P-glycoprotein (ABCB1) and Breast Cancer Resistance Protein (ABCG2) restrict brain accumulation of the active sunitinib metabolite N-desethyl sunitinib

Seng Chuan Tang, Nienke A.G. Lankheet, Birk Poller, Els Wagenaar, Jos H. Beijnen, and Alfred H. Schinkel

Authors’ Affiliations:
S.C.T., B.P., E.W., A.H.S.: Division of Molecular Biology, The Netherlands Cancer Institute, Amsterdam, The Netherlands;
N.A.G.L., J.H.B.: Department of Pharmacy and Pharmacology, Slotervaart Hospital, Amsterdam, The Netherlands.

Note: Supplementary data for this article are available
Running title

ABCB1 and ABCG2 limit N-desethyl sunitinib brain disposition

Address correspondence to: Alfred H. Schinkel, Division of Molecular Biology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands. Phone: +31-20-5122046; Fax: +31-20-6691383; Email: a.schinkel@nki.nl

ABBREVIATIONS: ABC, ATP-binding cassette; ANOVA, analysis of variance; AUC, area under the plasma concentration-time curve; BBB, blood-brain barrier; BCRP, breast cancer resistance protein; C\text{brain}, brain concentration; C\text{max}, maximum drug concentration in plasma; DMSO, dimethyl sulfoxide; LC-MS/MS, liquid chromatography coupled with tandem mass spectrometry; LLQ, lower limit of quantification; MDCKII, Madin-Darby canine kidney II; MDR2, multidrug resistance protein 2; Mdr1a/1b, genes encoding the murine P-glycoprotein; P\text{brain}, relative brain accumulation; P-gp, P-glycoprotein; T\text{max}, time to reach maximum drug concentration in plasma; TKI, tyrosine kinase inhibitor.
ABSTRACT (223/250):

N-desethyl sunitinib is a major and pharmacologically active metabolite of the tyrosine kinase inhibitor and anticancer drug sunitinib. Since the combination of N-desethyl sunitinib and sunitinib represents total active drug exposure, we investigated the impact of several multidrug efflux transporters on plasma pharmacokinetics and brain accumulation of N-desethyl sunitinib, following sunitinib administration to wild-type and transporter knockout mice. In vitro, N-desethyl sunitinib was a good transport substrate of human ABCB1 and ABCG2 and murine Abcg2, but not of ABCC2 or Abcc2. At 5 µM, ABCB1 and ABCG2 contributed almost equally to N-desethyl sunitinib transport. In vivo, the systemic exposure of N-desethyl sunitinib after oral dosing of sunitinib malate (10 mg/kg) was unchanged when Abcb1 and/or Abcg2 were absent. However, brain accumulation of N-desethyl sunitinib was markedly (13.7-fold) increased in Abcb1a/1b(-/-)/Abcg2(-/-) mice, but not in Abcb1a/1b(-/-) or Abcg2(-/-) mice. In the absence of the ABCB1 and ABCG2 inhibitor elacridar, brain concentrations of N-desethyl sunitinib were only detectable in Abcb1a/1b(-/-)/Abcg2(-/-) mice following sunitinib administration. Combined elacridar plus N-desethyl sunitinib treatment increased N-desethyl sunitinib plasma and brain exposures, but not brain-to-plasma ratios in wild-type mice. In conclusion, brain accumulation of N-desethyl sunitinib is effectively restricted by both Abcb1 and Abcg2. The effect of elacridar treatment in improving brain accumulation of N-desethyl sunitinib in wild-type mice was limited as compared with its effect on sunitinib brain accumulation.
Introduction

ATP-binding cassette (ABC) efflux transporters, such as P-glycoprotein (P-gp; ABCB1), breast cancer resistance protein (BCRP; ABCG2) and multidrug resistance protein 2 (MRP2; ABCC2) can have a significant impact on the absorption, distribution, excretion, and toxicity of xenobiotics (Glavinas et al., 2004). Several groups have shown that many tyrosine kinase inhibitors (TKIs) used in cancer therapy are substrates of both ABCB1 and ABCG2 (Burger et al., 2004; Dai et al., 2003; Bihorel et al., 2007; Chen et al., 2009; Lagas et al., 2009; Oostendorp et al., 2009; Lagas et al., 2010), and found that the interaction with these ABC transporters may affect oral availability and brain accumulation of TKIs.

