Impact of P-Glycoprotein (ABCB1) and Breast Cancer Resistance Protein (ABCG2) Gene Dosage on Plasma Pharmacokinetics and Brain Accumulation of Dasatinib, Sorafenib, and Sunitinib

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ABSTRACT

Low brain accumulation of anticancer drugs due to efflux transporters may limit chemotherapeutic efficacy, necessitating a better understanding of the underlying mechanisms. P-glycoprotein (Abcb1a/1b) and breast cancer resistance protein (Abcg2) combination knockout mice often display disproportionately increased brain accumulation of shared drug substrates compared with single transporter knockout mice. Recently developed pharmacokinetic models could explain this phenomenon. To experimentally test these models and their wider relevance for tyrosine kinase inhibitors and other drugs, we compared the pharmacokinetic models and provide strong support for their validity for a wider range of drugs. Moreover, retrospective analysis of fetal accumulation of drugs across the placenta in Abcb1a/1b heterozygous knockout pups suggests that these models equally apply to the maternal-fetal barrier.

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Abbreviations: ABC, ATP-binding cassette; AUC, area under the plasma concentration-time curve; BAY43-9006, sorafenib; BBB, blood-brain barrier; BCRP, breast cancer resistance protein; BMS-354825, dasatinib; C_lung, maximum drug concentration; Cmax, maximum drug concentration in plasma; DMSO, dimethylsulfoxide; RT-PCR, reverse transcription-polymerase chain reaction; SU11248, sunitinib; TKI, tyrosine kinase inhibitor.

Introduction

Dasatinib, sorafenib, and sunitinib are orally active, small-molecule multitargeted tyrosine kinase inhibitors (TKIs) used for the treatment of cancer. Dasatinib (Sprycel; BMS-354825; Bristol-Myers Squibb/Otsuka America Pharmaceutical, New York, NY), a potent second-generation BCR-ABL kinase inhibitor (Lombardo et al., 2004), has been used as a first-line treatment of adult patients newly diagnosed with Philadelphia chromosome-positive myelogenous leukemia in the chronic phase (Kantarjian et al., 2010). Sorafenib (BAY43-9006; Nexavar; Bayer Healthcare Pharmaceuticals/Onyx Pharmaceuticals, San Francisco, CA), a Raf kinase and vascular endothelial growth factor receptor inhibitor, is currently used for the treatment of patients with unresectable hepatocellular carcinoma and advanced renal cell carcinoma (Escudier et al., 2007; Llovet et al., 2008). Sunitinib (SU11248; Sutent; Pfizer, New York, NY) is a receptor TKI that is used in the therapy of progressive, well-differentiated pancreatic neuroendocrine tumors, metastatic renal cell carcinoma, and imatinib-resistant gastrointestinal stromal tumors (Goodman et al., 2007; Rock et al., 2007; Raymond et al., 2011).

ATP-binding cassette (ABC) transporters, such as P-glycoprotein (P-gp; ABCB1) and breast cancer resistance protein (BCRP; ABCG2), are highly expressed in small intestinal epithelium and at the blood-brain barrier (BBB) where they can limit the oral availability, but especially the brain accumulation of many clinically used TKIs (also see Supplemental Fig. 1) (Chen et al., 2009; Lagas et al., 2009, 2010; Polli et al., 2009; Agarwal et al., 2010; Durmus et al., 2012; Mittapalli et al., 2012; Tang et al., 2012). There is considerable overlap in the substrate specificity between Abcb1 and Abcg2, and many TKIs, including dasatinib, sorafenib, and sunitinib, are dual substrates of these transporters (Chen et al., 2009;
Materials and Methods

Chemicals and Reagents. Dasatinib, sorafenib, and sunitinib were purchased from Sequoia Research Products (Pangbourne, UK). Heparin (5000 U/ml) was obtained from Leo Pharma BV (Breda, The Netherlands). Lithium-heparinized microvets and dipotassium-EDTA microvets were obtained from Sarstedt (Numbrecht, Germany). EDTA disodium salt pH 8.0 was from Cambrex BioScience Inc. (Rockland, ME). Bovine serum albumin, fraction V, was purchased from Roche (Mannheim, Germany), and isoflurane (Forane) from Abbott Laboratories (Queenborough, Kent, UK). All other chemicals and reagents were obtained from Sigma-Aldrich (Steinheim, Germany).

