Involvement of Up-Regulation of Hepatic Breast Cancer Resistance Protein in Decreased Plasma Concentration of 7-Ethyl-10-hydroxycamptothecin (SN-38) by Coadministration of S-1 in Rats

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ABSTRACT:

The safety and efficacy of combination therapy with 7-ethyl-10-[4-\{1-piperidino\}-1-piperidino]carbonyloxytamptothecin (CPT-11, irinotecan) and S-1 composed of tegafur, a prodrug of 5-fluorouracil, gimeracil, and potassium oxonate, have been confirmed in patients with colorectal cancer. Previously, we showed that p.o. coadministration of S-1 decreased the plasma concentration of both CPT-11 and its metabolites in a patient with advanced colorectal cancer. The aim of this study was to clarify the mechanism of drug interaction using both in vivo and in vitro methods. Rats were administered S-1 p.o. (10 mg/kg) once a day for 7 consecutive days. On day 7, CPT-11 (10 mg/kg) was administered by i.v. injection. Coadministration of S-1 affected the pharmacokinetic behavior of CPT-11 and its metabolites, with a decrease in the C\text{max} and area under the plasma concentration curve (AUC) of the active metabolite 7-ethyl-10-hydroxyacamptothecin (SN-38) lactone form. Furthermore, the rate of biliary excretion of the SN-38 carboxylate form increased on coadministration of S-1. In the liver, the level of breast cancer resistance protein (BCRP), but not P-glycoprotein and multidrug resistance-associated protein 2, was elevated after administration of S-1. Enzymatic conversion of CPT-11 to SN-38 by carboxylesterase (CES) was unaffected by the liver microsomes of rats treated with S-1. In addition, components of S-1 did not inhibit the hydrolysis of p-nitrophenylacetate, a substrate of CES, in the S9 fraction of HepG2 cells. Therefore, the mechanism of drug interaction between CPT-11 and S-1 appears to involve up-regulation of BCRP in the liver, resulting in an increased rate of biliary excretion of SN-38 accompanied by a decrease in the C\text{max} and AUC of SN-38.

Irinotecan (CPT-11), an inhibitor of DNA topoisomerase I, is widely used in the treatment of several types of solid tumors, including colorectal cancer (Wiseman and Markham, 1996). CPT-11 exerts its antitumor activity after enzymatic transformation to its more active metabolite, SN-38, by hepatic carboxylesterases (CES) (Fig. 1) (Hamerickxhouse et al., 2000). In vitro study suggested that antitumor effects of SN-38 are 100 to 1000 times more potent than those of CPT-11 (Kawato et al., 1991). SN-38 is subsequently conjugated in the liver by UDP glucuronosyltransferase (UGT) 1A, thereby forming the inactive metabolite 7-ethyl-10-hydroxycampothecin (SN-38) lactone form. Furthermore, the rate of biliary excretion of the SN-38 carboxylate form increased on coadministration of S-1. In the liver, the level of breast cancer resistance protein (BCRP), but not P-glycoprotein and multidrug resistance-associated protein 2, was elevated after administration of S-1. Enzymatic conversion of CPT-11 to SN-38 by carboxylesterase (CES) was unaffected by the liver microsomes of rats treated with S-1. In addition, components of S-1 did not inhibit the hydrolysis of p-nitrophenylacetate, a substrate of CES, in the S9 fraction of HepG2 cells. Therefore, the mechanism of drug interaction between CPT-11 and S-1 appears to involve up-regulation of BCRP in the liver, resulting in an increased rate of biliary excretion of SN-38 accompanied by a decrease in the C\text{max} and AUC of SN-38.