The TKI sunitinib malate (SU11248; SUTENT) is an orally active, small-molecule ATP-competitive multi-targeted inhibitor of vascular endothelial growth factor receptors type 1 and 2, the platelet-derived growth factor receptors α and β, the stem cell factor receptor c-KIT, FMS-like TK-3 receptor, and the glial cell-line derived neurotrophic factor receptor (Chow and Eckhardt, 2007). Sunitinib is approved by the US Food and Drug Administration for the treatment of advanced or metastatic renal cell carcinoma (RCC) and imatinib-resistant gastrointestinal stromal tumors. Sunitinib displays an intrinsically high brain penetration among TKIs, and it is currently being tested in a phase II clinical trial of recurrent glioblastoma multiforme (http://clinicaltrials.gov/ct2/show/NCT00535379).

Following administration, sunitinib is primarily metabolized by cytochrome P450 3A4 to a major and pharmacologically active metabolite, N-desethyl sunitinib (supplemental Fig.1) (Houk et al., 2009). This is further metabolized by cytochrome P450 3A4 to an inactive compound (Adams and Leggas, 2007). In patients, plasma sunitinib and N-desethyl sunitinib accounted for 42% and 24%, respectively, of the total plasma radioactivity area under the plasma concentration-time curve (AUC)_{0-\infty} (Adams and Leggas, 2007). Given that N-desethyl sunitinib has a similar kinase inhibitory effect as sunitinib in vitro and similar plasma protein binding characteristics, the combination of sunitinib plus N-desethyl sunitinib represents the total pharmacodynamically active drug in plasma. N-desethyl sunitinib may thus well account for one
third of the therapeutic effect of oral sunitinib. Previously, we have shown that sunitinib is transported in vitro by human ABCB1, ABCG2, and by murine Abcg2, but not by human ABCC2 or murine Abcc2 (Tang et al., 2011). Simultaneous deficiency of Abcb1a/1b and Abcg2, but not single Abcb1a/1b or Abcg2 deficiency, resulted in highly increased brain levels of sunitinib in knockout mouse strains. We also demonstrated in wild-type mice that a clinically realistic oral coadministration of sunitinib and the dual ABCB1 and ABCG2 inhibitor elacridar could result in highly increased brain sunitinib levels (Tang et al., 2011). However, little is known so far about the interactions of \( N \)-desethyl sunitinib with ABC transporters in vitro, or in vivo after sunitinib treatment.

In view of their expression in pharmacokinetically important organs and broad substrate specificity, we wanted to establish to what extent \( N \)-desethyl sunitinib is transported by human ABCB1, ABCG2 and ABCC2 and by murine Abcg2 and Abcc2 in vitro, and what the consequences are for systemic availability and brain accumulation of \( N \)-desethyl sunitinib after oral administration of sunitinib as judged in knockout mouse models. We further tested whether these pharmacokinetic parameters of \( N \)-desethyl sunitinib could be improved by a clinically realistic coadministration of oral elacridar and oral sunitinib, with the ultimate aim of improving overall therapeutic efficacy of sunitinib and its metabolite. We also aimed to obtain better insight into the factors that determine the relative impact of Abcb1 and Abcg2 on the brain accumulation of \( N \)-desethyl sunitinib following sunitinib intravenous administration. This may help efforts to overcome the blood-brain barrier (BBB) for therapeutic purposes.
Materials and Methods

Chemicals and reagents

Sunitinib malate was purchased from Sequoia Research Products (Pangbourne, UK). N-desethyl sunitinib was purchased from Toronto Research Chemicals Inc. (North York, Ontario, Canada). Elacridar [GF120918; 1H-[4-[2-(1,2,3,4-Tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]-phenyl]-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide, hydrochloride] (Evers et al., 2000) was kindly provided by GlaxoSmithKline (Stevenage, UK). Zosuquidar [LY-335979; (R)-4-((1aR,6R,10bS)-1,2-difluoro-1,1a,6,10b-tetrahydrodibenzo-(a,e)cyclopropa(c)cycloheptan-6-yl)-α-((5-quinoloyloxy)methyl)-1-piperazine ethanol, trihydrochloride] (Eli Lilly, Indianapolis, IN, USA) was a kind gift of Dr. O. van Tellingen (The Netherlands Cancer Institute, Amsterdam, The Netherlands). Ko143 [(3S,6S,12aS)-1,2,3,4,6,7,12,12a-octahydro-9-methoxy-6-(2-methylpropyl)-1,4-dioxopyrazino[1',2':1,6]pyrido[3,4-b]indole-3-propanoic acid 1,1-dimethylethyl ester] was previously described (Allen et al., 2002). [14C]Inulin was obtained from Amersham (Little Chalfont, UK). Methoxyflurane (Metofane®) was supplied by Medical Developments Australia (Melbourne, Australia). Isoflurane (Forane®) was obtained from Abbott Laboratories Ltd (Queenborough, UK). Heparin (5000 IU/ml) was obtained from Leo Pharma BV (Breda, The Netherlands). Bovine serum albumin, fraction V was obtained from Roche (Mannheim, Germany). High-performance liquid chromatography grade acetonitrile and methanol were purchased from Biosolve (Valkenswaard, The Netherlands). Ammonia 25% was purchased from Merck (Darmstadt, Germany). The stable isotope-labeled sunitinib was purchased from AlsaChim (Illkirch, France). All other chemicals and reagents were obtained from Sigma-Aldrich (Steinheim, Germany).

