Panipenem Does Not Alter the Pharmacokinetics of the Active Metabolite of Irinotecan SN-38 and Inactive Metabolite SN-38 Glucuronide (SN-38G) in Rats

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Abstract. The present study has investigated the effect of panipenem, a widely used antibiotic, on the pharmacokinetics of an active metabolite of irinotecan (CPT-11), 7-ethyl-10-hydroxy-camptothecin (SN-38) and SN-38 glucuronide (SN-38G) produced by uridine-diphosphate glucuronosyltransferase (UGT) 1A isoform-mediated glucuronidation in rats. Rats received a 1 h infusion with panipenem at a loading dose of 10 mg/kg and a maintenance dose of 15 mg/min/kg once a day for 5 days. When the effect of pretreatment with panipenem on glucuronidation activities of substrates for hepatic UGT1A isoforms was investigated using substrates 4-methylumbelliferone (4MU), estradiol and SN-38, the rate of 4MU glucuronide formation was significantly increased, but that of estradiol glucuronide formation was unchanged. However, the rate of SN-38G formation showed a tendency to increase. One hour after the final infusion of panipenem or saline, SN-38 (2 mg/kg) was administered intravenously in rats with or without bile duct cannulation. Pretreatment with panipenem had no effect on the plasma concentration–time curves and biliary excretion of SN-38 and SN-38G in rats with and without bile duct cannulation. There were also no significant differences in the relative extent of glucuronidation of SN-38 to SN-38G (AUC2 h, SN-38G/AUC2 h, SN-38) between panipenem-treated and untreated rats. These findings suggest that pretreatment with panipenem does not alter the pharmacokinetics of SN-38 and SN-38G, suggesting the possibility that panipenem can be used safely for cancer patients undergoing irinotecan chemotherapy.

Irinotecan (CPT-11), a DNA topoisomerase I inhibitor, is widely used in the first- and second-line treatment of various types of cancer, including colorectal cancer and is rapidly hydrolyzed by carboxylesterase to an active metabolite, 7-ethyl-10-hydroxy-camptothecin (SN-38). SN-38, whose antitumor activity is very much stronger than that of irinotecan, is efficiently conjugated by uridine-diphosphate glucuronosyltransferase (UGT) 1A isoforms to form SN-38 glucuronide (SN-38G) (1, 2) as illustrated in Figure 1. Furthermore, irinotecan and its metabolites SN-38 and SN-38G are mainly excreted into bile via ATP-binding cassette (ABC) efflux pumps such as P-glycoprotein/ABCB1 and multidrug resistance-associated protein 2 (MRP2/ABCG2) (3, 4). SN-38G is deconjugated by β-glucuronidase derived from enterobacteria and undergoes enterohepatic recirculation (5-7).

Irinotecan is well-known to induce severe side-effects such as neutropenia and diarrhea, which are the major dose-limiting factors in its administration. It is reported that SN-38, but not irinotecan, plays an important key in the incidence of irinotecan-induced side-effects (8). Therefore, the pharmacokinetics of SN-38 contributes to the incidence of irinotecan-induced side-effects, in addition to the antitumor activity.

It is reported that oral administration of antibiotics which are not absorbed from the gastrointestinal tract does not alter the pharmacokinetics of irinotecan and SN-38, but inhibits β-glucuronidase activity, leading to a decrease

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in the exposure to SN-38 in the large intestine (4, 9). To our knowledge, the effect of systemic administration of antibiotics on the pharmacokinetic behavior of irinotecan and SN-38 has not yet been clarified in humans and animals, although there are numerous reports regarding the effect of drug transporter modulators and anticancer drugs on the pharmacokinetics of irinotecan and SN-38 (10-13).

It is clinically possible that antimicrobial agents, including carbapenem antibiotics, are administered prophylactically in rare cases to cancer patients who are scheduled to undergo anticancer drug chemotherapy. It has been reported that one of clinically widely used carbapenem antibiotics, panipenem, largely reduces plasma concentrations of the antiepileptic valproic acid, which is mainly converted into glucuronide via UGT1A isoforms in the liver, due mainly to the enhancement of glucuronidation by increasing the hepatic concentration of the cofactor, UDP-glucuronic acid (UDPGA), which is one of determinants for the conjugation reaction (14).

Considering that SN-38 undergoes glucuronidation by hepatic UGT1A isoforms (1, 2) and that repeated doses of antibiotics might reduce the production of β-glucuronidase by killing enterobacteria, pretreatment with panipenem might alter the pharmacokinetics of SN-38 and SN-38G. However, to our knowledge, there is no available information about the effect of pretreatment with panipenem on the pharmacokinetics of SN-38 or SN-38G.