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ABBREVIATIONS: CPT-11, irinotecan; 7-ethyl-10-[4-\{1-piperidino\}-1-piperidino]carbonyloxyacamptothecin; SN-38, 7-ethyl-10-hydroxyacamptothecin; CES, carboxylesterase(s); UGT, UDP glucuronosyltransferase; SN-38G, SN-38 glucuronide; P-gp, P-glycoprotein; MRP2, multidrug resistance-associated protein 2; BCRP, breast cancer resistance protein; FT, tegafur; CDHP, 5-chloro-2,4-dihydroxyxypiridine; Oxo, potassium oxonate; 5-FU, 5-fluorouracil; AUC, area under the plasma concentration curve; p-NPA, p-nitrophenylacetate; CMC, carboxyl methyl cellulose; PBS, phosphate-buffered saline.
The safety and efficacy of combination therapy with CPT-11 and S-1 (Ohtsu et al., 2000; Van den Brande et al., 2003; Shirao et al., 2004). and good compliance in patients with advanced colorectal cancer. treatment with S-1 had a high response rate, ranging from 19 to 39%, in the plasma and tumor cells by competitive inhibition of dihydro- pyrimidine dehydrogenase, which is responsible for the catabolism of 5-FU (Takechi et al., 2002). A side effect of 5-FU is diarrhea, which is caused by its phosphorylation in the intestine by orotate phosphoribosyltransferase. Oxo, a specific inhibitor of orotate phosphoribosyltransferase, is a protective agent against 5-FU–induced diarrhea (Shirasaka et al., 1996). In phase II studies, it was shown that a single treatment with S-1 had a high response rate, ranging from 19 to 39%, and good compliance in patients with advanced colorectal cancer (Ohsita et al., 2000; Van den Brange et al., 2003; Shirao et al., 2004). The safety and efficacy of combination therapy with CPT-11 and S-1 was investigated in a phase II study of patients with colorectal cancer, and its usefulness was confirmed (Goto et al., 2006). Overall response rate of combination therapy with CPT-11 and S-1 was 62.5%, and median progression-free survival was 8.0 months. Previous studies reported that the plasma concentration and area under the plasma concentration curve (AUC) of CPT-11 were elevated, and those of SN-38 reduced, in combined therapy with CPT-11 and 5-FU compared with a single treatment with CPT-11 in patients with colorectal cancer (Sasaki et al., 1994). Recently, we found that p.o. coadministration of S-1 appeared to affect the pharmacokinetic behavior of CPT-11, SN-38, and SN-38G in a patient with advanced colorectal cancer (Yokoo et al., 2006). In particular, coadministration of S-1 resulted in a significant decrease in the plasma concentration of SN-38 compared with a single treatment with CPT-11. However, the mechanisms of this drug interaction have not been elucidated. Therefore, we explored the effect of coadministration of S-1 on the pharmacokinetics of CPT-11 and its metabolites in rats.

Materials and Methods

Materials. CPT-11, SN-38, and SN-38G were provided by Daiichi Sankyo Co. Ltd. (Tokyo, Japan). S-1 was provided by Taiho Pharmaceutical Co. Ltd. (Tokyo, Japan). p-Nitrophenylacetate (p-NPA) and p-nitrophenol were purchased from Sigma-Aldrich (St. Louis, MO). All the other chemicals were commercially available products and of analytical grade.

Experimental Animals. Male Sprague-Dawley rats that had been administered CMC or S-1 (10 mg/kg). The livers were stored at -80°C until use. Hepatic microsomes were prepared by differential centrifugation. The rat liver homogenates were centrifuged at 9000 g for 20 min at 4°C. The supernatant was then centrifuged at 105,000 g for 1 h at 4°C. The microsomes were stored at -80°C in PBS (10 mg/ml) until use. Incubations of microsomes with CPT-11 were carried out in PBS at 37°C in 0.5-ml Eppendorf tubes. A stock solution of CPT-11 was prepared directly in PBS, and the pH was corrected to pH 7.4. An aliquot of the enzyme stock (25 µl) was added to 125 µl of PBS. After 5 min of equilibration at 37°C, the reaction was initiated by the addition of the required dilution of the CPT-11 stock (100 µl) to yield concentrations of CPT-11 ranging from 1 to 1000 µM in a total of 250 µl. The tubes were agitated in a shaking water bath maintained at 37°C. Aliquots (50 µl) were withdrawn at regular intervals and immediately mixed with 100 µl of ice-cold methanol to quench the reaction. Samples were then assayed for the total forms (carboxylate form plus lactone form) of SN-38 as detailed earlier.

