Effect of P-Glycoprotein Modulator, Cyclosporin A, on the Gastrointestinal Excretion of Irinotecan and Its Metabolite SN-38 in Rats

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Purpose. The purpose of this work was to investigate the role of the hepatic and intestinal P-glycoprotein (P-gp) and canalicular multispecific organic anion transporter /multidrug resistance-associated protein 2 (cMOAT/MRP2) on both biliary excretion and intestinal exsorption of irinotecan hydrochloride (CPT-11) and its metabolite, SN-38, in the lactone and carboxylate forms. Cyclosporin A (CsA) was used to modulate P-gp and cMOAT/MRP2.

Methods. The transcellular transport of CPT-11 and SN-38 was examined by using LLC-PK1 derivative cell lines transfected with murine mdr1a both in the absence or in the presence of CsA. The excretions of the compounds through the biliary and intestinal membrane routes were investigated by *in situ* perfusion technique.

Results. Basolateral-to-apical transport of CPT-11 lactone in L-mdr1a cells was significantly decreased by CsA (10 μM). The transcellular transport of SN-38 lactone showed similar behaviors as those of CPT-11 lactone. The biliary excretion and the intestinal exsorption of both forms of CPT-11 and SN-38 were significantly inhibited when the drug was co-administered with CsA.

Conclusions. The transports of CPT-11 and SN-38 via the biliary route seem to be essentially related with cMOAT/MRP2, whereas those of both compounds via the intestinal membrane seem to be related with P-gp.

KEY WORDS: irinotecan; cyclosporin A; intestinal exsorption; biliary excretion; P-glycoprotein.

INTRODUCTION

Irinotecan hydrochloride (CPT-11) is a semisynthetic water-soluble analogue of camptothecin (CPT) that exerts a potent antitumor activity by inhibiting topoisomerase I. CPT-11 exerts its antitumor activity after transform *in vivo* into its more active metabolite, 7-ethyl-10-hydroxycamptothecin (SN-38), which shows 100- to 1000-fold more potent antitumor activity *in vitro* than CPT-11 (1,2). One of the principal dose-limiting toxicities associated with CPT-11 therapy is severe diarrhea, although there is a great individual difference in the severity of diarrhea. The cause of the delayed diarrhea is considered to be as follows. CPT-11 is metabolized in the liver by carboxylesterase to SN-38, which is conjugated to SN-38-glucuronide to be excreted in the intestine via bile. The SN-38-glucuronide excreted in the gut is hydrolyzed to SN-38 by enterobacterial β-glucuronidase, and consequently it impairs the gut (3). These impairments in the gut have a close relationship with CPT-11 and its metabolites excreted into the gastrointestinal tract. Excretions of both CPT-11 and SN-38 also depend on the presence of drug-transporting protein, especially P-glycoprotein (P-gp) and canalicular multispecific organic anion transporter/multidrug resistance-associated protein 2 (cMOAT/MRP2), which is present on the bile canalicular membrane (4,5). In our previous report, we demonstrated that significant portions of both CPT-11 and SN-38 were excreted into the gastrointestinal lumen not only via the biliary route but also via the intestinal membrane route after intravenous dosing of CPT-11 to rats (6). Our results also suggested that the gastrointestinal impairment induced by CPT-11 might also occur as a consequence of the significant secretion (exsorption) of CPT-11 via the intestinal membrane in addition to the above-mentioned mechanism. Accordingly, we should take into consideration the intestinal excretion of both CPT-11 and SN-38 across the intestinal membrane as well as the biliary excretion. Several studies on active transport mechanism of CPT-11 and its metabolites have been made by using membrane vesicles (7), P-gp and/or cMOAT modulators (8–10), and mutated rats with a genetic deficiency of cMOAT/MRP2 (11). Most of them were aimed at elucidating the mechanism of the active biliary excretion. There are few *in vivo* studies on the active transport of CPT-11 including those on the gastrointestinal exsorption via the intestinal membrane.

The present study was aimed at investigating the role of the hepatic and intestinal P-gp and cMOAT/MRP2 on both biliary excretion and intestinal exsorption of CPT-11 and SN-38 in the lactone and carboxylate forms. CsA was used to modulate P-gp and cMOAT/MRP2, since CsA is known as an inhibitor of both transporters (12). The present study extends our previous study on the excretion of CPT-11 and SN-38 into the gastrointestinal lumen (6).

MATERIALS AND METHODS

Materials and Chemicals

Camptothecin, CPT-11 and SN-38 were obtained from Daiichi Pharmaceutical Co. Ltd.(Tokyo, Japan). CsA (Sandimmun®) injections were purchased from a local wholesaler. CPT-11 in the lactone form for injection (10 mg/mL) was dissolved in pyrogen-free distilled water (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) just prior to use and, if needed, adjusted with 0.01 N hydrochloric acid to pH 4.0. The conversion of the carboxylate form into lactone form was virtually complete at pH 4.0 (>99%) as determined by high-performance liquid chromatography (HPLC). All other chemicals were commercially available products and of analytical grade.

Cell Culture

Murine *mdr1a*-transfected LLC-PK1 cells (L-mdr1a cells) were generously donated by St. Jude Children's Re-

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search Hospital (Memphis, TN, USA). Cells were maintained in a complete medium consisting of Medium 199 supplemented with 10% fetal bovine serum, 1% penicillin and streptomycin, and 640 nM vincristine for L-mdr1a cells. Cells were grown under an atmosphere of 5% CO_2-95% air at 37°C, and were subcultured every 3 to 4 days using 0.1% trypsin.