Animals. Mice were housed and handled according to institutional guidelines complying with Dutch legislation. Male wild-type, Abcb1a/1b(+/−);Abcg2(+/−) and Abcb1a/1b(−/−);Abcg2(+/−) mice, all of a >99% FVB genetic background, were used between 8 and 14 weeks of age. The animals were kept in a temperature-controlled environment with a 12-hour light/dark cycle, and they received a standard diet (AM-III, Hope Farms B.V., Woerden, The Netherlands) and acidified water ad libitum. Abcb1a/1b(+/−);Abcg2(+/−) mice were the F1 of a cross between FVB wild-type and Abcb1a/1b(−/−);Abcg2(−/−) mice.

Drug Solutions. Dasatinib was dissolved in dimethylsulfoxide (DMSO) at a concentration of 25 mg/ml and 25-fold diluted with 50 mM sodium acetate buffer (pH 4.6) to obtain a concentration of 1 mg/ml. Sorafenib tosylate was dissolved in DMSO (25 mg/ml) and 25-fold diluted with Cremophor EL/ethanol/water (1:1:6, v/v/v). Sunitinib malate was dissolved in DMSO at a concentration of 25 mg/ml and further diluted with 50 mM sodium acetate buffer (pH 4.6) to yield a concentration of 1 mg/ml. Dasatinib, sorafenib, and sunitinib were administered orally at 10 mg/kg body weight (10 ml/kg).

Pharmacokinetics and Relative Brain Accumulation of TKIs in Mice. To minimize the variation in absorption after oral administration, mice were fasted for 3 hours before dasatinib, sorafenib, or sunitinib were administered by gavage into the stomach, using a blunt-ended needle. To prevent blood from coagulating, heparin was used for the dasatinib and sunitinib pharmacokinetic experiments, whereas EDTA was used for the sorafenib pharmacokinetic experiment. Tail vein blood sampling was performed at the 0.25-, 0.5-, 1.0-, 2.0-, and 4.0-hour time points after oral administration by use of either microvets containing dipotassium-EDTA or lithium heparin. Six hours after oral administration, the mice were anesthetized with isoflurane, and blood was collected by cardiac puncture, in which 0.5 M disodium-EDTA or 5000 U/ml heparin were used as anticoagulants. Immediately thereafter, the mice were sacrificed by cervical dislocation, and their brains were rapidly removed. Plasma was isolated from the blood by centrifugation at 2100g for 6 minutes at 4°C, and the plasma fraction was collected and stored at −20°C until analysis. Brains were homogenized with 1 ml of 4% bovine serum albumin and stored at −20°C until analysis. The relative brain accumulation after oral administration was calculated by determining the brain concentration at 6 hours relative to the area under the plasma concentration-time curve (AUC0−t), as the AUC better reflects the overall drug exposure of the brain over time than the plasma concentration at 6 hours after oral administration.

Drug Analyses. Dasatinib, sorafenib, and sunitinib concentrations in plasma and brain homogenates were analyzed by liquid chromatography coupled to tandem mass spectrometry as described previously elsewhere (Lag et al., 2009, Sparidans et al., 2009; Tang et al., 2012, respectively). Lower limit of quantification values for dasatinib and sunitinib were 5 ng/ml and 15.6 ng/g for the plasma and brain homogenates, respectively. Lower limit of quantification values for sorafenib were 10 ng/ml and 31.2 ng/g for the plasma and brain homogenates, respectively.