Western Blot Analysis. Livers were homogenized in a homogenization buffer composed of 230 mM sucrose, 5 mM Tris-HCl, pH 7.5, 2 mM EDTA, 0.1 mM phenylmethanesulfonyl fluoride, 1 mg/ml leupeptin, and 1 mg/ml pepstatin A. The homogenate was centrifuged twice at 3000 g for 15 min, and the supernatant was further centrifuged at 105,000 g for 30 min. The resultant pellet is referred to as the crude membrane fraction. After measurement of the protein content using a bicinchoninic acid protein assay reagent (Pierce, Rockford, IL), each sample was mixed in a loading buffer (2% SDS, 125 mM
Tris-HCl, pH 6.4, 20% glycerol, and 5% 2-mercaptoethanol) and heated at 100°C for 2 min. The samples were separated by 7.5% SDS-polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride membranes (Immobilon-P, Millipore, Bedford, MA) by semidy electroblotting. The blots were blocked overnight at 4°C with 2% enhanced chemiluminescence advanced blocking agents (GE Healthcare, Little Chalfont, Buckinghamshire, UK) in Tris-buffered saline containing 0.3% Tween 20 and incubated for 1 h at room temperature with primary antibody specific for P-gp (C219 monoclonal antibody, Signet Laboratories, Inc., Dedham, MA), MRP2 (MRP2 monoclonal antibody, CHEMICON International, Inc., Temecula, CA), and BCRP (ABCG2 polyclonal antibody, Santa Cruz Biotechnology, Inc., Santa Cruz, CA). The blots were washed with Tris-buffered saline containing 0.3% Tween 20 and incubated with the secondary antibody [horseradish peroxidase-linked anti-rabbit immunoglobulin F(ab)2 or horseradish peroxidase-linked antimouse immunoglobulin F(ab)2; both from GE Healthcare] for 1 h at room temperature. Immunoblots were visualized with an enhanced chemiluminescence system (ECL Advance Western Blotting Detection Kit, GE Healthcare). The relative amount of each band was determined densitometrically using Densitograph Imaging Software (ATTO Corporation, Tokyo, Japan). Densitometric ratios were normalized to the value obtained without S-1.

Statistical Analysis. The differences were analyzed statistically using Student's unpaired t test. A value of P < 0.05 was considered significant.

Results

Effect of S-1 on the Pharmacokinetics of CPT-11 and Its Metabolites. Figure 2 shows the plasma concentration-time profiles of the carboxylate form, lactone form, and total form (i.e., carboxylate form plus lactone form) of CPT-11, active metabolite SN-38, and inactive metabolite SN-38G after i.v. administration of CPT-11 at a dose of 10 mg/kg i.v. administration in the absence (○, △, □) or presence of S-1 (●, ▲, ■) at a dosage of 10 mg/kg/day. All the data are expressed as mean ± S.D., n = 5.

