Differential impact of P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2) on axitinib brain accumulation and oral plasma pharmacokinetics.


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Running title page

Running Title: Effects of ABCB1 and ABCG2 on axitinib pharmacokinetics

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Text pages:
Number of tables: 1
Number of figures: 3
Number of references: 34

Number of words in
Abstract: 249
Introduction: 624
Results and Discussion: 2023
Number of text pages: 11

Non-standard abbreviations: Blood-brain barrier (BBB), brain capillary endothelial cells (BCECs), tyrosine kinase inhibitor (TKI), ATP-binding cassette transporter (ABC transporter), renal cell carcinoma (RCC)
Abstract

The second-generation tyrosine kinase inhibitor and anticancer drug axitinib is a potent, orally active inhibitor of the vascular endothelial growth factor receptors 1, 2, and 3. Axitinib has clinical activity against solid tumors such as metastatic renal cell carcinoma and advanced pancreatic cancer. We studied axitinib transport using MDCKII cells overexpressing human ABCB1 or ABCG2 or murine Abcg2. Axitinib was a good substrate of ABCB1 and Abcg2, whereas transport activity by ABCG2 was moderate. These transporters may therefore contribute to axitinib resistance in tumor cells. Upon oral administration of axitinib, Abcg2−/− and Abcb1a/1b;Abcg2−/− mice displayed 1.7- and 1.8-fold increased axitinib plasma AUCs(0-4h) compared to wild-type mice. Plasma concentrations in Abcb1a/1b−/− mice were not significantly increased. In contrast, relative brain accumulation of axitinib in Abcb1a/1b−/− and Abcb1a/1b;Abcg2−/− mice was, respectively, 6.8- and 13.9-fold higher than in wild-type mice at 1 h and 4.9- and 20.7-fold at 4 h after axitinib administration. In Abcg2−/− mice we found no significant differences in brain accumulation compared to wild-type mice. Thus, Abcb1 strongly restricts axitinib brain accumulation, and completely compensates for the loss of Abcg2 at the blood-brain barrier, whereas Abcg2 can only very partially take over Abcb1-mediated axitinib efflux. Hence, Abcg2 has a stronger impact on axitinib oral pharmacokinetics, whereas Abcb1 is the more important transporter at the blood-brain barrier. These findings illustrate that in vitro transport data for ABCB1 and ABCG2 cannot always be simply extrapolated to the prediction of relative impact of these transporters on oral availability versus brain penetration.
Introduction

The ATP-binding cassette (ABC) transporters P-glycoprotein (P-gp/ABCB1) and breast cancer resistance protein (BCRP/ABCG2) affect the disposition of a variety of endogenous and exogenous compounds, including many anticancer drugs. Both transporters are expressed at the apical membranes of enterocytes, hepatocytes, and renal tubular epithelial cells, where they potentially limit gastrointestinal absorption or mediate direct intestinal, hepatic, or renal excretion of their substrates. Moreover, ABCB1- and ABCG2-mediated efflux activity in brain endothelial capillary cells (BCECs) of the blood-brain barrier (BBB) is crucial for the protection of the central nervous system from harmful compounds (Schinkel and Jonker, 2003; Vlaming et al., 2009). In addition, ABC transporters are expressed in many tumor types, mediating multidrug resistance against anticancer drugs (Borst and Oude Elferink, 2002).

Only recently, the combined role of ABCB1 and ABCG2 at the BBB in limiting brain accumulation of shared substrates was studied in detail using \textit{Abcb1a/1b;Abcg2/-} combination knockout mice. It was found that brain penetration of topotecan and several tyrosine kinase inhibitors (TKIs) including lapatinib, imatinib, dasatinib, sorafenib, gefitinib, and erlotinib was disproportionately increased in Abcb1a/1b;Abcg2/- knockout mice as compared to wild-type (WT) and single Abcb1a/1b/- and Abcg2/- knockout mice (de Vries et al., 2007; Polli et al., 2008; Lagas et al., 2009; Oostendorp et al., 2009; Agarwal et al., 2010; Kodaira et al., 2010; Lagas et al., 2010). These data suggested that the loss of either ABCB1 or ABCG2 at the BBB can often be largely compensated for by the complementary transporter, which is still present. For most of the above-mentioned drugs, brain penetration was mainly restricted by ABCB1, whereas only for sorafenib ABCG2 was the major factor limiting brain accumulation (Lagas et al., 2010).

