Effect of P-Glycoprotein and Breast Cancer Resistance Protein Inhibition on the Pharmacokinetics of Sunitinib in Rats

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ABSTRACT

The aim of this study was to elucidate the roles of P-glycoprotein (P-gp/ABCB1) and breast cancer resistance protein (BCRP/ABCG2) in the pharmacokinetics of sunitinib. Sunitinib concentrations in plasma, bile, liver, kidney, and brain of rats were significantly increased by pretreatment with PSC833 or pantoprazole. Each inhibitor markedly reduced the biliary excretion of sunitinib for 60 minutes after an intravenous administration and significantly increased the distribution of sunitinib to the liver as well as kidney. In addition, the brain distribution of sunitinib was significantly increased by PSC833 but not pantoprazole, and coadministration of both inhibitors further enhanced the accumulation of sunitinib in the brain. These results demonstrate that plasma concentrations of sunitinib and the biliary excretion and distribution to the kidney, liver, and brain of sunitinib are influenced by pharmacologic inhibition of P-gp and/or BCRP.

Introduction

Sunitinib is an orally active multitargeted tyrosine kinase inhibitor for various receptor tyrosine kinases such as vascular endothelial growth factor receptors, platelet-derived growth factor receptors, and the stem cell factor receptor c-KIT (Chow and Eckhardt, 2007). Sunitinib is widely used for the treatment of advanced renal cell carcinoma and imatinib-refractory gastrointestinal stromal tumors, and was recently approved for pancreatic neuroendocrine tumors by the U.S. Food and Drug Administration and European Medicines Agency. Several clinical trials have demonstrated that sunitinib significantly improves overall survival as well as progression-free survival in patients with advanced renal cell carcinoma (Motzer et al., 2007, 2009) and imatinib-refractory gastrointestinal stromal tumors (Demetri et al., 2006); however, patients taking sunitinib are often forced to reduce the dose or discontinue treatment because of frequent adverse events, such as thrombocytopenia, liver dysfunction, and hand-foot syndrome (Demetri et al., 2006; Motzer et al., 2007, 2009; Hong et al., 2009; Uemura et al., 2010; Yoo et al., 2010). In addition, tumor regrowth removed by metabolism in the liver and excretion into bile (Chow and Eckhardt, 2007; Speed et al., 2012). Studies in vitro have demonstrated that these transporters play important roles in the pharmacokinetics of several substrate drugs including tyrosine kinase inhibitors by limiting their intestinal absorption and tissue distribution (Oostendorp et al., 2009; Polli et al., 2009; Agarwal et al., 2010; Kodaira et al., 2010; Lagas et al., 2010). In addition, several pharmacokinetic studies have shown in vivo drug-drug interaction (DDI) via P-gp and BCRP. For instance, the ratio of renal digoxin clearance to creatinine clearance in one patient was lower during the concomitant administration of clarithromycin than that after cessation of clarithromycin administration (Wakasugi et al., 1998), and BCRP inhibition by gefitinib resulted in increased bioavailability of oral irinotecan (Furman et al., 2009).

P-glycoprotein (P-gp/ABCB1) and breast cancer resistance protein (BCRP/ABCG2) are ATP-binding cassette transporters involved in multidrug resistance in tumors (Glavinas et al., 2004). Both transporters are also expressed in the brain, kidney, small intestine, and liver, and mediate the efflux of substrate compounds (Glavinas et al., 2004). It has been demonstrated that these transporters play important roles in the pharmacokinetics of several substrate drugs including tyrosine kinase inhibitors by limiting their intestinal absorption and tissue distribution (Oostendorp et al., 2009; Polli et al., 2009; Agarwal et al., 2010; Kodaira et al., 2010; Lagas et al., 2010). In addition, several pharmacokinetic studies have shown in vivo drug-drug interaction (DDI) via P-gp and BCRP. For instance, the ratio of renal digoxin clearance to creatinine clearance in one patient was lower during the concomitant administration of clarithromycin than that after cessation of clarithromycin administration (Wakasugi et al., 1998), and BCRP inhibition by gefitinib resulted in increased bioavailability of oral irinotecan (Furman et al., 2009).