Effect of S-1 on the Biliary Excretion of CPT-11 and Its Metabolites. Figure 3 shows the cumulative biliary excretion curves of the carboxylate form, lactone form, and total forms (i.e., lactone form) of CPT-11, active metabolite SN-38, and inactive metabolite SN-38G after i.v. administration of CPT-11 at a dose of 10 mg/kg with or without S-1 (10 mg/kg/day) in rats. Coadministration p.o. of S-1 appeared to affect the pharmacokinetic parameters of CPT-11, SN-38, and SN-38G (Table 1). In particular, coadministration of S-1 resulted in a significant decrease in the plasma concentration of the SN-38 lactone form compared with a single treatment with CPT-11. The maximum plasma concentration (C_max) of the SN-38 lactone form decreased by 30.1% from 144.1 ± 15.8 ng/ml (without S-1) to 100.8 ± 8.9 ng/ml (with S-1). Furthermore, the AUC of the SN-38 lactone form decreased by 33.8% from 235.8 ± 43.6 ng · h/ml (without S-1) to 156.2 ± 15.0 ng · h/ml (with S-1). However, C_max and AUC of the SN-38 carboxylate form were unchanged by coadministration of S-1, whereas C_max and AUC of both CPT-11 and SN-38G showed a slight decrease in concentration on coadministration with S-1.
SN-38 carboxylate form, whereas SN-38G was excreted into bile mostly as the SN-38G lactone form. The biliary excretion of both forms of CPT-11 and SN-38 or the SN-38 lactone form was unaltered by administration of S-1. However, the biliary excretion of the SN-38 carboxylate form was significantly increased by coadministration of S-1. The amount of the SN-38 carboxylate form excreted via bile significantly increased from 9.7 ± 3.3% (without S-1) to 14.9 ± 3.7% (with S-1) 4 h after coadministration of S-1. In addition, the total amount of the total forms of SN-38 also significantly increased after coadministration of S-1 from 12.4 ± 2.8% (without S-1) to 17.6 ± 1.0% (with S-1).

### Effect of Each Component of S-1 and 5-FU on the Relative Inhibition of p-NPA Hydrolysis

Kinetic analysis of p-NPA hydrolysis at a range of concentrations (0.1–5.0 mM) was performed (Fig. 4A). The hydrolysis of p-NPA was best fitted by a single Michaelis-Menten equation with a Michaelis-Menten constant ($K_m$) of 599 μM and maximum hydrolysis rate ($V_{max}$) of 8.38 μM/min. The effects of CPT-11, FT, 5-FU, CDHP, and Oxo on p-NPA (500 μM) hydrolysis were examined by incubating with the S9 fraction of HepG2 cells (Fig. 4B). CPT-11 was found to be a potent inhibitor of p-NPA hydrolysis, whereas FT, 5-FU, CDHP, and Oxo did not inhibit p-NPA hydrolysis.

### Effect of S-1 on the Hydrolysis of CPT-11 by Rat Liver Microsomes

Production of SN-38 from 10 μM CPT-11 over a 2-h incubation period with rat liver microsomes in the absence of S-1 is shown in Fig. 5A. A linear regression of the concentration versus time plot from 15 min onward yielded a steady-state velocity ($r^2 = 0.99$). The kinetics of production of the total forms of SN-38 from CPT-11 at a range of concentrations (0.1–300 μM) was investigated at 1 h (Fig. 5B). The $K_m$ and $V_{max}$ values for hydrolysis of CPT-11 with rat liver microsomes were unchanged by administration of S-1 for 7 consecutive days. The $K_m$ and $V_{max}$ values for rat liver microsomes in the absence or presence of S-1 were 4.08 μM and 466 nM/h or 5.23 μM and 472 nM/h, respectively.

### Effect of S-1 on P-gp, MRP2, and BCRP Protein Expression in the Liver

Potential changes to the expression of P-gp, MRP2, and BCRP protein in the crude plasma membrane from rat liver in the absence or presence of S-1 were explored by Western blot analyses (Fig. 6A). The presence of S-1 significantly increased the level of BCRP protein in the crude plasma membrane preparation of the rat liver. The relative amount of BCRP with S-1 was about 1.6-fold higher than that without S-1. By contrast, administration of S-1 had no significant effect on the P-gp and MRP2 protein levels.

