

Research Article

Breast Cancer Resistance Protein and P-glycoprotein Limit Sorafenib Brain Accumulation

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Abstract

Sorafenib is a second-generation, orally active multikinase inhibitor that is approved for the treatment of patients with advanced renal cell carcinoma and patients with unresectable hepatocellular carcinoma. We studied active transport of sorafenib in MDCK-II cells expressing human P-glycoprotein (P-gp/ABCB1) or ABCG2 (breast cancer resistance protein) or murine *Abcg2*. Sorafenib was moderately transported by P-gp and more efficiently by ABCG2 and *Abcg2*. Because sorafenib is taken orally, we orally administered sorafenib to wild-type, *Abcb1a/1b*^{-/-}, *Abcg2*^{-/-}, and *Abcb1a/1b;Abcg2*^{-/-} mice, completely lacking functional *Abcb1a/1b*, *Abcg2*, or both, respectively, and we studied plasma pharmacokinetics and brain accumulation. The systemic exposure on oral administration was not different among all strains. However, brain accumulation was 4.3-fold increased in *Abcg2*^{-/-} mice and 9.3-fold increased in *Abcb1a/1b;Abcg2*^{-/-} mice. Moreover, when wild-type mice were treated with sorafenib in combination with the dual P-gp and ABCG2 inhibitor elacridar, brain accumulation was similar to that observed for *Abcb1a/1b;Abcg2*^{-/-} mice. These results show that the brain accumulation of sorafenib is primarily restricted by ABCG2. This contrasts with previous studies using shared ABCG2 and P-gp substrates, which all suggested that P-gp dominates at the blood-brain barrier, and that an effect of ABCG2 is only evident when both transporters are absent. Interestingly, for sorafenib, it is the other way around, that is, ABCG2, and not P-gp, plays the dominant role in restricting its brain accumulation. Clinically, our findings may be relevant for the treatment of renal cell carcinoma patients with central nervous system relapses, as a dual ABCG2 and P-gp inhibitor might improve the central nervous system entry and thereby the therapeutic efficacy of sorafenib. *Mol Cancer Ther*; 9(2); 319–26. ©2010 AACR.

Introduction

The ATP-binding cassette (ABC) drug transporters P-glycoprotein (P-gp, *ABCB1*) and breast cancer resistance protein (*ABCG2*) are localized at several so-called sanctuary site barriers, such as the blood-brain, blood-testis, and blood-placenta barriers. At these barriers, the ABC drug transporters restrict the accumulation of many harmful compounds, thereby protecting the sanctuary tissues (1). However, their protective function becomes a disadvantage when penetration of substrate drugs into sanctuary tissues is desired, for instance, for the treatment of brain tumors. An example that illustrates this disadvantage is the low brain accumulation of imatinib,

a first-generation, orally active inhibitor of BCR-ABL kinase that is used as frontline therapy for Philadelphia chromosome-positive leukemia. Because imatinib is a good substrate of both P-gp and ABCG2 (2, 3), its accumulation into the central nervous system is markedly restricted by both transporters (4–6).

Sorafenib (BAY 43-9006, Nexavar; Fig. 1A) is a second-generation, orally active multikinase inhibitor that is recently approved for the treatment of patients with advanced renal cell carcinoma and patients with unresectable hepatocellular carcinoma (7). Interactions of sorafenib with P-gp or ABCG2 are thus far not reported. However, a few case reports indicated that sorafenib can achieve partial remission in renal cell carcinoma patients with brain metastases (8, 9). Although brain metastases may contain leaky blood vessels due to neovascularization, they are often protected from adequate chemotherapy because they are still mostly behind an intact blood-brain barrier (BBB; reviewed in ref. 10). It is thus important to establish whether the entry of sorafenib into the brain is limited by P-gp and/or ABCG2 because this information may be used to further optimize the treatment of renal cell carcinoma patients with central nervous system metastases.

Thus far, interactions of sorafenib with either ABCG2 or P-gp have not been described. However, for a number of tyrosine kinase inhibitors (TKI), including imatinib

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Note: Supplementary material for this article is available at Molecular Cancer Therapeutics Online (<http://mct.aacrjournals.org/>).