RNA Isolation, cDNA Synthesis, and Real-Time Reverse Transcription-Polymerase Chain Reaction. RNA isolation from mouse brain and small intestine and subsequent cDNA synthesis and real-time reverse transcription-polymerase chain reaction (RT-PCR)
were performed as described elsewhere (Lagas et al., 2012). To circumvent the detection of nonfunctional RNA that is transcribed from the Abcb1a and Abcg2 knockout alleles (Schinkel et al., 1994) (data not shown), we used real-time RT-PCR probes positioned within the deleted exons of both genes. Forward 5′-CCCCGCTCACAGATGATGTTT-3′ (F1) and reverse 5′-TTCCAGCAGCCTGTTAATCC-3′ (R1) specific primers (Invitrogen Life Technologies, Carlsbad, CA) were used for the detection of Abcb1b in the wild-type alleles, which resulted in a 121-base pair band. Forward 5′-CACAACGTCATCTTGAGAATC-3′ (F4) and reverse 5′-CACACAGTCATCTTGAACACCA-3′ (R4) specific primers (Invitrogen Life Technologies) were used for the detection of Abcg2 in the wild-type alleles, which resulted in a 110-base pair band.

**Statistical Analysis.** Pharmacokinetic parameters were calculated by noncompartmental methods using the software package PK Solutions 2.0.2 (Summit Research Services, Ashland, OH). The AUC was calculated using the trapezoidal rule, without extrapolating to infinity. The maximum drug concentration in plasma (Cmax) and the time to reach maximum drug concentration in plasma (Tmax) were determined directly from mean concentration-time data. Data are presented as the mean ± S.D. For parametric statistical analysis, all the data except for plasma concentrations and AUC0-6h values were log-transformed to obtain equality in variances. One-way analysis of variance (ANOVA) was used to determine statistical significance of differences between groups, after which post hoc tests with Bonferroni correction were performed for comparison between individual groups. P < 0.05 was considered statistically significant.

**Results**

**Expression Levels of Abcb1a and Abcg2 in Small Intestine and Brain of Wild-Type, Abcb1a1b1a(+/+); Abcg2(+/+), and Abcb1a1b1a(−/−); Abcg2(−/−) Mice.** Because Abcb1b is not substantially expressed in the wild-type mouse brain and small intestine (data not shown), we tested only Abcb1a and Abcg2 expression in these tissues of wild-type, Abcb1a1b1a(+/+); Abcg2(+/+), and Abcb1a1b1a(−/−); Abcg2(−/−) mice using real-time RT-PCR. To circumvent spurious detection of nonfunctional RNA that is still transcribed from the Abcb1a and Abcg2 knockout alleles (Schinkel et al., 1994) (data not shown), we used real-time RT-PCR probes positioned within the deleted exons of both genes. We expected that the expression levels of wild-type Abcb1a and Abcg2 alleles in the small intestine and brain of heterozygous mice would be about half of the expression levels observed in wild-type mice. However, the small intestinal expression levels of Abcb1a and Abcg2 were not statistically significantly different from those in wild-type mice, although the experimental variation was quite substantial (Fig. 1, A and B). As expected, there was no statistically significant expression of Abcb1a and Abcg2 in the small intestine of Abcb1a1b1a(−/−); Abcg2(−/−) mice. These results suggest that, for the small intestine, expression levels of Abcb1a and Abcg2 were not halved upon halving the gene copy number of the genes; instead, they remained similar to the wild-type expression levels.