Transepithelial Transport

L-mdr1a cells were seeded on polycarbonate membrane filters inside transwell cell culture chambers (3414, 24 mm in diameter, 3.0-μm pore size, Costar) at a cell density of 2×10^6 cells/well. Cells were cultured in 2 mL of Medium 199 supplemented with 10% fetal bovine serum, 1% penicillin and streptomycin in each chamber in an atmosphere of 5% CO_2-95% air at 37°C for 3 days. The medium was replaced by a fresh medium after 2 days, and the cells were used in the transport studies at 3 days after plating. Two hours before transport experiments, all culture media were replaced with fresh medium. The cell monolayers were preincubated with 2 mL of Medium 199 on each side for 2 h at 37°C. Transepithelial transport of CPT-11 and SN-38 was determined using cell monolayers. Medium on either the basal or apical side of the monolayers was replaced with 2 mL of a fresh medium containing CPT-11 (10 μM) or SN-38 (2 μM), and that on the opposite side was replaced with 2 mL of fresh medium alone. In the inhibition study, CsA (10 μM) was added to the media of both sides. The monolayers were incubated in 5% CO_2-95% air at 37°C for up to 4 h, and 70-μL aliquots on the other side were taken at 4 h. The concentrations of CPT-11 and SN-38 lactone and carboxylate forms in each sample were measured by HPLC.

Animals

The present study was approved by the Animal Research and Use Committee of Kumamoto University. Male Wistar rats were purchased from Kyudou Co. Ltd. (Kumamoto, Japan). The body weight of rats ranged from 280 to 340 g. The rats were fasted overnight with free access to drinking water before experiments.

In Situ Perfusion Studies

In situ perfusion experiments were performed as described previously (13). Rats were anesthetized by intraperitoneal (i.p.) injection of ethyl carbamate (1.2 g/kg). The small intestine was exposed by placing a midline abdominal incision. The upper duodenum and the ileocecal junction were cannulated with a polyethylene tube. The small intestine was washed with saline maintained at 37°C and was perfused with lactated Ringer's solution at a rate of 1.3 ml/min from the duodenum through the small intestine to the ileocecal junction (14). CPT-11 was intravenously (i.v.) administered to rats at 10 mg/mL/kg *via* the femoral vein using about 1 min. Prior to injection of CPT-11, CsA (25 mg/0.5 mL/kg) or saline (0.5 mL/kg) was given intravenously to rats. After the injection, a 5-min perfusion period was allowed because of the lag phase of the equilibration period. Blood samples (0.2–0.3 mL) were then collected periodically every 15 min through a cannula introduced into the femoral artery. Perfusates were also collected every 15 min from the ileal outflow and the bile was collected separately every 15 min from the cannula introduced into the common bile duct. The serum was separated immediately by centrifugation at 10,000 g for 2 min at –10°C and stored at –80°C until assay. After completion of the blood collection periods, rats were sacrificed, and tissues (liver, kidney, intestine), were removed.

Pharmacokinetic Analysis

The area under the serum concentration-time curves (AUC) of CPT-11 and SN-38 lactone and carboxylate forms was calculated by the trapezoidal rule. The apparent biliary and intestinal clearance of CPT-11 and SN-38 were calculated by dividing the overall amount of the drugs excreted into the bile or exsorbed into the perfusate during 4 h by AUC from 0 h to 4 h, respectively. The unpaired *t* test was used to assess the pharmacokinetic parameters. A probability level of $p < 0.05$ was considered significant.

Analytical Method

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

RESULTS

Transcellular Transport Study

We investigated whether P-gp was involved in the transepithelial transport of CPT-11 and SN-38 in lactone and carboxylate forms using LLC-PK1 derivative cell lines transfected with murine *mdr1a* (L-mdr1a) either in the absence or in the presence of CsA. Figure 1 shows the transcellular transport of lactone and carboxylate forms of both CPT-11 (10 μM) and SN-38 (2 μM) after application of each on the basal side or the apical side of L-mdr1a cell monolayers in the absence or presence of CsA (10 μM). In the absence of CsA, the basolateral-to-apical transports of both forms of CPT-11 and SN-38 were remarkably higher than those of the apical-to-basolateral direction in L-mdr1a cells. In the presence of