Cell lines and transport assays

Polarized Mardin Darby canine kidney II (MDCKII) cells and its subclones transduced with human ABCB1, ABCC2, ABCG2, murine Abcc2 or Abcg2 cDNA were used and cultured as described previously (Bakos et al., 2000; Evers et al., 1998; Pavek et al., 2005; Zimmermann et al., 2008; Jonker et al., 2000). Recently, Poller et al. (2011) generated a MDCKII cell line simultaneously...
overexpressing both ABCB1 and ABCG2 to better study the interplay of both transporters in vitro. Transepithelial transport assays were performed as described previously with minor modifications (Pavek et al., 2005). Two hours before starting the experiment, cells were washed with prewarmed PBS and preincubated with 2 ml of Opti-MEM either alone or containing the dual ABCB1 and ABCG2 inhibitor elacridar (5 µM), the ABCB1 inhibitor zosuquidar (5 µM), the ABCG2 inhibitor Ko143 (1 µM) or a combination of zosuquidar and Ko143, which were present in both compartments during a 2-h preincubation period and during the transport experiment. Experiments were started (t = 0 h) by replacing the medium in one compartment (either basolateral or apical) with fresh Opti-MEM medium, either with or without inhibitor and containing 5 µM N-desethyl sunitinib. Cells were incubated at 37°C in 5% CO₂, and 50 µl aliquots were taken at t = 2 h and 4 h for determination of drug concentration. Transport was calculated as the fraction of drug found in the acceptor compartment relative to the total amount added to the donor compartment at the beginning of the experiment. Transport is given as mean percentage ± 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 (3.3 kBq/well) leakage. Leakage had to remain <1% of the total added radioactivity per hour. Active transport was expressed by the relative transport ratio (r), defined as r = percentage apically directed transport divided by basolaterally directed translocation after 4 h. Due to some inter-day variation in transport ratios, we only directly compared transport ratios determined on the same day.

**Animals**

Mice were housed and handled according to institutional guidelines complying with Dutch legislation. Male wild-type, Abcb1a/1b(-/-) (Schinkel et al., 1997), Abcg2(-/-) (Jonker et al., 2002), and Abcb1a/1b(-/-)/Abcg2(-/-) mice (Jonker et al., 2005) all of a >99% FVB genetic background, were used between 10 and 14 weeks of age. Animals were kept in a temperature-controlled environment with a 12-h light/12-h dark cycle and they received a standard diet (AM-II, Hope Farms) and acidified water ad libitum.
Drug solutions

Drug solutions for sunitinib and oral elacridar were prepared as previously described (Tang et al., 2011). For intravenous administration, elacridar hydrochloride was first dissolved in DMSO at 150 mg/ml and further diluted with a mixture of ethanol, polyethylene glycol 200, and 5% glucose (2:6:2, v/v) to obtain a concentration of 4 mg/ml. *N*-desethyl sunitinib was dissolved in DMSO at a concentration of 50 mg/ml and further diluted with 50 mM sodium acetate buffer (pH 4.6) to yield a concentration of 1 mg/ml. In all used drug formulations drugs and/or modulators were completely dissolved, also during administration to the mice.

Animal experiments

All the animal experiments in which sunitinib was given either orally or intravenously were carried out as previously described (Tang et al., 2011). After oral sunitinib administration, multiple blood samples (~50 µl) were collected from the tail vein at 15 and 30 min, and 1, 2 and 4 hr. At 6 hr, blood was collected by cardiac puncture under isoflurane anesthesia. This allowed determination of plasma concentration-time curves for each individual mouse.