Axitinib (AG013736) is a newly developed oral small molecule tyrosine kinase inhibitor. It selectively inhibits the vascular endothelial growth factor receptors (VEGFR)-1, -2 and -3 at picomolar levels and the platelet-derived growth factor receptor beta (PDGFR-β) at nanomolar levels (Hu-Lowe et al., 2008). In phase I/II studies axitinib showed efficacy against various tumor types, such as metastatic renal cell carcinoma (RCC) (Rixe et al., 2007; Rini et al., 2009), metastatic breast cancer (Rugo et al., 2005), thyroid cancer (Cohen et al., 2008), advanced non-small cell lung cancer (Schiller et al., 2009) and pancreatic cancer (Spano et al., 2008). Phases III studies testing the effect of axitinib in advanced...
pancreatic cancer and in metastatic RCC and pancreatic carcinoma are ongoing (ClinicalTrials.gov numbers NCT00920816, NCT00678392, NCT00471146).

Animal studies and early stage clinical trials have shown beneficial effects of VEGFR-targeting agents including the TKIs cediranib, sorafenib, sunitinib, and dasatinib for the treatment of malignant glioma (summarized in (Rahman et al., 2010)). A crucial characteristic for an anticancer drug in order to treat brain tumors or brain metastases is its ability to reach all the tumor cells, and therefore often its ability to cross the BBB. Many anticancer drugs are subject to ABCB1- and ABCG2-mediated efflux at the BBB, resulting in significantly reduced brain concentrations (Gottesman et al., 2002; Vlaming et al., 2009). Although therapeutic efficacy of axitinib against brain tumors has not yet been assessed, it is relevant to know the impact of ABC transporters on the axitinib brain accumulation with respect to possible future clinical applications.

To our knowledge no data are currently available regarding interactions of axitinib with ABCB1 and ABCG2. Therefore, the aim of this study was to investigate whether axitinib is a substrate of one or both of these transporters, and how this would impact on oral plasma pharmacokinetics and brain penetration of the drug. To assess the transport of axitinib in vitro we used M DCKII cells overexpressing human ABCB1 and ABCG2 as well as murine Abcg2. We next measured the axitinib plasma concentration profiles and brain accumulation in WT, Abcb1a/1b−/−, Abcg2−/− and Abcb1a/1b;Abcg2−/− mice upon oral administration.
Materials and methods

Chemicals

Axitinib and el acridar (GF120918) were purchased from Sequoia Research Products (Pangborne, UK). [\(^{14}\)C]Inulin (5.6 Ci/mol) was from Amersham Biosciences (Little Chalfont, UK). Zosuquidar (Eli Lilly, Indianapolis, IN, USA) was a kind gift of Dr. O. van Tellingen (The Netherlands Cancer Institute, Amsterdam, NL). All other chemicals were of analytical grade and obtained from Sigma-Aldrich (St. Louis, MO) unless mentioned otherwise.

Transport assays

For transepithelial transport assays we used the polarized Madin-Darby canine kidney cell line (MDCKII) and subclones transduced (using retroviral vectors) with human ABCB1, mouse Abcg2 (Bakos et al., 1998; Jonker et al., 2000) and a newly derived human ABCG2-overexpressing clone, recently generated by Poller et al. (submitted for publication). Transport assays were performed as previously described with minor modifications (Lagase et al., 2009). Two hours before starting the experiment cells were washed with PBS and preincubated with Opti-MEM (Invitrogen, Carlsbad, USA) alone or containing either elacridar (5 \(\mu\)M) or zosuquidar (5 \(\mu\)M). At \(t = 0\) h the medium in the donor compartment was replaced with Opti-MEM containing axitinib (1 \(\mu\)M) alone or in combination with an inhibitor. Aliquots of 100 \(\mu\)l were taken at 2 and 4 h. The percentage of axitinib appearing in the acceptor compartment relative to the total amount added to the donor compartment at the beginning of the experiment was calculated. All data are means (\(n = 3\)) ± standard deviation (SD). Transport ratios (\(r\)) were calculated by dividing apically directed by basolaterally directed axitinib translocation. Paracellular [\(^{14}\)C]inulin leakage (0.09 \(\mu\)Ci/well), which had to remain below 1% per hour, was measured in parallel in the same cells seeded and cultured in the same way to assure monolayer integrity. As axitinib is sensitive to light-induced isomerization (Sparidans et al., 2009) adequate precautions were taken throughout all experimental procedures to limit light exposure as much as possible (e.g. reduced lighting during sample handling, collection of samples in amber tubes).
Animals