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doi: 10.1158/1535-7163.MCT-09-0663

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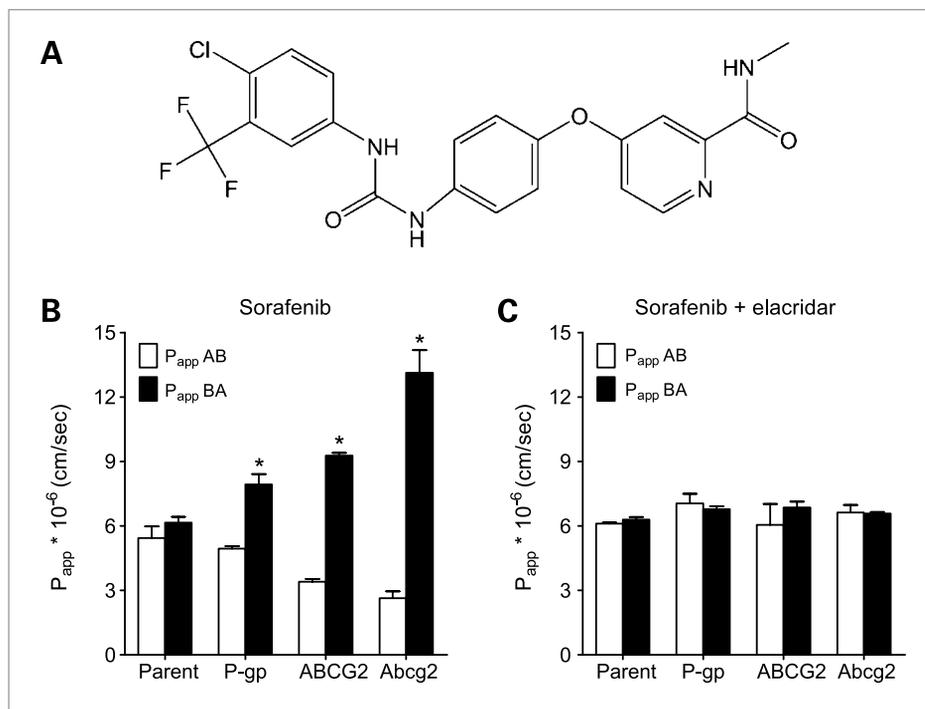


Figure 1. Molecular structure of sorafenib (A) and apparent permeability coefficients (B and C) for sorafenib in MDCK-II parent cells and in human P-gp- or ABCG2- or murine Abcg2-transduced subclones. P_{app} was calculated 4 h after the application of 5 $\mu\text{mol/L}$ sorafenib to the apical compartment (P_{app}^{AB}) or to the basolateral compartment (P_{app}^{BA}), either in the absence (B) or presence (C) of 5 $\mu\text{mol/L}$ elacridar. Note that the transporters in the transduced subclones are present in the apical membrane and that they are pumping their substrates toward the apical compartment. Data are mean $P_{app} \pm \text{SD}$ ($n = 3$). *, $P < 0.001$, when P_{app}^{BA} was compared with P_{app}^{AB} for parental cells or for each transduced subclone.

(2, 3), erlotinib (11), dasatinib (12, 13), and lapatinib (14), transport by ABCG2 and P-gp was reported, whereas other TKIs were shown to inhibit ABCG2- and/or P-gp-mediated transport [e.g., gefitinib (15–17), nilotinib (18, 19), and sunitinib (20)]. We therefore anticipated that sorafenib could be a substrate of P-gp and/or ABCG2 as well, and we studied the *in vitro* transport of sorafenib by human P-gp and ABCG2 and murine Abcg2. We next studied the oral plasma pharmacokinetics and brain accumulation of sorafenib in wild-type (WT), *Abcb1a/1b*^{-/-}, *Abcg2*^{-/-}, and *Abcb1a/1b;Abcg2*^{-/-} mice. We further tested whether we could boost the entry of sorafenib into the brain by blocking P-gp and/or ABCG2 at the BBB with the dual P-gp and ABCG2 inhibitor elacridar.

Materials and Methods

Chemicals

Sorafenib tosylate was purchased from Sequoia Research Products. Methoxyflurane (Metofane) was from Medical Developments Australia. Bovine serum albumin, fraction V, was from Roche. Cremophor EL was supplied by Fluka Biochemica. All other chemicals and reagents were of analytic grade or better and obtained from Sigma-Aldrich.