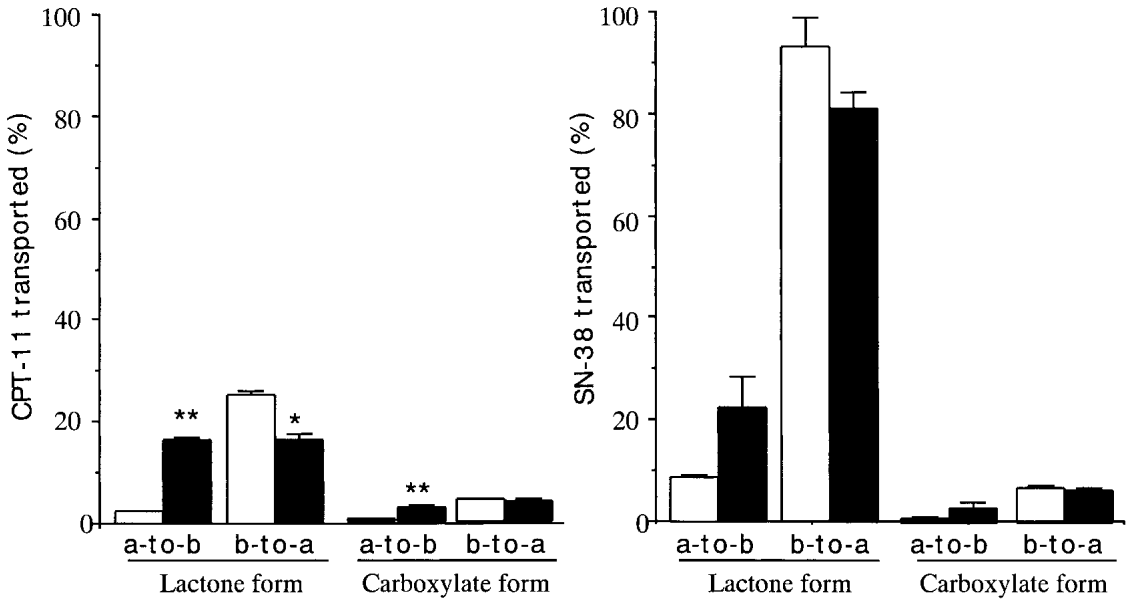

Fig. 1. Effect of cyclosporin A on transepithelial transport of CPT-11 and SN-38 in lactone and carboxylate forms across L-mdr1a cell monolayers. The apical to basal (a-to-b) transport and the basal to apical (b-to-a) transport in the presence of 10 μM cyclosporin A (closed column) was compared with those in the absence of cyclosporin A (open column) at 37°C for 4 h. Each value represents the mean ± SEM of three monolayers. *$p < 0.05$, **$p < 0.01$ (with cyclosporin A vs. without cyclosporin A).

CsA, the basolateral-to-apical transport of CPT-11 lactone was significantly decreased in L-mdr1a cells. The transcellular transport of SN-38 lactone showed behaviors similar to those of CPT-11 lactone, although these change did not reach statistically significant level.

In Situ Single-Pass Perfusion Study

Serum Concentration–Time Profile

We examined the effect of CsA on the gastrointestinal excretion of CPT-11 and SN-38 via the biliary and intestinal routes by using *in situ* single-pass perfusion technique. Figure 2 shows the serum concentration–time curves of the lactone and carboxylate of CPT-11 and SN-38 after i.v. administration of CPT-11 at a dose of 10 mg/kg with or without CsA (25 mg/kg) in rats. Serum levels of the carboxylate form of CPT-11 and the lactone and carboxylate forms of SN-38 were significantly increased in the presence of CsA. On the other hand, little difference was seen in the serum levels of CPT-11 lactone form in the presence of CsA.

Biliary Excretion

Figure 3 shows the cumulative biliary excretion curves of the lactone and carboxylate forms of both CPT-11 and SN-38 after i.v. administration of CPT-11 at a dose of 10 mg/kg with or without CsA in rats. After i.v. administration of CPT-11, both CPT-11 and SN-38 were excreted into the bile mostly in their carboxylate forms. Biliary excretion of CPT-11 and SN-38 in both forms was significantly inhibited by CsA. The amounts of CPT-11 lactone and carboxylate excreted via the bile were decreased by CsA from 7.02 and 59.8% to 2.05 and 27.2% of the dose in 4 h, respectively. Those of SN-38 lactone and carboxylate were also decreased CsA from 0.37 and 2.16% to 0.084 and 1.58% of the dose in 4 h, respectively.

Intestinal Exsorption

Figure 4 shows the cumulative exsorption curves of the lactone and carboxylate forms of CPT-11 and SN-38 from blood into the intestinal lumen across the intestinal membrane after i.v. administration of CPT-11 with or without CsA in rats. Both CPT-11 and SN-38 were exsorbed into the gastrointestinal lumen via the intestinal membrane to an appreciable extent. The exsorption rates of the lactone forms of CPT-11 were considerably higher than those of their carboxylate forms. The average amount of CPT-11 lactone absorbed into the intestinal lumen during the first 4 h after administration was 18.5% of the dose. However, the total amount of SN-38 absorbed in the lactone and carboxylate forms was less than 1% of the dose in the first 4 h. These results indicate that the excretion of CPT-11 into the gastrointestinal lumen occurs not only via the biliary route but also appreciably via the intestinal membrane route. The exsorption of CPT-11 lactone was significantly inhibited when CsA was co-administered. The average amount of the compound absorbed decreased from 18.5% to 8.84% of the dose in the presence of CsA. The exsorption of the CPT-11 in the carboxylate form and SN-38 in the lactone and carboxylate forms also tended to be inhibited by CsA.

Pharmacokinetic Analysis

Table I lists the AUC, biliary and intestinal clearance of CPT-11 and SN-38 following i.v. administration of CPT-11 in the presence or absence of CsA in rats. The AUC value of CPT-11 carboxylate during the period from 0 h to 4 h was increased approximately 2-fold by co-administration of CsA, whereas little change was seen in that of CPT-11 lactone. The AUC values of both lactone and carboxylate forms of SN-38 were increased by CsA approximately 1.5-fold. The increase in the AUC values may be attributable to the significant in-