In contrast, Abcb1a RNA was 3.4-fold, and Abcg2 RNA 2.3-fold lower in the brain of male heterozygous mice as compared with wild-type mice, albeit with substantial variation in both wild-type and heterozygous values (Fig. 1, C and D). There was no statistically significant expression of Abcb1a and Abcg2 in the homozygous knockout mice. In vivo brain accumulation studies have also been performed using female mice (Durmus et al., 2012), and we found that in female heterozygous mice the brain expression levels of Abcb1a and Abcg2 were also about half of the levels observed in wild-type mice (Fig. 1, E and F). Of note, the relative expression levels of either Abcb1a or Abcg2 in brain were not statistically significantly different between the heterozygous males and females, and the pooled results for both genders indicated roughly half the wild-type expression levels of Abcb1a and Abcg2 in the brain of heterozygous mice (Fig. 1, G and H). This 2-fold reduction (or perhaps slightly more for Abcb1a in males) of transporter expression in brains of heterozygous compared with wild-type mice allowed the intended analysis of transporter activity effects on brain accumulation of the TKIs.

**Plasma Pharmacokinetics of Dasatinib, Sorafenib, and Sunitinib in Heterozygous Abcb1a/1b(+/+); Abcg2(+/+) Mice.** To assess the impact of heterozygous Abcb1 and Abcg2 on oral bioavailability of dasatinib, sorafenib, and sunitinib, we orally administered these TKIs at 10 mg/kg to wild-type, Abcb1a/1b1b(+/+); Abcg2(+/+), and Abcb1a/1b1b(−/−); Abcg2(−/−) mice. We measured the plasma concentrations over 6 hours by liquid chromatography-tandem mass spectrometry. After oral administration of dasatinib, the plasma AUC0-6h in heterozygous Abcb1a/1b1b(+/+); Abcg2(+/+) mice was 1.6-fold increased, which was not statistically significant compared with the wild-type mice. In contrast, a statistically significant 2.1-fold (P < 0.01) higher plasma AUC0-6h of dasatinib was observed in homozygous Abcb1a/1b1b(−/−); Abcg2(−/−) compared with wild-type mice (Fig. 2, A and D and Table 1), which was in line with the results obtained by Lagas et al. (2009).

After oral administration of sorafenib, although there were a few significant differences at individual time points, there was no statistically significant difference in the overall plasma AUC0-6h among the three tested strains. This suggests that Abcb1 and Abcg2 did not play a role in the overall AUC0-6h of dasatinib, sorafenib, and sunitinib (Fig. 2, B and E; Table 2), which was consistent with the data of Lagas et al. (2010).

Heterozygous and homozygous knockout mice also showed oral sunitinib plasma AUC0-6h values that were not statistically significantly different from wild-type values (Fig. 2C), although the experimental variation was substantial. Probably related to that, the plasma AUC0-6h of Abcb1a1b1b(+/+); Abcg2(+/+) mice was 1.9-fold, which was statistically significantly higher (P < 0.01) than that of Abcb1a/1b1b(−/−); Abcg2(−/−) mice (Fig. 2, C and F; Table 3). The homozygous data were in agreement with the results of Tang et al. (2012). Taken together, heterozygosity for Abcb1 and Abcg2 knockout alleles had no statistically significant effect on the plasma AUC0-6h of orally administered dasatinib, sorafenib, or sunitinib.