Increased systemic exposure to sunitinib is associated with more frequent adverse events and poor tolerability of sunitinib treatment (Faivre et al., 2006; Mizuno et al., 2010, 2012). Sunitinib has a long elimination half-life of about 50 hours in humans, and is mainly removed by metabolism in the liver and excretion into bile (Chow and Eckhardt, 2007; Speed et al., 2012). Studies in vitro have demonstrated that sunitinib is transported by human P-gp (Hu et al., 2009; Tang et al., 2012) and BCRP (Mizuno et al., 2010; Tang et al., 2012). However, the influence of genetic disruption of these transporters on the plasma concentrations of sunitinib is controversial (Hu et al., 2009; Tang et al., 2012). In addition, the contributions of the transporters to the hepatic and renal disposition of sunitinib are not well studied.

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ABBREVIATIONS: AUC0–t, area under the concentration-time curve for 4 hours; BCRP, breast cancer resistance protein; CL, clearance; Cmax, maximum concentration; DDI, drug-drug interaction; F, oral bioavailability; P-gp, P-glycoprotein; PSC833, valsoparad; Vdss, volume of distribution at steady state.
Also, little information is available about the potential for DDI with sunitinib via these transporters.

In this study, we first examined the interactions of sunitinib with rat P-gp and BCRP by measuring the ATPase activity. Furthermore, we used PSC833 (valspodar) and pantoprazole as a potent inhibitor of P-gp (Fracasso et al., 2000; Tai, 2000) and BCRP (Muenster et al., 2008; Adkison et al., 2010), respectively, and the effects of the inhibitors on the plasma concentration, biliary excretion, and distribution to the liver, kidney, and brain of sunitinib were examined to demonstrate the contribution of these transporters to the pharmacokinetics of sunitinib and the potential for DDI via these transporters.

Materials and Methods

Materials. Sunitinib malate was purchased from LC Laboratories (Woburn, MA), and PSC833 was obtained from Novartis Pharmaceuticals Corporation (Tokyo, Japan). Pantoprazole sodium and Cremophor EL were obtained from LKT Laboratories (St. Paul, MN) and Nacalai Tesque (Kyoto, Japan), respectively. All other chemicals used were of the highest purity available.

ATPase Assay. The ability of sunitinib and pantoprazole to stimulate ATP hydrolysis was examined using the membranes-expressing rat Mdr1a or Bcrp (BD Biosciences, Woburn, MA) and BD Gentest ATPase Assay Kit (BD Biosciences). The method used to determine the drug-simulated ATPase activity was optimized based on the manufacturer’s protocol. Briefly, membranes were incubated at 37°C for 5 minutes in assay buffer in the presence or absence of test compounds. The reaction was initiated by the addition of 20 μl of 3 mM ATP (magnesium salt) and was stopped 20 minutes later by the addition of 30 μl of 10% sodium dodecyl sulfate. Color reagent (200 μl) was added to all wells and incubated at 37°C for 20 minutes. The absorbance at 800 nm was measured using a plate reader. The drug-stimulated ATPase activity (nmol/min/mg protein) was determined as the difference in absorbance at 800 nm was measured using a plate reader. The drug-stimulated ATPase activity was calculated as the difference between the amounts of inorganic phosphate released from ATP in the absence and presence of vanadate. Potassium phosphate standards were prepared in each plate, and verapamil or sulfasalazine served as the positive control for rat Mdr1a or Bcrp, respectively. The kinetic parameter Km was estimated by fitting of drug concentrations and ATP hydrolysis activity into the Michaelis-Menten equation.

Animals. Male Wister/ST rats (9 to 11 weeks old) were used. The animal experiments were performed in accordance with the Guidelines for Animal Experiments of Kyoto University, and the experimental protocol was approved by the Animal Research Committee of the Graduate School of Medicine of Kyoto University. The rats were fed a normal chow ad libitum. In all experiments, the animals had free access to water. In experiments for the intraintestinal administration of sunitinib, the rats were fasted at least 16 hours before the experiments.

