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ORIGINAL ARTICLE

Involvement of UDP-glucuronosyltransferase activity in irinotecan-induced delayed-onset diarrhea in rats

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Abstract We assessed the involvement of UDP-glucuronosyltransferase (UGT) activity in episodes of irinotecan hydrochloride (CPT-11)-induced delayed-onset diarrhea using a mutant rat strain with an inherited deficiency of UGT1A (Gunn rats). Gunn rats exhibited severe diarrhea after the intravenous administration of CPT-11 at a dose of 20 mg/kg, whereas Wistar rats did not. In the epithelium of the small intestine and cecum in Gunn rats, the shortening of villi, degeneration of crypts, and destruction of the nucleus were observed. The AUC, MRT, and $t_{1/2}$ of CPT-11, and the AUC of 7-ethyl-10-hydroxycamptothecin (SN-38) in plasma were, respectively, 1.6-fold, 1.5-fold, 1.7-fold, and 6.5-fold higher, and the cumulative biliary excretion rate of SN-38 was 2.3-fold higher, in Gunn rats than Wistar rats. SN-38 glucuronide excreted via bile in Wistar rats was not deconjugated in the small intestinal lumen. The SN-38 AUC values in small intestinal tissues were also 5.0 to 5.8-fold higher in Gunn rats than Wistar rats. In conclusion, Gunn rats developed severe delayed-onset diarrhea after i.v. administration of CPT-11 at a much lower dose. Severe intestinal impairments would be induced in Gunn rats through exposure to SN-38 at high levels for a long period mainly via the intestinal lumen and partially via the bloodstream. These results clarified that the deficiency of UGT activity contributed greatly to the induction of the CPT-11-induced delayed-onset diarrhea and epithelial impairment in the intestine. In the clinic, great care is needed when using chemotherapy with CPT-11 in patients with poor UGT activity.

Keywords Irinotecan hydrochloride · CPT-11 · Diarrhea · Gunn rat · UDP-glucuronosyltransferase

Abbreviations

CPT-11	Irinotecan hydrochloride
CPT	Camptothecin
SN-38	7-Ethyl-10-hydroxycamptothecin
SN-38G	SN-38 glucuronide
CES	Carboxylesterase
UGT	UDP-glucuronosyltransferase
LLOQ	Lower limit of quantification
C_{max}	Maximum concentration
AUC_{plasma}	Area under the plasma concentration-time curve
AUC_{tissue}	Area under the intestinal tissue concentration-time curve
AUMC	Area under the moment curve
MRT	Mean resident time
CL_{tot}	Total clearance
Vd_{ss}	Volume of distribution at steady state
$t_{1/2}$	Half-life
IS	Internal standard
i.v.	Intravenous(ly)

Introduction

Irinotecan hydrochloride (CPT-11), a water-soluble derivative of camptothecin (CPT) [6, 21], is used clinically to treat colorectal, gastric, lung, uterine cervical, and ovarian

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cancers, malignant lymphoma, and other malignancies [5, 27, 33, 34, 37]. However, CPT-11 sometimes causes severe adverse effects such as diarrhea and myelosuppression. These dose-limiting effects prevent the adoption of a more aggressive CPT-11-based chemotherapy [1, 5, 25, 26, 29, 33].

Diarrhea usually appears in acute and/or delayed-onset settings [31]. As the mechanism of acute diarrhea, it is assumed that the cholinergic activity of CPT-11 stimulates intestinal contractility as well as disturbs normal intestinal mucosal absorptive and secretory functions [7, 15, 27]. This diarrhea is short lived and can be prevented and rapidly suppressed with atropine [7, 31]. In contrast, delayed-onset diarrhea is so severe that it can be life threatening. 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 [11, 12, 16, 18, 27, 35, 36]. The pharmacokinetics of SN-38 is therefore considered to be mainly responsible for the induction of the delayed-onset diarrhea. But, this diarrhea remained unpredictable despite many studies until recently [20, 24, 30, 32, 36] because of the great inter-patient variability in pharmacokinetics, the severity of the diarrhea, and the effect of anti-diarrheal agents such as loperamide [1, 5, 10, 25, 31, 33].

Recently, the involvement of UDP-glucuronosyltransferase (UGT) in episodes of CPT-11-induced delayed-onset diarrhea has been earnestly studied in the clinic. CPT-11 is converted to SN-38 by carboxylesterase (CES) mainly in liver in humans, and in liver and plasma in rats and mice. SN-38 is detoxified to SN-38 glucuronide (SN-38G) by UGT, and is excreted via bile and urine [3, 14]. Thus UGTs, especially isoforms 1A1 and 1A9, are recognized as important enzymes for the elimination of SN-38 [8]. Clinically, severe adverse effects of CPT-11-based chemotherapies were reported in patients with Gilbert's syndrome [19], and several studies have been carried out analyzing the relationships among polymorphisms of UGT, the adverse effects of CPT-11, and the pharmacokinetics of SN-38 [17, 24, 28, 30].

To clarify the involvement of UGT activity in episodes of CPT-11-induced delayed-onset diarrhea, we studied the induction of diarrhea and the pharmacokinetics after the administration of CPT-11 in Gunn rats. This mutant strain has an inherited deficiency of UGT1A involved in bilirubin glucuronidation, and exhibits hyperbilirubinemia. Therefore, it is recognized as a phenotypic model of human Crigler–Najjar syndrome Type I, involving a defect in bilirubin UGT (BUGT) [4].

