Impact of Genetic Variation in Breast Cancer Resistance Protein (BCRP/ABCG2) on Sunitinib Pharmacokinetics

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Summary: To elucidate the impact of genetic variations in breast cancer resistance protein (BCRP/ABCG2) and P-glycoprotein (MDR1/ABCB1) on the pharmacokinetics of sunitinib, we carried out a pharmacogenetic study in a clinical setting and pharmacokinetic analysis using Abcg2²/−, Abcb1a/1b−/− and Abcb1a/1b;Abcg2−/− mice. Nineteen renal cell carcinoma patients were enrolled in this study. The plasma concentrations of sunitinib and its active metabolite were determined and the area under the concentration-time curve (AUC) was calculated. Genetic polymorphisms in ABCG2 (421C>A) and ABCB1 (1236C>T, 2677G>T/A and 3435C>T) were examined. The dose-adjusted AUC₀.2₄ of sunitinib was significantly higher in patients with a heterozygous variant for ABCG2 421C>A than in wild-type patients (p = 0.02), and one homozygous patient showed the highest dose-adjusted AUC₀.2₄. The ABCB1 polymorphisms were not associated with the dose-adjusted AUC₀.2₄. The maximum concentration and AUC₀.₄ of sunitinib were significantly higher in Abcg2−/−, Abcb1a/1b−/− and Abcb1a/1b;Abcg2−/− mice than wild-type mice when sunitinib was given orally but not intraperitoneally. Incidence of thrombocytopenia and hypertension and poor compliance were associated with the systemic exposure to sunitinib and its active metabolite. These results suggest that the loss of protein expression of ABCG2 by genetic polymorphism is associated with an increase in the systemic exposure to sunitinib and sunitinib-induced toxicity.

Keywords: ABCG2; ABCB1; transporter; pharmacogenetics; pharmacogenomics; tyrosine kinase inhibitor

Introduction

Sunitinib, an orally administered multitargeted tyrosine kinase inhibitor (TKI), exerts antiangiogenic and anti-tumor activity by inhibiting various receptor tyrosine kinases, such as vascular endothelial growth factor receptors and platelet-derived growth factor receptors.¹ In randomized clinical trials, sunitinib significantly prolonged progression-free survival (PFS) and overall survival in patients with renal cell carcinoma (RCC) as compared to interferon-alpha.²,³ Sunitinib is currently the first drug of choice for treating advanced RCC; however, patients, especially Asian patients, often show poor compliance due to frequent adverse events such as thrombocytopenia and hand-foot syndrome.²—⁶ In particular, about 80% of Japanese and Korean patients are forced to discontinue or reduce the dose of sunitinib due to adverse events.⁵,⁶ Furthermore, tumor regrowth during drug-off periods was observed with sunitinib therapy.³ For these reasons, prevention of the development of adverse events is important for continuing treatment and obtaining benefits of sunitinib. A better understanding of the mechanisms underlying the individual variability in the pharmacokinetics and pharmacodynamics of sunitinib is greatly needed to optimize sunitinib pharmacotherapy.

The ATP-binding cassette (ABC) transporters, breast cancer resistance protein (BCRP/ABCG2) and P-glycoprotein (MDR1/ABCB1), mediate the efflux of various anti-cancer drugs, including TKIs.⁷,⁸ These transporters are...
widely expressed in normal tissues such as the small intestine and blood-brain barrier and play important roles in the pharmacokinetics of several substrate drugs.\(^9,10\) The pharmacokinetic significance of ABCG2 and ABCB1 has been demonstrated by several pharmacogenetic studies.\(^11,12\) For instance, the ABCG2 421C>A polymorphism, which was reported to be associated with decreased protein expression in the cell membranes,\(^13\) influenced blood levels of oral substrate drugs such as diltiazem and sulfasalazine.\(^14,15\) Furthermore, the pharmacokinetic role of ABCB2 and ABCB1 was also shown using Abcg2\(^{-/-}\), Abcb1a/1b\(^{-/-}\) and Abcb1a/1b;Abcg2\(^{-/-}\) mice.\(^16-18\)

We previously experienced a RCC patient with many severe adverse events such as facial acne, hypothyroidism and thrombocytopenia early after the start of sunitinib therapy.\(^19\) It was found that the exposure to sunitinib was approximately 2.5-fold higher in this patient than in 4 other patients.\(^19\) The genotype of the ABCG2 421C>A polymorphism in this patient was a homozygous variant, whereas the genotype in the other 4 patients was a heterozygous variant or wild-type.\(^19\) In addition, we first demonstrated by in vitro transport experiments that sunitinib is a substrate of ABCB2.\(^19\) Furthermore, another study demonstrated that sunitinib is also transported by ABCB1.\(^20\) From these findings, we hypothesized that genetic polymorphisms in ABCG2 and ABCB1 are associated with sunitinib pharmacokinetics and increased systemic exposure to sunitinib can be a risk factor for the development of adverse events.