Transport Assays

Polarized canine kidney MDCK-II cell lines were used in transport assays. MDCK-II cells transduced with human P-gp and ABCG2 or murine Abcg2 were described previously (21–23). Transepithelial transport assays using transwell plates were carried out as described previously

(24). Experiments were done in the absence or presence of 5 $\mu\text{mol/L}$ elacridar, a dual inhibitor of P-gp and Abcg2. When elacridar was applied, it was present in both compartments during a 2-h preincubation period and during the transport experiment. After preincubation, experiments were started ($t = 0$) by replacing the medium in either the apical or the basolateral compartment with fresh DMEM (Invitrogen) containing 10% FCS and 5 $\mu\text{mol/L}$ sorafenib, either with or without 5 $\mu\text{mol/L}$ elacridar. Cells were incubated at 37°C in 5% CO₂, and 50- μL aliquots were taken at $t = 4$ h. The apparent permeability coefficient (P_{app}) was calculated using the following equation: P_{app} (cm/s) = $dC/dt \times 1/A \times V/C_o$ (cm/s), where dC/dt [$(\mu\text{mol/L}) \text{s}^{-1}$] represents the flux across the monolayer (permeability rate), A (cm²) the surface area of the monolayer, V (cm³) the volume of the receiver chamber, and C_o ($\mu\text{mol/L}$) the initial concentration in the donor compartment (25). Results are presented as mean $P_{app} \pm \text{SD}$ ($n = 3$). Membrane tightness was assessed in parallel, using the same cells seeded on the same day and at the same density, by analyzing transepithelial [¹⁴C]inulin (~3 kBq/well) leakage. Leakage had to remain <1% of the total added radioactivity per hour.

Animals

Mice were housed and handled according to institutional guidelines complying with Dutch legislation. Animals used were male WT mice; *Abcb1a/1b*^{-/-} mice, which lack both functional *Abcb1a* and *Abcb1b* genes (26); *Abcg2*^{-/-} mice (22); and *Abcb1a/1b;Abcg2*^{-/-} mice, which are knockout for all three genes (27). Note that

in the mouse, the *Abcb1a* and *Abcb1b* genes together seem to fulfill the same functions as the single human *ABCB1* gene. Mice were all of a >99% FVB genetic background and between 9 and 12 wk of age. Animals were kept in a temperature-controlled environment with a 12-h light:12-h dark cycle and received a standard diet (AM-II, Hope Farms) and acidified water *ad libitum*.

Plasma Pharmacokinetics and Brain Accumulation

Sorafenib tosylate was dissolved in DMSO (25 mg/mL), 25-fold diluted with Cremophor EL/ethanol/water (1:1:6, v/v/v), and orally administered at 10 mg/kg (10 μ L/g). To minimize variation in absorption, mice ($n = 5$ per group) were fasted 3 h before sorafenib was given by gavage into the stomach, using a blunt-ended needle. Multiple blood samples (~30 μ L) were collected from the tail vein at 15 and 30 min and 1, 2, 4, and 6 h, using heparinized capillary tubes (Oxford Labware). At the last time point, mice were anesthetized with methoxyflurane and blood was collected by cardiac puncture. Immediately thereafter, mice were sacrificed by cervical dislocation and brains were rapidly removed, homogenized on ice in 1 mL of 4% bovine serum albumin, and stored at -30°C until analysis. Blood samples were centrifuged at $2,100 \times g$ for 6 min at 4°C , and the plasma fraction was collected and stored at -20°C until analysis.

Calculation of the Relative Brain Accumulation

Brain concentrations were corrected for the amounts of drug in the brain vasculature, that is, 1.4% of the plasma concentration right before the brains were isolated (28). Brain accumulation after oral administration was calculated by determining the sorafenib brain concentration at $t = 6$ h relative to the area under the plasma concentration-time curve from 0 to 6 h (AUC_{0-6}). The AUC_{0-6} was used instead of plasma concentration at 6 h because the

AUC better reflects the overall sorafenib exposure of the brain.

Brain Accumulation of Sorafenib in Combination with Elacridar

WT mice ($n = 4$) were compared with *Abcb1a/1b;Abcg2*^{-/-} mice ($n = 5$). Elacridar (100 mg/mL in DMSO) was 25-fold diluted in a mixture of ethanol, polyethylene glycol 200, and 5% glucose (2:6:2, v/v/v) and i.v. injected into the tail vein at 10 mg/kg (2.5 mL/kg). Sorafenib tosylate (25 mg/mL in DMSO) was 25-fold diluted with Cremophor EL/ethanol/water (1:1:6, v/v/v) and i.v. injected into the tail vein at 5 mg/kg (5 mL/kg). Sorafenib was administered 15 min after injection of either elacridar or the elacridar vehicle. Blood and brains were isolated 60 min after sorafenib administration and processed and stored as described above.