Materials and methods

Materials and reagents

CPT-11 (Lot 115126), SN-38 (Lot 300917R), and SN-38G (Lot 970326) were provided by Yakult Honsha Co. (Tokyo, Japan). Camptothecin was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sodium 1-decanesulfonate was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). The water was of Milli-Q grade (Millipore Co., Bedfold, MA, USA) and all other chemicals were of analytical or HPLC grade obtained from commercial sources.

Animals

Male Gunn rats and Wistar rats (7 and 6-weeks old for monitoring of diarrhea, and 8–11 and 7-weeks old for the pharmacokinetics, respectively) were purchased from Japan SLC (Hamamatsu, Japan) and used for experiments after 1 week of acclimatization with free access to water and commercial animal chow (F-2, Funabashi Farm, Funabashi, Japan). Body weights of Gunn and Wistar rats at the start of experiments were 186–230 and 177–188 g for the diarrhea monitoring, and 261–328 and 204–241 g for the pharmacokinetics, respectively.

Monitoring of CPT-11-induced diarrhea

CPT-11 dissolved with saline as almost entirely the lactone form was administered i.v. at a dose of 20 mg/kg on Day 1 to Wistar and Gunn rats (Wistar-CPT20 and Gunn-CPT20), and the severity of diarrhea and body weights were monitored throughout the experimental period. On Day 1, diarrhea observed from 6 h after the administration was considered delayed-onset diarrhea. Acute symptoms other than diarrhea such as tremors and lacrimation continued for 3 h after the administration of CPT-11, and had almost subsided at 6 h in Gunn rats, whereas a relatively mild tremor was observed for a short time in Wistar rats. The severity of the diarrhea was scored as follows: 0, normal; 1, soft feces or small black feces; 2, muddy feces; 3, watery feces; 4, mucous feces. The diarrhea score was the median among the feces excreted for 24 h before the decision.

Histopathological experiments

In Gunn and Wistar rats left un-treated and at 1 and 3 days after the administration of CPT-11, the upper small intestine (duodenum and jejunum), lower small intestine (ileum), cecum, and colon, were removed immediately after exsanguination and fixed in 10% neutral-buffered formalin. After the fixation, the tissues were embedded in

paraffin, sectioned about 5 μm thick, and stained with hematoxylin–eosin for histopathological examination.

Pharmacokinetic experiments

To examine the plasma-concentration profiles and the biliary excretion of CPT-11, SN-38, and SN-38G after the i.v. administration of CPT-11 at a dose of 20 mg/kg to both Gunn and Wistar rats, cannulae (Intramedic PE-50 and PE-10 for blood vessels and bile ducts, respectively; Clay Adams, Parsippany, NJ, USA) were implanted under light ether anesthesia into the right femoral vein (for CPT-11 administration) and either the left femoral artery (for blood sampling) or the bile duct (for bile sampling). The animals were kept in bollman cages after the implantation and had free access to an ordinary diet and water. CPT-11 at a dose of 20 mg/kg was administered to rats via the femoral vein cannula after they completely awoke from the anesthesia, and the treatment was followed by flushing with physiological saline. Two hundred microliters of blood was collected at 2, 5, 10, and 30 min, and 1, 3, 6, 9, 12, and 24 h after the administration. Bile was collected at 15, 30, and 45 min, and 1, 3, 6, 9, 12, and 24 h after the administration. Either the SN-38 lactone form dissolved with dimethylsulfoxide: 10 mM H_3PO_4 = 9:1 or the SN-38 carboxylate form dissolved with 0.1 N NaOH at a dose of 2 mg/kg was administered to rats via the femoral vein cannula, and the bile was collected at 15, 30, and 45 min, and 1, 2, 4, and 6 h after the administration. Bile was kept on ice during sampling. CPT-11 and its two metabolites in bile were stable under these conditions.

To examine the concentration of CPT-11 and its metabolites in both intestinal tissues and intestinal contents after the administration of CPT-11, Wistar and Gunn rats were killed by exsanguination and the duodenum, jejunum, ileum, cecum, and colon were extirpated at 3, 6, 12, or 24 h after the i.v. administration of CPT-11 at a dose of 20 mg/kg via the tail. The intestinal contents were obtained from the extirpated intestinal tissues.

Sample preparations

The plasma was separated immediately after blood sampling, diluted fivefold with 0.15 M H_3PO_4, and then added to an equal volume of the internal standard (IS) solution (0.15 M H_3PO_4 containing 1 μg/ml of CPT as IS). The bile was diluted 500-fold with water and then added to an equal volume of IS solution. A portion of intestinal tissue (approximately 0.1 g) and the intestinal contents were homogenized with a fivefold volume of IS solution on ice with a sonicator (frequency; 20 kHz, amplitude; 250 μm, output; 10 W, time; 30 s. Model 450 Sonifier, Branson Ultrasonics Co., Danbury, CT, USA). The homogenate (100 μl) was mixed with methanol (400 μl) and centrifuged at 15,000 rpm for 5 min. The supernatant was diluted fivefold with 0.15 M H_3PO_4 and analyzed.

