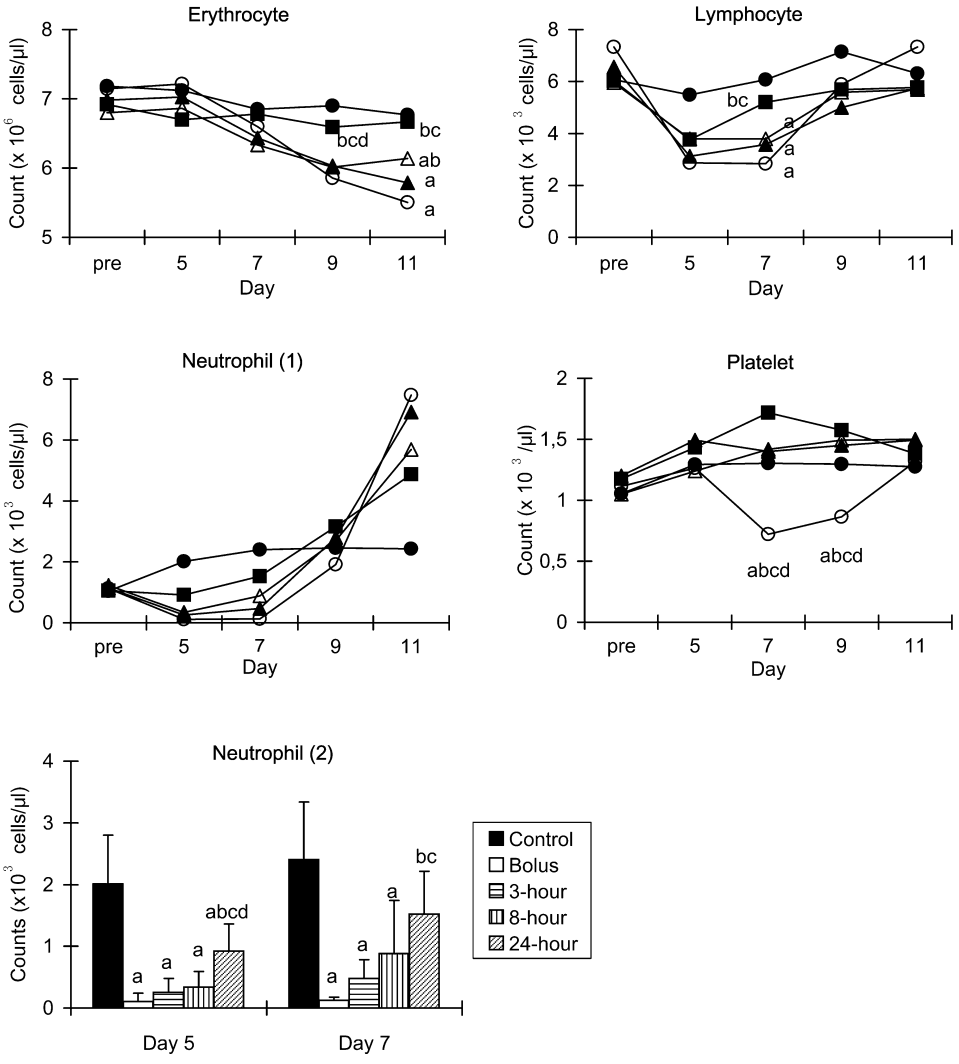

Fig. 2 Changes in the counts of erythrocytes, lymphocytes, neutrophils and platelets after i.v. administration of CPT-11 at a daily dose of 60 mg/kg for four consecutive days (days 1–4) to rats (● control, physiological saline 3 ml/kg, bolus injection; ○ bolus injection; ▲ 3-h infusion; △ 8-h infusion; ■ 24-h infusion). The graph *Neutrophil (2)* indicates the neutrophil counts on days 5 and 7. The values are the means from six (control), seven (3-h infusion) and eight (bolus injection, 8-h infusion and 24-h infusion) rats. *a, b, c, d* The mean was significantly different from that of the control (*a*), bolus injection (*b*), 3-h infusion (*c*) or 8-h infusion (*d*)

Table 1 Incidence of acute diarrhea after i.v. administration of CPT-11 at a daily dose of 60 mg/kg for four consecutive days (days 1–4) to rats. The diarrheal score was determined as follows: 0, normal; 1, slight; 2, moderate; 3, severe. The values are the number of animals with each score