Drug Analysis

Sorafenib concentrations in DMEM (Invitrogen) cell culture medium, plasma samples, and brain homogenates were determined using a sensitive and specific liquid chromatography-tandem mass spectrometry assay (29).

Pharmacokinetic Calculations and Statistical Analysis

Pharmacokinetic parameters were calculated by non-compartmental methods using the software package WinNonlin Professional version 5.0. The AUC was calculated using the trapezoidal rule, without extrapolating to infinity. The peak plasma concentration (C_{max}) and the time of the maximum plasma concentration (T_{max}) were estimated from the original data. To assess the statistical significance, we performed one-way ANOVA followed by Dunnett's multiple comparison test. Differences were

Figure 2. Plasma concentration-time curves (A) and relative brain accumulation (B) of sorafenib in male FVB WT, *Abcg2*^{-/-}, *Abcb1a/1b*^{-/-}, and *Abcb1a/1b;Abcg2*^{-/-} mice after oral administration of 10 mg/kg sorafenib. Relative brain accumulation was calculated by dividing brain concentration by the AUC_{0-6} . Data are means \pm SD ($n = 5$). *, $P < 0.001$, compared with WT mice, †, $P < 0.01$, compared with *Abcg2*^{-/-} mice.

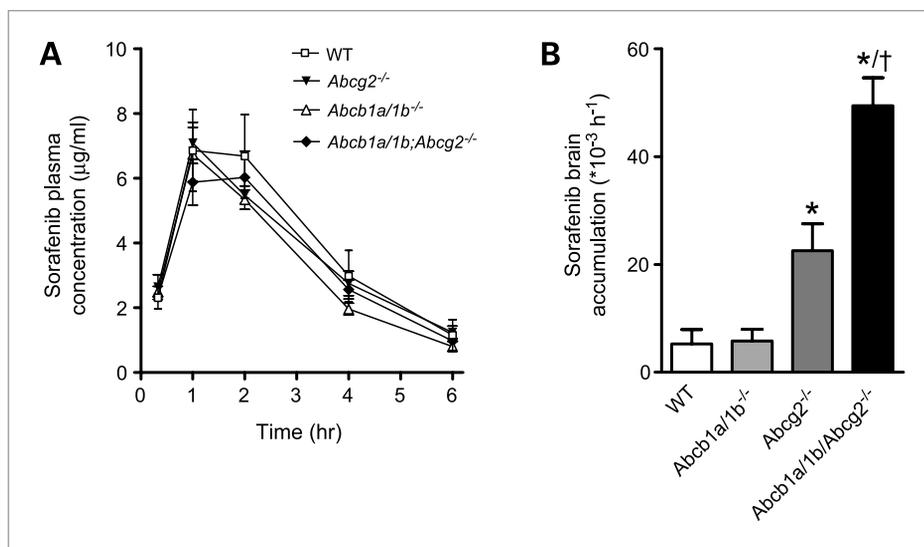


Table 1. Pharmacokinetic parameters, brain concentrations, and relative brain accumulation of sorafenib after oral administration at 10 mg/kg

	Genotype			
	WT	<i>Abcb1a/1b</i> ^{-/-}	<i>Abcg2</i> ^{-/-}	<i>Abcb1a/1b/Abcg2</i> ^{-/-}
AUC ₍₀₋₆₎ , µg/mL h	24.0 ± 4.8	19.6 ± 0.7	22.2 ± 1.5	21.2 ± 2.3
C _{max} , µg/mL	6.9 ± 1.3	6.7 ± 0.8	7.1 ± 0.6	6.0 ± 0.8
T _{max} , h	1	1	1	2
C _{brain} , µg/g	0.12 ± 0.04	0.11 ± 0.04	0.50 ± 0.13*	1.04 ± 0.08*†
Fold increase	1.0	0.9	4.2	8.7
P _{brain} (×10 ⁻³ h ⁻¹)	5.3 ± 2.7	5.8 ± 2.2	22.6 ± 5.0*	49.4 ± 5.2*†
Fold increase	1.0	1.1	4.3	9.3

NOTE: AUC₍₀₋₆₎, area under plasma concentration-time curve up to 6 h; C_{max}, maximum plasma concentration; T_{max}, time of maximal plasma concentration; C_{brain}, brain concentration at 6 h after oral administration; P_{brain}, relative brain accumulation at 6 h after oral administration, calculated by determining the sorafenib brain concentration relative to the AUC₀₋₆. Data are means ± SD (n = 5).