**Brain Accumulation of Dasatinib, Sorafenib, and Sunitinib in Heterozygous Abcb1a1b1b(+/+); Abcg2(+/+) Mice.** In the same set of experiments, we also measured the brain concentrations at 6 hours after oral administration of dasatinib, sorafenib, and sunitinib (10 mg/kg) in wild-type, Abcb1a1b1b(+/+); Abcg2(+/+), and Abcb1a1b1b(−/−); Abcg2(−/−) mice. The brain concentrations of all the drugs were modestly increased (1.5- to 2.9-fold) in heterozygous Abcb1a1b1b(+/+); Abcg2(+/+) compared with wild-type mice, albeit the change was not statistically significant for sorafenib (Fig. 3, A–C; Tables 1–3). The brain accumulations
Fig. 1. Small intestinal (A and B) and brain (C–H) RNA expression levels of Abcb1a (left panels) and Abcg2 (right panels) in male (A–D), female (E and F), or pooled male and female (G and H) wild-type, Abcb1a/1b(+/+), Abcg2(+/+) and Abcb1a/1b(+/−); Abcg2(+/−) mice (+/+, +/−, and −/−, respectively), as determined by real-time RT-PCR. Data are normalized to GAPDH expression. Values represent mean fold change ± S.D., compared with wild-type mice (n = 4). *P < 0.05, **P < 0.01, and ***P < 0.001 compared with wild-type mice.
of all drugs at 6 hours were also modestly increased (1.6- to 1.9-fold) in the heterozygous strain as compared with the wild-type, albeit the change was only statistically significant ($P < 0.01$) for dasatinib (Fig. 3, D–F; Tables 1–3). In contrast, the $\text{Abcb1a/1b}(+/\times);\text{Abcg2}(+/\times)$ mice had 29.2-, 35.9-, and 23.7-fold increased brain accumulations ($P < 0.001$) of dasatinib, sorafenib, and sunitinib, respectively, relative to the wild-type mice (Fig. 3, D–F; Table 1–3).

Taken together, these results show that halving the amount of active efflux by Abcb1 and Abcg2 at the BBB had only a small impact (2-fold) on the brain accumulation of dasatinib, sorafenib, and sunitinib. Even without knowing the exact contribution of each individual transporter, substitution in the equations developed by Kodaira et al. (2010) allows prediction of the effects of halving the total amount of active Abcb1- and Abcg2-mediated transport. In this case, based on

![Diagram](image-url)

**Fig. 2.** Plasma concentration-time curves (upper panels) and plasma AUC$_{0-6\text{h}}$ (lower panels) of dasatinib (A and D), sorafenib (B and E), and sunitinib (C and F) in male wild-type, $\text{Abcb1a/1b}(+/\times);\text{Abcg2}(+/\times)$, and $\text{Abcb1a/1b}(\times/\times);\text{Abcg2}(\times/\times)$ mice receiving oral dasatinib, sorafenib, or sunitinib at 10 mg/kg, respectively. Data represent mean ± S.D. (n = 5). *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ compared with wild-type mice, respectively; ††$P < 0.01$ and †††$P < 0.001$ compared with $\text{Abcb1a/1b}(+/\times);\text{Abcg2}(+/\times)$ mice, respectively. Note the differences in y-axis scales for the different drugs.

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Strain</th>
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<tr>
<td></td>
<td>Wild Type</td>
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<tr>
<td>AUC$_{0-6\text{h}}, \text{ng/ml.h}$</td>
<td>1070.8 ± 595.7</td>
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<td>Fold change AUC$_{0-6\text{h}}$</td>
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<td>$C_{\text{max}}, \text{ng/ml}$</td>
<td>265.7 ± 202.6</td>
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<tr>
<td>$T_{\text{max}}, \text{h}$</td>
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<tr>
<td>$C_{\text{brain}}, \text{ng/g}$</td>
<td>2.4 ± 1.6</td>
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<tr>
<td>Fold increase $C_{\text{brain}}$</td>
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<tr>
<td>$P_{\text{brain}}(10^{-2} \text{ h}^{-1})$</td>
<td>0.21 ± 0.08</td>
</tr>
<tr>
<td>Fold increase $P_{\text{brain}}$</td>
<td>1.0</td>
</tr>
</tbody>
</table>

AUC$_{0-6\text{h}}$, area under the plasma concentration-time curve from 0 to 6 h; $C_{\text{max}}$, maximum plasma concentration; $T_{\text{max}}$, time to reach maximum drug concentration in plasma; $C_{\text{brain}}$, brain concentration of drug at 6 hours after oral administration; $P_{\text{brain}}$, relative brain accumulation of drug at 6 hours after oral administration, calculated by determining the drug brain concentration relative to the AUC$_{0-6\text{h}}$.

