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 in rats treated with PSC833 (valspodar) and pantoprazole, potent inhibitors of P-gp and BCRP, respectively. The pharmacokinetics of sunitinib were examined 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 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.

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.

ABBREVIATIONS: AUC0-4, 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, valspodar; 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 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 \( K_c \) was estimated by fitting 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 (PE10; Becton Dickinson, Franklin Lakes, NJ) to examine 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 (SP-31; Natsume Seisakusho, Tokyo, Japan) for blood sampling. The rats were administered 5 mg/kg of PSC833, 40 mg/kg of pantoprazole, or both PSC833 and pantoprazole at the same dosage via the catheterized right femoral vein. After 15 minutes, rats were given 0.97 mg/kg of sunitinib as a bolus via the catheterized right femoral vein. 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 to evaluate 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 bile flow.

Figure 1. Effect of sunitinib on ATPase activity of rat Mdr1a and Bcrp. Membranes expressing rat Mdr1a (A) or Bcrp (B) were used for determining the ability of sunitinib to stimulate ATP hydrolysis. The method used to determine the drug-simulated ATPase activity was optimized based on the manufacturer’s protocol. The \( K_c \) was estimated by fitting sunitinib concentrations and ATPase activity into the Michaelis-Menten equation. Each point represents the mean ± S.E. (n = 3–4).

Figure 2. Effect of pantoprazole on ATPase activity of rat Mdr1a and Bcrp. Membranes expressing rat Mdr1a (A) or Bcrp (B) were used for determining the ability of pantoprazole to stimulate ATP hydrolysis. The method used to determine the drug-simulated ATPase activity was optimized based on the manufacturer’s protocol. The \( K_c \) was estimated by fitting pantoprazole concentrations and ATPase activity into the Michaelis-Menten equation. Each point represents the mean ± S.E. (n = 3).
adding 50 stock solutions of sunitinib (10, 50, 250, 500, 1000, and 2000 ng/ml) diluted with 50% methanol. Standard curves were prepared by dissolving in dimethylsulfoxide followed by stepwise dilution with 50% methanol. The intra-assay and inter-assay variability, and the accuracy bias were less than 10%. Stock solutions of sunitinib (LC Laboratories) and the cyclin-dependent kinase inhibitor roscovitine (LC Laboratories) used as an internal standard were prepared by dissolving in dimethylsulfoxide followed by stepwise dilution with 50% methanol. Standard curves were prepared by using 2 ml of stock solutions of sunitinib (10, 50, 250, 500, 1000, and 2000 ng/ml) diluted with 18 ml of blank matrix. Twenty ml of samples or standard samples were added with 10 µl of roscovitine solution (1 µg/ml), and were deproteinized by adding 50 µl of acetonitrile. After centrifugation, the supernatants were further diluted with 50 µl of 0.2% formic acid and were injected into a liquid chromatography with tandem mass spectrometry (LC-MS/MS) system after further dilution with 50% methanol. Blood samples were collected at 0.25, 0.5, 1.0, 2.0, 3.0, and 4.0 hours after the dose. Each point represents the mean ± S.D. (n = 4–7).

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 further dilution with 50% methanol. The tissue-to-plasma concentration ratio was calculated by dividing the tissue concentration with plasma concentration at each time point were significantly increased by pretreatment with PSC833 and pantoprazole compared with the control rats. The AUC0-4 from the intravenous administration experiments with the dose-normalized AUC0-4 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 Km (S.E.) of sunitinib for rat Mdr1a and Bcrp was estimated at 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 Km (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 AUC0-4 and Cmax 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

**TABLE 1**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pretreatment</th>
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<tr>
<td></td>
<td>Vehicle</td>
<td>PSC833</td>
</tr>
<tr>
<td><strong>Intraintestinal administration</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cmax (ng/ml)</td>
<td>59 ± 14</td>
<td>100 ± 86</td>
</tr>
<tr>
<td>AUC0–4 (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>
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<td></td>
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<tr>
<td>AUC0–4 (ng · h /ml)</td>
<td>175 ± 28</td>
<td>175 ± 40</td>
</tr>
<tr>
<td>Vdss (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).

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

c P < 0.05, statistically significantly different from the vehicle-treated group.
pretreatment with PSC833; however, the ratio in rats pretreated with pantoprazole was not significantly altered compared with that in the vehicle-treated rats (Fig. 6). We then assessed the influence of co-administration of PSC833 and pantoprazole on the brain distribution of sunitinib to clarify whether BCRP is involved in the penetration by sunitinib of the blood-brain barrier. As shown in Fig. 6, the brain distribution of sunitinib was 3 times increased by administration of both inhibitors 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 $K_m = 15.1$ 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$_{0\rightarrow \infty}$, $C_{\text{max}}$, and $F$ of sunitinib were significantly increased in rats pretreated with PSC833 or pantoprazole. After 15 minutes, the rats were given 0.97 mg/kg of sunitinib. Blood samples were collected at 0.25, 0.5, 1.0, 2.0, 3.0, and 4.0 hours after the dose. Each point represents the mean ± S.D. ($n = 3$–4). *$P < 0.05$; **$P < 0.01$; and ***$P < 0.001$, statistically significantly different from the vehicle-treated group.

**Effect of Inhibitors of P-gp and BCRP on the Biliary Excretion of Sunitinib Administered Intravenously.** The bile concentration of sunitinib was sequentially determined after intravenous administration of sunitinib to clarify whether BCRP is involved in the secretion of sunitinib into the bile. As shown in Fig. 5, bile concentrations of sunitinib of the blood-brain barrier. As shown in Fig. 6, the brain distribution of sunitinib was 3 times increased by administration of both inhibitors 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 P-gp and BCRP in a clinical setting. Therefore, clinical studies are necessary to clarify the DDI of sunitinib with substrates or 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 Abcl1a/1b 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) (Bratten 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

**TABLE 2**

<table>
<thead>
<tr>
<th>Ratio</th>
<th>Pretreatment</th>
<th>Fold Increase</th>
<th>Pretreatment</th>
<th>Fold Increase</th>
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<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>PSC833</td>
<td>Vehicle</td>
<td>Pantoprazole</td>
</tr>
<tr>
<td>Liver-to-plasma ratio</td>
<td>24 ± 6.5</td>
<td>34 ± 4.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.5</td>
<td>19 ± 1.4</td>
</tr>
<tr>
<td>Kidney-to-plasma ratio</td>
<td>26 ± 5.6</td>
<td>33 ± 2.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.3</td>
<td>32 ± 5.7</td>
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</table>

<sup>a</sup> Data are shown as the mean ± S.D. (n = 5–7).
<sup>b</sup> P < 0.05, statistically significantly different from the vehicle-treated group.
<sup>c</sup> P < 0.01, statistically significantly different from the vehicle-treated group.
<sup>d</sup> P < 0.001, statistically significantly different from the vehicle-treated group.
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: Kunimoto, Mizuno, Fukudo, Katsura.

Conducted experiments: Kunimoto, Mizuno, Fukudo.

Performed data analysis: Kunimoto, Mizuno, Fukudo.

Wrote or contributed to the writing of the manuscript: Kunimoto, Mizuno, Fukudo, Katsura.

References