*P < 0.001, compared with WT mice.

†P < 0.01, compared with *Abcg2*^{-/-} mice.

considered statistically significant when $P < 0.05$. Data are presented as mean ± SD.

Results

We studied the *in vitro* transepithelial transport of sorafenib in polarized monolayers of MDCK-II cells and subclones of these cells stably transduced with human P-gp or ABCG2 or murine *Abcg2*. In the parental cells, the P_{app} was not different for apically or basolaterally applied sorafenib, indicating that sorafenib was not actively transported by these cells (Fig. 1B). In MDCK-II cells transduced with P-gp, the P_{app} for basolaterally applied sorafenib was 1.6-fold higher than that for apically applied sorafenib, whereas for ABCG2- and *Abcg2*-transfected cells, this ratio was respectively 2.7- and 5.0-fold higher ($P < 0.001$; Fig. 1B). In the presence of the dual P-gp and ABCG2/*Abcg2* inhibitor elacridar, the transport of sorafenib in all transfected subclones was completely inhibited (Fig. 1C). Sorafenib thus seems to be actively transported by P-gp, ABCG2, and *Abcg2*. The P_{app} values indicate that sorafenib is a moderate P-gp substrate, whereas sorafenib is more efficiently transported by ABCG2 and *Abcg2*.

Because sorafenib is taken orally by cancer patients, we next studied oral sorafenib plasma pharmacokinetics and we investigated whether the entry of sorafenib into the brain was restricted by either one or both transporters. We orally administered 10 mg/kg sorafenib to male WT, *Abcb1a/1b*^{-/-}, *Abcg2*^{-/-}, and *Abcb1a/1b/Abcg2*^{-/-} mice. No signs of toxicity were observed in any of the mouse strains. As shown in Fig. 2A and in Table 1, plasma concentrations and AUCs were not different among all strains. This suggests that *Abcg2* and P-gp do not limit oral uptake or contribute notably to first-pass elimination on oral administration of sorafenib in mice. Furthermore,

the brain accumulation of sorafenib, 6 hours after oral administration, was not different between *Abcb1a/1b*^{-/-} and WT mice (Fig. 2B). In contrast, *Abcg2*^{-/-} mice had a 4.3-fold increased brain accumulation ($P < 0.001$; Fig. 2B). Moreover, *Abcb1a/1b/Abcg2*^{-/-} mice had an even further increase in relative brain accumulation, which was 2.2-fold higher than that in *Abcg2*^{-/-} mice ($P < 0.01$) and 9.3-fold increased compared with that in WT mice ($P < 0.001$; Fig. 2B; Table 1). These results show that primarily *Abcg2* restricts the entry of sorafenib into the brain, both in P-gp-proficient and P-gp-deficient mice. In contrast, single loss of P-gp does not lead to a higher brain accumulation. However, P-gp can partly take over the function of *Abcg2* at the BBB in *Abcg2*^{-/-} mice, which becomes evident when the increase in brain accumulation in *Abcg2*^{-/-} mice (4.3-fold) is compared with that in *Abcb1a/1b/Abcg2*^{-/-} mice (9.3-fold; $P < 0.01$). In contrast, *Abcg2* can fully compensate for the loss of P-gp because *Abcb1a/1b*^{-/-} and WT mice have similar sorafenib concentrations in their brains. Uncorrected brain concentrations and values for the relative brain accumulation (i.e., corrected for the plasma AUC₀₋₆ of sorafenib) essentially yielded the same results (Table 1).