Preparation of Drug Solutions. PSC833 was dissolved in a solution of Cremophor EL and ethanol (1:1), which was further diluted with 9 times its volume of saline to a final concentration of 5 mg/ml. In the experiments for intraintestinal administration, sunitinib malate and pantoprazole sodium were dissolved in saline to a concentration of 1.9 mg/ml as sunitinib and 20 mg/ml as pantoprazole, respectively. In the experiments for intravenous administration, sunitinib malate and pantoprazole sodium were dissolved in saline to a concentration of 0.97 mg/ml as sunitinib and 40 mg/ml as pantoprazole, respectively.

Pharmacokinetic Study in Rats. In the experiments for the intraintestinal administration of sunitinib, the femoral artery was cannulated with a polyethylene tube (SP-31; Natsume Seisakusho, Tokyo, Japan) for blood sampling. The abdominal cavity was opened via a middle incision, and the upper duodenum was ligated with silk sutures (4-0 Nescosuture; Nihon-Shoji, Osaka, Japan). Rats were administered 10 mg/kg of PSC833 or 40 mg/kg of pantoprazole via the duodenum using a 25-gauge needle. After 15 minutes, rats were given 3.87 mg/kg of sunitinib via the duodenum using a 25-gauge needle. Blood samples were collected at 0.25, 0.5, 1.0, 2.0, 3.0, and 4.0 hours after the dose from the left femoral artery.

We conducted two different experiments for intravenous administration in rats. The right femoral vein and the left femoral artery were cannulated with a polyethylene tube (PE10; Becton Dickinson, Franklin Lakes, NJ) to examine the pharmacokinetic profile of sunitinib. In different experiments, we cannulated the common bile duct with a polyethylene tube (PE10; Becton Dickinson, Franklin Lakes, NJ) to examine...
the biliary excretion of sunitinib. Bile samples were collected for each interval (0–10, 10–20, 20–30, 30–40, 40–50, and 50–60 minutes) after sunitinib administration. One hour after the administration of sunitinib, the rats were sacrificed to obtain plasma, liver, kidney, and brain samples. The excised tissues were gently washed with saline, weighed, and homogenized in ice-cold saline, the volume of which was 9, 9, and 2 times that of the liver, kidney, and brain, respectively. The homogenates of liver and kidney were further diluted with 9 times their volume of saline.

In all experiments, the control rats were administered with the same volume of vehicle before the administration of sunitinib. Blood samples were centrifuged for 5 minutes at 14,000 rpm to separate plasma. The concentrations of sunitinib in plasma, bile, and tissues including liver, kidney, and brain were determined by liquid chromatography with tandem mass spectrometry (LC-MS/MS) system after chromatography with tandem mass spectrometry (LC-MS/MS) as previously described elsewhere (Mizuno et al., 2012). Briefly, gradient elution was carried out at a flow rate of 0.2 ml/min using a mobile phase containing 0.1% formic acid and acetonitrile. The intra-assay and inter-assay variability, and the accuracy bias were less than 10%.

The maximum concentration (C\text{max}) was obtained from the concentration-time curve of sunitinib. The area under the concentration-time curve for 4 hours (AUC\text{0-4}) from the intravenous administration of sunitinib was calculated by the trapezoidal rule. The tissue-to-plasma concentration ratio was calculated by dividing the tissue concentration with plasma concentration at 60 minutes after the intravenous administration of sunitinib. The clearance (CL) and volume of distribution at steady state (V\text{dss}) were estimated by noncompartmental analysis with WinNonlin software (Pharsight Corporation, Tokyo, Japan). Oral bioavailability (F) was calculated by dividing the dose-normalized AUC\text{0-4} from the intraintestinal administration experiments with the dose-normalized AUC\text{0-4} from the intravenous administration experiments.

**Statistical Analysis.** Results are expressed as the mean ± S.D. unless otherwise specified. Data were analyzed statistically using an unpaired t test or Welch’s test, as applicable. P < 0.05 was considered statistically significant.

