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ORIGINAL ARTICLE

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Modified irinotecan hydrochloride (CPT-11) administration schedule improves induction of delayed-onset diarrhea in rats

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Abstract *Purpose*: Clinically, diarrhea is the major dose-limiting toxicity of irinotecan hydrochloride (CPT-11). Using a rat model, we attempted to decrease the incidence of delayed-onset diarrhea by modifying the administration schedule of CPT-11, and studied the pharmacokinetics in this model in relation to the incidence of diarrhea. *Methods*: CPT-11 (total dose, 240 mg/kg) was administered intravenously (i.v.) to rats according to various schedules, and the incidence of delayed-onset diarrhea was monitored. *Results*: Administration of CPT-11 at a dose of 60 mg/kg once daily for four consecutive days induced severe diarrhea, while at 30 mg/kg twice daily at an interval of 9 h (daily dose 60 mg/kg) for four consecutive days alleviated the diarrheal symptoms, and at 30 or 40 mg/kg once daily for eight or six consecutive days, respectively, diarrhea was hardly induced. With the first schedule, mucosal impairment of the cecal epithelium was observed, including wall thickening, edema, decrease in crypt number and size, and formation of pseudomembrane-like substance, whereas these changes were less severe with the second schedule and were hardly observed with the other two schedules. The areas under the plasma and cecal tissue concentration-time curves (AUC_{pla} and AUC_{cec}), the maximum plasma concentrations (C_{max}) and the biliary excretions of CPT-11 and its metabolites, 7-ethyl-10-hydroxycamptothecin (SN-38) and SN-38 glucuronide (SN-38G) in rats depended on the daily dose of CPT-11. Exceptionally, CPT-11 C_{max} was significantly lower and SN-38 AUC_{cec} was larger in the animals treated at 30 mg/kg twice daily than in those treated at 60 mg/kg once daily. *Conclusion*: These results suggested that the duration of exposure to both CPT-11 and SN-38 of the intestinal epithelium and CPT-11 plasma C_{max} are closely related to the incidence and severity of CPT-11-induced delayed-onset diarrhea in rats.

Key words Irinotecan hydrochloride · CPT-11 · SN-38 · Delayed-onset diarrhea · Pharmacokinetics

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Introduction

Irinotecan hydrochloride (CPT-11) is a water-soluble derivative of camptothecin (CPT) [12, 25] and is used clinically to treat colorectal, gastric, lung, uterine cervical and ovarian cancers, malignant lymphoma and other malignancies [10, 32, 39, 40, 46]. However, at higher dosage, CPT-11 sometimes causes severe diarrhea, which is recognized as the dose-limiting toxicity and limits the use of more aggressive CPT-11 therapy [1, 10, 28, 31, 33, 39]. The diarrhea is of two types, acute or delayed onset, either or both of which can occur [36].

It is assumed that in the acute diarrhea the cholinergic activity of CPT-11 stimulates intestinal contractility and disturbs normal intestinal mucosal absorptive and secretory functions [13, 21, 42]. This diarrhea is of short duration and can be prevented or rapidly suppressed by atropine [13, 36]. In contrast, delayed-onset diarrhea is so severe that it can be life-threatening. Moreover, it is unpredictable despite the many pharmacokinetic studies done on it [15, 24, 31, 37]. Antidiarrheal agents such as loperamide and acetorphan may be effective in treating this diarrhea, but they are not effective in controlling the symptoms in all patients [1, 14, 36]. The great interpatient variability in both the severity of the diarrhea and the effect of the antidiarrheal agents makes it difficult to elucidate the mechanism involved in producing this diarrhea [1, 10, 28, 39].

It is possible that the mitotic activity of CPT-11 and its active metabolite, 7-ethyl-10-hydroxycamptothecin (SN-38), damages the gastrointestinal tract both structurally and functionally [15, 16, 42, 43, 44]. The pharmacokinetics of SN-38 are considered to be the main cause of the induction of the diarrhea, as SN-38 is much

more cytotoxic than CPT-11 [22, 23]. The major metabolic pathway of CPT-11 is shown in Fig. 1. CPT-11 is hydrolyzed to SN-38 by carboxylesterase. Most of the SN-38 is subsequently conjugated to SN-38 glucuronide (SN-38G) by UDP-glucuronosyltransferase in the liver. SN-38G is excreted in the bile with the other major components, CPT-11 and SN-38 [3, 18]. Most of the SN-38G excreted in the bile is deconjugated to SN-38 by β-glucuronidase of the intestinal microflora [18, 45]. Inhibition of β-glucuronidase by coadministration of antibiotics decreases the SN-38 concentration in the intestinal tissue and alleviates diarrheal symptoms [44, 45]. Therefore, β-glucuronidase is considered to be an important factor in the induction of the diarrhea [35, 43, 44, 45, 46]. Delay in SN-38 disposition might exacerbate the diarrheal symptoms [15, 16].