### Discussion

The efficacy of combination therapy with various anticancer agents has now been generally recognized. However, there is little information on the pharmacokinetic interaction of combination chemotherapy. Recently, we reported the pharmacokinetic changes of CPT-11 and its metabolites in a patient with colorectal cancer given S-1 p.o. (Yokoo et al., 2006). Here, we investigated the effect of S-1 on the pharmacokinetics of CPT-11 and its metabolites in rats to explore the possible mechanism of drug interaction.

Our results show that p.o. coadministration of S-1 causes a significant decrease in the plasma concentration of an active metabolite, SN-38. In particular, $C_{max}$ and AUC of the SN-38 lactone form were decreased by coadministration of S-1 (Table 1). Furthermore, coadministration of S-1 resulted in a significant increase in the biliary excretion of the total form of SN-38 (Fig. 3). The predominant biliary excretion of SN-38 was in the SN-38 carboxylate form rather than the SN-38 lactone form. The rate of biliary excretion of the SN-38 carboxylate form increased on coadministration of S-1; however, that of the SN-38 lactone form was not increased. Accordingly, an increase in biliary excretion of the total form of SN-38 could have resulted from the increased biliary excretion of the carboxylate form. By contrast, the total volume of bile secretion was unchanged between rats given CPT-11 alone or in combination with S-1 (data not shown, $P = 0.84$). On the other hand, P-gp, MRP2, and BCRP expressed at the bile canalicular membrane appear to be responsible for the biliary excretion of CPT-11 and its metabolites (Sugiyama et al., 1998; Nakatomi et al., 2001). Thus, S-1–mediated up-regulation of one or more of these membrane proteins may explain the increased biliary excretion of SN-38. A previous pharmacokinetic study of patients with colon cancer showed that cyclosporin, a modulator of P-gp and MRP2, reduces the clearance of CPT-11 and increases the AUC of CPT-11 after coadministration (Chester et al., 2003). Furthermore, it

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Without S-1</th>
<th>With S-1</th>
<th>Percentage of Change</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPT-11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carboxylate form</td>
<td>$C_{max}$ (ng/ml)</td>
<td>1172.4 ± 403.5</td>
<td>773.9 ± 239.0</td>
<td>-34.0</td>
</tr>
<tr>
<td>AUC (ng · h/ml)</td>
<td>3347.9 ± 1148.3</td>
<td>1955.0 ± 920.9</td>
<td>-41.6</td>
<td>0.067</td>
</tr>
<tr>
<td>Lactone form</td>
<td>$C_{max}$</td>
<td>6167.5 ± 1351.9</td>
<td>4838.4 ± 698.2</td>
<td>-21.6</td>
</tr>
<tr>
<td>AUC</td>
<td>6953.4 ± 1864.1</td>
<td>5428.9 ± 1282.8</td>
<td>-21.9</td>
<td>0.146</td>
</tr>
<tr>
<td>Total form</td>
<td>$C_{max}$</td>
<td>8363.3 ± 3178.1</td>
<td>5206.7 ± 821.7</td>
<td>-37.7</td>
</tr>
<tr>
<td>AUC</td>
<td>11037.6 ± 1470.0</td>
<td>7383.9 ± 2160.1</td>
<td>-33.1</td>
<td>0.014</td>
</tr>
<tr>
<td>SN-38</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carboxylate form</td>
<td>$C_{max}$</td>
<td>118.8 ± 65.0</td>
<td>112.4 ± 23.9</td>
<td>-5.4</td>
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<tr>
<td>AUC</td>
<td>219.3 ± 26.8</td>
<td>236.2 ± 59.9</td>
<td>7.7</td>
<td>0.581</td>
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<tr>
<td>Lactone form</td>
<td>$C_{max}$</td>
<td>144.1 ± 15.8</td>
<td>100.7 ± 8.9</td>
<td>-30.1</td>
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<tr>
<td>AUC</td>
<td>235.8 ± 43.6</td>
<td>156.2 ± 15.0</td>
<td>-33.8</td>
<td>0.005</td>
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<tr>
<td>Total form</td>
<td>$C_{max}$</td>
<td>259.6 ± 63.9</td>
<td>203.0 ± 28.6</td>
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<tr>
<td>AUC</td>
<td>468.2 ± 74.8</td>
<td>390.4 ± 72.7</td>
<td>-16.6</td>
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<tr>
<td>SN-38G</td>
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<td>Carboxylate form</td>
<td>$C_{max}$</td>
<td>75.0 ± 21.54</td>
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<td>AUC</td>
<td>244.0 ± 647</td>
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<tr>
<td>Lactone form</td>
<td>$C_{max}$</td>
<td>216.3 ± 113.4</td>
<td>134.4 ± 27.1</td>
<td>-37.9</td>
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<td>AUC</td>
<td>410.8 ± 181.7</td>
<td>337.2 ± 82.3</td>
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<td>232.4 ± 72.6</td>
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<td>AUC</td>
<td>644.1 ± 249.2</td>
<td>490.0 ± 146.7</td>
<td>-23.9</td>
<td>0.268</td>
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The differences were analyzed statistically using Student’s unpaired t test.
was reported that the biliary excretion and the intestinal exsorption of CPT-11 or SN-38 were significantly inhibited by coadministration of cyclosporin in rats (Arimori et al., 2003).