**Results**

**Effect of Sunitinib and Pantoprazole on ATP Hydrolysis by Rat P-gp and BCRP.** We first examined whether sunitinib is a substrate for rat P-gp and BCRP by measuring the ATPase activity. The mean K\text{m} (S.E.) of sunitinib for rat Mdr1a and Bcrp were estimated 19.5 (10.2) μM and 0.28 (0.16) μM, respectively (Fig. 1). To evaluate the selectivity of pantoprazole for rat P-gp and BCRP, we compared the effect of pantoprazole on the ATPase activity of these transporters. The mean K\text{m} (S.E.) of pantoprazole for rat Mdr1a and Bcrp were 125 (15) μM and 0.10 (0.04) μM, respectively (Fig. 2). Thus, pantoprazole was revealed to interact more selectively with rat BCRP than P-gp with a similar affinity to sunitinib.

**Effect of Inhibitors of P-gp and BCRP on the Plasma Concentrations of Sunitinib.** Figure 3 shows plasma concentration profiles of sunitinib administered intraintestinally in rats pretreated with PSC833 or pantoprazole. The plasma concentrations of sunitinib at each time point were significantly increased by pretreatment with PSC833 or pantoprazole compared with the control rats. The AUC\text{0-4} and C\text{max} of sunitinib were significantly higher in the rats treated with PSC833 or pantoprazole than in the vehicle-treated rats (Table 1). PSC833 and pantoprazole increased the bioavailability of sunitinib to 1.8- and 2.1-fold, respectively (Table 1).

Figure 4 shows the plasma concentration profiles of sunitinib administered intravenously in rats pretreated with PSC833 or pantoprazole. The plasma concentrations of sunitinib at each time point were not significantly influenced by pretreatment with PSC833 or pantoprazole compared with the control rats. In addition, there was no significant difference in the apparent clearance, volume of distribution, and area under the concentration-time curve of sunitinib between the PSC833 or pantoprazole pretreated rats and the control rats (Table 1). Pretreatment with PSC833 or pantoprazole increased the bioavailability of sunitinib to 1.8- and 2.1-fold, respectively (Table 1).

**Table 1.** Plasma pharmacokinetics of sunitinib in rats pretreated with PSC833 or pantoprazole.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pretreatment</th>
<th>Pretreatment</th>
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<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>PSC833</td>
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<tr>
<td><strong>Intraintestinal administration</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C\text{max} (ng/ml)</td>
<td>59 ± 14</td>
<td>100 ± 8</td>
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<tr>
<td>AUC\text{0-4} (ng·h/ml)</td>
<td>165 ± 35</td>
<td>298 ± 37</td>
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<tr>
<td>F</td>
<td>0.27</td>
<td>0.47</td>
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<tr>
<td><strong>Intravenous administration</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC\text{0-4} (ng·h/ml)</td>
<td>175 ± 28</td>
<td>175 ± 40</td>
</tr>
<tr>
<td>V\text{dss} (ml)</td>
<td>3090 ± 484</td>
<td>3320 ± 631</td>
</tr>
<tr>
<td>CL (ml/h)</td>
<td>1410 ± 242</td>
<td>1380 ± 499</td>
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* Data for parameters except F are shown as the mean ± S.D. (n = 3–7).

1 P < 0.001, statistically significantly different from the vehicle-treated group.

2 P < 0.05, statistically significantly different from the vehicle-treated group.
The distribution of sunitinib to the liver and kidney were also significantly increased by each inhibitor. To our knowledge, this is the first report demonstrating the contribution of P-gp and BCRP to the biliary excretion of sunitinib and its accumulation in the liver and kidney.