All mice were housed and handled according to institutional guidelines complying with Dutch legislation. Animals used for this study were male WT, Abcb1a/1b\(^{-/-}\) (Schinkel et al., 1997), Abcg2\(^{-/-}\) (Jonker et al., 2002) and Abcb1a/1b;Abcg2\(^{-/-}\) (Jonker et al., 2005) knockout mice of a >99% FVB genetic background, between 8 and 12 weeks of age. Animals were kept in a temperature-controlled environment with a 12 hour light / 12 hour dark cycle and received a standard diet (AM-II, Hope Farms, Woerden, The Netherlands) and acidified water \textit{ad libitum}.

Plasma pharmacokinetics and brain accumulation of axitinib

Axitinib was dissolved in Tween/ethanol (1:1, v/v) (3.3 mg/l) and 3.3-fold diluted with NaCl 0.9% (w/v). Axitinib was administered orally at 10 mg/kg (10 ml/kg). To reduce variation of absorption mice (n = 4 - 5 per group) were fasted at least 3 h before axitinib was given by gavage into the stomach using a blunt-ended needle. Multiple blood samples (~30 µl) were collected from the tail vein either at 15, 30 and 60 min or at 15, 30, 60, 120 and 240 min using heparinized capillary tubes (Oxford labware, St. Louis, USA). At the last time points (60 or 240 min) mice were sacrificed by cardiac puncture under isoflurane anesthesia followed by cervical dislocation. Brains were rapidly removed and homogenized on ice in 1 ml of 4% (w/v) bovine serum albumin. Plasma was obtained by centrifugation of blood samples at 5200 \(x\) \(g\) for 6 min at 4°C. All procedures were performed under limited light exposure of axitinib-containing samples.

Axitinib analysis

Axitinib concentrations in Opti-MEM, plasma and brain homogenate samples were analyzed by a sensitive and specific liquid chromatography-tandem mass spectrometry assay as described (Sparidans et al., 2009). Axitinib-containing solutions and samples were protected from light throughout all experimental procedures.
**Calculation of AUC, relative brain accumulation and statistical analysis**

The area under the plasma concentration-time curve (AUC) was calculated using the trapezoidal rule, without extrapolating to infinity. One-way ANOVA was used for statistical analysis and data obtained from knockout mice were compared with data from WT mice. Axitinib brain concentrations were corrected by the amount of drug in the brain vasculature, corresponding to 1.4% of the plasma concentration at the last time point (Dai et al., 2003). Relative brain accumulation (P\text{brain}) was calculated by dividing brain concentrations at either t = 1 h or t = 4 h by the area under the plasma concentration-time curve from 0 to 1 h (AUC\text{0-1h}) or 0 to 4 h (AUC\text{0-4h}), respectively. For statistical analysis of brain accumulation data, the individual values were log-transformed to obtain normal distribution and one-way ANOVA was performed. Differences were considered statistically significant when p < 0.05. All data are given as means ± SD.
Results and Discussion