We investigated the mechanisms of CPT-11-induced delayed-onset diarrhea using a rat model. As in humans, CPT-11 induces two types of diarrhea, acute and delayed-onset, in rats. Takasuna et al. [42] have reported that the conventional antidiarrheal agents, atropine, ondansetron, clonidine and morphine, can prevent the acute diarrhea, but exacerbate the delayed-onset diarrheal symptoms associated with intestinal mucosal disruption. This suggests that commonly used antidiarrheal agents are unsuitable for treating CPT-11-induced delayed-onset diarrhea [42, 43].

In this study, we attempted to reduce the incidence of delayed-onset diarrhea by modifying the CPT-11 administration schedule, and studied the relationship of the pharmacokinetics of CPT-11 and its metabolites to the incidence and the severity of delayed-onset diarrhea in rats.

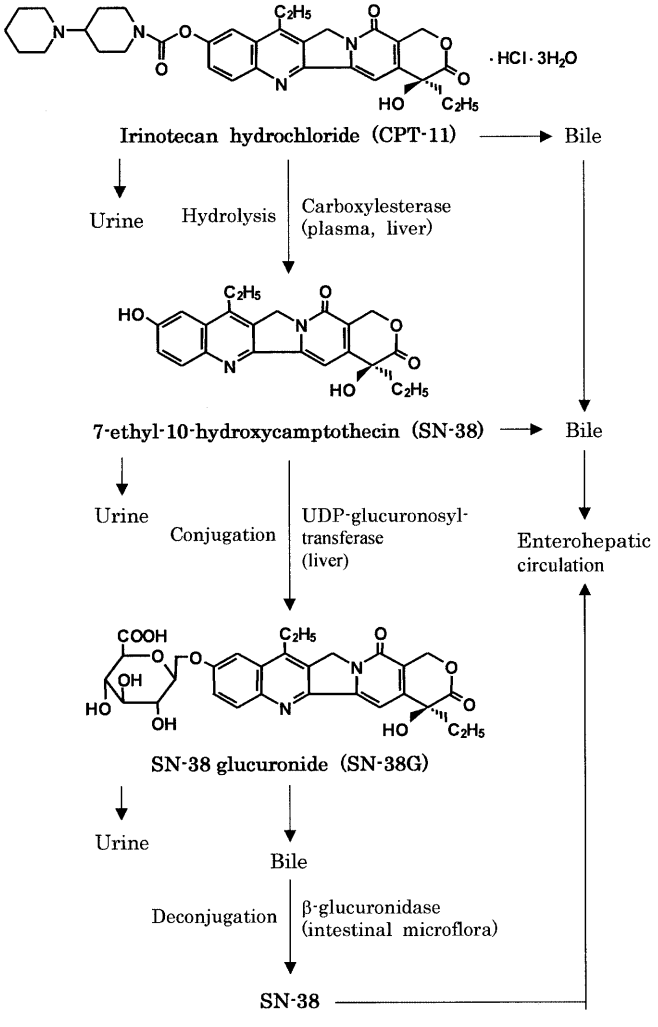

Fig. 1 Major metabolic pathway of irinotecan hydrochloride (CPT-11) in the rat

Materials and methods

Materials and reagents

CPT-11 (lot 115002), SN-38 (lot 30091R) and SN-38G (lot 970326) were provided by Yakult Honsha Co. (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 was of Milli-Q grade (Millipore Co., Bedfold, Mass.) and all other chemicals were of analytical or HPLC grade 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 230–260 g were used in all experiments except for monitoring the incidence of diarrhea after consecutive administrations of CPT-11, for which rats weighing 165–185 g were used.

Administration schedules

Saline or CPT-11 (total dose 240 mg/kg) was administered intravenously (i.v.) to the animals via the tail vein in one of five administration schedules (control, S1, S2, S3, S4) as listed in Table 1 between 8:00 and 9:00 a.m. for the first administration and between 5:00 and 6:00 p.m. for the second administration of S2.

Monitoring of CPT-11-induced diarrhea

The severity of diarrhea and body weight changes were monitored throughout the experimental period (11 days from the first administration). Diarrhea observed after the final administration was considered to be delayed-onset diarrhea. The severity of the

Table 1 CPT-11 i.v. administration schedules. The total dose of CPT-11 in each schedule was 240 mg/kg

Schedule	Compound	Daily dose	Administration period (days)
Control	Physiological saline	3 ml/kg once daily	1–4
S1	CPT-11	60 mg/kg once daily	1–4
S2	CPT-11	30 mg/kg twice daily at a 9-h interval	1–4
S3	CPT-11	30 mg/kg once daily	1–8
S4	CPT-11	40 mg/kg once daily	1–6

diarrhea was scored as follows: 0 (normal, normal stool or absent); 1 (slight, slightly wet and soft stool); 2 (moderate, wet and unformed stool with moderate perianal staining of the coat); and 3 (severe, watery stool with severe perianal staining of the coat).