Treatment group	*n*	Diarrheal score																			
		Day 1					Day 2					Day 3					Day 4				
		0	1	2	3	Mean	0	1	2	3	Mean	0	1	2	3	Mean	0	1	2	3	Mean
Control[a]	6	6				0.0	6				0.0	6				0.0	6				0.0
Bolus injection	8	2	2	4		1.3	4	3	1		0.6		1	3	4	2.4				8	3.0
Infusion																					
3 h	7	5		2		0.6	5	2			0.3	5	2			0.3*	5		2		0.6*
8 h	8	5	2	1		0.5	8				0.0*	8				0.0*	8				0.0*
24 h	8	7	1			0.1*	8				0.0*	8				0.0*	8				0.0*

*$P < 0.05$ vs bolus injection
[a]Physiological saline 3 ml/kg, bolus injection

microscopic edema in the submucosa, a decrease in crypt number and size, and a marked increase in the number of crypts covered with flattened epithelial cells were observed in all animals. These impairments, however, were mild in the infusion groups.

Pharmacokinetics of CPT-11 and its metabolites

The CPT-11 plasma concentration in the bolus injection group decreased biexponentially (Fig. 4). In the infusion groups, it reached a maximum during the infusion and

Table 2 Incidence of delayed-onset diarrhea after i.v. administration of CPT-11 at a daily dose of 60 mg/kg for four consecutive days (days 1–4) to rats. The diarrheal score was determined as follows: 0, normal; 1, slight; 2, moderate; 3, severe. The values are the number of animals with each score

Treatment group	*n*	Diarrheal score														
		Day 5					Day 6					Day 7				
		0	1	2	3	Mean	0	1	2	3	Mean	0	1	2	3	Mean
Control[a]	6	6				0.0	6				0.0	6				0.0
Bolus injection	8			2	6	2.8		2	4	2	2.0		6	2		1.3
Infusion																
3 h	7	1	3	3		1.3*	3	3	1		0.7*	3	4			0.6
8 h	8	4	4			0.5*	4	4			0.5*	6	2			0.3*
24 h	8	6	2			0.3*,**	7	1			0.1*	7	1			0.1*

*$P < 0.05$ vs bolus injection; **$P < 0.05$ vs 3-h infusion
[a]Physiological saline 3 ml/kg, bolus injection

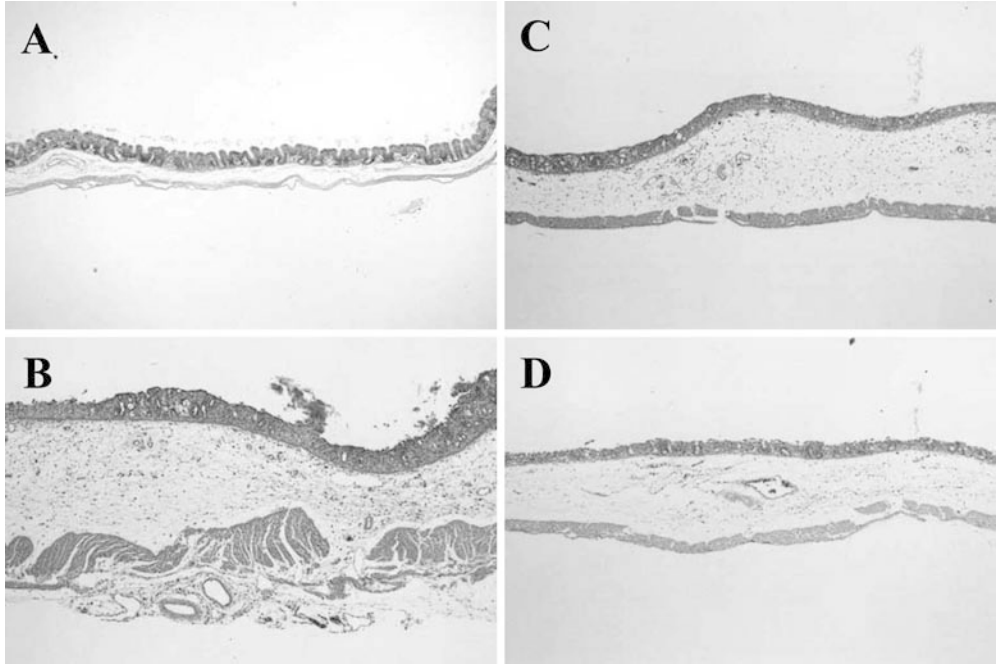

Fig. 3A–D Micrographs (×40) of cecal epithelium on day 5 after i.v. administration of CPT-11 at a daily dose of 60 mg/kg for four consecutive days (days 1–4) to rats. **A** Control (physiological saline 3 ml/kg/day, bolus injection); **B** bolus injection; **C** 3-h infusion; **D** 8-h infusion