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

A previously reported high-performance liquid chromatographic method with a fully automated on-line solid phase extraction system (PROSPEKT; Spark Holland, Emmen, the Netherlands) [22] was used. Briefly, 100, 100, 200, and 200 μl of the plasma, bile, intestinal tissue, and intestinal content samples, respectively, were used for the solid phase extraction with a Cartridge-C18 Analytichem (Spark Holland). A C_{18} reverse-phase column (Symmetry Column C18, 150 mm × 4.6 mm I.D., 5 μm, Waters, Milford, MA, USA) 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 and 3.8–8.5 min, and 380 and 540 nm for 2.7–3.8 min. The mobile-phase consisted of 0.05 M KH_2PO_4:acetonitrile (70:30, v/v) containing 4 mM sodium 1-decanesulfonete (pH 3.5 with H_3PO_4) and the flow rate was 1.5 ml/min. The retention time of SN-38G, SN-38, CPT(IS), and CPT-11 was approximately 1.5, 3.3, 4.2, and 7.2 min, respectively. The lower limits of quantitation (LLOQ) of CPT-11, SN-38 and SN-38G in each sample were 2.5, 2.5, and 5 ng/ml for plasma, 100, 20, and 100 ng/ml for bile, and 50, 50, and 100 ng/g content for intestinal content, respectively. The LLOQ of both CPT-11 and SN-38 in intestinal tissue samples was 10 ng/g tissue.

Pharmacokinetic analysis

Plasma and intestinal tissue concentration-time curves were analyzed with a noncompartmental model. The areas under the concentration–time curve of plasma (AUC_{pla}) and that of intestinal tissue (AUC_{int}) were calculated by the trapezoidal rule with an estimation of AUC from the last sampling time to infinity using Eq. A.

$$\int_{\text{last}}^{\infty} Cdt = C_{\text{last}}/\text{last log} - \text{linear phase slope} \qquad \text{(A)}$$

where C_{last} is the concentration at the last sampling time. Total clearance (CL_{tot}), mean resident time (MRT), and volume of distribution at steady state (Vd_{ss}) were calculated as follows.

CL_{tot} =Dose/AUC_{pla}
MRT =AUMC/AUC (AUMC: area under the moment curve)

$Vd_{ss} = CL_{tot} \times MRT$.

Statistical analysis

Differences were considered significant based on Student's *t* test except for the pharmacokinetic analysis.

Results

Incidence of CPT-11-induced diarrhea

CPT-11 was administered i.v. to Wistar and Gunn rats on Day 1, and each animal's body weight (Fig. 1) and diarrheal symptoms (Table 1) were monitored. In the Wistar-CPT20 group, neither a decline in body weight nor any diarrheal symptom was observed. In the Gunn-CPT20 group, feeding, body weights, and the number of feces decreased day by day after the administration of CPT-11 till Day 5–7, and then recovered very slowly. Feces that were small and black, not soft, were excreted till Day 3, after which serious symptoms appeared (muddy, watery, or mucous feces). Most of the Gunn rats hardly fed and excreted yellow mucous feces along with watery feces for days 4–5. One of them continued to excrete mucous feces and died on Day 6.

Intestinal tissue histopathology

Wistar and Gunn rats were dissected pre-dosing (control) and 1 and 3 days after the i.v. administration of CPT-11 at a dose of 20 mg/kg, and their intestinal damage was evaluated (Fig. 2). In Wistar rats, there was no damage in any part of the intestinal tract. On the other hand, in the small intestine and cecum of Gunn rats, the destruction of nuclei was observed from 1 day after the administration, and the shortening of villi and degeneration of crypts were evident 3 days after the administration. The degeneration of crypts was more serious in the small intestine than in the cecum. No significant change was observed in the colon or rectum.

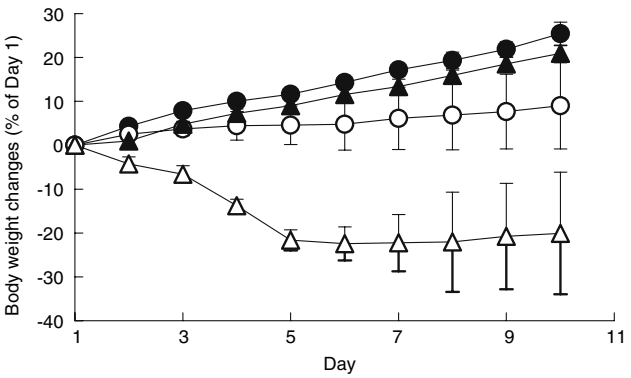

Fig. 1 Body weight changes after i.v. administration of CPT-11 to Wistar and Gunn rats. CPT-11 was given i.v. at a dose of 20 mg/kg on Day 1. *Filled circle* Wistar-control, *filled triangle* Wistar-CPT20, *open circle* Gunn-control, *open triangle* Gunn-CPT20. Data points, mean and SD of 3 (each control), 5 (Wistar-CPT20), or 8 rats (Gunn-CPT20). One animal of the Gunn-CPT20 group died on Day 6

Pharmacokinetics of CPT-11 and its metabolites

The pharmacokinetics of CPT-11 and its metabolites were examined after the i.v. administration of CPT-11 at a dose of 20 mg/kg to Wistar and Gunn rats.

In plasma (Fig. 3; Table 2), the maximum concentration (C_{max}) of CPT-11 was similar between the two strains. However, the elimination of CPT-11 was slower in Gunn rats, and the AUC_{plasma}, MRT, and $t_{1/2}$ values were 1.6-fold, 1.5-fold, and 1.7-fold higher than those in Wistar rats, respectively. The concentration of SN-38 in plasma was maintained at a higher level for a longer period in Gunn rats than in Wistar rats, and the AUC_{plasma} was 6.5-fold higher in the former. SN-38G was not detected in the plasma samples of Gunn rats.

Regarding biliary excretion (Fig. 4), that of CPT-11 was slightly higher in Gunn rats than Wistar rats. The excretion rate of SN-38 in Gunn rats was significantly greater than that in Wistar rats, but somewhat smaller than the total excretion rate of SN-38 and SN-38G in Wistar rats.