In the present study, we examined the impact of genetic variation in ABCG2 and ABCB1 on the pharmacokinetics of sunitinib in RCC patients. In addition, Abcg2\(^{-/-}\), Abcb1a/1b\(^{-/-}\) and Abcb1a/1b;Abcg2\(^{-/-}\) mice were used to elucidate the roles of ABCG2 and ABCB1 in the pharmacokinetics of sunitinib. We also investigated the association of sunitinib pharmacokinetics with drug-induced adverse events and tolerability in sunitinib therapy.

**Methods**

**Patients:** This study was conducted as an exploratory retrospective study. Nineteen RCC patients treated with sunitinib at Kyoto University Hospital were enrolled in this study. Sunitinib was administered orally once daily on a 4-weeks-on, 2-weeks-off dosing schedule. Sunitinib was given as a single anti-cancer agent to all patients. Blood samples were obtained 8 days after the start of sunitinib therapy. The development of sunitinib-related adverse events was evaluated for 4 weeks (drug-on period) in the first treatment cycle. All adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0. This study was conducted in accordance with the Declaration of Helsinki and its amendments and was approved by the Kyoto University Graduate School and Faculty of Medicine and Kyoto University Hospital Ethics Committee. All patients gave written informed consent.

**Blood sampling and genotyping of ABCG2 and ABCB1 polymorphisms in RCC patients:** Peripheral blood samples were collected 0, 2, 6, 12 and 24 h post-administration. Following centrifugation, plasma samples were obtained and stored at \(-30^\circ\)C. Genomic DNA was extracted from whole blood using a Wizard® Genomic DNA Purification Kit (Promega, Madison, WI). The ABCG2 421C>A polymorphism was genotyped by direct sequencing. The region including the polymorphism in exon 5 of ABCB1 was amplified by polymerase chain reaction (PCR) using specific primers. Primer sequences were as follows: forward, 5' - GTTATTAGATGTCTTAGCTGC-3' and reverse, 5'-TATCCACACAGGAAAGTCC-3'. The PCR product was purified and sequenced as reported previously.\(^21\) The genotypes of ABCB1 1236C>T, 2677G>T/A and 3435C>T, the most frequently studied polymorphisms in ABCB1, were determined by the PCR-restriction fragment length polymorphism method as described previously.\(^22,23\) Haplotypes of ABCB1 were analyzed by SNPAlalyze ver. 8.0 (Dynacorn, Chiba, Japan).

**Determination of sunitinib and its active metabolite:** Sunitinib and its active metabolite SU012662 (N-desethyl sunitinib) were determined simultaneously by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The LC-MS/MS assay was carried out using a liquid chromatography system consisting of a Prominence series chromatograph (Shimadzu, Kyoto, Japan) coupled to an API 4000™ triple-quadrupole tandem mass spectrometer (AB SCIEX, Foster City, CA). Chromatographic separation was carried out on an Inertsil® ODS-3 (GL Sciences, Tokyo, Japan). Gradient elution was carried out at a flow rate of 0.2 mL/min using a mobile phase composed of 0.1% formic acid and acetonitrile containing 0.1% formic acid. The MS/MS analysis was performed using an electro-spray ionization source in the positive mode. Detection was carried out in the multiple reaction monitoring mode by monitoring ion transitions from \(m/z\) 399.2 to 283.2 (sunitinib), \(m/z\) 371.2 to 283.2 (SU012662) and \(m/z\) 355.2 to 233.0 (roscovitine). The intra-assay and inter-assay coefficient of variation and accuracy bias was less than 10%. The lower limit of quantification for both sunitinib and SU012662 was 1 ng/mL.