We first studied the interaction between axitinib and ABC transporters *in vitro* by measuring axitinib (1 μM) translocation through polarized monolayers of the MDCKII parental cell line and subclones overexpressing human ABCB1 or ABCG2, or mouse Abcg2. As shown in Fig. 1A, we observed moderate apically directed axitinib transport in the parental cell line (transport ratio r = 1.4), which was abrogated by treatment with the ABCB1/ABCG2 inhibitor elacridar and the relatively ABCB1-specific inhibitor zosuquidar (Fig. 1B). This suggests that this background transport was mediated by endogenous canine ABCB1 present in the MDCKII cells (Goh et al., 2002). In MDCKII cells transduced with human ABCB1, we observed active apically directed transport with an r of 5.3 (Fig. 1A), which was completely blocked by elacridar, indicating that axitinib is a good substrate of human ABCB1. In subsequent transport experiments using MDCKII cells overexpressing human or mouse ABCG2, zosuquidar was included to block the background transport mediated by endogenous canine ABCB1 (Fig. 1B). While axitinib was moderately transported by human ABCG2 (r = 1.3) we observed substantial apically directed translocation by the mouse Abcg2 (r = 3.4). Axitinib transport by human and mouse ABCG2 was efficiently blocked by elacridar. Axitinib was not significantly transported by human ABCC2 or mouse Abcc2 expressed in MDCKII cells (data not shown). To the best of our knowledge, this is the first report demonstrating active transport of axitinib by ABCB1 and ABCG2/Abcg2.

We subsequently studied the single and combined effects of Abcb1 and Abcg2 on axitinib plasma pharmacokinetics and brain accumulation using WT, *Abcb1a/1b−/−*, *Abcg2−/−*, and *Abcb1a/1b;Abcg2−/−* mice. As axitinib is given orally to patients, we administered axitinib orally at a dose of 10 mg/kg. In *Abcg2−/−* and *Abcb1a/1b;Abcg2−/−* mice we found 1.7- and 1.8-fold, statistically significant increases in AU C_{0-4h} compared with WT mice (Fig. 2, Table 1). In contrast, the AU C_{0-4h} in *Abcb1a/1b−/−* mice was not significantly increased (P = 0.12). Qualitatively similar results were obtained by measuring the axitinib plasma AUC_{0-1h} in an independent experiment, with 1.6- and 1.3-fold increased AUCs_{0-1h} in *Abcg2−/−* and *Abcb1a/1b;Abcg2−/−* mice, but no increase in *Abcb1a/1b−/−* mice compared to WT (Table 1). The virtually identical axitinib plasma concentration-time curves in *Abcg2−/−* and *Abcb1a/1b;Abcg2−/−* mice as shown in Fig. 2 also suggest a substantial impact of Abcg2 on axitinib plasma pharmacokinetics, whereas the effect of Abcb1 on axitinib plasma concentrations appears to be minor or negligible. This finding seems to...
be at odds with the observed efficient axitinib transport activity by ABCB1 \textit{in vitro}. However, it is a fairly common observation that many good \textit{in vitro} ABCB1 substrates show little or no alteration in oral AUC in Abcb1a/1b\textsuperscript{-/-} mice. For instance, no effect of either Abcb1 or Abcg2 on (oral) plasma concentrations in mice was observed for the shared ABCB1 and ABCG2 substrates sorafenib, gefitinib and lapatinib (Polli et al., 2008; Agarwal et al., 2010; Lagas et al., 2010). Possibly, the high intestinal luminal concentrations of these drugs obtained after oral administration lead to saturation of the intestinal efflux transporters. In contrast, the oral AUC of dasatinib in mice is somewhat reduced by Abcb1, although not by Abcg2 (Lagas et al., 2009). The increased axitinib plasma concentrations in \textit{Abcg2\textsuperscript{-/-}} and \textit{Abcb1a/1b;Abcg2\textsuperscript{-/-}} mice are most likely caused by increased drug absorption from the gastrointestinal tract and/or reduced hepatobiliary excretion when Abcg2 is absent. Although predictions from animal data to the situation in humans can be complicated, the impact of ABCG2 on plasma AUCs might be relevant for potential drug-drug interactions. As an absolute oral axitinib bioavailability of 58\% was reported in cancer patients (Pithavala et al., 2010), our results suggest a potentially increased risk for adverse drug reactions due to higher exposure, when axitinib is given concomitantly with an efficient ABCG2-inhibiting drug such as pantoprazol (Oostendorp et al., 2009).