The aim of the present study was to create a guideline for the safe use of carbapenem antibiotics, including panipenem, for cancer patients who are scheduled to receive irinotecan chemotherapy. To investigate whether panipenem alters the pharmacokinetics of irinotecan in rats, SN-38 was used in the present study to exclude the complicated metabolic and excretory pathways of irinotecan.

**Materials and Methods**

**Chemicals.** Panipenem was used in the form of a commercial preparation for injection (Carbenin; Daiichi Sankyo Co. Ltd., Tokyo, Japan). SN-38 was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). The metabolite of SN-38 (SN-38G) was kindly donated by Daiichi Sankyo Co. Ltd. 4-Methylumbelliferone (4MU), 4MU β-D-glucuronide (4MU-G), β-estradiol, β-estradiol 3-O-glucuronide (estradiol-G), and UDP-glucuronic acid (UDPGA) were purchased from Sigma Chemicals (St. Louis, MO, USA). All other chemicals were commercially available and were of the highest purity available. SN-38 was prepared by dissolution in 2 mol/l NaOH solution and adjustment to pH 9 with 1 mol/l HCl solution for in vivo experiments.

**Animals and experiments.** Nine- to ten-week-old male SD rats weighing 290 to 310 g were used for all experiments. The rats were housed under controlled environmental conditions (approximately 25°C) with a commercial diet and water freely available. All animal experiments were carried out in accordance with the guidelines of Aichi Medical University for the care and use of laboratory animals. Before the experiments, rats under light anesthesia by intraperitoneal injection of sodium pentobarbital (25 mg/kg) were cannulated with polyethylene tubes in the right jugular vein for administration of panipenem and blood collection. After surgical preparation, rats received a bolus injection of panipenem at a loading dose of 10 mg/kg, followed by a constant-rate infusion, with a Harvard infusion pump (PHD 2000; Harvard, South Natick, MA, USA), of a saline solution at a dose of 1.5 mg/min/kg for 1 h once a day for 5 days. The loading and maintenance doses of panipenem were determined by using pharmacokinetic parameters obtained from the single intravenous injection experiment. The control group was treated with isotonic saline in place of panipenem.

One hour after finishing this dosage regimen, in order to investigate the effect of panipenem on plasma concentration–time curves of SN-38 and SN-38G, the rats received a single intravenous injection of SN-38 (2 mg/kg). For biliary excretion experiments, after finishing the final infusion of panipenem or saline, rats were immediately cannulated with polyethylene tubes in the right carotid artery and bile duct for blood sampling and bile collection.
respectively. After surgical preparation, rats received a single intravenous injection of SN-38 (2 mg/kg).

Blood samples (approximately 0.3 ml) were collected at designated intervals (5, 10, 20, 30, 60, 90, and 120 min after the administration of SN-38). Plasma samples were obtained by immediate centrifugation of blood samples. Bile samples were collected three times at 30-min intervals for 90 min. The volume of bile samples was measured gravimetrically with specific gravity assumed to be 1.0. Plasma and bile samples were stored at −80°C until analysis. In all experiments, the rats were placed in plastic metabolic cages under light anesthesia with pentobarbital, and the body temperature of the animals was maintained at 37°C throughout the experiments with the assistance of a heat lamp.

In vitro experiments. Glucuronidation activities in livers of rats were determined according to the methods described elsewhere (15, 16) with some slight modifications. Livers were eviscerated from control rats (saline) and panipenem-treated rats. Microsomes were prepared according to the prior published methods (17) with some modifications. Briefly, liver (approximately 1 g) was homogenized with a tight homogenizer (20 strokes up and down) using 1.15% KCl. The homogenate was centrifuged at 12,000 xg for 25 min at 4°C. The supernatant was further centrifuged at 100,000 xg for 60 min at 4°C to obtain the microsomal fraction. The pellet obtained was suspended in 1.15% KCl. The protein concentration of the microsomal fraction was measured by Bio-Rad Protein Assay kit (Bio-Rad Laboratories, Richmond, CA, USA), using bovine serum albumin (Sigma Chemicals) as a standard.