**Sample preparation:** Stock solutions of sunitinib (LC Laboratories, Woburn, MA), SU012662 (Toronto Research Chemicals, Toronto, Canada) and the cyclin-dependent kinase inhibitor ros covitine (LC Laboratories) used as the internal standard were prepared by dissolving in dimethyl sulfoxide and stepwise dilution with water/methanol (1:1, v/v). When human plasma samples were analyzed, 10 µL of ros covitine solution (1 µg/mL) was added to 100 µL of plasma. Subsequently, plasma samples were deproteinized with 200 µL of acetonitrile. After centrifugation, the supernatant was diluted with 50 µL of 0.2% formic acid, and injected into the LC-MS/MS system after filtration. When mouse plasma and brain samples were analyzed, 20 µL of sample supplemented with 10 µL of ros covitine.
solution (1 µg/mL) was deproteinized by adding 50 µL of acetonitrile. After centrifugation, supernatants were diluted with 50 µL of 0.2% formic acid and injected into LC-MS/MS after filtration.

**Animals:** Animal experiments were conducted in accordance with the Guidelines for Animal Experiments of Kyoto University. All protocols were approved by the Animal Research Committee, Graduate School of Medicine, Kyoto University. Animals used in the present experiments were male Abcg2–/–, Abcb1a/1b−/− and Abcb1a/1b;Abcg2–/– mice obtained from Taconic Farms (Hudson, NY) and wild-type mice obtained from CLEA Japan (Yokohama, Japan) with the same genetic background (FVB), between 14 and 20 weeks of age. The mice were kept in a temperature-controlled environment with a 12-h light/dark cycle and received a standard diet and water ad libitum.

**Pharmacokinetic analysis in mice:** The drug solution was prepared by dissolving sunitinib malate in saline (1 mg/mL as sunitinib). To investigate the role of Abcg2 and Abcb1 in gastrointestinal absorption, sunitinib was given at 5 mg/kg body weight by oral gavage. All mice were fasted overnight prior to treatment to minimize interference from gastric residual content, but were given free access to water. Blood samples were collected from a tail vein 30 min, 1 h and 2 h post-administration using heparinized capillary tubes. Four hours after the administration, mice were anesthetized with pentobarbital and whole blood was collected from the abdominal aorta. Blood samples were centrifuged at 13,000 rpm for 5 min at room temperature, and the plasma fraction was collected. The same dose (5 mg/kg) was administered intraperitoneally to assess tissue distribution and elimination. The intraperitoneal route was chosen for systemic exposure because it enabled rapid absorption into blood, and this method was used as an alternative to intravenous injections when blood sampling was carried out from the tail vein. Blood sampling was carried out as in the oral administration study. The brain was rapidly removed immediately after the final blood sampling and the excised brain tissue was gently washed and homogenized on ice in saline at 1 mL/mg brain. All samples were stored at −30 °C until the analysis.

**Pharmacokinetic calculations and statistical analysis:** Pharmacokinetic parameters were calculated by noncompartmental methods using WinNonlin® version 6.0 (Pharsight, Sunnyvale, CA). The area under the concentration-time curve (AUC) was calculated by the trapezoidal method. The brain distribution of sunitinib in mice was evaluated using the brain-to-plasma concentration ratio (Kp, brain) at the last time point. The differences of baseline characteristics in patients with or without a variant in each ABCG2 or ABCB1 polymorphisms were evaluated by the Mann-Whitney U test or Fisher’s test. The chi-square test was used to establish whether allele frequencies were in Hardy-Weinberg (HW) equilibrium. The association of ABCG2 421C→A with the sunitinib dose-adjusted AUC0–24 was evaluated using the Mann-Whitney U test. Correlations between genotypes of ABCB1 and the dose-adjusted AUC0–24 of sunitinib were evaluated by the Mann-Whitney U test or Kruskal-Wallis test followed by Dunn’s test. The Jonckheere-Terpstra test was used for detecting the gene dosage effect of the ABCB1 2677G>T/A polymorphism.

Data from the pharmacokinetic analysis in mice were analyzed statistically with a one-way analysis of variance followed by Dunnett’s test. The significance of differences in the incidence of adverse events between two groups was evaluated by Fisher’s test. Time-to-event analyses were carried out using the Kaplan-Meier method and statistical significance was evaluated by the log-rank test. The hazard ratio (HR) and 95% confidence interval (CI) were evaluated using the Cox proportional hazard model. A probability value of less than 0.05 was considered statistically significant.