Table 3 Histological findings in the cecal epithelium on day 5 after i.v. administration of CPT-11 at a daily dose of 60 mg/kg for four consecutive days (days 1–4) to rats. The scores were defined as follows: – no significant change, ± slight, + mild, + + moderate, + + + severe

Treatment group	*n*	Macroscopic																Microscopic											
		Wall thickening						Formation of a pseudomembrane-like substance						Hemorrhage				Degeneration of crypts						Edema					
		–	±	+	+ +	+ + +		–	±	+	+ +	+ + +		–	±	+	+ +	+ + +		–	±	+	+ +	+ + +		–	±	+	+ +	+ + +	
Bolus injection	6	4	1	1				5	1					6						6							1	1		4	
Infusion																															
3 h	5	5						4	1					5				1	1	2		1				2	2	1			
8 h	6	6						6						6				1	4			1			1	1	2	2			

decreased exponentially at the end of the infusion. The C_{max} of CPT-11 decreased with increasing infusion time (Table 4). The AUC_{pla}, MRT, CL_{tot} and $T_{1/2}$ of CPT-11 were similar among the bolus injection group, and the 3-h and 8-h infusion groups. However, the values of these parameters in the 24-h infusion group were different from those in the other groups. SN-38 plasma concentrations in all groups decreased immediately after

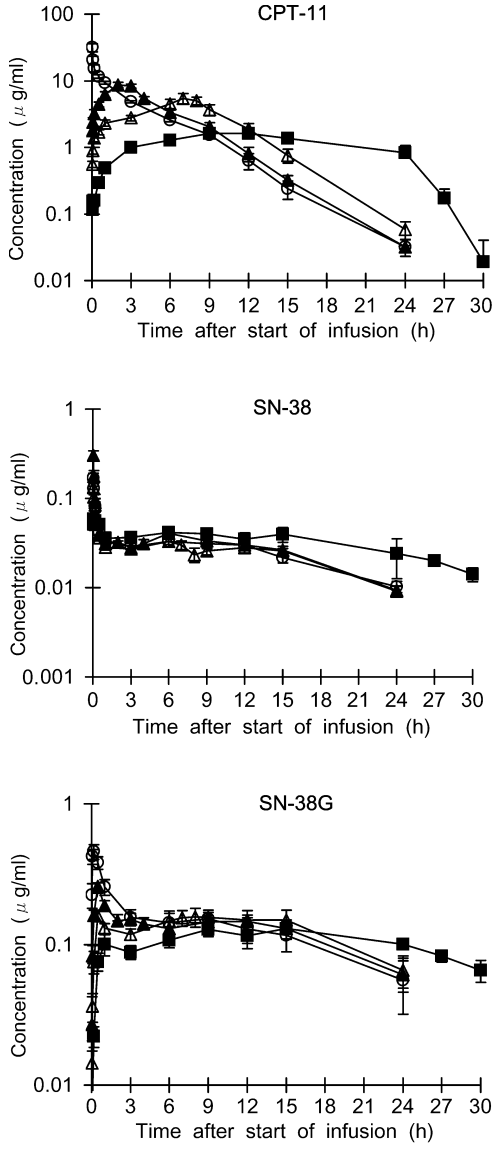

Fig. 4 Plasma concentration profiles of CPT-11, SN-38 and SN-38G after i.v. administration of CPT-11 at a dose of 60 mg/kg to rats (○ bolus injection, ▲ 3-h infusion, △ 8-h infusion, ■ 24-h infusion). The values are the means (±SD *bars*) from four rats

the start of administration, remained constant for several hours and then decreased gradually. The pharmacokinetic parameters of SN-38 were similar among the three groups except the 24-h infusion group. SN-38G C_{max} values decreased with increasing infusion time, but the values for AUC_{pla} and $T_{1/2}$ of SN-38G were similar among all groups.