We next studied the impact of Abcb1 and Abcg2 on axitinib brain accumulation in the different mouse strains. Oral administration of 10 mg/kg axitinib to \textit{Abcg2\textsuperscript{-/-}} mice did not result in significantly altered brain concentrations, either at 1 or at 4 h, as compared to WT animals (Fig. 3A, B; Table 1). In contrast, brain concentrations in \textit{Abcb1a/1b\textsuperscript{-/-}} mice showed statistically significant increases by 6.3- and 7.9-fold at 1 and 4 h, respectively, compared to WT mice. We further found markedly (and significantly) higher axitinib brain concentrations (20- and 42-fold at 1 and 4 h, respectively) in \textit{Abcb1a/1b;Abcg2\textsuperscript{-/-}} than in WT mice. Correcting the axitinib brain concentrations for the corresponding plasma AUCs also failed to reveal increased axitinib accumulation in brains of \textit{Abcg2\textsuperscript{-/-}} compared to WT mice, whereas brain accumulation in \textit{Abcb1a/1b\textsuperscript{-/-}} mice at 1 h was 6.8-fold higher than in WT mice (p < 0.001), and 4.9-fold at 4 h (Fig. 3C, D; Table 1). In \textit{Abcb1a/1b;Abcg2\textsuperscript{-/-}} mice we found highly significant 14- and 21-fold higher brain accumulation at 1 and 4 h, respectively (Fig. 3C, D; Table 1). In general, axitinib brain concentrations and brain accumulation were roughly 10- and 50-fold lower, respectively, at 4 h than at 1h. This pattern was consistently observed among all mouse strains. These data suggest that in all strains during the first hour
of exposure a more or less steady-state situation in axitinib brain-plasma ratios has been established that
is in part determined by Abcb1 and/or Abcg2 activity, and that does not dramatically change between 1
and 4 hours, as the plasma levels of axitinib drop. Thus, from 1 hour on, the disappearance of axitinib
from the brain was only modestly delayed compared to the disappearance from plasma. Brain-to-plasma
ratios, which were quite similar between 1 and 4 h, further supported this observation (Fig. 3E, F). Our
results demonstrate that Abcb1 strongly reduces axitinib brain accumulation, and can fully compensate
for the loss of Abcg2 at the mouse BBB, since axitinib brain levels in Abcg2−/− mice were virtually identical
to those in WT mice. On the other hand, Abcg2 can only very partially compensate for the loss of the
Abcb1-mediated efflux activity, in view of the increased axitinib brain accumulation in the absence of
Abcb1. The 2- to 4-fold further increase in brain accumulation in Abcb1a/1b;Abcg2−/− mice as compared to
Abcb1a/1b−/− mice demonstrates that Abcg2 can contribute to the BBB for axitinib, but this is only clearly
detectable in the absence of Abcb1.

Our brain accumulation data for axitinib are similar to published results in Abcb1 and Abcg2 knockout
mice for TKIs such as imatinib, dasatinib, lapatinib, gefitinib and erlotinib, all shared substrates of both
efflux transporters (Lagas et al., 2009; Oostendorp et al., 2009; Polli et al., 2009; Agarwal et al., 2010;
Kodaira et al., 2010). Abcb1 and Abcg2 limit the brain accumulation of these TKIs in concert, but with
Abcb1 providing the major contribution. In contrast, for sorafenib, which is only a very moderate ABCB1
substrate in vitro, Abcg2 was found to be the major determinant for limiting brain accumulation (Lagas et
al., 2010; Agarwal et al., 2011). The disproportionately high brain penetration of the indicated TKIs found
in Abcb1a/1b;Abcg2−/− mice compared to mice deficient for only one transporter raised the question of the
underlying mechanisms. Various explanations have been proposed, including adaptive increases in the
complementary transporter expression in the BBB of single Abcg2 or Abcb1a/1b knockout strains (Zhou
et al., 2009), or potential synergistic interactions between ABCB1 and ABCG2 (Polli et al., 2009). However, we
have found that Abcg2 RNA expression in brain and small intestine of FVB background Abcb1a/1b knockout
mice was not different from WT mice, and that Abcb1 RNA expression in brain and small intestine was also not altered in FVB background Abcg2−/− mice (Jonker et al., 2000; de Vries et al.,
2007; Lagas et al., 2009; Lagas et al., 2010). This makes adaptive changes in transporter expression in
the FVB knockout strains used here unlikely. In the course of this study, Kodaira and coworkers published
a straightforward kinetic analysis of brain penetration data of a wide selection of drugs (Kodaira et al., 2010). The authors concluded that the disproportionately increased drug concentrations in the brains of *Abcb1a/1b;Abcg2−/−* mice could be readily explained solely by the additive ABCB1- and ABCG2-mediated net efflux at the BBB, primarily because each in itself is considerably larger than the remaining clearance from the brain in the absence of these transporters. Hence, there is no need to postulate a synergistic interaction between ABCB1 and ABCG2 at the BBB in order to explain the observed brain accumulation data.