Histological studies

Intestinal tissues (ileum, cecum and colon) were removed after exsanguination the day after the final administration, and fixed in 10% neutral buffered formaldehyde. Segments were embedded in paraffin wax and stained with hematoxylin-eosin for light microscopy.

Pharmacokinetic studies

Animals were cannulated in the right femoral vein and the left femoral artery (Intramedic PE-50; Clay Adams, Parsippany, N.J.) 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.

CPT-11 was administered via the right femoral vein cannula according to the schedules S1 to S4 shown in Table 1 after the animals had completely recovered from the anesthesia, 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, 30, and 60 min and every 3 h from 3 h up to 24 h after the first administration. In S2 rats, blood was also collected at 2, 5, 10, 30, and 60 min after the second administration. Bile was collected for 24 h after the first administration and kept on ice. We have confirmed that CPT-11 and the two metabolites are stable under these conditions.

In different experiments, animals were killed by exsanguination at 1, 3, 6, 9 and 24 h after the first administration and the cecum was removed. In S2 rats, the cecum was also removed at 1, 3, 6 and 9 h after the second administration.

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 internal standard (IS) solution (0.146 *M* H_3PO_4 containing 1 μg/ml of CPT). The bile was diluted 500-fold with water and then added to an equal volume of IS solution. The cecal tissue (approximately 100 mg) was homogenized with 600 μl IS solution on ice with a sonicator (frequency 20 kHz, amplitude 250 μm, output 10 W, 1 min; Model 450 Sonifier, Branson Ultrasonics Co., Danbury, Ct.). Methanol (2 ml) was added to the homogenate and the mixture was centrifuged at 15,000 rpm for 5 min. The supernatant was diluted fivefold with 0.146 *M* H_3PO_4 and analyzed.

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

A previously reported high-performance liquid chromatographic method with a fully automated online solid phase extraction system (PROSPEKT; Spark Holland, Emmen, The Netherlands) [26] was used. Briefly, 100, 100 and 500 μl of the plasma, bile and cecal tissue samples, respectively, were used for the solid-phase extraction with a C18 Analytichem cartridge (Spark Holland). A C_{18} reversed-phase column (Symmetry Column C18, 150 × 4.6 mm, ID 5 μm; Waters, Milford, Mass.) was used at 50 °C. The fluorescence detector (470 scanning fluorescence detector; Waters) was set at 373 nm and 428 nm (excitation and emission, respectively) for 0–2.7 min, at 380 nm and 540 nm for 2.7–3.8 min, and at 373 nm 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, 5 and 2.5 ng/ml for plasma, 0.5, 0.5 and 0.25 μg/ml for bile and, 50, 10 and 10 ng/g for cecal tissue, respectively.

Pharmacokinetic analysis

Plasma and tissue concentration-time curves were analyzed by noncompartmental models. The areas under the plasma and the cecal tissue concentration-time curves (AUC_{pla} and AUC_{cec}) were calculated by the trapezoidal rule with estimation of AUC from the last sampling time to infinity by the following equation:

$$\int_{last}^{\infty} C dt = C_{last}/\text{last log-linear phase slope}$$

where C_{last} is the concentration at the last sampling time. Total clearance (CL_{tot}) was calculated as follows:

$$CL_{tot} = \text{Dose}/AUC_{pla}$$

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.

Results

Incidence of CPT-11-induced diarrhea

CPT-11 (total dose 240 mg/kg) was administered i.v. following the various schedules listed in Table 1. In S1 rats, the body weight decreased and reached a nadir on day 5 or 6. In the animals receiving the other schedules, there was little decrease during the administration periods (Fig. 2). The incidence of delayed-onset diarrhea is shown in Table 2. Moderate or severe delayed-onset diarrhea was observed in S1 rats. The symptoms were milder in S2 than in S1 rats, and they were hardly observed in S3 and S4 rats. Moreover, the S1 schedule induced severe acute diarrhea which was observed within 3 h of administration on days 3 and 4, but the

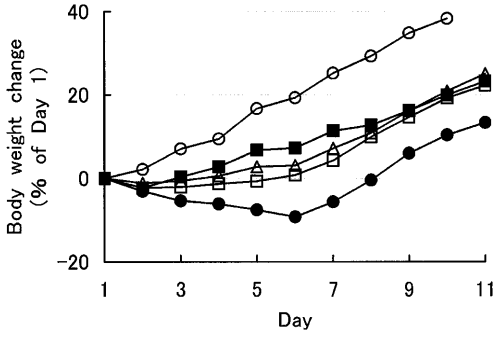

Fig. 2 Body weight changes after i.v. administration of CPT-11. CPT-11 was given as follows. ○ Control (physiological saline 3 ml/kg once daily for four consecutive days, days 1–4), ● S1 (60 mg/kg once daily for four consecutive days, days 1–4), □ S2 (30 mg/kg twice daily at 9-h intervals for four consecutive days, days 1–4), ■ S3 (30 mg/kg once daily for eight consecutive days, days 1–8), △ S4 (40 mg/kg once daily for six consecutive days, days 1–6). Symbols represent the means from ten (S1 and S2) or five (control, S3 and S4) rats