CPT-11 was quickly excreted into the bile for several hours after the start of administration and then very gradually in the bolus injection group, and the 3-h and 8-h infusion groups (Fig. 5), but the rate of biliary excretion in the early stage declined dependent on the infusion time, and that in the 24-h infusion group was constant for 24 h. The cumulative excretion ratios of CPT-11 in bile were similar among the bolus injection group, and the 3-h and 8-h infusion groups, while that in the 24-h infusion group was significantly lower (Table 5). SN-38 and SN-38G had similar profiles of biliary excretion. The biliary excretion rates in the bolus injection group, and the 3-h and 8-h infusion groups were nearly constant for several hours after the start of CPT-11 administration and then decreased slightly. On the other hand, the rate in the 24-h infusion group remained constant for 30 h. Biliary cumulative excretion ratios of SN-38 and SN-38G did not differ among the bolus injection group, and the 3-h and 8-h infusion groups, although the values in the 24-h infusion group were relatively large.

CPT-11 concentrations in bone marrow reached a maximum at the end of infusion and then decreased exponentially in all groups (Fig. 6). The AUC_{mar} and C_{max} values of CPT-11 decreased with increasing infusion time (Table 6). SN-38 concentrations in bone

Table 4 Pharmacokinetic parameters after i.v. administration of CPT-11 at a dose of 60 mg/kg to rats. AUC was calculated from 0 to infinity. Values are the means ± SD from four rats

Metabolite	Parameter	Treatment group			
		Bolus injection	Infusion		
			3 h	8 h	24 h
CPT-11	AUC (μg·h/ml)	48.8 ± 2.8	50.2 ± 4.5	46.4 ± 6.6	30.0 ± 1.7*,**,***
	T_{max} (h)	0.033	2.00	7.25 ± 0.50	10.50 ± 1.73
	C_{max} (μg/ml)	31.69 ± 4.31	8.67 ± 0.75*	5.47 ± 0.96*	1.71 ± 0.10*,**
	MRT (h)	3.90 ± 0.28	3.36 ± 0.17	3.56 ± 0.29	0.89 ± 0.41*,**,***
	CL_{tot} (l/h/kg)	1.23 ± 0.07	1.20 ± 0.11	1.31 ± 0.16	2.01 ± 0.11*,**,***
	$T_{1/2}$ (h)	2.80 ± 0.15	2.66 ± 0.03	2.47 ± 0.18	1.14 ± 0.40*,**,***
SN-38	AUC (μg·h/ml)	0.73 ± 0.06	0.76 ± 0.06	0.68 ± 0.04	1.22 ± 0.20*,**,***
	T_{max} (h)	0.033	0.033	0.033	0.067 ± 0.067
	C_{max} (μg/ml)	0.169 ± 0.021	0.303 ± 0.039*	0.131 ± 0.005 [b]	0.059 ± 0.002*,**,***
	MRT (h)	9.87 ± 1.31	9.26 ± 1.11	9.89 ± 1.07	6.57 ± 1.81*c
	$T_{1/2}$ (h)	7.96 ± 0.95	6.90 ± 1.04	7.16 ± 1.20	10.86 ± 1.54*,**,***
SN-38G	AUC (μg·h/ml)	3.98 ± 1.02	3.84 ± 0.58	3.98 ± 0.82	4.35 ± 1.07
	T_{max} (h)	0.167	0.50	4.25 ± 4.33	13.50 ± 3.00
	C_{max} (μg/ml)	0.458 ± 0.056	0.260 ± 0.027*	0.164 ± 0.016*,**	0.132 ± 0.015*,**
	$T_{1/2}$ (h)	9.19 ± 1.99	9.25 ± 1.22	9.36 ± 1.13	8.26 ± 2.19

*$P < 0.05$ vs bolus injection
**$P < 0.05$ vs 3-h infusion
***$P < 0.05$ vs 8-h infusion