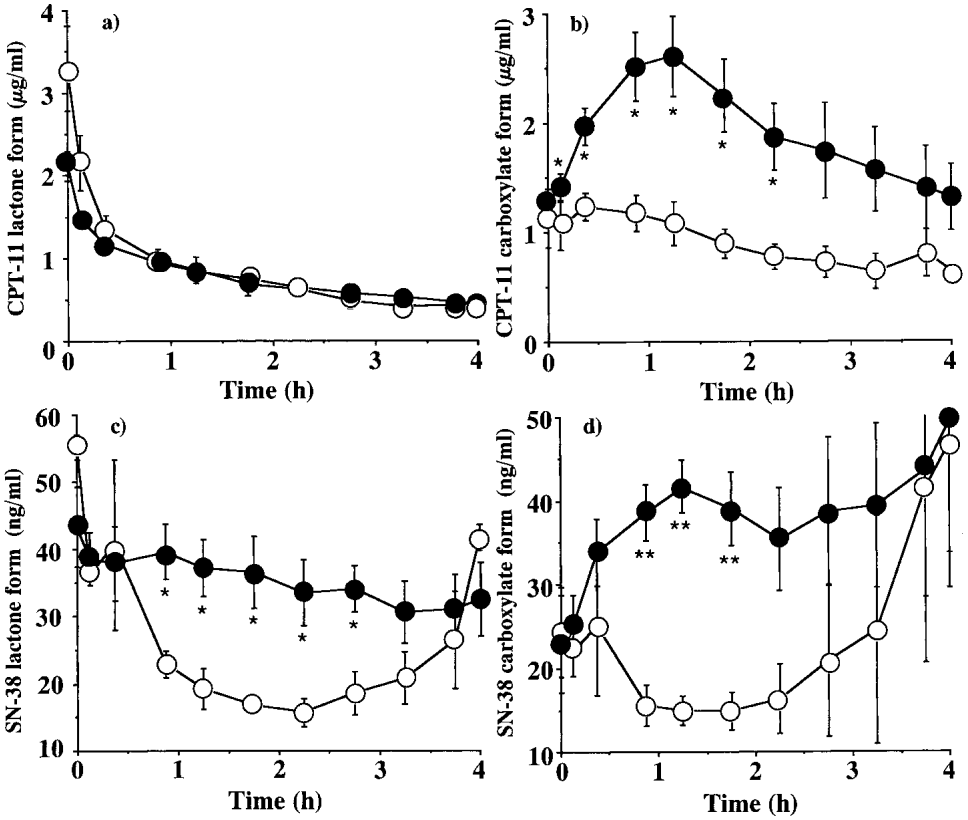

Fig. 2. Serum concentration–time profiles of CPT-11 lactone (a), CPT-11 carboxylate (b), SN-38 lactone (c), and SN-38 carboxylate (d) after i.v. administration of CPT-11 at a dose of 10 mg/kg in the presence (closed circles) or absence (open circles) of cyclosporin A (25 mg/kg) in rats. Each value represents the mean ± SEM of three to four rats. *$p < 0.05$, **$p < 0.01$ (with cyclosporin A vs. without cyclosporin A).

hibition of the biliary excretion and the intestinal exsorption of CPT-11 and SN-38 by CsA.

To evaluate the ability of a system to excrete both forms of CPT-11 and SN-38, apparent biliary and intestinal clearances were calculated. The biliary clearance values of CPT-11 and SN-38 were 7.5- and 6.3-fold higher in carboxylate forms than those in lactone forms, respectively. On the contrary, the intestinal clearance values of CPT-11 and SN-38 were 8.8- and 5.7-fold higher in their lactone forms as compared to those in the carboxylate forms, respectively. The average biliary clearance values of the lactone and carboxylate forms of CPT-11 were significantly decreased from 66.9 and 499 mL/h to 21.8 and 124 mL/h by co-administration of CsA, respectively. The corresponding values for lactone and carboxylate forms of SN-38 were from 123 and 777 mL/h to 23.0 and 326 mL/h, respectively. The average intestinal clearance values of CPT-11 lactone and carboxylate were also significantly decreased from 174 and 19.7 mL/h to 93.9 and 9.65 mL/h by co-administration of CsA and those of SN-38 lactone and carboxylate from 160 and 28.1 mL/h to 17.8 and 5.26 mL/h, respectively.

Tissue Distribution

We determined the tissue levels of the compounds in the liver, kidney, and intestine after completing the *in situ* perfusion study. Figure 5 shows tissue levels of CPT-11 and SN-38 at 4 h after i.v. administration of CPT-11 to rats in the absence or presence of CsA. CPT-11 existed mostly as the parent compound (CPT-11) in tissues rather than as the active metabolite, SN-38. Furthermore, the levels of CPT-11 lactone in tissues were higher than those of CPT-11 carboxylate. The tissue levels of CPT-11 and SN-38 at 4 h after i.v. dosing of CPT-11 were found to be increased after co-administration of CsA.

DISCUSSION

CPT-11 and SN-38 have been reported to be the substrates for P-gp and to have moderate affinity to P-gp (8,16). To confirm the participation of P-gp, we examined the transport of CPT-11 and SN-38 in their lactone and carboxylate forms by L-mdr1a cells that overexpress P-gp on the apical membrane. Our results showed that the basolateral-to-apical transports of CPT-11 and SN-38 by L-mdr1a cells were all higher than those in the opposite direction (Fig. 1). In addition, CsA as a P-gp modulator significantly inhibited the basolateral-to-apical transport of CPT-11 lactone, and significantly enhanced the apical-to-basolateral transport of the lactone and carboxylate forms of CPT-11 in L-mdr1a cells. These results suggest that CPT-11 and SN-38 in both forms are the substrates for P-gp. The enhanced basolateral directional transport of CPT-11 by CsA may be attributable to the inhibition of the expulsion of CPT-11 across the apical membranes via P-gp that is confined to the apical membrane domain of the epithelial cells (17). Thus, it is considered that P-gp is more or less involved in the active transport of both lactone and carboxylate forms of CPT-11 and SN-38.