It was previously shown that sunitinib is transported by human P-gp and BCRP (Hu et al., 2009; Mizuno et al., 2010; Tang et al., 2012). However, it was unclear whether sunitinib is a substrate for rat P-gp and BCRP. In the present study, it has been demonstrated that sunitinib stimulates the ATPase activity by rat Mdr1a and Bcrp (Fig. 1). The affinity of sunitinib for rat P-gp (apparent $K_m = 19.5 \mu M$) and BCRP (apparent $K_m = 0.28 \mu M$) were comparable to that of human orthologs (apparent $K_d = 15.1$ and $0.18 \mu M$, respectively) (Shukla et al., 2009). Although there are several limitations in using ATPase assays with respect to transport kinetics and search for potential inhibitors because a competitive inhibitor can also stimulate ATPase activity, our results would indirectly suggest that sunitinib is transported by rat P-gp as well as BCRP and that pantoprazole can alter the pharmacokinetics of sunitinib in vivo by interacting with P-gp and BCRP (Hu et al., 2009; Mizuno et al., 2010; Tang et al., 2012). In the present study, both the AUC$_{0-\infty}$, $C_{max}$, and $F$ of sunitinib were significantly decreased by administration of pantoprazole compared with the single administration of PSC833.

Discussion

In the present study, we have clarified the controversial effect of P-gp and BCRP on the pharmacokinetics of sunitinib in vivo by using inhibitors of the respective transporters. This study demonstrated that potent inhibitors of P-gp and BCRP affect the pharmacokinetics of sunitinib in rats. The systemic exposure to sunitinib administered intravenously but not intravenously was significantly increased by pretreatment with PSC833 or pantoprazole. In addition, we showed that P-gp and BCRP are involved in the biliary excretion of sunitinib.

It was previously shown that sunitinib is transported by human P-gp and BCRP (Hu et al., 2009; Mizuno et al., 2010; Tang et al., 2012). However, it was unclear whether sunitinib is a substrate for rat P-gp and BCRP. In the present study, it has been demonstrated that sunitinib stimulates the ATPase activity by rat Mdr1a and Bcrp (Fig. 1). The affinity of sunitinib for rat P-gp (apparent $K_m = 19.5 \mu M$) and BCRP (apparent $K_m = 0.28 \mu M$) were comparable to that of human orthologs (apparent $K_d = 15.1$ and $0.18 \mu M$, respectively) (Shukla et al., 2009). Although there are several limitations in using ATPase assays with respect to transport kinetics and search for potential inhibitors because a competitive inhibitor can also stimulate ATPase activity, our results would indirectly suggest that sunitinib is transported by rat P-gp as well as BCRP and that pantoprazole can alter the pharmacokinetics of sunitinib in vivo by interacting with BCRP rather than P-gp (apparent $K_m = 0.10$ and $125 \mu M$, respectively; Fig. 2).

P-gp and BCRP are expressed at the brush-border membrane of intestinal epithelial cells, and function as a barrier to the absorption of substrate drugs administered orally (Glavinas et al., 2004). In this study, both the AUC$_{0-\infty}$, $C_{max}$, and $F$ of sunitinib were significantly decreased by administration of pantoprazole compared with the single administration of PSC833.
increased by pretreatment with PSC833 or pantoprazole in the intestine, while the AUC_{0-4}, C_{max}, and CL were not changed by each inhibitor administered intravenously. These results indicate that P-gp and BCRP are involved in limiting the intestinal absorption of sunitinib. In fact, our previous study showed that a genetic polymorphism in ABCG2 (421 C>A) influenced the systemic exposure to sunitinib (Mizuno et al., 2010, 2012). These findings suggest the potential for DDI between sunitinib and substrates/inhibitors of P-gp and BCRP.

Several reports have indicated pharmacokinetic interaction between sunitinib and drugs (Di Gion et al., 2011) or foods (Bello et al., 2006; Ge et al., 2011; van Erp et al., 2011). In particular, a clinical study demonstrated that coadministration of rifampicin, an inducer of CYP3A4, significantly decreased systemic exposure to sunitinib (Di Gion et al., 2011). In contrast, the AUC of sunitinib was increased by ketoconazole, an inhibitor of CYP3A4 (Di Gion et al., 2011). However, there is no report that demonstrates the DDI of sunitinib via CYP3A4, significantly decreased systemic exposure to sunitinib (Mizuno et al., 2010, 2012). These findings suggest the potential for DDI between sunitinib and substrates/inhibitors of P-gp and BCRP.