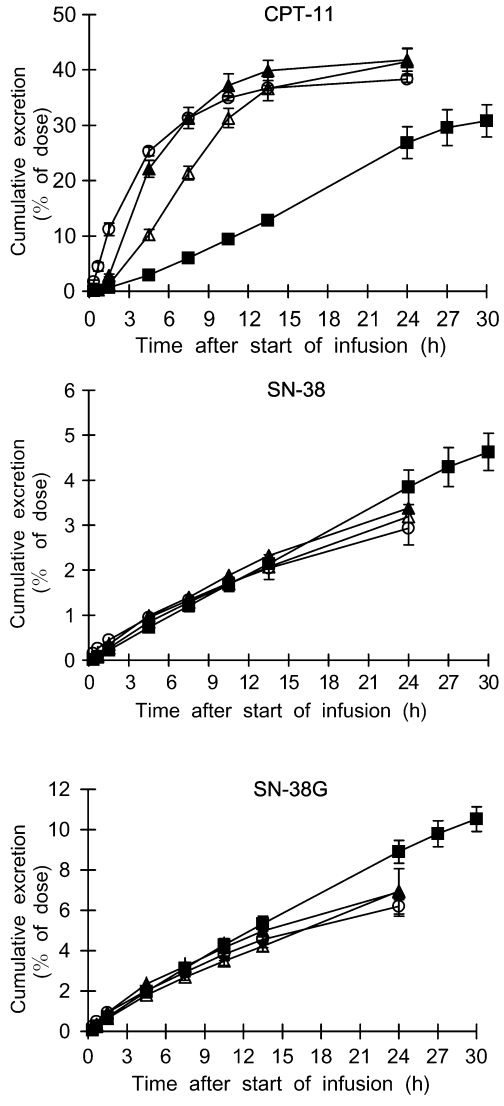

Fig. 5 Biliary excretion of CPT-11, SN-38 and SN-38G after i.v. administration of CPT-11 at a dose of 60 mg/kg to rats (○ bolus injection, ▲ 3-h infusion, △ 8-h infusion, ■ 24-h infusion). The values are the means (±SD *bars*) from four rats

marrow reached a maximum at 8 h (bolus injection group, and the 3-h and 8-h infusion groups) or 12 h (24-h infusion group) after the start of CPT-11 administration, and C_{max} values were similar. In the bolus injection group, and the 3-h and 8-h infusion groups, SN-38 concentrations at 24 h were below the limit of quantitation (2 ng/g tissue) and AUC values could not be calculated. Although the C_{max} values for the concentration of SN-38G in bone marrow were lower in the infusion groups than in the bolus injection group, AUC_{mar} values differed little among the groups.

CPT-11 concentrations in cecal tissues reached a maximum at the end of infusion, and then decreased exponentially in all groups (Fig. 7). The AUC_{cec} and C_{max} values of CPT-11 decreased with increasing infusion time (Table 7). SN-38 and SN-38G concentrations in cecal tissues peaked at 8 or 12 h after the start of CPT-11 administration and then decreased rapidly in the bolus injection group, and the 3-h and 8-h infusion groups. In the 24-h infusion group, the concentration of SN-38 remained constant for 36 h and that of SN-38G remained constant for 24 h and then decreased gradually. C_{max} values of SN-38 and SN-38G were similar among all groups, but AUC_{cec} values were significantly larger in the 24-h infusion group than the other groups.

Discussion

Clinically, CPT-11 can have severe side effects including diarrhea and myelosuppression, which limits the adoption of CPT-11 in aggressive therapy [1, 12, 34, 40, 46]. In the case of 5-fluorouracil, infusion time has been prolonged in an effort to maximize clinical efficacy and decrease toxicity. Thus, a similar approach has recently been adopted in CPT-11 chemotherapy [20, 37, 53]. However, it is difficult to assess the clinical advantage of the prolonged continuous infusion of CPT-11 because of interpatient variability. Therefore, we attempted to assess the alleviating effect of prolonged infusion on the adverse reactions to CPT-11 using a rat model.

The adverse reactions induced, including loss of body weight, decrease in neutrophil, lymphocyte, erythrocyte and platelet numbers, and acute and delayed-onset diarrhea, were alleviated, with the degree of alleviation being dependent on the infusion time.

Table 5 Biliary excretion after i.v. administration of CPT-11 at a dose of 60 mg/kg to rats. Bile was collected for 24 hours (bolus injection, 3-h infusion and 8-h infusion) or for 30 h (24-h infusion) from the start of infusion. Values are the means ± SD from four rats

Treatment group	Biliary excretion ratio (% of CPT-11 dose)			
	CPT-11	SN-38	SN-38G	Total
Bolus injection	38.37 ± 0.62	2.93 ± 0.37	6.21 ± 0.51	47.50 ± 0.56
Infusion				
3 h	41.84 ± 2.06	3.38 ± 0.42	6.91 ± 0.37	52.13 ± 2.23**
8 h	41.50 ± 2.31	3.19 ± 0.24	6.95 ± 0.37	50.98 ± 2.76
24 h	30.79 ± 2.94*	4.63 ± 0.42*	10.53 ± 0.61*	45.95 ± 2.60***

*$P < 0.05$ vs the other groups
**$P < 0.05$ vs the bolus injection group
***$P < 0.05$ vs both the 3-h infusion and the 8-h infusion groups