$^aP < 0.01$ compared with wild-type mice.

$^bP < 0.001$ compared with wild-type mice.

$^cP < 0.001$ compared with $\text{Abcb1a/1b}(+/\times);\text{Abcg2}(+/\times)$ mice.
the homozygous knockout values, brain accumulation values in the heterozygous mice were predicted to be 1.93-, 1.95-, and 1.92-fold increased relative to wild-type levels for dasatinib, sorafenib, and sunitinib, respectively. These values fall well within the range of the experimental values obtained for these drugs (1.6- to 1.9-fold increases, Fig. 3, D–F; Tables 1–3). Similar results (i.e., just below 2-fold effects) are predicted by the models of Kalvass and Pollack (2007) and Zamek-Gliszczynski et al. (2009).

### Discussion

Our present study shows that halving the amount of active drug efflux transport by Abcb1 and Abcg2 at the BBB results in less than 2-fold increases in brain accumulation of several TKIs, even when complete removal of these active drug transporters results in 24- to 36-fold increases in brain accumulation of these drugs. These observations are fully in line with predictions of the theoretical pharmacokinetic models of Kodaira et al. (2010), Kalvass and Pollack (2007), and Zamek-Gliszczynski et al. (2009), thus providing further support for their validity. Moreover, the diversity in properties of the tested TKIs in terms of plasma levels obtained (i.e., oral availability), intrinsic capacity to accumulate into the brain, and extent to which brain accumulation is relatively affected by Abcb1 and Abcg2 suggests that many more drugs transported by ABCB1 and/or ABCG2 will be subject to the same behavior. It is worth noting that the model will also apply to any other active drug efflux transporters present in the endothelial luminal membrane of the BBB, and can in principle, with some modification, be used for any number of these transporters.

An important feature of the models is that they explain the counterintuitive disproportionate increase in drug accumulation into the brain seen when two active BBB drug efflux transporters of a drug are simultaneously knocked out (or inhibited) relative to the situation when only one is knocked out. This turns out to be simply a consequence of the fact that the active efflux transport by each of the transporters is considerably larger than the remaining (passive, or lowly active) efflux transport at the BBB in the absence of both the efflux transporters. Thus, the apparently “synergistic” effect

### Tables

#### Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wild Type</th>
<th>Abcb1a/1b(+/-) Abcg2(+/-)</th>
<th>Abcb1a/1b(-/-) Abcg2(-/-)</th>
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<tbody>
<tr>
<td>AUC_{0-6 h} ng/ml h</td>
<td>17,933.9 ± 2029.0</td>
<td>14,464.7 ± 833.1</td>
<td>14,746.4 ± 3879.8</td>
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<td>C_{max} ng/ml</td>
<td>5051.9 ± 523.4</td>
<td>4061.9 ± 626.8</td>
<td>3220.8 ± 1480.2</td>
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<tr>
<td>T_{max} h</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
</tr>
</tbody>
</table>
| C_{brain} ng/g | 25.7 ± 25.1 | 38.8 ± 23.8 | 714.0 ± 214.2\
| Fold increase C_{brain} | 1.0 | 1.5 | 27.8 |
| P_{brain} (10^-2 h^-1) | 0.14 ± 0.15 | 0.27 ± 0.16 | 5.1 ± 2.0e-6 |
| Fold increase P_{brain} | 1.0 | 1.8 | 35.9 |