Figure 3 shows that the rate of biliary excretion of the SN-38 carboxylate form increased on coadministration of S-1. The total amount of SN-38 carboxylate form excreted in the absence or presence of S-1 was 9.7 and 14.9% of the dose 4 h after administration, respectively ($P < 0.01$). Figure 6 shows that the BCRP protein level in crude plasma membrane from the liver with S-1 was 1.6-fold higher than that without S-1 ($P < 0.05$). On the other hand, the mRNA level of BCRP was measured in both the intestine and kidney with or without S-1, and we found that the mRNA level was not affected by administration of S-1 in both tissues (data not shown). Therefore, an increase in the level of BCRP protein may result in increased biliary excretion of the SN-38 carboxylate form. In previous studies, sulfasalazine, various aryl hydrocarbon receptor agonists, and progesterone were found to significantly increase the level of BCRP protein in vitro (van der Heijden et al., 2004; Ebert et al., 2005; Wang et al., 2006). By contrast, 17β-estradiol significantly decreases the level of BCRP protein in vitro (Wang et al., 2006). However, there are few previous reports of drug-related regulation of BCRP protein levels in vivo (Han and Sugiyama, 2006). Here, we report the S-1–mediated induction of BCRP protein in vivo. Our study shows that the rate of biliary excretion of the SN-38 carboxylate form is significantly increased by coadministration of S-1, whereas that of the SN-38 lactone form is unaltered. These results suggest BCRP may display a higher affinity for the SN-38 carboxylate form relative to the SN-38 lactone form. Further studies are required to confirm this hypothesis.

Although the rate of biliary excretion of the SN-38 carboxylate form increased on coadministration of S-1, $C_{\text{max}}$ and AUC of the
SN-38 lactone form decreased. The predominant CPT-11 metabolite in rat liver is the SN-38 carboxylate form rather than the SN-38 lactone form. Four hours after i.v. administration of CPT-11 in the absence of S-1, the concentration of the SN-38 carboxylate and lactone forms in the liver was $0.7 \pm 0.2$ and $0.2 \pm 0.1$ ng/g of tissue, respectively. Thus, the ratio of the carboxylate or lactone forms of SN-38 to the total amount of SN-38 was 77.8 and 22.2%, respectively. Similar results were obtained after coadministration of CPT11 with S-1 (data not shown). These results suggest that the induction of the BCRP protein level by coadministration of S-1 increased the rate of biliary excretion of the SN-38 carboxylate form, followed by hydrolytic cleavage of the lactone, yielding the carboxylate form of SN-38 in the liver. The lower rate of secretion of the SN-38 lactone form from liver to blood compared with that of the SN-38 carboxylate form could explain the lower $C_{\text{max}}$ and AUC of the SN-38 lactone form.