In intestinal tissues (Fig. 5; Table 3), the concentrations of CPT-11 in the duodenum and jejunum were significantly higher in Gunn rats than Wistar rats (AUC_{tissue} ratio: 2.7 and 3.2, respectively), while those at other sites were slightly higher in Gunn rats (AUC_{tissue} ratio: 1.3–2.0). The SN-38 concentrations in intestinal tissues were markedly higher (AUC_{tissue} ratio: 5.0–5.8), and the concentration in cecal tissue was slightly higher (AUC_{tissue} ratio: 1.4), than those in Wistar rats.

At 6 h after the administration of CPT-11, the ratio of SN-38 to SN-38G in the small intestinal contents was constant (1:3.4–3.5 by molecular concentration), and only SN-38 was detected in the cecum and colon in Wistar rats (Fig. 6). SN-38 concentrations in the small intestinal contents were markedly higher in Gunn rats than Wistar rats, but those in the cecum and colon were similar between the two strains.

Regarding biliary excretion after the i.v. administration of either the lactone or carboxylate form of SN-38 (Fig. 7), in Wistar rats, the cumulative excretion ratio till 6 h was 79.9 and 54.9% of the dose for the lactone and carboxylate form, respectively. Most of the SN-38 administered in the lactone form was excreted as SN-38G, whereas much of the carboxylate form was excreted without glucuronidation. In Gunn rats, the cumulative excretion ratio after the

Table 1 Incidence of delayed-onset diarrhea after i.v. administration of CPT-11 in Wistar and Gunn rats. CPT-11 was given i.v. at a dose of 20 mg/kg on day 1 (CPT20)

Group	*n*	Diarrheal score																			
		Day 1					Day 2					Day 3					Day 4				
		0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
Wistar-control	3	3					3					3					3				
Wistar-CPT20	5	5					5					5					5				
Gunn-control	3	3					3					3					3				
Gunn-CPT20	8	7	1				8					8							6	2	
Group	*n*	Diarrheal score																			
		Day 5					Day 6					Day 7					Day 8				
		0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
Wistar-control	3	3					3					3					3				
Wistar-CPT20	5	5					5					5					5				
Gunn-control	3	3					3					3					3				
Gunn-CPT20	8		1	2	3	2		2	3	1	1[a]	3		3		1	4		1	1	1

Diarrheal score was defined as follows: 0, normal feces; 1, soft feces or small black feces; 2, muddy feces; 3, watery feces; 4, mucous feces

[a] One animal in the Gunn-CPT20 group died on Day 6

administration of each form of SN-38 was markedly lower than that in Wistar rats.

Discussion

The Gunn rat is a mutant with an inherited deficiency of UGT1A [4]. In a preliminary study, no glucuronidation of SN-38 was detected in the liver microsomes of Gunn rats, whereas the level of activity of CES to convert CPT-11 as a substrate in the plasma and the tissue homogenates of liver and intestine was not so different from that in Wistar rats (data not shown). From these findings, we considered that the Gunn rat would be a good experimental model for studying the contribution of UGT1A activity to both the adverse effects and the pharmacokinetics of CPT-11. Therefore, we examined the induction of delayed-onset diarrhea and the pharmacokinetics of CPT-11 in this mutant strain.

Gunn rats exhibited delayed-onset diarrhea following the i.v. administration of CPT-11 at a very low dose, whereas at this dose neither a diarrheal symptom nor epithelial damage in intestinal tissues was observed in Wistar rats. The diarrhea was aggravated in Gunn rats, with a change from small black feces to watery and mucous feces, and histologically the destruction of nuclei, shortening of villi, and degeneration of crypts were observed in the small intestine and cecum. On the other hand, in the conventional diarrheal model using normal rats [23], a change from soft to watery feces, edema in the submucosa, a decrease in crypt number and size, the degeneration of crypts, and the formation of a pseudomembrane-like substance in the cecum were observed after the i.v. administration of CPT-11 at a dose of 60 mg/kg for four consecutive days. The apparent differences in both the diarrheal symptoms and the epithelial impairments in the intestine suggested strongly that the mechanism of delayed-onset diarrhea in Gunn rats was different from that in the conventional diarrheal model using normal rats. We assumed that the difference in UGT1A activity changed the pharmacokinetics after the administration of CPT-11, and consequently caused different diarrheal symptoms in the two strains.

In Gunn rats, CPT-11 and SN-38 concentrations in plasma and intestinal tissues were higher than those in Wistar rats. Notably, SN-38 concentrations were markedly higher in plasma and small intestinal tissues and the AUC values were 6.5-fold and 5.0 to 5.8-fold greater than those in Wistar rats, respectively. The results in the present study also demonstrated that the lactone form of SN-38 was predominantly conjugated to SN-38G and was rapidly excreted via bile. Tallman et al. reported that UGT conjugated the lactone form more potently than the carboxylate form [38]. Furthermore, it was speculated that the lactone form of SN-38 arrived more easily at the endoplasmic reticulum where UGTs exist than did the carboxylate form, because of high lipophilicity. In Gunn rats, SN-38 lactone would remain in blood without glucuronidation and following excretion as SN-38G, and sequentially be transferred to each tissue. In a preliminary study, the equilibrium between the lactone and carboxylate forms of