Table 2 Incidence of delayed-onset diarrhea after i.v. administration of CPT-11 by various schedules. The diarrheal scores were defined as: 0, normal; 1, slight; 2, moderate; 3, severe. Data represent the number of animals showing each score

Schedule	*n*	Diarrheal score													
		One day[a]					Two days[a]					Three days[a]				
		0	1	2	3	Mean	0	1	2	3	Mean	0	1	2	3	Mean
Control	5	5	0	0	0	0.0	5	0	0	0	0.0	5	0	0	0	0.0
S1	10	0	2	6	2	2.0	1	6	3	0	1.2	1	6	3	0	1.2
S2	10	4	2	4	0	1.0*	6	3	1	0	0.5*	7	3	0	0	0.3*
S3	5	4	1	0	0	0.2*	5	0	0	0	0.0*	5	0	0	0	0.0*
S4	5	5	0	0	0	0.0*	5	0	0	0	0.0*	5	0	0	0	0.0*

*$P < 0.05$ vs S1
[a] Days after final administration by various schedules

acute diarrhea in other groups was moderate (data not shown).

Histological analysis of intestinal tissues

Little histological change in the ileum and the colon on the day after the final administration was observed irrespective of treatment schedule. Marked pathological differences were observed in the cecum in relation to the schedule (Fig. 3, Table 3). In S1 rats, wall thickening, and slight hemorrhage were observed macroscopically. Microscopic findings were edema in the submucosa, decrease in crypt number and size, and a marked increase in the crypts covered with flattened epithelial cells. Moreover, pseudomembrane-like substance consisting of fibrin, cell debris and enterobacteria formed on the mucosa. These pathological changes were milder in S2 than S1 rats, and hardly observed in S3 and S4 rats.

Pharmacokinetics of CPT-11 and its metabolites

CPT-11 plasma concentrations in rats after a single administration of CPT-11 decreased biexponentially (Fig. 4). AUC_{pla} and the maximum plasma concentration

Fig. 3A–E Micrographs (×130) of cecal epithelium on the day after the final administration of CPT-11 by various schedules. CPT-11 was given i.v. following the S1 (**B**), S2 (**C**), S3 (**D**) or S4 (**E**) schedule. Physiological saline was given to the control rats (**A**) instead of CPT-11. Histological slides were stained with hematoxylin-eosin. Edema, a decrease in crypt numbers and the formation of a pseudomembrane-like substance consisting of fibrin, cell debris and enterobacteria are apparent in **B** and **C**. These changes can hardly be seen in **D**, and slight villous shortening and a decrease in crypt number are apparent in **E**