Several drugs targeting the VEGF receptors demonstrated promising results in early stage clinical trials or animal experiments (summarized in (Rahman et al., 2010)). However, after an initial response tumors often quickly became drug-resistant, potentially due to poor tumor cell exposure (Tredan et al., 2007). Preclinical co-administration of various TKIs such as dasatinib, gefitinib and sorafenib to WT mice with the dual ABC B1 and ABC G2 inhibitor elacridar has resulted in significantly enhanced brain accumulation, to levels similar to those observed in *Abcb1a/1b;Abcg2−/−* mice (Lagas et al., 2009; Agarwal et al., 2010; Lagas et al., 2010). Furthermore, using the ABCB1- and ABC G2-inhibiting drug gefitinib, enhanced topotecan tumor penetration was demonstrated in mice bearing orthotopic human gliomas (Carcaboso et al., 2010). Clinical activity of axitinib was observed in a phase I I study in patients with cytokine-refractory metastatic RCC with a response rate of 44.2% (Rixe et al., 2007). However, in 52% of the initial responders the disease progressed due to unknown reasons. Independent studies, analyzing ABCB1 expression in tumors of RCC patients, revealed expression of ABCB1 at different levels in 100% of the analyzed tumors (Mignogna et al., 2006; Walsh et al., 2009). In addition, ABCB1 was suggested as a prognostic marker for RCC since an association between high ABCB1 expression and poor survival was described (Mignogna et al., 2006). As the intracellular ATP-binding site of the VEGF receptor is the molecular target for axitinib (Hu-Lowe et al., 2008), high ABC B1 levels in tumor cells might prevent axitinib from crossing the plasma membrane, resulting in insufficient intracellular concentrations and thus resistance to axitinib. Concomitant administration of axitinib with inhibitors of ABCB1 and/or ABCG2 might thus be a promising approach to enhance axitinib brain and brain tumor cell accumulation or to overcome ABCB1-mediated drug resistance in RCC therapy.
Interestingly, our experiments revealed a major role of Abcb1 in limiting axitinib brain accumulation compared to Abcg2, whereas in contrast only Abcg2, and not Abcb1, had a significant (albeit modest) impact on oral axitinib plasma concentrations. A possible explanation for this discrepancy might be different relative expression levels of Abcb1 and Abcg2 in intestinal enterocytes and BCECs at the BBB. Indeed, several studies suggest higher expression of Abcb1 than Abcg2 in the mouse BBB (discussed in Zhou et al., 2009), which might explain why brain penetration data of drugs which are not extremely good Abcg2 substrates (dasatinib, lapatinib, axitinib), generally show a major role of Abcb1 at the BBB. Regarding (oral) plasma AUCs, effects of ABC transporters are generally small compared to the effects observed at the BBB. This might be caused by higher background permeability for drugs at the intestinal wall, most likely also involving protein-mediated uptake processes, which could considerably reduce or even completely offset the impact of ABC transporter-mediated efflux. This may be the case for TKIs such as sorafenib, gefitinib and lapatinib, where neither Abcb1 nor Abcg2 have an impact on plasma kinetics. In contrast, dasatinib oral plasma AUCs are increased in Abcb1-deficient mice, while topotecan and axitinib oral AUCs are mainly influenced by Abcg2. As the relative efficiency of uptake processes may well differ between individual TKIs, and between intestine and BBB, this could also contribute to differential impacts of Abcb1 and Abcg2 in intestine and BBB. Differential saturation conditions (intestinal drug concentrations are generally much higher than systemic concentrations upon oral drug administration) for both drug transporters with a specific drug might further play an important role. Our results illustrate that in vitro transport data for ABCB1 and ABCG2 cannot yet be simply extrapolated to the prediction of relative impact of these transporters on both oral availability and brain penetration, with especially oral availability being complicated. This underscores that we are still far from fully understanding all factors determining penetration of drugs across the important biological and pharmacological barriers involved.
Acknowledgements