Because ABCG2 and P-gp together markedly restricted the brain accumulation of sorafenib, we used the dual P-gp and ABCG2 inhibitor elacridar to investigate whether inhibition of both efflux transporters at the BBB would result in an increased uptake of sorafenib into the brain. WT and *Abcb1a/1b/Abcg2*^{-/-} mice were treated with either i.v. elacridar or vehicle before sorafenib was administered. Plasma concentrations, 1 hour after i.v. sorafenib administration, were not different among all treatment groups, indicating that neither P-gp nor ABCG2 notably contributes to the plasma elimination of sorafenib (Fig. 3A). In contrast, elacridar increased the brain concentrations of sorafenib in WT mice by 7-fold ($P < 0.001$), resulting

in similar brain levels as observed for *Abcb1a/1b;Abcg2^{-/-}* mice (Fig. 3B). The fact that elacridar did not affect the brain concentration of sorafenib in *Abcb1a/1b;Abcg2^{-/-}* mice (Fig. 3B) indicates that other systems that could potentially influence the entry of sorafenib into the brain were not affected by elacridar. Overall, these results show that elacridar can specifically and completely block the BBB activity of Abcg2 and P-gp toward sorafenib.

Discussion

We show that the second-generation TKI sorafenib is efficiently transported *in vitro* by ABCG2 and Abcg2 and moderately by P-gp. Although the oral availability of sorafenib is not affected by murine P-gp and/or Abcg2, we could show that the brain accumulation of sorafenib is primarily restricted by Abcg2, and that P-gp can only partly take over this protective function at the BBB when Abcg2 is absent. Consequently, when both efflux transporters are absent, brain accumulation of sorafenib is highly increased. Finally, we show that the dual P-gp and ABCG2 inhibitor elacridar can completely block the activity of Abcg2 and P-gp at the BBB, leading to markedly increased sorafenib concentrations in the brain.

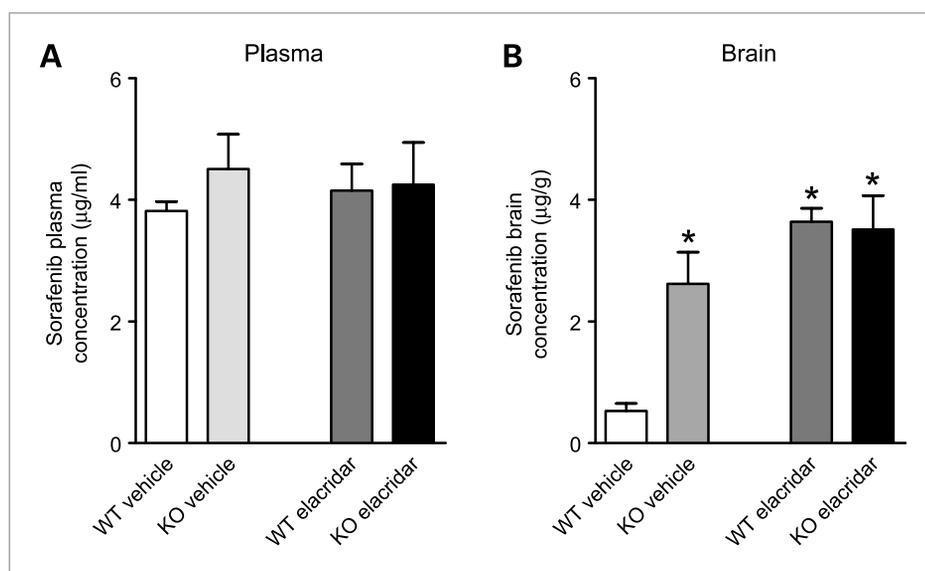
The apparent discrepancy that the loss of P-gp and Abcg2 does not affect the oral uptake of sorafenib whereas the brain accumulation is highly increased has also been observed for the TKI imatinib (6). It could be that there are efficient uptake transporter(s) for these drugs in the gut that are absent from the BBB and which overwhelm the efflux activity of P-gp and Abcg2 in the gut. Another explanation may be that passive diffusion of these compounds occurs much more easily across the intestinal wall than through the BBB. It is of interest to note that Abcg2 RNA was not differentially expressed in the brains or the small intestine of P-gp-deficient and WT mice (30). In ad-

dition, the RNA expression of Mdr1a P-gp was not altered in the small intestines of *Abcg2^{-/-}* mice (13). In the present study, we checked the Mdr1a P-gp RNA expression in the brains of *Abcg2^{-/-}* mice by real-time quantitative PCR and found no difference compared with WT mice (data not shown). Thus, the relative contribution of P-gp or Abcg2 at the BBB or in the small intestine of Abcg2- or P-gp-deficient mice, respectively, seems not to be obscured by altered expression of either P-gp or Abcg2.