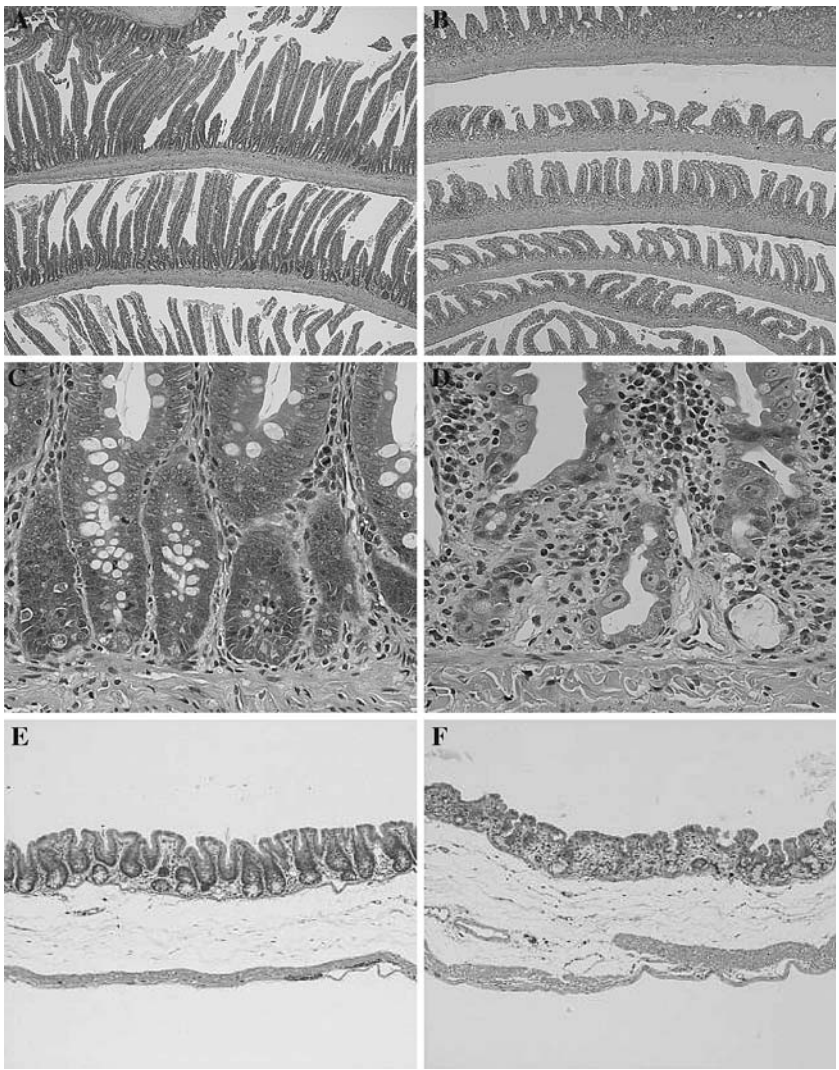

Fig. 2 Micrographs of the epithelium of the upper small intestine (duodenum and jejunum) and cecum on Day 4 after i.v. administration of CPT-11 to Gunn rats. CPT-11 was given i.v. at a dose of 20 mg/kg on Day 1. Histological slides were stained with hematoxylin-eosin. **a–d** Upper small intestine, **a** Control (×40), **b** CPT20 (×40), **c** control (×400), **d** CPT20 (×400). **e**, **f** cecum, **e** Control (×40), **f** CPT20 (×40)

SN-38 in plasma was similar in both Wistar and Gunn rats (the mean proportion of the lactone form relative to all SN-38 from 0.5 till 3 h after the i.v. administration of CPT-11 at 20 mg/kg (n = 2) was 70.8 and 72.2%, respectively).

The concentration of CPT-11 in plasma was also higher in Gunn rats than Wistar rats. Because the biliary excretion of CPT-11 as well as the plasma concentration profile was slightly higher in Gunn rats than Wistar rats, the hepatic capability to extract and/or excrete CPT-11 between the two strains wouldn't be so different.

In Gunn rats, much SN-38 was excreted into bile. On the other hand, in Wistar rats, much SN-38G was excreted into bile and little was deconjugated in the small intestinal lumen. Takasuna et al reported that the β-glucuronidase activity was much lower in the luminal content of the small intestine than cecum and colon in rats [36]. The SN-38 tissue concentrations in the small intestine (duodenum, jejunum, and ileum) were much higher in Gunn rats than in Wistar rats, whereas the concentration in the cecum was not so different between the two strains and the concentration in the luminal content was nearly the same. These results indicated that the biliary excretion of much SN-38 caused extensive exposure in the small intestinal tissues, and the consequent epithelial impairments in Gunn rats.

The lactone form of SN-38 is conjugated to SN-38G by UGT more easily than the carboxylate form [38]. In the present study, the lactone form was largely excreted into bile as SN-38G, and the carboxylate form was mostly excreted without glucuronidation in Wistar rats. From these findings, the ratio of the lactone to carboxylate form of SN-38 excreted into bile after the administration of CPT-11 would be higher in Gunn rats than Wistar rats, and the small intestinal epithelium in Gunn rats would be exposed to a high concentration of the lactone form with markedly potent cytotoxic activity compared to the carboxylate form.