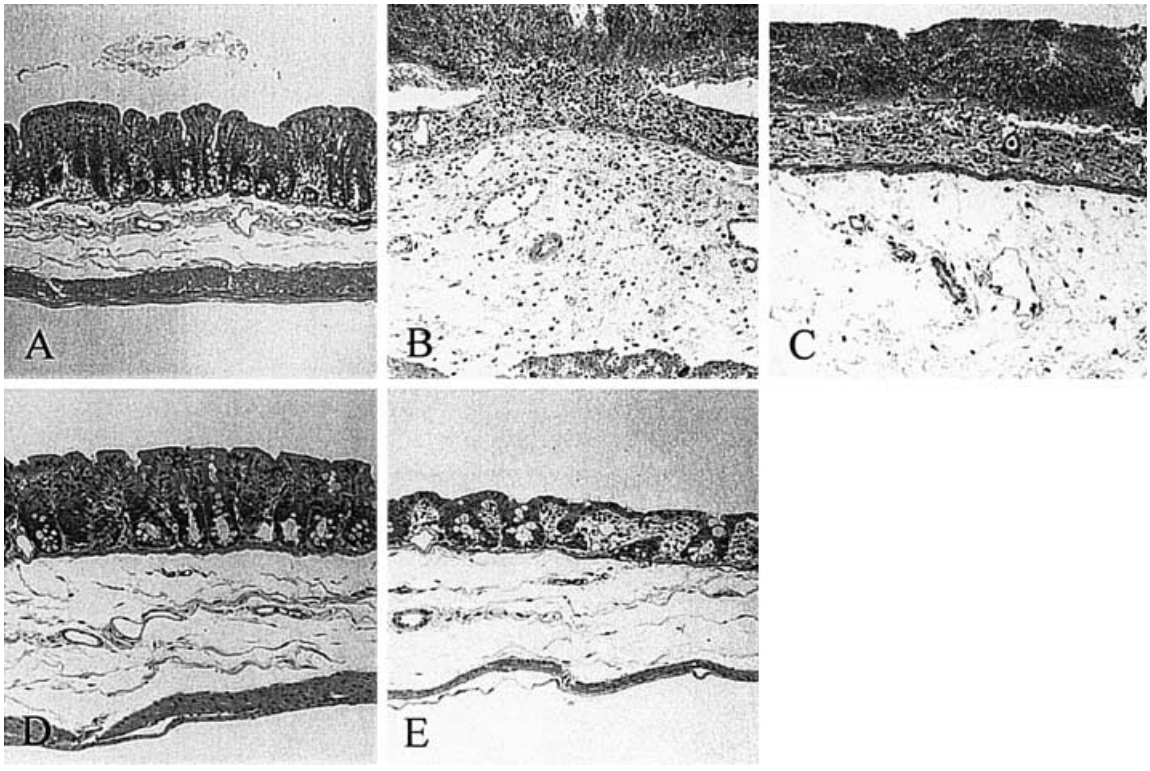

Table 3 Histological findings of cecal epithelium the day after final administration of CPT-11 by various schedules. The scores were defined as: – no significant changes, ± slight, + mild, ++ moderate, +++ severe

Schedule	*n*	Macroscopic													Microscopic										
		Wall thickening					Hemorrhage					Formation of pseudo-membrane-like substance					Edema					Decrease in crypt number				
		–	±	+	++	+++	–	±	+	++	+++	–	±	+	++	+++	–	±	+	++	+++	–	±	+	++	+++
Control	5	5	0	0	0	0	5	0	0	0	0	5	0	0	0	0	5	0	0	0	0	5	0	0	0	0
S1	10	2	2	2	4	0	7	2	1	0	0	4	2	4	0	0	0	1	3	6	0	0	0	2	6	2
S2	10	5	2	2	1	0	8	2	0	0	0	7	2	1	0	0	1	2	5	2	0	0	4	4	1	1
S3	5	5	0	0	0	0	5	0	0	0	0	5	0	0	0	0	5	0	0	0	0	5	0	0	0	0
S4	5	5	0	0	0	0	5	0	0	0	0	4	1	0	0	0	5	0	0	0	0	3	0	2	0	0

Fig. 4 Plasma concentration profiles of CPT-11, SN-38 and SN-38G after i.v. administration of CPT-11 (● S1, □ S2, ■ S3, △ S4). Symbols and bars represent the means and SD from four rats

(C_{max}) of CPT-11 depended on the CPT-11 daily dose (Table 4). In S2 rats, the CPT-11 concentration increased immediately after the second administration, and then decreased in the same profile as that of the first administration. CPT-11 C_{max} was much lower in S2 than in S1 animals, although the AUC_{pla} was comparable between S1 and S2 animals.

SN-38 plasma concentrations in S1, S3 and S4 rats decreased immediately after administration, remained constant for several hours and then decreased gradually. The time during which the SN-38 concentration remained constant, depended on the CPT-11 daily dose. In S2 rats, the SN-38 plasma concentration increased transiently after the second administration, then returned to the level before the second dosing, but the C_{max} after the second administration was half of the first C_{max}. SN-38 AUC_{pla} depended not on the schedule but the CPT-11 daily dose.

The SN-38G plasma concentrations in all schedules reached a maximum 10 min after administration and then decreased, first sharply and then gradually. In S2 rats, the SN-38G concentration increased soon after the

Table 4 Pharmacokinetic parameters after i.v. administration of CPT-11 by various schedules. AUC_{pla} was calculated from 0 to infinity. C_{max} of CPT-11 and SN-38 were the concentrations at t = 0 calculated by exponential fitting. Values are the means ± SD from four rats