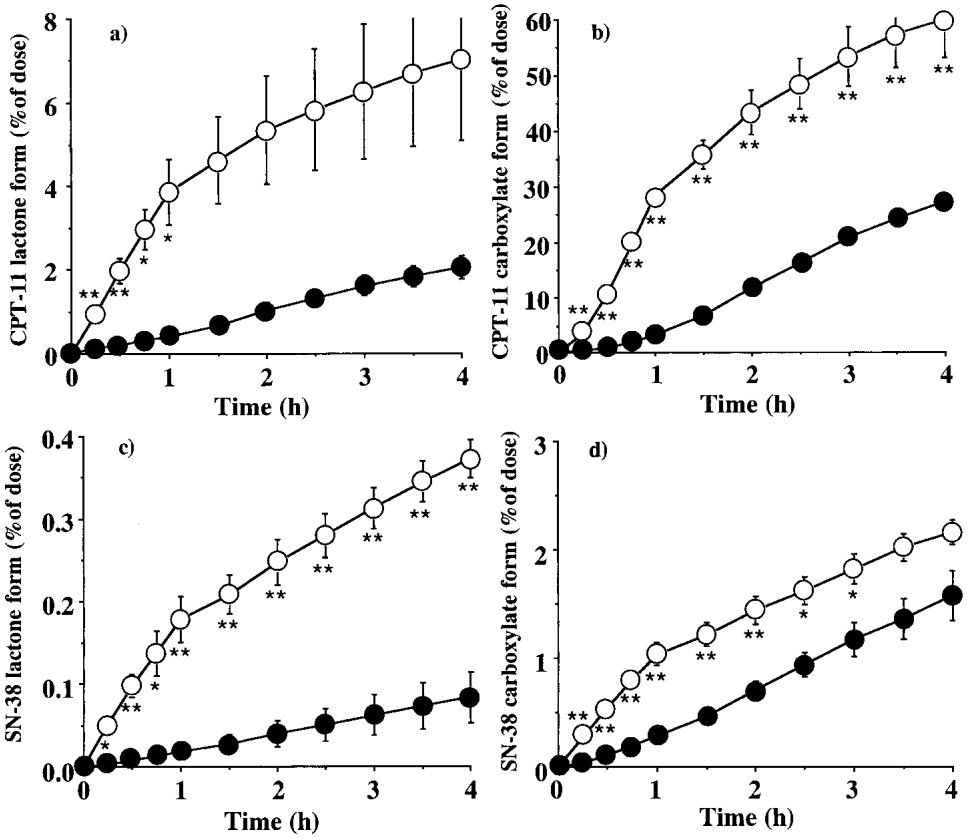

Fig. 3. Cumulative biliary excretion curves of CPT-11 lactone (a), CPT-11 carboxylate (b), SN-38 lactone (c), and SN-38 carboxylate (d) after i.v. administration of CPT-11 at a dose of 10 mg/kg in the presence (closed circle) or absence (open circle) of cyclosporin A (25 mg/kg) in rats. Each value represents the mean ± SEM of three to four rats. *$p < 0.05$, **$p < 0.01$ (with cyclosporin A vs. without cyclosporin A).

CsA is known as an inhibitor of both P-gp and cMOAT/MRP2 (12). P-gp and cMOAT/MRP2 are expressed in the apical domain of various organs such as the kidney, liver, brain and gastrointestinal tract (18,19), and function as an ATP-dependent efflux transport pump for a variety of drugs. P-gp contributes substantially to the transport of a variety of cationic and non-ionic drugs from the body through intestinal and hepatobiliary secretion (20,21), whereas cMOAT/MRP2 is responsible for the transport of organic anions and glucuronide conjugates (19). There were significant alterations in the pharmacokinetics of CPT-11 and SN-38 after co-administration of 25 mg/kg CsA. Serum levels of CPT-11 carboxylate and SN-38 lactone and carboxylate were significantly elevated by CsA dosing (Fig. 2). The elevated serum levels may be attributable to the reduced excretions of CPT-11 and SN-38 via the biliary route and/or the intestinal membrane route. On the other hand, the serum levels of CPT-11 lactone were little changed by CsA dosing (Fig. 2, Table I). This may be the result of the greater conversion rates of CPT-11 from the lactone form to the carboxylate form in the serum compared with the excretion rates of the lactone through the biliary and intestinal membrane routes since half-life of the lactone form of CPT-11 to its carboxylate form at 37°C was 13.7 min in aqueous solution (22).

The biliary excretion rates and the biliary clearance values of the lactone and carboxylate forms of CPT-11 and SN-38 were significantly decreased by co-administration of CsA. Recently, the biliary excretion mechanism of CPT-11 and SN-38 has been elucidated (7,9,11,23,24). The carboxylate forms of both CPT-11 and SN-38 have a high affinity to the canalicular multispecific organic anion transporter (cMOAT/MRP2), which is responsible for the biliary excretion of their carboxylate forms in rats (7,11,24). Thus, since most of CPT-11 and SN-38 were excreted into the bile in carboxylate forms, cMOAT/MRP2 may play an essential role in the biliary excretion of the carboxylate forms of both CPT-11 and SN-38. On the other hand, P-gp may be involved in the biliary excretion of the lactone forms of CPT-11 and SN-38 since both lactone forms have non-ionic charges.