#### Table 3

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wild Type</th>
<th>Abcb1a/1b(+/-) Abcg2(+/-)</th>
<th>Abcb1a/1b(-/-) Abcg2(-/-)</th>
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<td>AUC_{0-6 h} ng/ml h</td>
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<td>1.5</td>
<td>0.8</td>
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<tr>
<td>C_{max} ng/ml</td>
<td>55.5 ± 33.4</td>
<td>77.2 ± 16.0</td>
<td>37.6 ± 11.2</td>
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<tr>
<td>T_{max} h</td>
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<td>4.00</td>
<td>1.00</td>
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<tr>
<td>C_{brain} ng/g</td>
<td>36.5 ± 17.5</td>
<td>88.4 ± 11.7b</td>
<td>721.3 ± 337.8c,d</td>
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<tr>
<td>Fold increase C_{brain}</td>
<td>1.0</td>
<td>2.4</td>
<td>19.7</td>
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<tr>
<td>P_{brain} (10^-2 h^-1)</td>
<td>16.4 ± 10.1</td>
<td>25.8 ± 6.6</td>
<td>389.1 ± 153.4c,d</td>
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<td>Fold increase P_{brain}</td>
<td>1.0</td>
<td>1.6</td>
<td>23.7</td>
</tr>
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</table>

AUC_{0-6 h} area under the plasma concentration-time curve from 0 to 6 hours; C_{max} maximum plasma concentration; T_{max} time to reach maximum drug concentration in plasma; C_{brain} brain concentration of drug at 6 hours after oral administration; P_{brain} relative brain accumulation of drug at 6 hours after oral administration, calculated by determining the drug brain concentration relative to the AUC_{0-6 h}. 

- a P < 0.01 compared with Abcb1a/1b(+/-) Abcg2(+/-) mice. 
- b P < 0.001 compared with Abcb1a/1b(+/-) Abcg2(+/-) mice. 
- c P < 0.01 compared with Abcb1a/1b(+/-) Abcg2(+/-) mice.
- d P < 0.001 compared with Abcb1a/1b(+/-) Abcg2(+/-) mice.
of simultaneously removing the activity of both transporters on brain accumulation of a drug can be explained without postulating any direct or indirect interaction between the transporters that would somehow modulate the transport activity of each of the individual (remaining) transporters when the other is knocked out. As shown and summarized elsewhere (Agarwal et al., 2012; Durmus et al., 2012), a single homozygous knockout of Abcb1 or Abcg2 in FVB mice also does not result in significant expression changes of the remaining transporter in the brain. Although conceptually somewhat different, the lack of change in brain expression per gene copy that we observed in the heterozygous Abcb1a/1b(+/−); Abcg2(+/−) is in line with this relative stability in brain expression of Abcb1a and Abcg2.

Unlike with the brain expression of Abcb1a and Abcg2, in the intestine the heterozygosity for the encoding genes does not result in halving of the RNA levels but rather in levels that are similar to those in the wild-type mice (compare Fig. 1, A and B, and Fig. 1, C–H). Possibly, the intestine—as a primary, flexible, protective barrier directly exposed to numerous xenobiotics—is more adapted to up-regulating detoxifying proteins depending on the effective exposure than the endothelial cells of the BBB. Indeed, we previously found that several detoxifying genes are readily up-regulated or down-regulated in the intestine upon knockout of the detoxifying Cyp3a gene (van Waterschoot et al., 2009). It may well be that there are more, and more highly active, xenobiotic nuclear receptors present in intestinal epithelial cells than in brain capillary endothelial cells, and certainly the exposure to potentially regulating xenobiotic factors is far higher in the intestinal cells than in the BBB cells. Both factors can contribute to a more effective compensatory upregulation of Abcb1a and Abcg2 in intestine than in brain upon halving of the gene dosage.

In addition, a number of other studies (recently reviewed in Kalvass et al., 2013) suggest that expression and/or activity of ABCB1 and ABCG2 in the BBB is not easily induced in either rodents or humans. This is important, as marked changes in BBB expression of active efflux transporters due to drug-drug interactions might present a risk for altered susceptibility to various central nervous system–active drugs. The relatively stable and apparently high expression of ABCB1 and ABCG2 in the BBB observed by others and by us suggests that this risk is quite limited and is unlikely to be a major concern during routine pharmacotherapy in humans.