	Parameter	Administration schedule			
		S1	S2	S3	S4
CPT-11	AUC_{pla} (μg h/ml)	43.47 ± 3.72	41.73 ± 1.73	18.03 ± 0.79*	24.72 ± 2.25*
	C_{max} (first) (μg/ml)	47.83 ± 11.54	23.95 ± 2.98*	22.14 ± 4.09*	28.66 ± 0.30*
	C_{max} (second) (μg/ml)	–	29.15 ± 5.17*	–	–
	CL_{tot} (l/h/kg)	1.39 ± 0.11	1.44 ± 0.06	1.67 ± 0.07*	1.63 ± 0.14*
	$t_{1/2}$ (h)	2.90 ± 0.03	2.30 ± 0.16*	2.25 ± 0.09*	2.39 ± 0.16*
SN-38	AUC_{pla} (μg · h/ml)	0.70 ± 0.12	0.75 ± 0.10	0.42 ± 0.06*	0.52 ± 0.08*
	C_{max} (μg/ml)	0.28 ± 0.04	0.25 ± 0.04	0.18 ± 0.03*	0.21 ± 0.04*
	$t_{1/2}$(h)	4.72 ± 0.52	5.30 ± 0.37	4.18 ± 0.40	4.13 ± 0.61
SN-38G	AUC_{pla} (μg · h/ml)	3.06 ± 0.19	4.03 ± 0.44*	1.96 ± 0.10*	2.50 ± 0.33
	t_{max} (h)	0.167	0.167	0.167	0.167
	C_{max} (μg/ml)	0.38 ± 0.04	0.34 ± 0.03	0.28 ± 0.01*	0.34 ± 0.03
	$t_{1/2}$ (h)	8.56 ± 1.21	8.27 ± 1.28	7.54 ± 1.76	9.84 ± 3.34

*$P < 0.05$ vs S1

second administration and then decreased in the same profile as that after the first dosing, but the C_{max} after the second administration was two-thirds of the first C_{max}. SN-38G AUC_{pla} was larger in S2 than in S1 animals.

Biliary excretion profiles of CPT-11, SN-38 and SN-38G are shown in Fig. 5. CPT-11 was excreted quickly for several hours after administration and then gradually in S1, S3 and S4 rats. In S2 rats, it was approximately half of that in S1 rats for 9 h after the first administration, and increased again after the second dosing, and the cumulative excretion for 24 h was similar to that in S1 rats. About 40% of the doses were excreted unchanged in the bile independently of the schedule (Table 5). SN-38 and SN-38G were excreted with similar profiles for several hours after administration in all schedules and the excretion became gradually dependent on the CPT-11 daily dose. In S2 rats, they were excreted rapidly again after the second administration, and their cumulative excretion weights were a little larger than those in S1 rats.

The CPT-11 cecal tissue concentration in S1, S3 and S4 rats decreased exponentially (Fig. 6). In S2 rats, it increased again after the second administration and then decreased exponentially. CPT-11 AUC_{cec} depended on the CPT-11 daily dose irrespective of the schedule (Table 6). The SN-38 and SN-38G cecal tissue concentrations in S1, S3 and S4 rats increased for 9 h after administration and then decreased gradually (Fig. 6). In S2 rats, they increased further for 3 h after the second administration and then decreased gradually. SN-38 and SN-38G AUC_{cec} depended on the CPT-11 daily dose except in S2 rats in which SN-38 AUC_{cec} was larger than in S1 rats (Table 6).

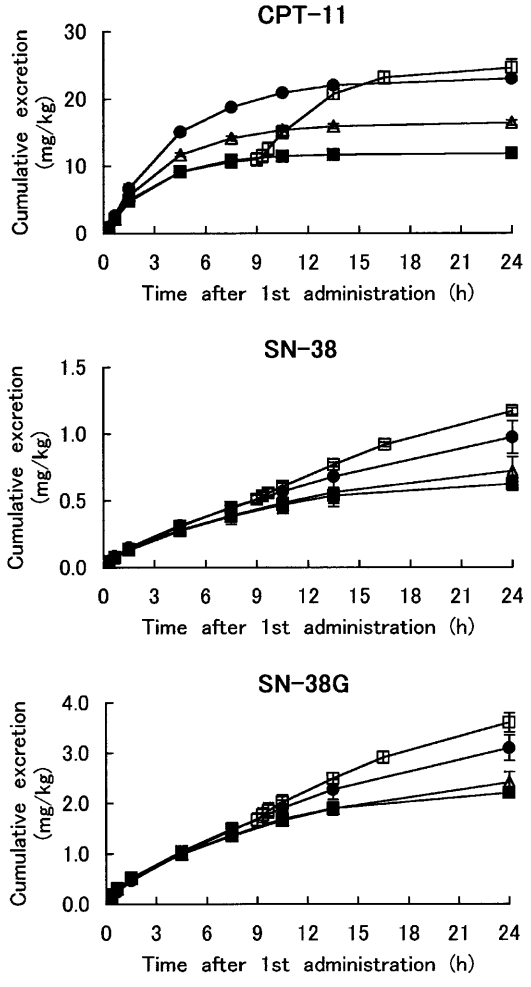

Fig. 5 Biliary excretion of CPT-11, SN-38 and SN-38G after i.v. administration of CPT-11 (● S1, □ S2, ■ S3, △ S4). Symbols and bars represent the means and SD from four rats

Discussion

In S2 rats, the incidence of both acute and delayed-onset diarrhea was lower and the degree of histological