4MU and estradiol were dissolved in water and methanol, respectively. The reaction mixture contained 4MU or estradiol (0.1 mmol/l each), microsome from rat livers (50 μg/ml), MgCl2 (10 mmol/l), UDPGA (2 mmol/l) and Tris-HCL buffer (pH 7.4) (50 mmol/l). The reaction was initiated by the addition of UDPGA after preincubation at 37°C for 2 min. The reaction mixture was incubated at 37°C for 10 min (4MU glucuronidation assay), 15 min (estradiol glucuronidation assay) and 20 min (SN-38 glucuronidation assay), and the reaction was terminated with 50 μl of ice-cold 15% (w/v) perchloric acid. After centrifugation at 12,000 xg for 10 min at 4°C, the supernatant was filtered with a PTFE membrane filter of 0.45 μm pore size (Millipore, Bedford, MA, USA). The concentrations of 4MU-G, estradiol-G and SN-38G in the filtrated samples were analyzed by high-performance liquid chromatography (HPLC). The blank samples contained all components except for UDPGA, which was added after termination of the reaction.

Drug analysis. The concentrations of SN-38 and SN-38G in plasma, bile and in vitro filtrated samples were determined by HPLC according to reported methods (18) with slight modifications. The apparatus used for HPLC was a Shimadzu LC-20AB system (Kyoto, Japan) equipped with a fluorescence detector (F-10AXL; Shimadzu) and UV spectrophotometric detector (SPD-6A, Shimadzu) consisting of an LC-20AB liquid pump and an SIL-20AC autoinjector. The conditions were as follows: column, a Cosmosil 5C18 column (4.6 by 150 mm; Nacalai Tesque, Kyoto, Japan); mobile phase, 50 mM KH2PO4 containing 3 mM sodium 1-octanesulfonate–acetonitrile–methanol (68:26:6, v/v/v); wavelength, 355 nm (exitation) and 515 nm (emission); column temperature, 40°C (OTC-6A; Shimadzu); flow rate, 1.0 ml/min.

Bile samples were diluted two-fold with saline. Each sample (50 μl) was added to 50 μl of 0.2 mol/l NaH2PO4, 0.2 mol/l citric acid and 70% perchloric acid, then mixed vigorously and were centrifuged at 12,000 xg for 10 min at 4°C. The supernatant (80 μl) and 4 mol/l CH3CO2Na (50 μl) were mixed and injected into the HPLC system.

The concentrations of 4MU-G and estradiol-G were determined by HPLC according to reported methods (19, 20). HPLC conditions were as follows: mobile phase, 20 mmol/l triethylamine (pH 2.1)–acetonitrile (80:20, v/v) for 4MU-G and 1 mmol/l perchloric acid–acetonitrile [75:25, v/v] for estradiol-G; column temperature, 40°C; flow rate, 1.0 ml/min; wavelength, 318 nm for 4MU-G and 280 nm (excitation) and 310 nm (emission) for estradiol-G.

These assays were shown to be linear for the concentrations tested, with a correlation coefficient of 0.998. No interference with the peaks of SN-38 and SN-38G was observed in any samples. The within- and between-day coefficients of variation for these assays were less than 8%. The detection limit for SN-38 and SN-38G was 0.01 μg/ml and that for 4MU-G and estradiol-G was 0.05 μg/ml and 0.01 μg/ml, respectively.

Data analysis. Plasma concentration–time data for SN-38 and SN-38G in each rat after a single intravenous administration were analyzed individually using a noncompartmental model. The area under the plasma concentration–time curve (AUC) and the area under the first-moment curve (AUMC) were calculated by the trapezoidal method until the last measurable concentration in plasma and were extrapolated to infinity. The systemic clearance (CLSYS) was calculated as dose/AUC. The mean residence time (MRT) was calculated as MRT=AUMC/AUC. The steady-state volume of distribution (VSS) was calculated as VSS=CLSYS × MRT.

For biliary clearance experiments, the biliary clearance (CLBILE) was calculated by dividing the total amount of drug excreted into the bile during the collection period (120 min) by the corresponding AUC (AUC120). Each parameter of SN-38 and SN-38G was calculated by correcting for molar weight.

Statistical analysis. The results are expressed as means±standard deviation (SD) for the indicated numbers of experiments. Statistical analysis was performed by analysis of variance (ANOVA). When F values were significant (p<0.05), Scheffe’s post-hoc tests between the two groups were used and p-values of <0.05 were considered statistically significant.