Our results show that sorafenib is a good Abcg2 substrate and a moderate P-gp substrate. Interestingly, single P-gp deficiency did not affect the entry of sorafenib into the brain, whereas absence of Abcg2 resulted in a markedly higher brain accumulation. This observation is of particular interest because previous reports, exploring brain accumulation of shared P-gp and ABCG2 substrates in *Abcb1a/1b^{-/-}*, *Abcg2^{-/-}*, and compound *Abcb1a/1b;Abcg2^{-/-}* mice, all indicated that P-gp, and not Abcg2, plays a dominant role at the BBB. This was first shown for the anticancer agent topotecan (31). Although topotecan is a comparatively weak P-gp substrate and a good substrate for Abcg2 (32, 33), its brain accumulation is primarily restricted by P-gp (31). This is striking because sorafenib is also a moderate P-gp substrate and a good Abcg2 substrate, and yet opposite results are found.

We also recently studied the *in vitro* transport and brain accumulation of the TKI dasatinib (13). In Supplementary Fig. S1, transport results of dasatinib and sorafenib are compared. *In vitro*, dasatinib is a good substrate for Abcg2 and, in contrast to sorafenib, also a good substrate for P-gp (Supplementary Fig. S1B; ref. 13). Although dasatinib seems to be a very good substrate of Abcg2 *in vitro*, loss of Abcg2 in mice does not result in a higher brain accumulation (Supplementary Fig. S2B). Apparently, in the case of dasatinib, P-gp fully compensates for the loss of Abcg2. When the brain accumulation of sorafenib is compared

Figure 3. Plasma (A) and brain (B) concentrations of sorafenib for male FVB WT and *Abcb1a/1b;Abcg2^{-/-}* (KO) mice 1 h after i.v. administration of 5 mg/kg sorafenib. Sorafenib was given 15 min after the i.v. administration of either vehicle or elacridar (10 mg/kg) as indicated. Data are means \pm SD ($n = 4-5$). *, $P < 0.001$, compared with WT mice treated with vehicle.



with that of dasatinib (Supplementary Fig. S2), it is evident that Abcg2 is the dominant transporter for sorafenib, whereas for dasatinib this is P-gp. Nonetheless, *Abcb1a/1b;Abcg2^{-/-}* mice have the highest brain accumulation for both TKIs, indicating that in each case, the nondominant transporter can (partly) compensate for the loss of the dominant one. We note that sorafenib and dasatinib differ in hydrophobicity, as the experimental LogP is ~ 3.8 for sorafenib and ~ 1.8 for dasatinib.⁴ This difference might explain why WT mice have higher plasma and brain concentrations of sorafenib compared with dasatinib (13), despite the fact that the same dose was applied. However, the P_{app} values in MDCK-II cells (Supplementary Fig. S1) and the relative brain accumulation of both compounds in WT and *Abcb1a/1b;Abcg2^{-/-}* mice (Supplementary Fig. S2) are of the same order, suggesting that differences in penetration properties between these compounds do not explain their difference in affinity toward P-gp and Abcg2.

Similar results as for dasatinib on the dominant role of P-gp at the BBB were found for the TKIs imatinib and lapatinib, which are good substrates for both P-gp and ABCG2 (6, 34). Deficiency of Abcg2 alone did not lead to an increased brain accumulation, whereas single P-gp knockout mice had higher brain levels than WT mice. Again, *Abcb1a/1b;Abcg2^{-/-}* mice had the highest imatinib and lapatinib brain concentrations. And finally, Zhou et al. (35) reported a study in which they tried to gain insight into the role of ABCG2 at the BBB. In that study, more than 1,000 compounds were screened *in vitro* for their affinity for P-gp and ABCG2 to find a substrate with good affinity for ABCG2/Abcg2 and negligible affinity for P-gp. Only one compound, PF-407288, was classified as a specific ABCG2/Abcg2 substrate. Nonetheless, the brain accumulation was not considerably increased in single *Abcg2^{-/-}* or *Abcb1a/1b^{-/-}* mice and only about 2-fold higher in *Abcb1a/1b;Abcg2^{-/-}* mice.