The increased exposure to CPT-11 and SN-38 via the bloodstream probably contributes to the adverse effects of CPT-11. In the diarrhea-monitoring experiment, the

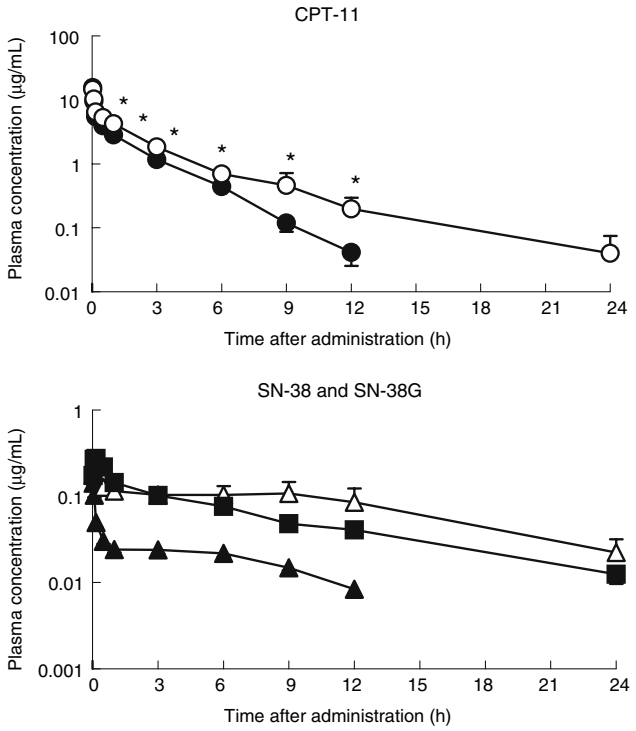

Fig. 3 Plasma concentration profiles after i.v. administration of CPT-11 at a dose of 20 mg/kg to Wistar and Gunn rats. *Closed symbols* Wistar rats, *open symbols* Gunn rats. *Filled circle*, *open circle*: CPT-11, *filled triangle*, *open triangle* SN-38, *filled square*: SN-38G. Data points, mean and SD of 4 Wistar rats and 5 Gunn rats. *The mean was significantly different from that of Wistar rats ($P < 0.05$ by Student's *t* test). The mean concentration of SN-38 was significantly different between the two strains at each time point ($P < 0.05$ by Student's *t* test)

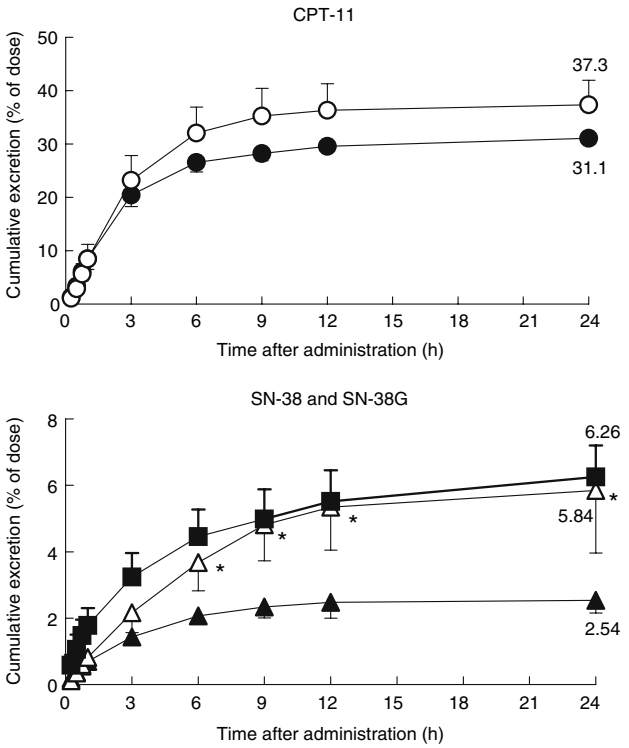

Fig. 4 Cumulative biliary excretion profiles after i.v. administration of CPT-11 at a dose of 20 mg/kg to Wistar and Gunn rats. *Closed symbols* Wistar rats, *open symbols* Gunn rats. *Filled circle*, *open circle*: CPT-11, *filled triangle, open triangle* SN-38, *filled square*: SN-38G. Data points, mean and SD of 4 Wistar rats and 5 Gunn rats. *The mean was significantly different from that of Wistar rats ($P < 0.05$ by Student's *t* test)

Table 2 Pharmacokinetic parameters after i.v. administration of CPT-11 at a dose of 20 mg/kg to Wistar and Gunn rats

	Parameter	Wistar	Gunn
CPT-11	AUC_{inf} (μg·h/ml)	11.7 ± 0.9	18.7 ± 1.8**
	C_{max} (μg/ml)	15.5 ± 1.3	14.5 ± 1.3
	MRT (h)	2.33 ± 0.18	3.50 ± 0.92*
	CL_p (l/h/kg)	1.71 ± 0.13	1.08 ± 0.11**
	Vd_{ss}(l/kg)	3.96 ± 0.09	3.70 ± 0.65
	$t_{1/2}$ (h)	1.79 ± 0.17	2.97 ± 0.93*
SN-38	AUC_{inf} (μg h/ml)	0.303 ± 0.039	1.96 ± 0.72**
	C_{max} (μg/ml)	0.142 ± 0.005	0.184 ± 0.022*
	MRT (h)	5.04 ± 1.13	6.97 ± 1.71
	$t_{1/2}$ (h)	4.49 ± 1.16	5.61 ± 0.85
SN-38G	AUC_{inf} (μg h/ml)	1.39 ± 0.11	–
	C_{max} (μg/ml)	0.285 ± 0.030	–
	MRT (h)	1.28 ± 0.68	–
	$t_{1/2}$ (h)	6.33 ± 0.88	–

Each value represents the mean ± SD of 4 animals. AUC_{inf} values were calculated from time zero to infinity

*,** The mean was significantly different from that of the Wistar group (*$P < 0.05$, **$P < 0.01$ by Student's *t* test)