Brain accumulation of *N*-desethyl sunitinib in combination with intravenous elacridar treatment

Values of lower limit of quantification (LLQ) for *N*-desethyl sunitinib were 7.5 ng/g for the oral sunitinib in combination with oral elacridar brain accumulation experiment and 13.5 ng/g for the BBB efflux transport saturation experiment. This difference was due to an improvement in the detection method to enhance sensitivity during the course of our studies. To circumvent detection problems of *N*-desethyl sunitinib in brain homogenates, we injected *N*-desethyl sunitinib (5 mg/kg) directly into the tail vein of wild-type and knockout mice, 15 min after an intravenous injection of either elacridar or vehicle in solution form. Blood and brain were isolated 60 min after *N*-desethyl sunitinib administration and processed as previously described (Tang et al., 2011).

Drug analysis
Determination of N-desethyl sunitinib and the internal standard, sunitinib-$^2$H$_{10}$ was performed on a sensitive and specific liquid chromatography coupled with tandem mass spectrometry assay. The analytical method that was previously described (Lankheet et al., 2011; Tang et al., 2011) was used for the detection of sunitinib and N-desethyl sunitinib in plasma and brain homogenates. N-desethyl sunitinib was detected at the transition from m/z 371 to 283 with a retention time of 3.8 min. In case the (usually minor) E-isomer of N-desethyl sunitinib was detected in addition to the main Z-isomer, we report the combined concentration of both isomers.

**Pharmacokinetic calculations and statistical analysis**

Pharmacokinetic parameters were calculated by noncompartmental methods using the software package PK Solutions 2.0.2 (Summit, Research Services, Ashland, OH, USA). The AUC was calculated using the trapezoidal rule, without extrapolating to infinity. The AUC and maximum drug concentration in plasma (C$_{\text{max}}$) were determined directly from individual concentration-time data. Data are presented as means ± SD. For parametric statistical analysis, the individual values of Fig. 6C-F, Fig. 7A and D were log-transformed to obtain equality in variances. One-way analysis of variance (ANOVA) was used to determine significance between groups, after which post-hoc tests with Bonferroni correction were performed for comparison between individual groups. Between-group comparisons of genotype or elacridar effect were made using the two-tailed unpaired Student’s $t$-test. Differences were considered statistically significant when $p < 0.05$. 
Results

**In vitro transport of N-desethyl sunitinib**

Transepithelial drug transport was tested using polarized monolayers of MDCKII parental cells and various ABC transporter-overexpressing derivative cell lines. *N*-desethyl sunitinib was modestly transported in the apical direction in the parental MDCKII cell line (transport ratio *r* of 2.1, Fig. 1A), presumably by the low-level endogenous canine ABCB1. In cells overexpressing human ABCB1, there was clear apically directed transport of *N*-desethyl sunitinib, with an *r* of 15.1 (Fig. 1B). *N*-desethyl sunitinib was also actively transported by human ABCG2 or murine Abcg2, with transport ratios of 6.0 and 6.6, respectively (Fig. 1C and D). Addition of elacridar, a dual inhibitor of ABCB1 and ABCG2, completely inhibited polarized transport in MDCKII parental and MDCKII-ABCB1 cells (Fig. 1E and F). However, elacridar at 5 µM did not completely inhibit the apical transport of *N*-desethyl sunitinib in MDCKII-ABCG2 cells (*r* reduced from 6.0 to 1.8) (Fig. 1G), and had only a minimal effect in MDCKII cells overexpressing mouse Abcg2 (Fig. 1H). 5 µM elacridar does not modulate ABCC2/Abcc2 activity (Evers et al., 2000), and was therefore used to suppress any transport by endogenous canine ABCB1 in cells overexpressing human ABCC2 or murine Abcc2. Under these conditions, we did not observe polarized transport of *N*-desethyl sunitinib by either ABCC2 or Abcc2 (Fig 1I and J).

As we did not observe complete inhibition of ABCG2- and Abcg2-mediated *N*-desethyl sunitinib transport by elacridar, we investigated whether transport of *N*-desethyl sunitinib in these cell lines could be completely inhibited with the specific and high-affinity ABCG2 inhibitor Ko143. The specific ABCB1 inhibitor zosuquidar was added to suppress any contribution of endogenous canine ABCB1 (Fig. 2A). *N*-desethyl sunitinib was actively transported in cells overexpressing human ABCG2 and murine Abcg2, with transport ratios of 13.5 and 20.3 vs 1.0 in parental cells (Fig. 2B and C). Addition of Ko143 resulted in extensive inhibition of polarized transport in all these cell lines (Fig. 2D and E). Collectively, *N*-desethyl sunitinib was a good transport substrate of human ABCB1, ABCG2, and murine Abcg2, but not of human ABCC2 and murine Abcc2. 5 µM elacridar could only partially inhibit *N*-desethyl sunitinib transport by human ABCG2 and
especially mouse Abcg2, a rather unusual observation, as in our experience in vitro transport of most Abcg2 substrates can be completely inhibited by 5 µM elacridar.