The alteration of plasma concentrations of sunitinib administered intraintestinally by the pharmacologic inhibition of each transporter was consistent with our previous study using Abcb1alb and Abcg2 knockout mice (Mizuno et al., 2012). On the other hand, two other groups showed that systemic exposure to sunitinib administered orally was not affected by the loss of P-gp and BCRP (Hu et al., 2009; Tang et al., 2012). These different results may be associated with the dosages of sunitinib used. In our studies, the dosages were chosen to give a similar plasma concentration to that in patients treated with the standard dosage of sunitinib (50 mg/d) (Britten et al., 2008; Mizuno et al., 2012), whereas the dosages used by the other groups were approximately 2- to 5-fold higher than ours (Hu et al., 2009; Tang et al., 2012). It is known that P-gp and BCRP-mediated efflux of sunitinib is saturable at high concentrations in vitro (Poller et al., 2011). One possible explanation for the discrepancy is that P-gp and BCRP may be saturated by a large amount of sunitinib in the intestinal epithelial cells. Further study is necessary to clarify the potential saturation of efflux transport in vivo by examining the influence of increasing doses of sunitinib on its intestinal absorption.

P-gp and BCRP, located at the canalicular membrane of the liver and the brush-border membrane of proximal tubules, mediate the secretion of compounds into bile and urine, respectively (Glavinas et al., 2004). In our previous study, no influence of the loss of P-gp and BCRP on systemic clearance of sunitinib was observed (Mizuno et al., 2012); however, the involvement of P-gp and BCRP in the secretion of sunitinib into bile and urine remains unclear. Our study has demonstrated for the first time that the biliary excretion of sunitinib is mediated by both P-gp and BCRP (Fig. 5). In addition, the distribution of sunitinib to the liver was significantly increased by treatment with PSC833 or pantoprazole (Table 2). An increase in the distribution of sunitinib to the kidney was also observed on treatment with PSC833 or pantoprazole (Table 2). Thus, our data indicate that the pharmacologic inhibition of P-gp and BCRP has the potential to cause the extensive accumulation of sunitinib in the liver and kidney although the clinical relevance of this is unclear. Further studies are necessary to clarify whether P-gp and BCRP are responsible for the hepatotoxicity and nephrotoxicity of sunitinib.

In our present study, the brain distribution of sunitinib was significantly increased by PSC833 but not pantoprazole (Fig. 6). The lack of apparent increase in the brain distribution of sunitinib in rats pretreated with pantoprazole may be due to the potential compensation by P-gp at the blood-brain barrier. To clarify whether BCRP is involved in the penetration by sunitinib of the brain, we examined the brain accumulation of sunitinib in rats treated with both inhibitors. As shown in Fig. 6, the brain distribution of sunitinib was much higher when both transporters were inhibited than when only P-gp was inhibited. It was previously reported that pantoprazole increased the brain penetration of imatinib in P-gp knockout mice (Breedveld et al., 2005). Based on this finding, it can be postulated that pantoprazole did not potentiate the PSC833-mediated inhibition of rat P-gp but rather increased the brain penetration of sunitinib by inhibiting BCRP at the
blood-brain barrier. Our results indicate that not only P-gp but also BCRP is involved in the penetration by sunitinib of the brain. These findings are consistent with previous reports using P-gp and/or BCRP knockout mice (Hu et al., 2009; Mizuno et al., 2012; Tang et al., 2012). Based on these findings, the coadministration of inhibitors of P-gp and BCRP increased the cerebral accumulation of sunitinib, which may have the potential to enhance its therapeutic effect on brain metastasis.

In conclusion, we have demonstrated that the plasma concentration, biliary excretion, and accumulation in the kidney, liver, and brain of sunitinib are significantly influenced by inhibitors of P-gp and BCRP. These findings should improve our understanding of the pharmacokinetics of sunitinib and indicate the potential for DDI between sunitinib and substrates or inhibitors of P-gp and BCRP.

Authorship Contributions

Participated in research design: Kunimatsu, Mizuno, Fukudo, Katsura.
Conducted experiments: Kunimatsu, Mizuno, Fukudo.
Performed data analysis: Kunimatsu, Mizuno, Fukudo.
Wrote or contributed to the writing of the manuscript: Kunimatsu, Mizuno, Fukudo, Katsura.

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