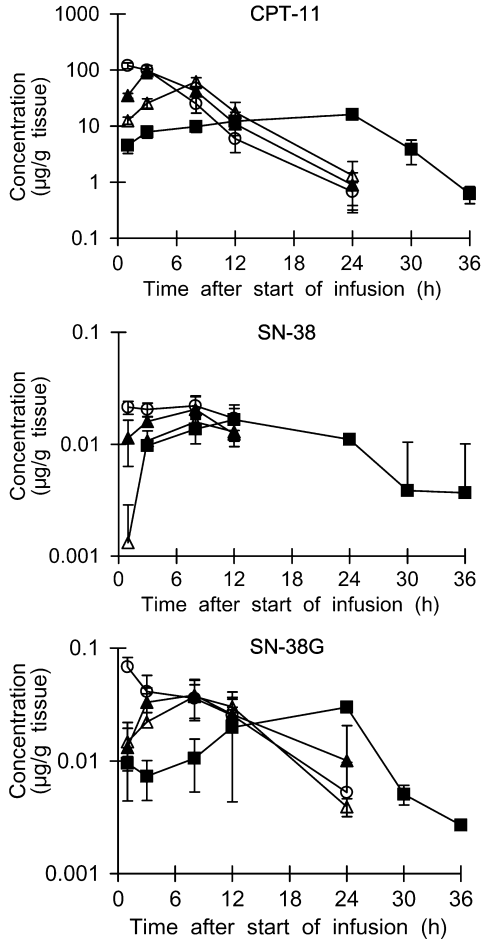

Fig. 6 CPT-11, SN-38 and SN-38G concentration profiles in bone marrow after i.v. administration of CPT-11 at a dose of 60 mg/kg to rats (○ bolus injection, ▲ 3-h infusion, △ 8-h infusion, ■ 24-h infusion). The values are the means (± SD *bars*) from four rats

Impairments in the cecal epithelium such as edema and degeneration of crypts were also ameliorated by infusion. There was little difference in the plasma concentrations and biliary excretion of CPT-11, SN-38 and SN-38G between the bolus injection group, and the 3-h and 8-h infusion groups, except for plasma CPT-11 C_{max}, which decreased with increasing the infusion time. The plasma concentration and biliary excretion of CPT-11 during several hours after the start of infusion greatly differed among the bolus injection group, and the 3-h and 8-h infusion groups. However, for SN-38 there was hardly any difference among the groups, probably because of the saturation of the enzymatic hydrolysis of CPT-11 to SN-38 by carboxylesterase [55].

In rats of all groups, the neutrophil and lymphocyte counts decreased significantly and reached a nadir on day 5 or 7 following administration of CPT-11 for four consecutive days, and then recovered rapidly. The degree of the decrease gradually improved with increasing infusion time, although exposure to CPT-11 and SN-38 in bone marrow was extended by infusion. CPT-11 concentrations were tenfold higher in bone marrow than in plasma, while SN-38 concentrations were lower. This indicates that CPT-11 was rapidly distributed into the bone marrow but SN-38 was not, and CPT-11 was hardly hydrolyzed to SN-38 at all in the bone marrow. CPT-11 concentrations were much higher in bone marrow than SN-38 concentrations. CPT-11 has DNA topoisomerase I-inhibitory activity, although it is a much weaker inhibitor than SN-38 [24, 26]. Consequently, it was considered that CPT-11, as well as SN-38, greatly contributed to the myelosuppression, and that the prolonged infusion of CPT-11 alleviated the adverse reaction caused by the decrease in CPT-11 C_{max} in the bone marrow, because the DNA topoisomerase I-inhibitory activity of CPT-11 depended on both the concentration and the duration of exposure [9].

CPT-11-induced acute and delayed-onset diarrhea and impairment in cecal epithelium were markedly alleviated by infusion. As it is assumed that acute diarrhea is induced by the cholinergic activity of CPT-11 [15, 23, 48], the decrease in CPT-11 plasma C_{max} by infusion might contribute to the prevention of diarrhea. Delayed-onset diarrhea is thought to be due to structural and functional damage to the gastrointestinal tract caused by the cytotoxicity of CPT-11 and SN-38 [18, 19, 48, 49, 50]. In the bolus injection group, and the 3-h and 8-h infusion groups, the profiles of