We gratefully acknowledge the technical assistance of Anita van Esch and Ahmed Elbatsh as well as the statistical advice of Dr. Marta Lopez Yurda.
Authorship contributions

*Participated in research design:* Poller, Iusuf, Beijnen and Schinkel

*Conducted experiments:* Poller, Iusuf, Sparidans and Wagenaar

*Performed data analysis:* Poller, Iusuf, Sparidans and Schinkel

*Wrote or contributed to the writing of the manuscript:* Poller, Iusuf, Sparidans, Beijnen and Schinkel
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Footnotes

* These authors (B.P., D.I.) contributed equally to this work

Financial support: This work was supported by the Swiss National Science Foundation [Grant PBBS3-128567]; and the Dutch Cancer Society grant [Grant NKI 2007-3764].
Legends for Figures

**Fig. 1.** Transepithelial transport of 1 μM axitinib through monolayers of MDCKII parental cells and human ABCB1- (A) or human ABCG2- or mouse Abcg2- (B) transduced sublines. Transport was measured in the absence of an inhibitor or in the presence of 5 μM elacridar or 5 μM zosuquidar. Data for parental cells in presence of elacridar are identical in (A) and (B). Open circles represent translocation from the apical to the basolateral compartment, filled circles translocation from the basolateral to the apical compartment. Results are expressed as mean values (n = 3) of relative transport (%) ± SD. The transport ratio (r) was calculated as the quotient of apically directed and basolaterally directed transport at 4 h. C: Molecular structure of axitinib.

**Fig. 2.** Plasma concentration-time curves of axitinib in male WT (closed circles), Abcg2−/− (open diamonds), Abcb1a/1b−/− (closed triangles) and Abcb1a/1b;Abcg2−/− (open squares) mice after oral administration of 10 mg/kg axitinib. Data are given as means ± SD (n = 5).

**Fig. 3.** Brain concentrations (A, B), relative brain accumulation (C, D) and brain-to-plasma ratios (E, F) of axitinib in male WT, Abcg2−/−, Abcb1a/1b−/− and Abcb1a/1b;Abcg2−/− mice after oral administration of 10 mg/kg axitinib at t = 1 h and t = 4 h. Relative brain accumulation was calculated by dividing brain concentrations by the axitinib plasma AUC0-1h and AUC0-4h, respectively. Data are means ± SD (n = 5, except n = 4 for Abcg2−/−, Abcb1a/1b;Abcg2−/− at t = 1 h). *: p < 0.05, **: p < 0.01 compared with WT mice; †: p < 0.05, ††: p < 0.01 compared with Abcg2−/− mice; ‡: p < 0.05, ‡‡: p < 0.01 compared with Abcb1a/1b−/− mice, using log-transformed data to normalize the SDs between study groups.
Table 1. Plasma AUC, brain concentrations and relative brain accumulation of axitinib in mice at 1 h and 4 h after oral administration at 10 mg/kg.