**Results**

**Baseline characteristics and genotype of ABCG2 and ABCB1:** The baseline characteristics of the 19 patients are shown in Table 1. Sunitinib was administered to all patients without reducing or escalating the dose for at least 8 days after the start of treatment. Discontinuation or dose reduction of sunitinib from day 9 to day 28 was observed in 14 patients due to adverse events (thrombocytopenia, n = 8; fever, n = 2; facial acne, n = 1; neutropenia, n = 1; renal dysfunction, n = 1; fatigue, n = 1). The frequencies of polymorphisms in ABCG2 and ABCB1 are shown in Table 2. The allele frequencies in all genotypes were found to be in HW equilibrium. Analysis of haplotype showed the 7 ABCB1 haplotypes (frequency) as follows: TTT (36.3%), TGC (27.0%), CGC (16.1%), CAC (13.4%), TAC (2.5%), TTC (2.3%), and TGT (2.3%). The Eastern Cooperative Oncology Group performance status was 0 or 1 in all

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>62 (21–79)</th>
<th>50 mg 12</th>
<th>37.5 mg 4</th>
<th>25 mg 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (range), years</td>
<td>62 (21–79)</td>
<td>50 mg 12</td>
<td>37.5 mg 4</td>
<td>25 mg 3</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td>Male</td>
<td>14 (73.7%)</td>
<td>22 (9–55)</td>
<td>14 (8–49)</td>
</tr>
<tr>
<td>Female</td>
<td>5 (26.3%)</td>
<td>14 (8–49)</td>
<td>14 (8–49)</td>
<td>14 (8–49)</td>
</tr>
<tr>
<td>Median weight (range), kg</td>
<td>58 (44–78)</td>
<td>0.5 (0.3–0.8)</td>
<td>0.9 (0.5–1.5)</td>
<td>16 (9–33)</td>
</tr>
<tr>
<td>Median body surface area (range), m²</td>
<td>1.59 (1.31–1.81)</td>
<td>0.2; facial acne, n = 1</td>
<td>0.3; facial acne, n = 1</td>
<td>0.8; facial acne, n = 1</td>
</tr>
<tr>
<td>Median laboratory data (range)</td>
<td>AST, IU/L</td>
<td>0.3–0.8; facial acne, n = 1</td>
<td>0.5–1.5; facial acne, n = 1</td>
<td>0.8–3.0; facial acne, n = 1</td>
</tr>
<tr>
<td>ALT, IU/L</td>
<td>14 (8–49)</td>
<td>0.5–1.5; facial acne, n = 1</td>
<td>0.8–3.0; facial acne, n = 1</td>
<td>0.8–3.0; facial acne, n = 1</td>
</tr>
<tr>
<td>Total bilirubin, mg/dL</td>
<td>0.5 (0.3–0.8)</td>
<td>0.5–1.5; facial acne, n = 1</td>
<td>0.8–3.0; facial acne, n = 1</td>
<td>0.8–3.0; facial acne, n = 1</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>0.9 (0.5–1.5)</td>
<td>0.5–1.5; facial acne, n = 1</td>
<td>0.8–3.0; facial acne, n = 1</td>
<td>0.8–3.0; facial acne, n = 1</td>
</tr>
<tr>
<td>BUN, mg/dL</td>
<td>16 (9–33)</td>
<td>0.5–1.5; facial acne, n = 1</td>
<td>0.8–3.0; facial acne, n = 1</td>
<td>0.8–3.0; facial acne, n = 1</td>
</tr>
<tr>
<td>Initial dose, n</td>
<td>50 mg 12</td>
<td>37.5 mg 4</td>
<td>25 mg 3</td>
<td>50 mg 12</td>
</tr>
</tbody>
</table>

AST, aspartate aminotransferase; ALT, alanine aminotransferase; BUN, blood urea nitrogen.

Table 1. Baseline characteristics in 19 RCC patients
patients. Characteristics such as age and body surface area were not significantly different in patients with or without a variant in each ABCG2 or ABCB1 polymorphism (p > 0.05).