Table 6 AUC and C_{max} of CPT-11, SN-38 and SN-38G in bone marrow after i.v. administration of CPT-11 at a dose 60 mg/kg to rats. AUC was calculated for 24 h (bolus injection, 3-h infusion and 8-h infusion) or for 36 h (24-hour infusion). Each C_{max} value is the mean ± SD from three or four animals

Parameter	Metabolite	Treatment group			
		Bolus injection	Infusion		
			3 h	8 h	24 h
AUC (μg·h/g tissue)	CPT-11	634	606	466	333
	SN-38	_[a]	_[a]	_[a]	0.336
	SN-38G	0.607	0.554	0.477	0.525
C_{max} (μg/g tissue)	CPT-11	119.5 ± 14.2	95.5 ± 25.7	61.9 ± 11.3	16.0 ± 0.8
	SN-38	0.022 ± 0.004	0.020 ± 0.007	0.016 ± 0.001	0.016 ± 0.006
	SN-38G	0.068 ± 0.015	0.038 ± 0.014	0.037 ± 0.014	0.030 ± 0.002

[a]AUC values could not be calculated because the SN-38 concentration at 24 h was below the limit of quantitation

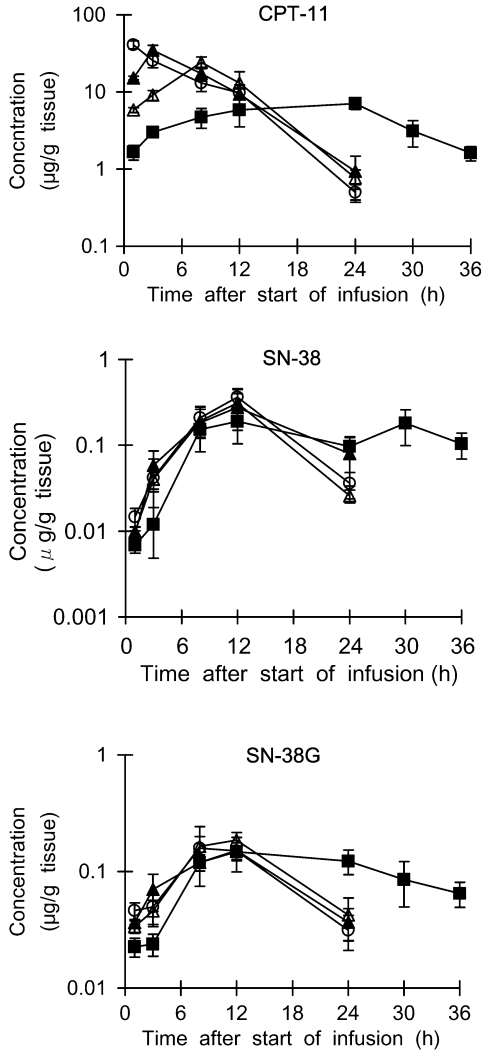

Fig. 7 CPT-11, SN-38 and SN-38G concentration profiles in cecal tissues after i.v. administration of CPT-11 at a dose of 60 mg/kg to rats (○ bolus injection, ▲ 3-h infusion, △ 8-h infusion, ■ 24-h infusion). The values are the means (±SD *bars*) from four rats

CPT-11 concentrations in cecal tissues were similar to those in plasma, and the C_{max} values decreased with increasing infusion time. On the other hand, the profiles of SN-38 concentrations in cecal tissues were quite different to those in plasma. SN-38 concentrations in cecal tissues exhibited similar profiles among the three groups, and the values of AUC_{cec} and C_{max} for SN-38 in cecal tissues were similar, perhaps because they were affected by accumulation of SN-38 via the conversion of CPT-11 by carboxylesterase in cecal tissue and the reabsorption from the intestinal lumen as well as distribution from the blood. These results suggest that not only exposure to SN-38 but also the C_{max} values of CPT-11 in cecal tissues contribute to the induction of delayed-onset diarrhea by CPT-11, and the prolonged infusion method alleviated the diarrheal symptoms by lowering these values [30].