In contrast with the biliary excretion of CPT-11 and SN-38, the exsorption of CPT-11 and SN-38 from the blood across the intestinal membrane was higher in their lactone forms than that in their carboxylate forms. The lactone forms of CPT-11 and SN-38 are nonionic in the blood, and the non-ionized forms would be expected to permeate freely through capillary walls to the gastrointestinal tract. Kobayashi *et al.* (25) reported from experiments using hamster intestinal cells that the lactone forms of CPT-11 and SN-38 were transported passively, whereas their carboxylate forms were absorbed actively. However, our *in vitro* study confirmed that both the lactone and carboxylate forms of CPT-11 and SN-38 were more or less the substrates for P-gp (Fig. 1). Furthermore, *in situ* perfusion studies showed that the transport of CPT-11 lactone via the intestinal membrane was significantly inhib-

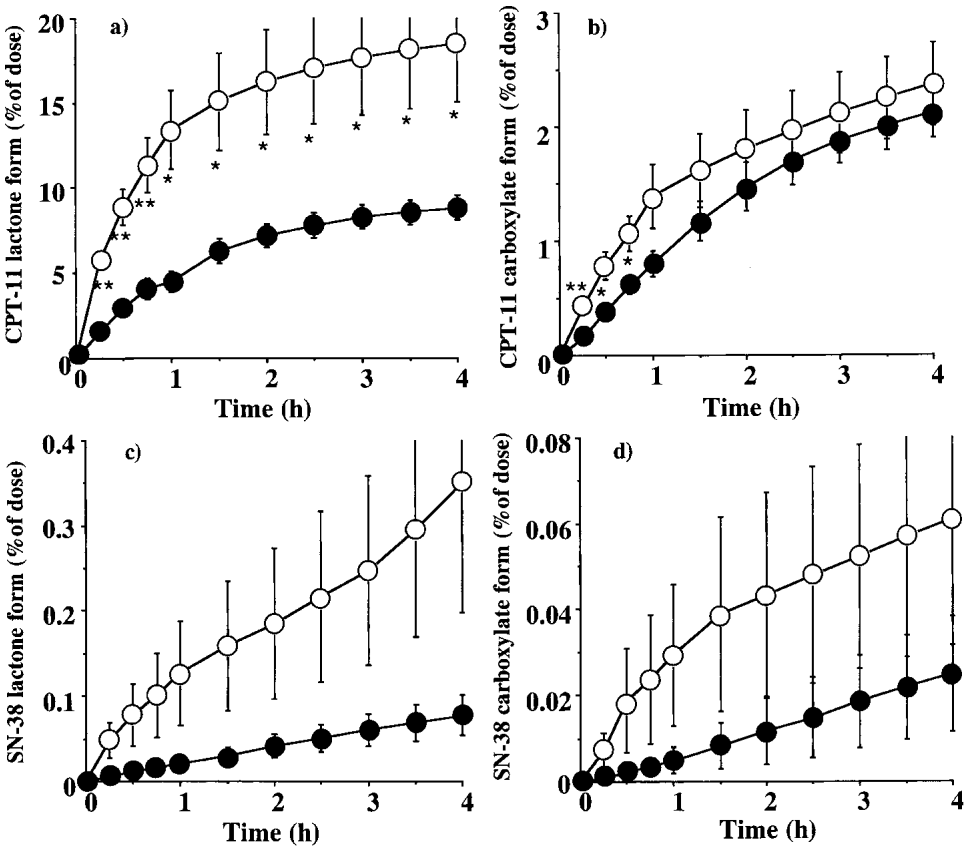

Fig. 4. Cumulative intestinal excretion curves of CPT-11 lactone (a), CPT-11 carboxylate (b), SN-38 lactone (c), and SN-38 carboxylate (d) after i.v. administration of CPT-11 at a dose of 10 mg/kg in the presence (closed circle) or absence (open circle) of cyclosporin A (25 mg/kg) in rats. Each value represents the mean ± SEM of three to four rats. *$p < 0.05$, **$p < 0.01$ (with cyclosporin A vs. without cyclosporin A).

ited by P-gp and cMOAT/MRP2 modulator, CsA (Fig. 4). From these points of view, it appears that P-gp is more involved in the exsorption of both lactone forms across the intestinal membrane than cMOAT/MRP2.

The tissue levels of CPT-11 and SN-38 in the liver, kidney and intestine tended to be increased in 4 h after coadministration of CsA (Fig. 5). P-gp and cMOAT/MRP2 are expressed in the apical domain of various organs such as hepatocytes, proximal renal tubular cells and enterocytes of the proximal small intestine (18,19). Therefore, both transporters may be involved in the transport of CPT-11 and SN-38 to these tissues. The increase in CPT-11 and SN-38 levels in tissue may be associated with increases in the pharmacological effect and/or clinical toxicity.

In conclusion, CsA, a P-gp and cMOAT/MRP2 modulator, was found to inhibit the transport of CPT-11 and SN-38 into the gastrointestinal lumen via the biliary and intestinal routes, keeping their serum and tissue levels high. The trans-

Table I. Area under the Curve (AUC), Biliary, and Intestinal Clearance Values of CPT-11 and SN-38 after Intravenous Administration of CPT-11 (10 mg/kg) Solutions with or without Cyclosporin A (CsA; 25 mg/kg) to Rats

	CPT-11		SN-38	
	Lactone	Carboxylate	Lactone	Carboxylate
AUC (μg h/mL)				
With saline	3.20 ± 0.49	3.61 ± 0.45	0.0945 ± 0.010	0.0916 ± 0.033
With CyA	3.10 ± 0.18	7.72 ± 1.28*	0.141 ± 0.018	0.153 ± 0.024
Biliary CL (mL/h)				
With saline	66.9 ± 15	499 ± 7.3	123 ± 20.2	777 ± 87
With CyA	21.8 ± 3.3*	124 ± 17**	23.0 ± 3.70*	326 ± 58**
Intestinal CL (mL/h)				
With saline	174 ± 33	19.7 ± 3.0	160 ± 12.2	28.1 ± 9.6
With CyA	93.9 ± 6.8*	9.65 ± 1.6*	17.8 ± 3.9**	5.26 ± 2.4*

Note: Each value represents the mean ± SEM of three to four rats.
* $p < 0.05$, **$p < 0.01$ (with saline vs. with CyA).