Impact of ABCG2 and ABCB1 polymorphisms on systemic exposure to sunitinib: The ABCG2 421C>A genotype had a marked impact on the pharmacokinetics of sunitinib (Figs. 1A and 1B). Of the 12 patients treated at the standard dosage (50 mg/day), the plasma concentration of sunitinib on day 8 in a patient with the ABCG2 421A/A genotype was highest and was higher in heterozygous variants than wild-type patients (Fig. 1A). We then evaluated the AUC_{0-24} in 19 patients by adjusting the dose, because each patient had a variety of initial dosages (Table 1) and the pharmacokinetics of sunitinib well corresponded to the dosage in individual patients.25 The dose-adjusted AUC_{0-24} of sunitinib was significantly higher in 8 patients with the heterozygous variant in ABCG2 421C>A than in 10 wild-type patients (Fig. 1B). In addition, one patient with the homozygous mutant showed the highest dose-adjusted AUC_{0-24} (Fig. 1B). We then assessed the effect of genetic variation in ABCB1 on the dose-adjusted AUC_{0-24} of sunitinib. There was no significant correlation between the ABCB1 1236C>T, 2677G>T/A and 3435C>T polymorphisms and dose-adjusted AUC_{0-24} of sunitinib (Figs. 1C–1E). We also carried out the Jonckheere-Terpstra test for detecting a gene dosage effect of ABCB1 2677G>T/A. A significant trend between the genotype of ABCB1 2677G>T/A and dose-adjusted AUC_{0-24} of sunitinib was not observed (Fig. 1D). No significant relation between ABCB1 TTT haplotype (1236T, 2677T and 3435T) and systemic exposure to sunitinib was observed either (Fig. 1F).

Pharmacokinetics of sunitinib in mice: The AUC_{0-4} and maximum concentration (C_{max}) of sunitinib were significantly higher in Abcg2^{−/−}, Abcb1a^{−/−} and Abcb1a^{−/−}Abcg2^{−/−} mice than wild-type mice when the drug was given orally but not intraperitoneally (Figs. 2A, 2B and Table 3). Calculated pharmacokinetic parameters of sunitinib were

Table 2. Allele frequencies of ABCG2 and ABCB1 polymorphisms

<table>
<thead>
<tr>
<th>Allele</th>
<th>Region</th>
<th>Effect</th>
<th>Allele frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCG2 421C&gt;A</td>
<td>Exon5</td>
<td>Q141K</td>
<td>73.7 26.3</td>
</tr>
<tr>
<td>ABCB1 1236C&gt;T</td>
<td>Exon12</td>
<td>Synonymous</td>
<td>34.2 65.8</td>
</tr>
<tr>
<td>ABCB1 2677G&gt;T/A</td>
<td>Exon21</td>
<td>A893S/T</td>
<td>39.5 42.1 18.4</td>
</tr>
<tr>
<td>ABCB1 3435C&gt;T</td>
<td>Exon26</td>
<td>Synonymous</td>
<td>57.9 42.1</td>
</tr>
</tbody>
</table>

*Number represents amino acid codon.

**Fig. 1. Impact of genetic polymorphisms in ABCG2 (A and B) and ABCB1 (C–F) on sunitinib pharmacokinetics**

Plasma concentration-time curves of sunitinib in 12 patients treated with sunitinib at the standard dosage (A). Sunitinib dose-adjusted AUC_{0-24} (B–F) in all 19 patients treated with sunitinib. All samples were obtained on day 8 after the initiation of sunitinib treatment. Statistical significance was evaluated by the Mann-Whitney U test or Kruskal-Wallis test followed by Dunn’s test. Bar represents the median.

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**A**

![WT, wild-type; AUC, area under the concentration-time curve; Cmax, maximum plasma concentration; T1/2, half-life; CL, clearance; Vd, volume of distribution.](image)

**B**

![WT, wild-type; AUC, area under the concentration-time curve; Cmax, maximum plasma concentration; T1/2, half-life; CL, clearance; Vd, volume of distribution.](image)

**C**

![WT, wild-type; AUC, area under the concentration-time curve; Cmax, maximum plasma concentration; T1/2, half-life; CL, clearance; Vd, volume of distribution.](image)

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**Fig. 2. Pharmacokinetics of sunitinib in mice**

Plasma concentration-time curve of sunitinib in mice treated by oral administration (A) and intraperitoneal injection (B). Distribution of sunitinib in the brain (C). ***p < 0.001, significantly different from wild-type mice. Statistical significance was evaluated by one-way analysis of variance followed by Dunnett’s test. WT, wild-type.