Results

Glucuronidation activities in livers of rats pretreated with or without panipenem. The effect of panipenem on glucuronidation activities in livers of rats is illustrated in Figure 2. Panipenem significantly increased the rate of 4MU-G formation (4.51±0.61 to 6.35±1.54 nmol/min/mg protein). There was a tendency for an increase in the rate of SN-38G formation (64±9 to 76±10 nmol/min/mg protein), although statistical analysis did not detect a statistically significant difference. However, there was no significant difference in the rate of estradiol-G formation between rats treated with and without panipenem (0.18±0.07 to 0.18±0.04 nmol/min/mg protein).
Effect of panipenem on pharmacokinetics of SN-38 and SN-38G after a single intravenous injection of SN-38 in rats without bile duct cannulation (normal rats). The effect of pretreatment with panipenem on the pharmacokinetics of SN-38 and SN-38G was examined. Semilogarithmic plots of plasma concentration–time data for SN-38 and SN-38G after a single intravenous injection of SN-38 (2 mg/kg) in rats without bile duct cannulation are illustrated in Figure 3A. SN-38 was biphasically eliminated from plasma after intravenous injection. There were no significant differences in the plasma concentration–time curves of SN-38 and SN-38G between panipenem-treated and untreated rats. SN-38G reached its peak concentration time approximately 10 min after injection of SN-38 in both panipenem-treated and untreated rats, indicating that SN-38 was very rapidly metabolized to SN-38G. The corresponding pharmacokinetic parameters of SN-38 and SN-38G are summarized in Tables I and II, respectively. Moreover, no significant differences in
Table I. Effect of pretreatment with panipenem on pharmacokinetic parameters of SN-38 after a single intravenous administration of SN-38 (2 mg/kg) in rats without bile duct cannulation. Pharmacokinetic parameters were calculated as described in the Materials and Methods.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Without PAPM</th>
<th>With PAPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLSYS (l/h/kg)</td>
<td>0.98±0.22</td>
<td>1.01±0.20</td>
</tr>
<tr>
<td>VSS (l/kg)</td>
<td>0.18±0.07</td>
<td>0.16±0.07</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>0.18±0.04</td>
<td>0.16±0.03</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>0.36±0.07</td>
<td>0.37±0.09</td>
</tr>
</tbody>
</table>

PAPM, Panipenem; CLSYS, systemic clearance; VSS, volume of distribution at steady state; MRT, mean residence time; t1/2, terminal half-life. Each value represents the mean±SD (n=4 and 5, respectively).

Table II. Effect of pretreatment with panipenem on pharmacokinetic parameters of SN-38G after a single intravenous administration of SN-38 (2 mg/kg) in rats without bile duct cannulation. Pharmacokinetic parameters were calculated as described in the Materials and Methods.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Without PAPM</th>
<th>With PAPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC2 h, SN-38G (μg min/ml)</td>
<td>49.8±12.6</td>
<td>54.6±17.2</td>
</tr>
<tr>
<td>REG</td>
<td>0.41±0.14</td>
<td>0.45±0.15</td>
</tr>
<tr>
<td>Cmax (μg/ml)</td>
<td>0.94±0.17</td>
<td>0.93±0.09</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>0.56±0.12</td>
<td>0.51±0.03</td>
</tr>
</tbody>
</table>

PAPM, Panipenem; AUC2 h, area under concentration-time curve from time zero to the last sampling time point (2 h); Cmax, maximum concentration; t1/2, terminal half-life, REG, relative extent of glucuronidation of SN-38 to SN-38G (AUC2 h, SN-38G/AUC2 h, SN-38), which was corrected for molar weights. Each value represents the mean±SD (n=4 and 5, respectively).

Discussion

Irinotecan (CPT-11) is used for the treatment of cancer sites such as colorectum, lung, ovary, stomach and breast. However, it causes clinically severe side-effects, such as neutropenia and diarrhea (8, 21). This anticancer drug appears to have the most complicated pharmacokinetic characteristics among other commercially available anticancer drugs. That is, irinotecan is inactivated by cytochrome P450 CYP3A4- and CYP3A5-mediated oxidation in the liver (22) and is transformed to SN-38 by carboxylesterase-mediated hydrolysis (23). Furthermore, both irinotecan and SN-38 are transported via Mrp2 and P-glycoprotein and other sensitive efflux transporters which are expressed in hepatic bile canalicular membranes.
of enteric injury caused by SN-38 (3-5, 26, 28). It is considered that the pharmacokinetic behavior of SN-38 might play an important role in the incidence of diarrhea by irinotecan therapy. Therefore, in the present study, we investigated the pharmacokinetics and hepatobiliary excretion of SN-38 and SN-38G after a single intravenous injection of SN-38 in rats in place of irinotecan.