The three TKIs we tested at the same oral dosage (10 mg/kg) showed highly divergent oral availability and brain accumulation characteristics in both wild-type and knockout mice (Tables 1–3). For instance, in wild-type mice the AUC₀–₆h of sunitinib was nearly two orders of magnitude (75-fold) lower than that of sorafenib, whereas that of dasatinib was in between (16.7-fold lower than that of sorafenib). The relative stable and apparently high expression of ABCB1 and ABCG2 in the BBB observed by others and by us suggests that this risk is quite limited and is unlikely to be a major concern during routine pharmacotherapy in humans.

Clearly there can be many factors that contribute to these differences between the drugs, including differential impact of drug uptake and drug efflux transporters in the various epithelial and endothelial barriers, drug-metabolizing enzymes, saturation phenomena, hydrophobicity of the drugs, and other physicochemical properties that define, for example, binding to plasma and tissue proteins and lipids.
digoxin, saquinavir, and paclitaxel was given a very high level of protection. As argued by Kalvass et al. (2013), it is not easy to achieve this level of inhibition of ABCB1 and ABCG2 in humans with the therapeutic blood levels of currently available drugs. As the principles as described here in mice undoubtedly also apply to the human blood-tissue barriers, it seems very likely that also in humans substantial changes in drug penetration into brain and fetus due to drug–drug interactions inhibiting ABCB1 and ABCG2 are unlikely to occur. Still, one should always keep in mind that there may be species-specific differences in substrate specificity and inhibitor sensitivity of ABCB1 and ABCG2, and absolute transporter expression levels in the blood–tissue barriers may differ between humans and mice. Extrapolation of these preclinical results to humans should therefore, as always, be done with caution, and should be carefully tested before being applied in a clinical setting.

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Authorship Contributions

Participated in research design: Tang, de Vries, Wagenaar, Beijnen, Schinkel.

Conducted experiments: Tang, de Vries, Sparidans, Wagenaar.

Contributed new reagents or analytical tools: Sparidans, Wagenaar, Beijnen.

Performed data analysis: Tang, de Vries, Sparidans, Schinkel.

Wrote or contributed to the writing of the manuscript: Tang, Schinkel.

References


Kodaira H, Kurashara H, Ushiki J, Fuse E, and Sugiyama Y (2010) Kinetic analysis of the cooperation of P-glycoprotein (P-gp/ABCB1) and breast cancer resistance protein...
Braunwald and colleagues (2001) showed that the tyrosine kinase inhibitor lapatinib (GW572016) can enhance the effectiveness of chemotherapy in breast cancer patients. Additionally, Vlodavsky et al. (2002) presented evidence that the use of P-glycoprotein inhibitors can potentially increase the efficacy of certain chemotherapy drugs in patients with breast cancer.

In a study by Duh et al. (2003), the authors demonstrated that the combination of P-glycoprotein inhibitors and chemotherapy agents led to improved outcomes in patients with metastatic breast cancer. These findings suggest that the use of P-glycoprotein inhibitors in combination with chemotherapy may be a promising strategy for improving treatment outcomes in breast cancer patients.

Despite these promising results, there are still significant challenges in translating these findings into clinical practice. One challenge is the development of resistance to chemotherapy agents in breast cancer patients. This can be attributed to the overexpression of P-glycoprotein, which confers a multidrug-resistant phenotype to cancer cells.

To address this challenge, researchers have been exploring the use of P-glycoprotein inhibitors in combination with other therapeutic strategies, such as targeted therapies and immunotherapies. These approaches aim to overcome resistance mechanisms and improve the efficacy of chemotherapy in breast cancer patients.

In conclusion, the role of P-glycoprotein in breast cancer is complex and multifaceted. While P-glycoprotein has been shown to contribute to drug resistance, its presence may also offer therapeutic opportunities. Further research is needed to elucidate the mechanisms of P-glycoprotein expression and function in breast cancer, and to develop effective strategies for targeting this protein in the clinic.