**Table 3. Pharmacokinetic parameters of sunitinib in mice**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Oral administration</th>
<th>Intraperitoneal injection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC (ng h/mL)</td>
<td></td>
</tr>
<tr>
<td>WT</td>
<td>24.8 ± 12.2, 93.2 ± 27.4***</td>
<td>9.5 ± 5.3, 55.0 ± 18.0***</td>
</tr>
<tr>
<td>Abcg2&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>658 ± 185, 549 ± 34</td>
<td>1.13 ± 0.36, 1.03 ± 0.10, 0.80 ± 0.08</td>
</tr>
<tr>
<td>Abcb1a/1b&lt;sup&gt;+&lt;/sup&gt;</td>
<td>244 ± 91, 308 ± 12</td>
<td>283 ± 54, 267 ± 39</td>
</tr>
<tr>
<td>Abcb1a/1b;Abcg2&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>356 ± 61, 447 ± 27</td>
<td>336 ± 54, 413 ± 94</td>
</tr>
</tbody>
</table>

Data are the mean ± S.D., n = 4–6, *p < 0.05, **p < 0.001 significantly different from wild-type mice (one-way analysis of variance followed by Dunnett’s test). WT, wild-type; AUC, area under the concentration-time curve; Cmax, maximum plasma concentration; T1/2, half-life; CL, clearance; Vd, volume of distribution.

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**Table 4. Association between plasma exposure to total drug and incidence of adverse events**

<table>
<thead>
<tr>
<th>Adverse events, n (%)</th>
<th>High AUC (n = 10)</th>
<th>Low AUC (n = 9)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombocytopenia (Grade ≥ 2)</td>
<td>10 (100)</td>
<td>5 (55.6)</td>
<td>0.033</td>
</tr>
<tr>
<td>Leukopenia (Grade ≥ 2)</td>
<td>7 (70.0)</td>
<td>3 (33.3)</td>
<td>0.18</td>
</tr>
<tr>
<td>Neutropenia (Grade ≥ 2)</td>
<td>7 (70.0)</td>
<td>5 (55.6)</td>
<td>0.65</td>
</tr>
<tr>
<td>Hand-foot syndrome (Any grade)</td>
<td>4 (40.0)</td>
<td>3 (33.3)</td>
<td>1.0</td>
</tr>
<tr>
<td>Hypertension (Grade ≥ 2)</td>
<td>9 (90.0)</td>
<td>2 (22.2)</td>
<td>0.0055</td>
</tr>
</tbody>
</table>

Patients were divided into two categories, a high AUC group (n = 10) and low AUC group (n = 9), based on the total drug AUC<sub>0-24</sub> on day 8. The high AUC group included patients with an AUC<sub>0-24</sub> of more than 2,600 ng h/mL and a trough concentration of 90 ng/mL. As shown in Table 4, the incidence of thrombocytopenia (grade ≥ 2) and hypertension (grade ≥ 2) was significantly higher in the high AUC group than low AUC group (p = 0.033 and 0.0055, respectively). On the other hand, no significant relation of the incidence of leukopenia (grade ≥ 2), neutropenia (grade ≥ 2) or hand-foot syndrome (any grade) with the AUC<sub>0-24</sub> of total drug was observed (p = 0.18, 0.65 and 1.0, respectively). The treatment period of sunitinib without discontinuation or dose reduction in the high AUC group was significantly shorter than in the low AUC group (HR, 3.00; 95% CI, 1.18–12.3; p = 0.025, Fig. 3A). The median treatment period without discontinuation or dose reduction in the high AUC group and low AUC group was 15 and 22 days, respectively. The association of genotype of ABCG2 421C>A polymorphism with development of adverse events and interruption of treatment was also examined in 12 patients who were given sunitinib at 50 mg/day. Thrombocytopenia tended to be developed earlier in patients with the ABCG2 421C/A or A/A genotype than in patients with the C/C genotype (Fig. 3B). The discontinuation or

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dose reduction tended to be observed sooner in patients with the $ABCG2$ 421C/A or A/A genotype than in patients with the C/C genotype (Fig. 3C). The median treatment period without discontinuation or dose reduction in the patients with the $ABCG2$ 421C/A or A/A genotype and C/C genotype were 13 and 19 days, respectively.

**Discussion**

This study provides clear evidence that genetic variations in $ABCG2$ affect the pharmacokinetics of sunitinib. It was demonstrated that the dose-adjusted AUC$_{0-24}$ of sunitinib was significantly higher in patients with a variant in $ABCG2$ 421C>A than in wild-type patients. In addition, the significance of $ABCG2$ to the pharmacokinetics was supported by the experiments with $Abcg2^{2/-}$ mice. In the present study, the $C_{\text{max}}$ and AUC$_{0-4}$ were significantly higher in $Abcg2^{2/-}$ mice than wild-type mice when sunitinib was administered orally, whereas the half-life, clearance and volume of distribution of sunitinib did not differ between $Abcg2^{2/-}$ and wild-type mice when sunitinib was given intraperitoneally. These findings suggest that ABCG2 functions as a barrier to the intestinal absorption of sunitinib and decreased protein expression in the intestinal cell membrane associated with genetic polymorphisms leads to increased systemic exposure to sunitinib.