Table 5 Biliary excretion after i.v. administration of CPT-11 by various schedules. Bile was collected for 24 h from the first administration. Values are the means ± SD (n = 4 rats) of the biliary excretion ratio (percent of the CPT-11 dose)

	Administration schedule			
	S1	S2	S3	S4
CPT-11	38.37 ± 0.62	41.07 ± 2.12	39.78 ± 2.62	41.04 ± 0.92
SN-38	2.93 ± 0.37	3.51 ± 0.07	3.75 ± 0.31*	3.26 ± 0.46
SN-38G	6.21 ± 0.51	7.21 ± 0.38	8.83 ± 0.37*	7.25 ± 1.34*

*$P < 0.05$ vs S1

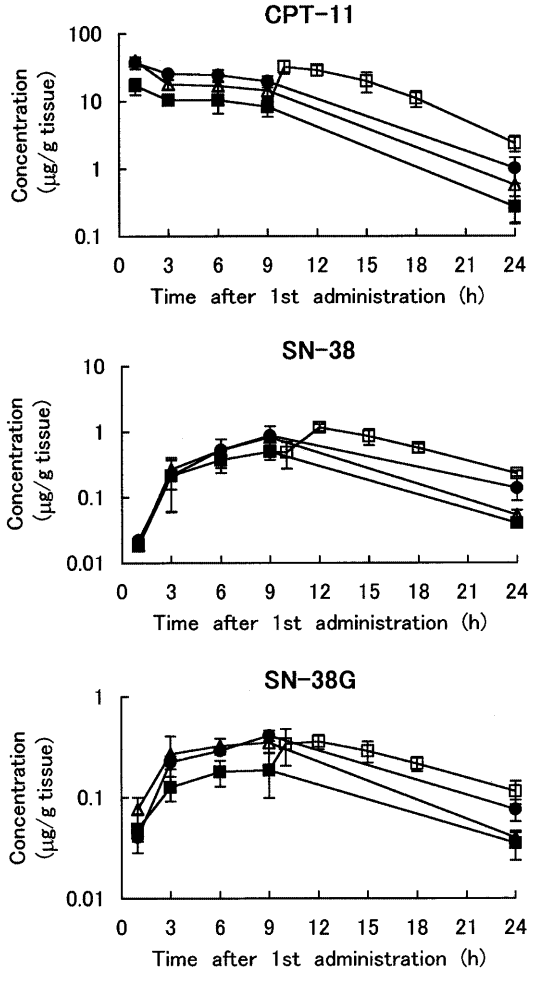

Fig. 6 Cecal tissue concentration profiles of CPT-11, SN-38 and SN-38G after i.v. administration of CPT-11 (● S1, □ S2, ■ S3, △ S4). Symbols and bars represent the means and SD from four rats

Table 6 Area under the cecal tissue concentration-time curve (AUC_{cec}) after i.v. administration of CPT-11 by various schedules. Values are AUC_{cec} (μg · h/g tissue) calculated from 0 to 24 h from the mean values (n = 4) at each time-point for each curve

	Administration schedule			
	S1	S2	S3	S4
CPT-11	317.64	326.28	130.24	239.16
SN-38	9.32	11.74	5.05	7.58
SN-38G	5.01	4.82	2.55	4.38

impairment of the cecal epithelium was milder than in S1 rats even though the CPT-11 daily dose was the same. In S3 and S4 rats, symptoms of acute and delayed-onset diarrhea and impairment of cecal tissue were hardly observed. The AUC_{pla}, AUC_{cec}, plasma C_{max} and biliary excretion of CPT-11, SN-38 and SN-38G were dependent on the CPT-11 daily dose in S1, S3 and S4 rats (Tables 4, 5 and 6). Moreover, the time during which the SN-38 plasma concentration was maintained constant because of saturation of the hydrolysis of CPT-11 to SN-38 by carboxylesterase [48] depended on the daily dose of CPT-11 (Fig. 4). In contrast, the CPT-11 plasma C_{max} was significantly lower and the SN-38 AUC_{cec} was larger in S2 than in S1 rats, while other pharmacokinetic parameters in S2 rats were similar to those in S1 rats.

The duration of exposure to CPT-11 and SN-38 may affect the severity of intestinal cytotoxicity more than the drug concentration [19], because DNA topoisomerase I inhibitors appear to be cell-cycle specific [8], and SN-38 is much more cytotoxic than CPT-11 [22, 23]. Therefore, SN-38 exposure is assumed to be the principal cause of delayed-onset diarrhea. In this study, both the incidence of delayed-onset diarrhea and the degree of impairment of the cecal epithelium depended on the duration of exposure to both CPT-11 and SN-38 (AUC_{pla} and AUC_{cec}) in S1, S3 and S4 rats (Tables 2, 3, 4 and 6). These results suggest that exposure to these two agents is the main cause of the diarrhea.