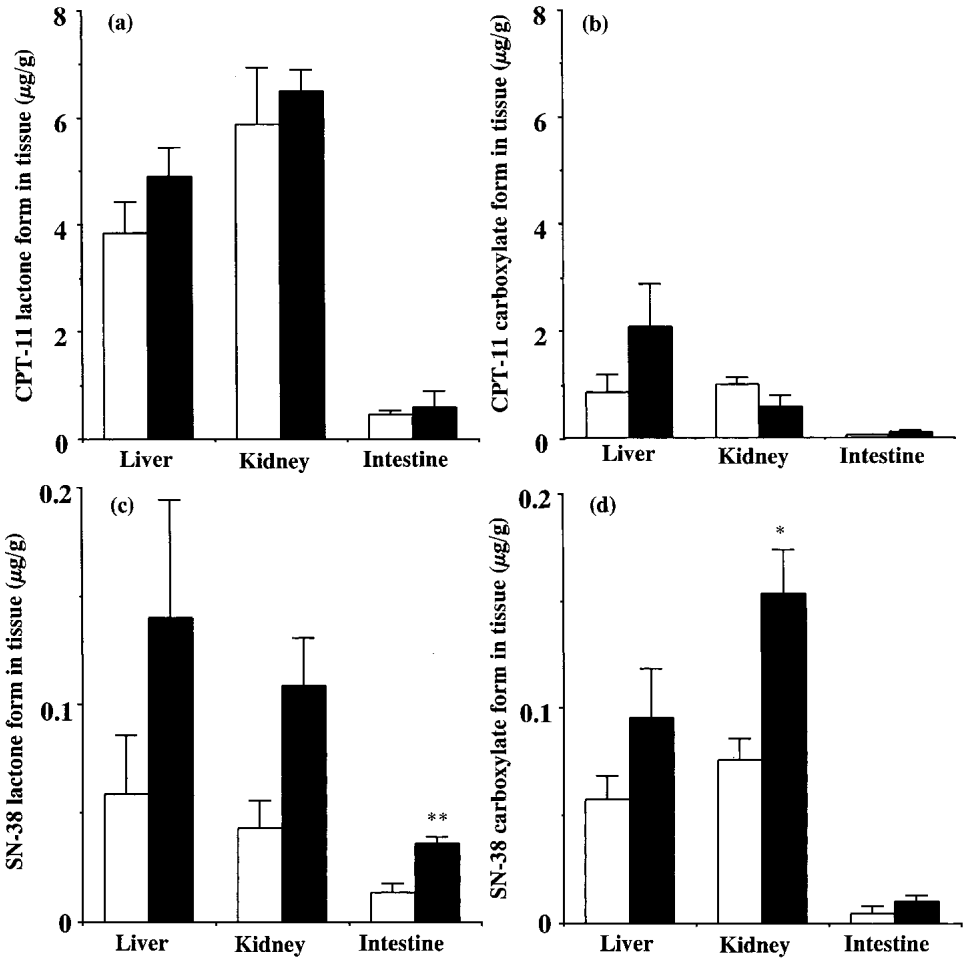

Fig. 5. Distribution of CPT-11 lactone (a), CPT-11 carboxylate (b), SN-38 lactone (c), and SN-38 carboxylate (d) at 4 h after i.v. administration of CPT-11 at a dose of 10 mg/kg in the presence (closed columns) or absence (open columns) of cyclosporin A (25 mg/kg) in rats. Each value represents the mean ± SEM of three to four rats. *$p < 0.05$, **$p < 0.01$ (with cyclosporin A vs. without cyclosporin A).

ports of CPT-11 and SN-38 via the biliary route seem to be essentially related with cMOAT/MRP2, whereas those of both compounds via the intestinal membrane route seem to be rather related with P-gp. Thus, it can be expected that reduction in the excretion of CPT-11 and SN-38 into the gastrointestinal lumen by P-gp and/or cMOAT/MRP2 inhibitors may reduce the gastrointestinal toxicity.