Firstly, we investigated the effect of pretreatment with panipenem on hepatic glucuronidation activities using substrates for UGT1A isoforms in vitro. The in vitro results obtained from the present study showed that pretreatment with panipenem significantly increased the activity of UGT1A6, as shown by the rate of 4MU-G formation (10, 14) and showed a tendency to increase the glucuronidation of SN-38 to SN-38G. These results confirmed that panipenem increases the activity of UGT1A6. However, no changes in the activity of UGT1A1, which is represented here as the rate of estradiol-G formation (29), were observed. It is reported that hepatic UGT1A1 is primarily involved in the glucuronidation of SN-38 in humans (30, 31). On the other hand, it has been reported that the contribution of UGT1A7 to the glucuronidation of SN-38 is higher than that of UGT1A1 in humans and rats (32, 33), suggesting that other UGT1A isoforms in addition to UGT1A1 are involved in the glucuronidation of SN-38 (34). These findings suggest the possibility that the pharmacokinetics of SN-38 may be altered by pretreatment with panipenem.

Secondly, we investigated the effect of pretreatment with panipenem on the pharmacokinetics of SN-38 and SN-38G in rats with and without bile duct cannulation. The in vivo experiments demonstrated that pretreatment with panipenem had no effect on the plasma concentration–time curves and pharmacokinetic parameters of SN-38 and its inactive metabolite SN-38G after a single intravenous injection of SN-38 in both rats with and without bile duct cannulation were unchanged. These results are consistent with results obtained from the in vitro experiments and suggest that pretreatment with panipenem does not alter the pharmacokinetic behavior of SN-38 and SN-38G, including hepatobiliary excretion. The present study found that 65% of the systemic clearance of SN-38 was accounted for the biliary clearance, suggesting that SN-38 is mainly excreted into bile via SN-38-sensitive efflux transporters, including Mrp2. SN-38G is also excreted into bile via SN-38G-sensitive efflux transporters, including Mrp2, as well as SN-38, and undergoes enterohepatic recirculation (7). It is most likely that panipenem does not competitively or directly inhibit SN-38- and SN-38G-sensitive transporters because biliary excretion is negligible and urinary excretion is a major excretion route of panipenem (35, 36). If panipenem inhibits the enterohepatic recirculation of SN-38, differences in the plasma concentrations of SN-38 should be observed between rats with and those without bile duct cannulation. However, no clear difference was observed in this study. Although not proven, it is likely that the contribution of enterohepatic recirculation of SN-38 to the plasma concentration of SN-38 is small.

It is well known that the concomitant use of carbapenem antibiotics, including panipenem, significantly reduces the plasma concentration and increases the systemic clearance of valproic acid (14). It is suggested that this is most likely caused as a result of carbapenem antibiotics enhancing the glucuronidation of valproic acid in liver (14). However, most likely, a newly-discovered mechanism of drug interaction between carbapenem antibiotics and valproic acid exists since it has recently been reported that carbapenem antibiotics specifically inhibit acylpeptidehydrolase, which is mainly involved in hydrolysis of valproic acid glucuronide to valproic acid in the liver (37). We recently found in rats (unpublished data) that pretreatment with panipenem at the same dosage schedule as the present study did not alter the pharmacokinetics of acetaminophen which is metabolized by UGT1A7-mediated glucuronidation in rats (38). These observations may, at least in part, support the present findings. Unlike valproic acid, it is considered that hepatic acylpeptidehydrolase is not involved in the hydrolysis of SN-38G to SN-38.

In conclusion, the present study is the first to indicate that pretreatment with panipenem does not alter the pharmacokinetics of SN-38 and its inactive metabolite SN-

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Table IV. Effect of pretreatment with panipenem on biliary excretion of SN-38 and SN-38G after a single intravenous administration of SN-38 (2 mg/kg) in rats with bile duct cannulation. Pharmacokinetic parameters were calculated as described in the Materials and Methods.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cumulative biliary excretion (μg/2 h)</th>
<th>CL_BILE, SN-38 (ml/min/kg)</th>
<th>CL_BILE, SN-38G (ml/min/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SN-38</td>
<td>SN-38G</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>378±67.1</td>
<td>350±64.0</td>
<td>8.43±2.70</td>
</tr>
<tr>
<td>PAPM</td>
<td>331±1.60</td>
<td>337±109</td>
<td>6.40±2.96</td>
</tr>
</tbody>
</table>

PAPM, Panipenem; SN-38G, SN-38 glucuronide conjugate; CLM, metabolic clearance of SN-38 to SM-38G; CL BILE, SN-38, biliary clearance of SN-38; CL BILE, SN-38G, biliary clearance of SN-38G. Each value represents the mean±SD (n=4 and 5, respectively).
38G. Although the data obtained from this study cannot be extrapolated directly to humans, the present findings may provide useful information for irinotecan chemotherapy in cancer patients.

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