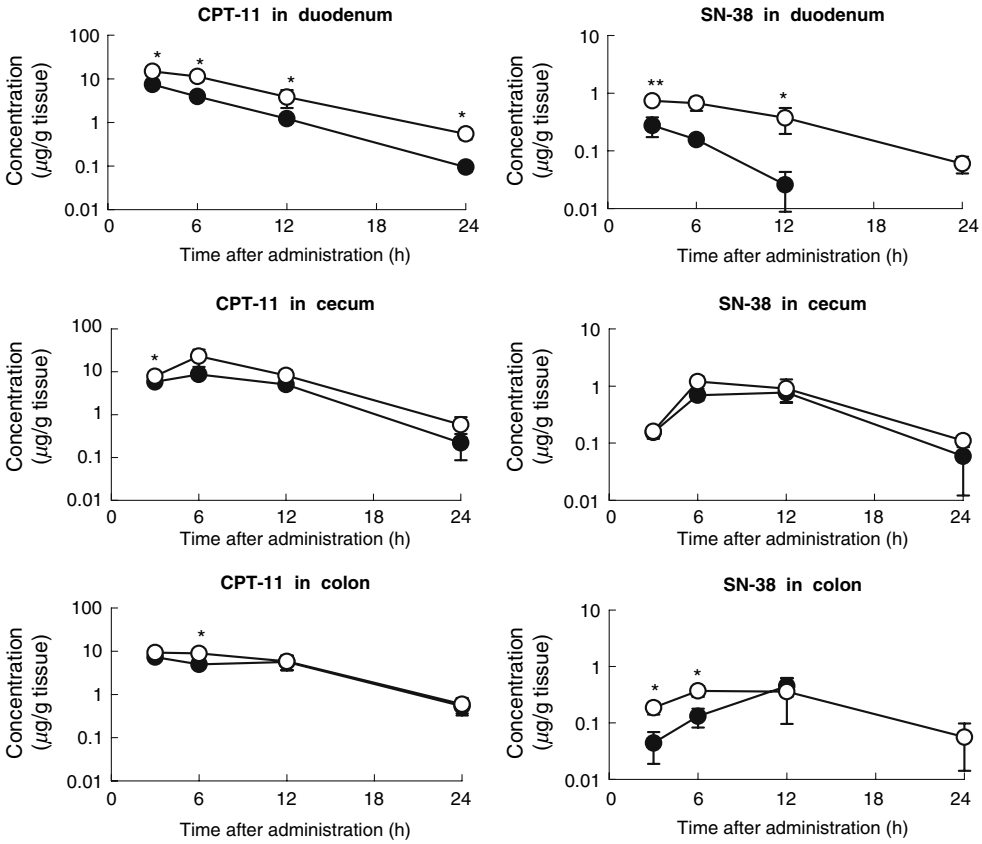

Fig. 5 Concentration profiles in intestinal tissues after i.v. administration of CPT-11 at a dose of 20 mg/kg to Wistar and Gunn rats. *Filled circle* Wistar rats, *open circle* Gunn rats. Data points, mean and SD of 4 animals. *The mean was significantly different from that of Wistar rats ($P < 0.05$ by Student's t test)

Table 3 AUC values of CPT-11 and SN-38 in intestinal tissues after i.v. administration of CPT-11 at a dose of 20 mg/kg to Wistar and Gunn rats

		AUC (μg·h/g tissue or μg h/ml)					
		Duodenum	Jejunum	Ileum	Cecum	Colon	Plasma
CPT-11	Wistar	47.2	49.9	300	90.1	89.5	11.7
	Gunn	127	161	466	178	115	18.7
	G/W ratio	2.7	3.2	1.6	2.0	1.3	1.6
SN-38	Wistar	1.59	2.56	3.84	9.50	–[a]	0.303
	Gunn	8.81	14.7	19.0	13.5	6.02	1.96
	G/W ratio	5.5	5.8	5.0	1.4	–	6.5

AUC values were calculated from time zero to infinity

[a] The AUC value couldn't be calculated because the concentration at 24 h was below the 'LLOQ'

hematological toxicity induced by CPT-11 was monitored using a satellite group of each strain ($n = 3$), and in Gunn rats, a significant decrease in neutrophil (0.73 ± 0.09 to $0.05 \pm 0.01 \times 10^3$ cells/μl) and lymphocyte (3.13 ± 0.13 to $0.90 \pm 0.21 \times 10^3$ cells/μl) numbers was observed at Day 4. From these results, it is understood that exposure via the bloodstream would influence the CPT-11-sensitive tissues. Moreover, the intestinal tissue in rats was reported to have carboxylesterase activity [36], and the production of SN-38 from CPT-11 in the homogenate of the small intestinal epithelium of both strains was confirmed in our preliminary study, whereas the carboxylesterase activity was not detected in the marrow homogenate. Accordingly, exposure to CPT-11 and SN-38 via the bloodstream and the SN-38 produced in the intestinal tissues would be also partially involved in the epithelial impairments in the intestinal tract.

The deficiency of UGT activity in intestinal tissue may have contributed to the epithelial impairments in Gunn rats. Tallman et al. reported that the intestinal tissues as well as hepatocytes in humans and Sprague–Dawley rats had UGT activity and that the lactone form was converted to SN-38G

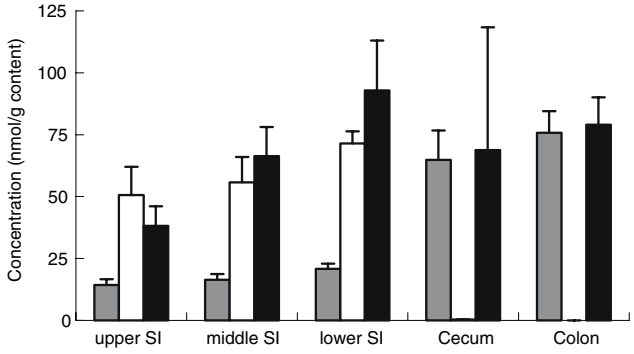

Fig. 6 Concentration of SN-38 and SN-38G in intestinal contents at 6 h after i.v. administration of CPT-11 at a dose of 20 mg/kg to Wistar and Gunn rats. *Grey square*: Wistar-SN-38, *white square* Wistar-SN-38G, *black square*: Gunn-SN-38. Data points, mean and SD of 4 animals