REFERENCES

1. Y. Kawato, M. Aonuma, Y. Hirota, H. Kuga, and K. Sato. Intracellular roles of SN-38, a metabolite of the camptothecin derivative CPT-11, in the antitumor effects of CPT-11. *Cancer Res.* **51**:4187–4191 (1991).
2. A. Kojima, T. Shinkai, and N. Saijo. Cytogenetic effects of CPT-11 and its active metabolite, SN-38 on human. *Jpn. J. Clin. Oncol.* **23**:116–122 (1993).
3. M. Narita, E. Nagai, H. Hagiwara, M. Aburada, T. Yokoi, and T. Kamataki. Inhibition of beta-glucuronidase by natural glucuronides of kampo medicines using glucuronide of SN-38 (7-ethyl-10-hydroxycamptothecin) as a substrate. *Xenobiotica* **23**:5–10 (1993).
4. R. H. Mathijssen, R. J. van Alphen, J. Verweij, W. J. Loos, K. Nooter, G. Stoter, and A. Sparreboom. Clinical pharmacokinetics and metabolism of irinotecan (CPT-11). *Clin. Cancer Res.* **7**: 2182–2194 (2001).
5. W. Yamamoto, J. Verweiji, P. de Bruijin, M. J. de Jonge, and H. Takano. M. Nishiyama, M. Kurihara, and A. Sparreboom. Active transepithelial transport of irinotecan (CPT-11) and its metabolites by human intestinal Caco-2 cells. *Anticancer Drugs* **12**:419–432 (2001).
6. K. Arimori, N. Kuroki, A. Kumamoto, N. Tanoue, M. Nakano, E. Kumazawa, A. Tohgo, and M. Kikuchi. Excretion into Gastrointestinal tract of irinotecan lactone and carboxylate forms and their pharmacodynamics in rodents. *Pharm. Res.* **18**:814–822 (2001).
7. X.-Y. Chu, Y. Kato, K. Ueda, H. Suzuki, K. Niinuma, C. A. Tyson, V. Weizer, J. E. Dabbs, R. Froehlich, C. E. Green, and Y. Sugiyama. Biliary excretion mechanisms for CPT-11 and its metabolites in humans: involvement of primary active transporters. *Cancer Res.* **58**:5137–5143 (1998).
8. E. Gupta, A. R. Safa, X. Wang, and M. J. Ratain. Pharmacokinetic modulation of irinotecan and metabolites by cyclosporin A. *Cancer Res.* **56**:1309–1314 (1996).
9. X.-Y. Chu, Y. Kato, and Y. Sugiyama. Possible involvement of P-glycoprotein in biliary excretion of CPT-11 in rats. *Drug Metab. Dispos.* **27**:440–441 (1999).
10. M. Horikawa, Y. Kato, C. A. Tyson, and Y. Sugiyama. The potential for an interaction between MRP2 (ABCC2) and various therapeutic agents: probenecid as a candidate inhibitor of the biliary excretion of irinotecan metabolites. *Drug Metabol. Pharmacokinet.* **17**:23–33 (2002).
11. X.-Y. Chu, Y. Kato, K. Niinuma, K.-I. Sudo, H. Hakusui, and Y. Sugiyama. Multispecific organic anion transporter is responsible for the biliary excretion of the camptothecin derivative irinotecan and its metabolites in rats. *J. Pharmacol. Exp. Ther.* **281**:304–314 (1997).

12. Y. Liu, L. Huang, T. Hoffman, M. Gosland, and M. Vore. MDR1 substrates/modulators protect against beta-estradiol- 17beta-D-glucuronide cholestasis in rat liver. *Cancer Res.* **56**:4992–4997 (1996).
13. A. Tomimaru, K. Arimori, N. Inotsume, and M. Nakano. Effect of activated charcoal and atropine on absorption and/or exsorption of organophosphorus compounds. *J. Pharm. Pharmacol.* **48**: 351–356 (1996).
14. K. Arimori and M. Nakano. Transport of theophylline from blood to the intestinal lumen following i.v. administration to rats. *J. Pharmacobio-Dyn.* **8:**324–327 (1985).
15. L. P. Rivory, and J. Robert. 1994. Reversed-phase high- performance liquid chromatographic method for the simultaneous quantitation of the carboxylate and lactone forms of the camptothecin derivative irinotecan, CPT-11, and its metabolite SN-38 in plasma. *J. Chromatogr. B Biomed. Appl.* **661:**133–141 (1994).
16. X.-Y. Chu, H. Suzuki, K. Ueda, Y. Kato, and Y. Sugiyama. Active efflux of CPT-11 and its metabolites in human KB-derived cell lines. *J. Pharmacol. Exp. Ther.* **288**:735–741 (1999).
17. K. Ueda, N. Okamura, M. Hirai, Y. Tanigawara, T. Saeki, N. Kioka, and R. Hori. Human P-glycoprotein transports cortisol, aldosterone, and dexamethasone, but not progesterone. *J. Biol. Chem.* **267**:24248–24252 (1992).
18. F. Thiebaut, T. Tsuruo, H. Hamada, M. M. Gottesman, I. Pastan, and M. C. Willingham. Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc. Natl. Acad. Sci. USA* **84**:7735–7738 (1987).
19. P. M. Gerk and M. Vore. Regulation of expression of the multidrug resistance-associate protein 2 (MRP2) and its role in drug disposition. *J. Pharmacol. Exp. Ther.* **302**:407–415 (2002).
20. J. W. Smit, A. H. Schinkel, M. Muller, B. Weert, and D. K. Meijer. Contribution of the murine mdr1a P-glycoprotein to hepatobiliary and intestinal elimination of cationic drugs as measured in mice with an mdr1a gene disruption. *Hepatology* **27**: 1056–1063 (1998).
21. K. Arimori and M. Nakano. Drug exsorption from blood into the gastrointestinal tract. *Pharm. Res.* **15:**371-376 (1998).
22. K. Akimoto, A. Kawai, and K. Ohya. Kinetic studies of the hydrolysis and lactonization of camptothecin and its derivatives, CPT-11 and SN-38, in aqueous solution. *Chem. Pharm. Bull.* **42**: 2135–2138 (1994).
23. Y. Sugiyama, Y. Kato, and X-X. Chu. 1998. Multiplicity of biliary excretion mechanisms for the camptothecin derivative irinotecan (CPT-11), its metabolite SN-38, and its glucuronide: role of canalicular multispecific organic anion transporter and P-glycoprotein. *Cancer Chemother. Pharmacol.* **42(**Suppl):S44–S49 (1998).
24. X.-Y. Chu, Y. Kato, and Y. Sugiyama. Multiplicity of biliary excretion mechanisms for irinotecan, CPT-11, and its metabolites in rats. *Cancer Res.* **57**:1934–1938 (1997).
25. K. Kobayashi, B. Bouscarel, Y. Matsuzaki, S. Ceryak, S. Kudoh, and H. Fromm. PH-dependent uptake of irinotecan and its active metabolite, SN-38, by intestinal cells. *Int. J. Cancer* **83**:491–496 (1999).