Previous studies of drug interaction between CPT-11 and 5-FU suggested that 5-FU inhibits the enzymatic hydrolysis of CPT-11 to SN-38 in humans and rats (Sasaki et al., 1994; Umezawa et al., 2000). However, the hydrolysis of p-NPA was not inhibited by components of S-1 and 5-FU (Fig. 4). Furthermore, administration of S-1 did not affect the hydrolysis activity of rat liver microsomes (Fig. 5). Therefore, inhibition of the hydrolysis activity by coadministration of S-1 cannot account for the pharmacokinetic parameters of SN-38.

The piperidine ring of CPT-11 is oxidized to 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecin and 7-ethyl-10-(4-amino-1-piperidino)carbonyloxycamptothecin by CYP3A4 and CYP3A5 in the liver (Santos et al., 2000). A decrease in the $C_{\text{max}}$ and AUC of SN-38 lactone form could be caused by enhanced activity of CYP3A on coadministration of S-1. Indeed, a previous study showed that chronic p.o. administration of FT (100 mg/kg daily for 20 days) resulted in the induction of CYP3A in rat liver (Yamazaki et al., 2001). However, no increase in the concentration of CYP3A-derived metabolites of CPT-11 could be detected (data not shown). Therefore, we conclude that the effect of coadministration of S-1 on CYP3A activity can be ignored.

SN-38 is conjugated with glucuronic acid by hepatic UGT1A to form the inactive metabolite SN-38G (Rivory and Robert, 1995). Induction or activation of UGT1A by treatment with S-1 is a potential mechanism for the observed drug interaction. If this is the case, we may anticipate an increase in the plasma concentration of SN-38G. However, we found that the plasma concentration of SN-38G was

Fig. 5. Production of SN-38 incubation time profiling and enzyme kinetics in rat liver microsomes. A, generation of SN-38 by incubating CPT-11 (10 \muM) with rat liver microsomes in the absence of S-1. B, production of SN-38 from CPT-11 in rat liver microsomes in the absence (△) or presence of S-1 (▲) at a dosage of 10 mg/kg/day. All the data are expressed as mean ± S.D., n = 3.

SN-38 lactone form decreased. The predominant CPT-11 metabolite in rat liver is the SN-38 carboxylate form rather than the SN-38 lactone form. Four hours after i.v. administration of CPT-11 in the

Fig. 6. Effect of S-1 on the protein concentration of P-gp, MRP2, and BCRP in the crude membrane fraction of rat liver. A, Western blot analyses for P-gp, MRP2, and BCRP in the crude membrane fraction of rat liver in the absence or presence of S-1 at a dosage of 10 mg/kg/day. B, the quantitative data mean ± S.D. of densitometry measurements of the crude membrane fraction of rat liver in the absence (open bars) or presence of S-1 (filled bars) at a dosage of 10 mg/kg/day. The values observed in the absence of S-1 were arbitrarily defined as 100%. All data are expressed as mean ± S.D. for four rats in each group. The differences were analyzed statistically using Student’s unpaired $t$ test. *: $P < 0.05$. 
lower on coadministration of S-1, suggesting that S-1 has no effect on UGT1A activity.

In summary, we have shown significant drug interaction between CPT-11 and S-1 in rats. In particular, Cmax and AUC of the SN-38 lactone form were markedly reduced by coadministration of S-1. Induction of BCRP protein was observed in the rat liver with S-1, resulting in an increased rate of biliary excretion of the SN-38 carbboxylate form. We suggest our data reveal the underlying mechanism of drug interaction between CPT-11 and S-1 that has been observed in previous clinical case reports. Further studies are needed to establish the most effective regimen for combination therapy based on the pharmacokinetic and pharmacodynamic data.

References


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