<table>
<thead>
<tr>
<th>Strain</th>
<th>WT</th>
<th>Abcg2&lt;sup&gt;−/−&lt;/sup&gt;</th>
<th>Abcb1a/1b&lt;sup&gt;−/−&lt;/sup&gt;</th>
<th>Abcb1a/1b;Abcg2&lt;sup&gt;−/−&lt;/sup&gt;</th>
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<tr>
<td>AUC&lt;sub&gt;(0-1h)&lt;/sub&gt; (μg / ml * h)</td>
<td>1.09 ± 0.58</td>
<td>1.76 ± 0.58&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.95 ± 0.35</td>
<td>1.40 ± 0.53</td>
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<tr>
<td>c&lt;sub&gt;brain&lt;/sub&gt; (μg / g)</td>
<td>0.10 ± 0.07</td>
<td>0.08 ± 0.03</td>
<td>0.64 ± 0.34&lt;sup&gt;***&lt;/sup&gt;/††† 1.</td>
<td>98 ± 1.26&lt;sup&gt;***&lt;/sup&gt;/†††/‡</td>
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<td>P&lt;sub&gt;brain&lt;/sub&gt; (* 10&lt;sup&gt;−3&lt;/sup&gt; * h&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>94.8 ± 27.0</td>
<td>47.7 ± 12.7&lt;sup&gt;*&lt;/sup&gt;</td>
<td>643.6 ± 183.2&lt;sup&gt;***&lt;/sup&gt;/†††</td>
<td>1315.2 ± 374.9&lt;sup&gt;***&lt;/sup&gt;/†††/‡‡</td>
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<td>Fold increase</td>
<td>1.0 0.</td>
<td>8.6.</td>
<td>3</td>
<td>19.6</td>
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<tr>
<td>t = 4 h</td>
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<tr>
<td>AUC&lt;sub&gt;(0-4h)&lt;/sub&gt; (μg / ml * h)</td>
<td>2.68 ± 1.36</td>
<td>4.67 ± 1.83&lt;sup&gt;*&lt;/sup&gt;</td>
<td>3.95 ± 1.81</td>
<td>4.95 ± 1.96&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>c&lt;sub&gt;brain&lt;/sub&gt; (μg / g)</td>
<td>0.006 ± 0.004</td>
<td>0.007 ± 0.003</td>
<td>0.047 ± 0.042&lt;sup&gt;††&lt;/sup&gt;0.</td>
<td>25 ± 0.20&lt;sup&gt;***&lt;/sup&gt;/†††/‡</td>
</tr>
<tr>
<td>P&lt;sub&gt;brain&lt;/sub&gt; (* 10&lt;sup&gt;−3&lt;/sup&gt; * h&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>2.3 ± 1.0</td>
<td>1.5 ± 0.7</td>
<td>11.2 ± 10.5&lt;sup&gt;‡†&lt;/sup&gt;4</td>
<td>7.6 ± 29.6&lt;sup&gt;***&lt;/sup&gt;/†††/‡‡</td>
</tr>
<tr>
<td>Fold increase</td>
<td>1.0 0.</td>
<td>7.4.</td>
<td>9</td>
<td>20.7</td>
</tr>
</tbody>
</table>

Data are means (n = 4 - 5) ± SD. One-way ANOVA analysis was performed for all AUCs and log-transformed brain penetration data obtained for WT and knockout mice. Abbreviations: AUC<sub>(0-1h)</sub>, AUC<sub>(0-4h)</sub>, area under plasma concentration-time curve up to 1 and 4 h, respectively; c<sub>brain</sub>, brain concentration; P<sub>brain</sub>, relative brain accumulation; *: p < 0.05, **: p < 0.001 compared with WT mice; †: p < 0.05, ††: p < 0.01, †††: p < 0.001 compared with Abcg2<sup>−/−</sup> mice; ‡: p < 0.05, ‡‡: p < 0.01 compared with Abcb1a/1b<sup>−/−</sup> mice. Part of these data is also presented in Figures 2 and 3.
Figure 1
Figure 2

This figure shows the plasma concentration (μg/ml) over time (h) for different genotypes: WT, Abcg2−/−, Abcb1a/1b−/−, and Abcb1a/1b−/−; Abcg2−/−. The graph illustrates the peak concentration at different time points and the subsequent decline.
Figure 3