On the other hand, both the diarrheal symptoms and the impairment of cecal epithelium were less severe in S2 rats than in S1 rats. If the assumption made above is correct, the toxicological observations in S2 rats would be at least the same as those in S1 rats, because the CPT-11 and SN-38 AUC_{cec} in S2 rats were equal to and larger than those in S1 rats, respectively. These results suggest that factors other than exposure to both CPT-11 and SN-38 are involved in the induction of delayed-onset diarrhea.

The only parameter which was significantly different between S1 and S2 rats was CPT-11 plasma C_{max} (Table 4). This result suggests that this parameter is related to diarrhea. To test this, we administered CPT-11 to rats by i.v. infusion and confirmed that both acute and delayed-onset diarrheal symptoms were ameliorated by extending the infusion time. The plasma C_{max} of CPT-11, SN-38 and SN-38G decreased with infusion time, while the AUC_{pla} and $t_{1/2}$ were similar to those in the S1 rats in this study (manuscript in preparation).

CPT-11 has several pharmacologic effects, one of which is a cholinergic effect [13] assumed to be the main

mechanism involved in the onset of acute diarrhea [42]. CPT-11 also induces a transient increase in prostaglandin E_2 (PGE_2) in the intestinal tissue [20]. PGE_2 is secreted by the mucosa and smooth muscle of the small intestine [6], and induces diarrhea by stimulating colonic secretion and hyperperistalsis of the gut [4, 5]. Moreover, PGE_2 inhibits Na^+,K^+-ATPase, thereby affecting the absorption of electrolytes [27, 38]. In addition, CPT-11 induces Cl^- secretion in the subepithelial tissue by stimulating eosinoid production, e.g. thromboxane A_2 [34]. These effects of CPT-11 may exacerbate the diarrheal symptoms and the impairment of cecal tissue. The effects would be stronger under the S1 schedule than the S2 schedule, because the CPT-11 plasma C_{max} was much higher with the S1 schedule.

These effects of CPT-11 may alter the intestinal luminal environment and induce colonization by indigenous microflora and the overgrowth of pathogenic bacteria [49]. Adverse bacteria or their toxins may exacerbate the epithelial impairments caused by the bacterium-induced inflammation. The intestinal microflora appears to be involved in the onset of CPT-11-induced delayed-onset diarrhea because coadministration of antibiotics ameliorates the diarrhea [44].

The cell cycle may also be responsible for the difference in diarrheal symptoms between S1 and S2 rats. Ohdo et al. [30] have reported that CPT-11 is less toxic to mice if given when DNA synthesis and topoisomerase I activity are low in the bone marrow cells, and vice versa. These results suggest that the toxicity of CPT-11 is cell-cycle dependent. The second administration in the S2 schedule may have a less-toxic effect than the first in relation to the cell cycle, and consequently the total toxicity may be lower with the S2 than with the S1 schedule.

The hematologic toxicity as the dose-limiting toxicity of CPT-11 administration would also be involved in the induction of delayed-onset diarrhea. Serious leukopenia induced by CPT-11 administration would assist the adverse bacteria in infecting the intestinal epithelium. Clinically, granulocyte colony-stimulating factor can be coadministered to inhibit the exacerbation of the hematologic toxicity induced by dose-escalating CPT-11 [2, 7, 9, 29], and may alleviate the diarrheal symptoms by inhibiting opportunistic infection of the intestinal tissue by adverse bacteria. We are now investigating the relevance of the hematologic toxicity to the CPT-11 administration schedule and the diarrheal symptoms.

The antitumor activity of each schedule was not assessed in this study. However, at the daily doses and schedules used in this study, CPT-11 will inhibit tumor growth almost completely, and so the antitumor activity would not be able to be compared among schedules, according to Furuta et al. [12]. Against mouse L1210 leukemia and Meth A fibrosarcoma, two administration schedules in which CPT-11 was administered i.v. on days 1, 5 and 9 or 1–3, 6–8 and 11–13 have shown similar antitumor activities at the same total dose [11]. Moreover, in our preliminary studies, CPT-11 administered three times daily for 5 days at a total dose of 25 and 50 mg/kg had more antitumor activity against mouse Meth A fibrosarcoma than when given once daily (data not shown).

From the results of this study, we conclude that an administration schedule which decreases the CPT-11 plasma C_{max}, i.e. low-dose CPT-11 administered on consecutive days [17, 41] and an extension in infusion time [47], would be effective for cancer chemotherapy [17, 47]. These regimens might be less effective than conventional regimens, but the improvement in the quality of life accompanying amelioration of the side effects would make continuous chemotherapy possible [41].

In conclusion, both the duration of exposure to both CPT-11 and SN-38 of the intestinal epithelium and the CPT-11 plasma C_{max} were indicated to be closely related to the incidence and the severity of delayed-onset diarrhea in rats.

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