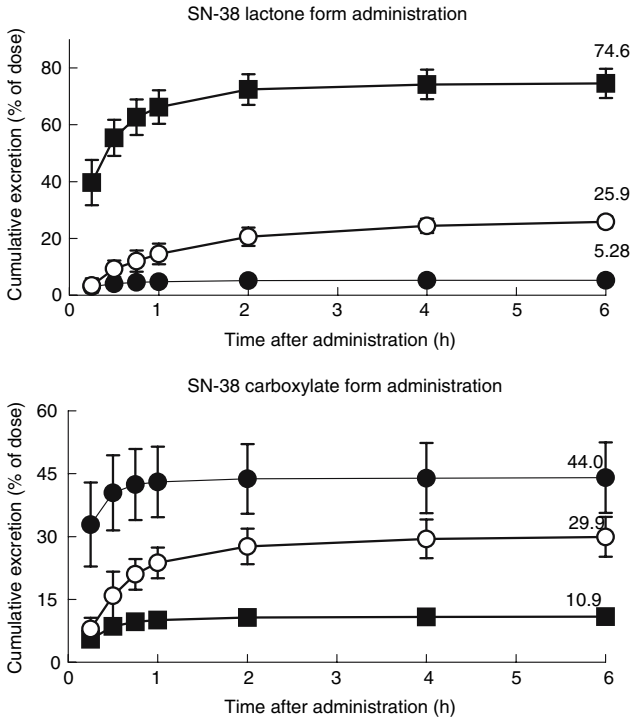

Fig. 7 Cumulative biliary excretion profiles after i.v. administration of the lactone or carboxylate form of SN-38 at a dose of 2 mg/kg to Wistar and Gunn rats. *Filled circle* Wistar-SN-38, *filled squre* Wistar-SN-38G, *open circle* Gunn-SN-38. Data points, mean and SD of 3–4 animals

much more predominantly than the carboxylate form, and that the presence of UGT in intestinal tissues would be critical for the gastrointestinal protection [38, 39]. The UGT activity was confirmed to be present in the epithelial homogenate of the upper small intestine in Wistar rats, but not in the lower small intestine in Wistar rats or at any sites in Gunn rats (data not shown).

It was indicated from these results that the epithelium of the small intestine in Gunn rats was exposed to SN-38 at a much higher level, mainly from the intestinal lumen and partially from the bloodstream. The ratio of local exposure to SN-38 in Gunn rats to Wistar rats would be higher in the intestinal epithelium than intestinal tissues (AUC_{tissue} ratio: 5.0–5.8). Intensive exposure to SN-38 would consequently induce severe epithelial impairments in the small intestine in Gunn rats.

Impairments of the cecal epithelium were apparent in Gunn rats after the administration of CPT-11, but not at all in Wistar rats, although levels of exposure, e.g., SN-38 concentrations in both the cecal tissues and contents, were not so different between the two strains. These results might indicate that the intestinal epithelium of Gunn rats was more sensitive to SN-38 than that of Wistar rats. Further examination is required to pursue the cause of these phenomena.

Although the mechanism of the epithelial impairments in the intestine on the administration of CPT-11 in Gunn rats was mostly elucidated, that of delayed-onset diarrhea remains unclear. The epithelial impairments in the small intestine may induce hypofunction, e.g., the depression of absorption of nutrition and bile acid, and peristaltic movement, and indirectly influence the function of the large intestine. Exposure to CPT-11 and SN-38 at high levels for a long period in the gastrointestine, especially the cecum and colon, due to a deficiency of UGT1A activity might influence intestinal functions such as absorption/secretion of water and secretion of mucous. It was reported that overmedication with CPT-11 induced a marked enhancement of mucous production in the colon in rats [9]. This effect might contribute to the excretion of mucous feces observed only in Gunn rats. Further examination is required to elucidate the mechanism of the delayed-onset diarrhea in Gunn rats.

In the clinic, some polymorphisms of the UGT gene (e.g., UGT1A1*28 and –3156G>A genotypes) were reported to be associated with severe toxicity (neutropenia and/or diarrhea) of CPT-11 [2, 13]. Severe neutropenia was more common than severe diarrhea. The usefulness of the gene diagnosis of UGT1A1 polymorphisms prior to CPT-11 chemotherapy, and if necessary, the reduction of CPT-11 dose or the alteration of the chemotherapy using other anticancer agents was recommended in order to prevent the CPT-11-induced adverse effects.

In conclusion, Gunn rats exhibited severe diarrheal symptoms after the i.v. administration of CPT-11 at a very low dose. Epithelial impairments were observed in the small intestine and cecum of Gunn rats after the administration of CPT-11. It was thought that the epithelium of the small intestine was exposed to SN-38 at higher levels from both the intestinal lumen and probably from the blood-

stream, and epithelial damage was consequently induced in Gunn rats. These results clarified that the deficiency of UGT activity contributed greatly to the induction of CPT-11-induced delayed-onset diarrhea and epithelial impairment in the intestine. In the clinic, great care is needed when using chemotherapy with CPT-11 in patients with poor UGT activity.

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