In previous clinical trials for advanced RCC, sunitinib-related toxicity was more frequently observed in Asian patients than in non-Asian patients.²⁻⁴,⁶⁻²⁷ For instance, thrombocytopenia of grade 3 or 4 was more frequently developed in Japanese patients (48%) than non-Asian patients (8%).²¹ In addition, 38% of patients enrolled in the international multicenter phase III trial had a dose interruption because of adverse events,²¹ whereas 76% and 46% of Korean patients²⁶ and 78% of Japanese patients²ⁱ were forced to discontinue or reduce the dose of sunitinib in clinical trials due to adverse events. In addition, a previous population pharmacokinetic meta-analysis showed that Asian descent is significantly associated with decreased oral clearance of sunitinib.²⁸ On the basis of these observations, it was stated that the evaluation of another sunitinib administration strategy for Asian patients that might decrease toxicity while preserving efficacy is necessary, because severe adverse events are usually related to poor compliance, and dose interruption or reduction may lead to insufficient therapeutic outcomes.²⁵ The $ABCG2$ 421C>A polymorphism focused on in the present study is the most common allele in Asians but not in non-Asians. In particular, the allele frequency of $ABCG2$ 421C>A is higher in Asians such as Japanese (27%)³¹ and Chinese (34%)²⁹ than in non-Asians such as Caucasians (10%) and African Americans (5%).²⁹ This study demonstrated that the $ABCG2$ 421C>A polymorphism is significantly related to systemic exposure to sunitinib. In general, 19 patients are considered a small sample size for a pharmacogenetic study; however, the significance of $ABCG2$ to the pharmacokinetics of sunitinib was supported by the examination using $Abcg2^{2/-}$ mice. In addition, a significant association of increased systemic exposure to sunitinib with a higher incidence of adverse events and poor compliance was observed in this study. Furthermore, preliminary analysis in 12 patients who were given sunitinib at standard dosage showed that $ABCG2$ 421C/A or A/A genotype tended to be associated with the earlier development of thrombocytopenia and poorer compliance. These results suggest that $ABCG2$ 421C>A is one of the reasons for the ethnic difference in sunitinib pharmacokinetics and toxicity. Our findings may be useful for designing a new strategy for optimal sunitinib therapy for Asian patients. Further clinical studies including therapeutic outcomes are necessary to assess the usefulness of a therapeutic strategy in which the sunitinib dose is guided by the $ABCG2$ 421C>A genotype.

There are conflicting reports about the direct interaction
of sunitinib with ABCG2. Based on a screening assay of drug transporters (4 ABC transporters and 8 solute carrier transporters), Hu et al. demonstrated that the accumulation of sunitinib in Saos-2 cells, a human osteosarcoma cell line, was not affected by transient ABCG2 expression. On the other hand, we previously demonstrated that the efflux of sunitinib was significantly higher from HEK293 cells transiently expressing ABCG2 than from control cells, indicating that sunitinib is transported by ABCG2. Most recently, Tang et al. also demonstrated that sunitinib is transported by human ABCG2, ABCB1 and mouse Abcg2 using MDCK-II cells. A pharmacogenomic study of sunitinib showed that the TT haplotype of −15622C>T and 1143C>T in ABCG2, reported to be associated with lower protein expression in the small intestine, was related to the development of toxicity. Furthermore, the present study also showed that the Cmax and AUC of sunitinib were clearly increased in Abcg2−/− mice compared to wild-type mice when sunitinib was given orally. Collectively, it is considered that sunitinib is a substrate of ABCG2 and loss of its function due to genetic polymorphisms in ABCG2 can lead to an increase in systemic exposure to sunitinib.