Taken together, all previous studies in *Abcb1a/1b;Abcg2^{-/-}* mice and many other studies in single *Abcb1a/1b^{-/-}* and/or *Abcg2^{-/-}* mice suggested that P-gp, and not Abcg2, plays a dominant role at the BBB for shared P-gp and Abcg2 substrates. Moreover, in all reports on shared P-gp and ABCG2 substrates, an effect of Abcg2 deficiency on brain accumulation only becomes evident when P-gp is absent too (i.e., in *Abcb1a/1b;Abcg2^{-/-}* mice; reviewed in ref. 36). To the best of our knowledge, the present study is the first to show that the brain accumulation of a shared P-gp and ABCG2 substrate is not noticeably affected by single P-gp deficiency, whereas Abcg2 plays a leading role in restricting the entry into the brain. A possible explanation for this result could be that sorafenib is by comparison one of the weakest P-gp substrates of the shared substrates tested thus far. This can readily explain the data observed in Fig. 2B. For instance, one could assume that P-gp and Abcg2 are equivalent transporters

for sorafenib, but with Abcg2 being 8-fold more efficient than P-gp. If we take the brain accumulation of sorafenib in the *Abcb1a/1b;Abcg2* knockouts as the starting point (Fig. 2B; Table 1), when P-gp is added (*Abcg2* knockout), there is an ~ 2 -fold reduction in brain accumulation (or brain-plasma ratio). In contrast, when Abcg2 is added to the combination knockout (*Abcb1a/1b* knockout), an 8-fold more efficient efflux is obtained and an ~ 8 -fold reduction in brain accumulation is observed. When both transporters are added (the WT situation), this would be equivalent to adding 1/8th of the amount of Abcg2 present in the *Abcb1a/1b* knockout, which should result in an ~ 9 -fold reduction in brain accumulation compared with the *Abcb1a/1b;Abcg2* knockout. A difference between an 8-fold (*Abcb1a/1b* knockout) and a 9-fold (WT) reduction in brain penetration will easily be lost in the experimental background variation, considering the SDs in Table 1. Although this model is relatively simple, more preclinical research is warranted to provide further mechanistic insights.

Apart from the basic mechanistic aspects of the individual contribution of these efflux transports at the BBB, the complete inhibition of both transporters by elacridar may provide a rationale for combining sorafenib with a dual P-gp and ABCG2 inhibitor in cancer patients. This approach may result in increased brain accumulation and improved therapeutic efficacy in patients with central nervous system relapses. It is interesting to note that, at the dose used, we did not observe any sign of acute sorafenib toxicity in the WT or knockout mouse strains, either with or without elacridar treatment. However, much more extensive toxicity testing should precede any attempt to extend these findings to humans. Based on the interactions of many TKIs with P-gp and ABCG2, and strengthened by the recently reported data on the highly increased brain accumulation of TKIs in *Abcb1a/1b;Abcg2^{-/-}* mice (6, 13, 34), we expect that this concept might be applicable to many other TKIs as well. Our observation that the systemic exposure to sorafenib is not affected by the complete loss of Abcg2 and P-gp might indicate that a dual ABCG2/P-gp inhibitor would not interfere with the amount of drug circulating in the blood, thus limiting the need for dose adaptation. As many tumors are known to (over)express P-gp and/or ABCG2, which can lead to drug resistance, inhibition of P-gp and/or ABCG2 might also render these tumors more sensitive to sorafenib. However, as always, extrapolation of mouse results to humans should be done with caution because we cannot exclude that there may be relevant differences between human and mouse transport (er) properties in the BBB and elsewhere in the body. We also note that it remains to be determined whether higher drug concentrations in the brain (and hence possibly in the tumor) will ultimately lead to a more favorable response, as there are other factors apart from ABC transporters at the BBB that can render tumor cells resistant to anticancer drugs or otherwise limit therapeutic efficacy. This will provide a challenging area of future research.

⁴ <http://www.drugbank.ca>

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Evita van de Steeg and Dilek Iusuf for critical reading of the manuscript.

Grant Support

Dutch Cancer Society grants NKI-2003-2940 and 2007-3764 and Technical Sciences Foundation of the Netherlands Organization for Scientific Research (NWO/STW) grant BFA.6165.

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Received 2/27/09; revised 10/15/09; accepted 12/16/09; published OnlineFirst 1/26/10.

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Mol Cancer Ther 2010;9:319-326. Published OnlineFirst January 26, 2010.

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