CPT-11 has several pharmacologic effects as well as a cholinergic effect [15]. It induces a transient increase in prostaglandin E_2 (PGE_2) in intestinal tissue [22]. PGE_2 is secreted by the mucosa and smooth muscle of the small intestine [7], and induces diarrhea by stimulating colonic secretion and hyperperistalsis of the gut [5, 6]. Moreover, PGE_2 inhibits Na^+,K^+-ATPase, thereby affecting the absorption of electrolytes [31, 45]. In addition, CPT-11 induces Cl^- secretion in the subepithelial tissue by stimulating the production of eicosanoid, e.g. thromboxane A_2 [41]. These effects of CPT-11 may not only exacerbate the diarrheal symptoms and the impairment of cecal tissue but also alter the intestinal luminal environment and induce colonization by indigenous microflora and an overgrowth of pathogenic bacteria [56]. Adverse bacteria or their toxins may exacerbate the epithelial impairment caused by the bacterium-induced inflammation. The intestinal microflora appear to be involved in the onset of CPT-11-induced delayed-onset diarrhea because the coadministration of antibiotics ameliorates the diarrhea [50].

In the 24-h infusion group, the adverse reactions induced by CPT-11 were much milder than those in the other infusion groups. The AUC_{pla}, AUC_{mar}, AUC_{cec} and biliary excretion of CPT-11 were lower, and the AUC_{pla}, AUC_{cec} and biliary excretion of SN-38, and AUC_{cec} and biliary excretion of SN-38G were inversely higher in the 24-h infusion group than in the other infusion groups. It is considered that the low plasma concentration of CPT-11 on prolonged infusion decreased the transfer of CPT-11 to the myeloid, cecal and other tissues, and moreover prevented the saturation of metabolic and excretory mechanisms, e.g. carboxylesterase,

Table 7 AUC and C_{max} of CPT-11, SN-38 and SN-38G in cecal tissues after i.v. administration of CPT-11 at a dose 60 mg/kg to rats. AUC was calculated for 24 h (bolus injection, 3-h infusion and 8-h infusion) or for 36 h (24-h infusion). Each C_{max} value is the mean ± SD from three or four animals

Parameter	Metabolite	Treatment group			
		Bolus injection	Infusion		
			3 h	8 h	24 h
AUC (μg·h/g tissue)	CPT-11	265	282	223	178
	SN-38	3.59	4.19	3.02	5.42
	SN-38G	2.36	2.41	2.77	4.78
C_{max} (μg/g tissue)	CPT-11	40.97 ± 4.86	34.95 ± 5.09	24.29 ± 4.30	7.02 ± 1.07
	SN-38	0.365 ± 0.093	0.276 ± 0.126	0.311 ± 0.134	0.191 ± 0.086
	SN-38G	0.159 ± 0.084	0.152 ± 0.025	0.186 ± 0.031	0.147 ± 0.048

UDP-glucuronosyltransferase (UGT) and relative transporters, leading to a more efficient enzymatic conversion of CPT-11 to SN-38, consecutive glucuronidation to SN-38G and biliary excretion [21, 53].

We have demonstrated that twice-daily administration and prolonged infusion of CPT-11 alleviates the adverse reactions induced with CPT-11 in a rat model [29]. However, side effects have been observed in some patients given CPT-11 in similar schedules [21, 32, 53]. There may be great interpatient variability in the mechanism by which CPT-11 induces adverse reactions, especially delayed-onset diarrhea, because many factors are thought to be involved in the diarrhea, such as UGT activity [3, 4], concurrent chemotherapy and radiotherapy [8, 20, 33], microflora (β-glucuronidase activity and opportunistic infection) [22, 25, 49, 50], and cyclooxygenase-2 activity [8, 54]. Therefore, modification of the CPT-11 administration regimen would not be effective in controlling the adverse reactions on its own, but may alleviate side effects in combination with other strategies, for example, concomitant G-CSF [2, 10, 11, 35], neomycin [25], kampo medicine (hangeshasin-to) [22, 36, 49], or glutamine [44], and oral alkalization combined with control of defecation [51], and loperamide [17] or octreotide [39].

The antitumor activity of CPT-11 for each method of administration was not determined in this study. Based on the findings of Furuta and Yokokura [13], it was speculated that CPT-11 might completely inhibit tumor growth at the daily dose used in this study, and the antitumor activity could not be compared. Clinically, the prolonged infusion of CPT-11 exhibits antitumor activity [21, 53]. Because CPT-11 is an S-phase cell cycle-specific cytotoxic agent, prolonged low-dose exposure may be theoretically more effective than the equivalent AUC exposure in the commonly used administration schedules [16].

In conclusion, it was confirmed that CPT-11-induced side effects including myelosuppression and diarrhea were alleviated by infusion of CPT-11 in rats. We speculate that prolonged infusion of CPT-11 would be an excellent method to alleviate the adverse reactions and maximize the clinical efficacy of CPT-11.

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