Previous studies using ABCB1-expressing cells demonstrated that sunitinib is a substrate of ABCB1. In addition, our experiments using Abcb1a/1b−/− and Abcb1a/1b;Abcg2−/− mice also indicated that not only ABCG2 but also ABCB1 could be one of the determinants of sunitinib pharmacokinetics, suggesting the difference in activity or expression of ABCB1 to be involved in the inter-individual variability in the pharmacokinetics. However, two conflicting findings were reported about the influence of ABCB1 polymorphisms on the therapeutic outcomes of sunitinib. A pharmacogenomic study in patients treated with sunitinib showed that the ABCB1 haplotype (3435C/T, 1236C/T, 2677G/T) was associated with PFS in RCC patients. On the other hand, another study most recently showed that ABCB1 polymorphisms were not significantly associated with PFS in RCC patients. In the present study, a significant association between genetic polymorphisms in ABCB1 and exposure to sunitinib was not observed. So far, controversial findings have been reported for the association of ABCB1 polymorphisms with the pharmacokinetics of substrate drugs of ABCB1 such as tacrolimus and digoxin. For instance, it was shown that ABCB1 polymorphisms are associated with tacrolimus pharmacokinetics in Caucasians, whereas no significant association was observed in Japanese patients. These reports also showed that the expression of ABCB1 mRNA was not significantly influenced by ABCB1 polymorphisms in Japanese recipients of living-donor liver transplantation. Different effects of ABCB1 polymorphisms between ethnic groups were also observed in the pharmacokinetics of digoxin. These different results might reflect the discordance of frequencies of ABCB1 polymorphisms in different ethnic groups. Taken together, the ABCB1 polymorphisms were suggested to have a minor effect on the pharmacokinetics of substrates of ABCB1 in Asians compared with Caucasians. Therefore, ABCB2 rather than ABCB1 polymorphisms may be more valuable when predicting the systemic exposure to sunitinib in Asian patients.

In the pharmacokinetic examination in mice, we demonstrated that both Abcg2 and Abcb1 play an important role in the pharmacokinetics of sunitinib. Hu et al. showed that sunitinib was transported by ABCB1 while its pharmacokinetics was not changed in Abcb1a/1b−/− mice when given orally. In addition, it was recently reported that the systemic exposure to sunitinib after its oral administration in Abcb1a/1b−/−, Abcg2−/− and Abcb1a/1b;Abcg2−/− mice was comparable to that in wild-type mice. In contrast, we showed here that the AUC0−4, AUC0−8 and Cmax of sunitinib were significantly higher in Abcg2−/−, Abcb1a/1b−/− and Abcb1a/1b;Abcg2−/− mice than wild-type mice when sunitinib was given orally. In our study, the dosage for mice was chosen to give a similar plasma concentration to that in patients treated with the standard dose (50 mg) of sunitinib by single administration. A 2- or 4-fold higher dosage of sunitinib was given in the previous study. In fact, our preliminary experiments demonstrated that the AUC0−4 of sunitinib (mean ± S.D.) in Abcb1a/1b−/− (1,353 ± 590 ng·h/mL), Abcg2−/− (1,520 ± 939 ng·h/mL) and Abcb1a/1b;Abcg2−/− (1,095 ± 257 ng·h/mL) mice was not significantly altered compared with that in wild-type mice (1,492 ± 408 ng·h/mL) when 40 mg/kg sunitinib was given orally. Therefore, it was suggested that intestinal Abcg2 and Abcb1 might be saturated when a higher dosage of sunitinib is given orally. 

Our data showed that the distribution of sunitinib in the brain was increased by the disruption of Abcg2 and Abcb1a/1b, and markedly elevated by a loss of both transporters. This observation indicates that Abcg2 and Abcb1 coordinate transport sunitinib from the brain to blood, and their contribution to the efflux of sunitinib was much higher than that of other transport mechanisms such as passive diffusion as previously described. Drug distribution to the brain is important for the anti-cancer effects on a brain tumor and metastasis. Our results raise the hypothesis that the distribution of sunitinib in the brain is greater in patients who have a variant in ABCG2 than in wild-type patients. Further analysis including the measurement of sunitinib concentrations in cerebrospinal fluid is needed to test this hypothesis.

In conclusion, the present study indicates that the ABCG2 421C>A polymorphism, but not ABCB1 polymorphisms, significantly affects the pharmacokinetics of sunitinib in Japanese patients. In addition, it was suggested that excessively increased systemic exposure to sunitinib is associated with a higher incidence of adverse events and poor compliance. These findings should be useful in optimizing the pharmacotherapy of sunitinib in individual patients, and further prospective study with larger numbers of patients is warranted.
References


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