**N-desethyl sunitinib transport in MDCKII-ABCB1/ABCG2 cells**

We tested the relative contributions of human ABCB1 and ABCG2 to N-desethyl sunitinib transport at 5 and 20 µM in the absence or presence of Ko143 and zosuquidar in MDCKII cells simultaneously overexpressing ABCB1 and ABCG2 (Poller et al., 2011). Without inhibitor, we measured transport ratios of 13.3 and 16.5 at 5 µM and 20 µM, respectively (supplemental Fig. 2A and B). The ABCG2-mediated N-desethyl sunitinib transport in the presence of zosuquidar was reduced from an r of 9.7 at 5 µM to 5.8 at 20 µM (supplemental Fig. 2C and D). Upon blocking ABCG2 with Ko143, we observed ABCB1-mediated N-desethyl sunitinib transport ratios of 6.7 and 6.0 at 5 µM and 20 µM, respectively (supplemental Fig. 2E and F). Active transport of N-desethyl sunitinib at both 5 and 20 µM was completely abolished when both Ko143 and zosuquidar were present (supplemental Fig. 3G and H). Taken together, the contribution of ABCG2 to N-desethyl sunitinib transport is almost equal to that of ABCB1 at 5 µM and 20 µM, although some initial ABCG2 saturation may occur at 20 µM.

**Impact of Abcb1 and Abcg2 on plasma pharmacokinetics of N-desethyl sunitinib following oral sunitinib treatment**

Since the parent compound sunitinib is given orally to cancer patients, we first studied the plasma concentration of N-desethyl sunitinib over time following oral sunitinib malate administration at 10 mg/kg to wild-type, *Abcb1a/1b(-/-), Abcg2(-/-),* and *Abcb1a/1b(-/-)/Abcg2(-/-)* mice. As shown in Fig. 3A and Table 1, there were no statistically significant differences in oral AUC or C<sub>max</sub> of N-desethyl sunitinib between the strains. The metabolite/parent drug AUC ratios ranged between 33% and 40% (Table 1), and also did not differ significantly between the strains. These ratios are similar to those observed in humans (Shirao et al., 2010). These results indicate that Abcb1 and Abcg2 do not have a substantial role in, or effect on, the availability, metabolism or elimination of N-desethyl sunitinib after oral sunitinib administration.
Impact of Abcb1 and Abcg2 on brain accumulation of N-desethyl sunitinib following oral sunitinib administration

As shown in Fig. 3B, the relative brain accumulation of N-desethyl sunitinib, determined 6 h after oral administration of sunitinib and corrected for the plasma AUC₀⁻₆h, was not significantly different in Abcb1a/1b(-/-) and Abcg2(-/-) mice as compared with wild-type mice. In contrast, Abcb1a/1b(-/-)/Abcg2(-/-) mice had a 13.7–fold increased brain accumulation (p < 0.05; see also Table 1). This indicates that brain accumulation of N-desethyl sunitinib was primarily restricted by both Abcb1 and Abcg2. Apparently, each of these transporters can largely take over the function of the other transporter at the BBB when knocked out. Only when both transporters are simultaneously absent, a large increase in brain accumulation of N-desethyl sunitinib can occur.

Influence of elacridar on N-desethyl sunitinib brain accumulation following oral sunitinib administration

We wanted to assess the effect of elacridar on plasma pharmacokinetics and brain accumulation of N-desethyl sunitinib following oral sunitinib administration, given that elacridar markedly increased the brain accumulation of sunitinib, although not its oral bioavailability (Tang et al., 2011). In view of the potential clinical importance of oral application for both sunitinib and elacridar, we administered elacridar (100 mg/kg) orally 2 h prior to oral sunitinib malate (10 mg/kg) to the wild-type and Abcb1a/1b(-/-)/Abcg2(-/-) strains, and assessed plasma and brain N-desethyl sunitinib levels 1 h later, i.e., around the sunitinib Tₘₐₓ (supplemental Table 1). As shown in Fig. 4A, N-desethyl sunitinib plasma concentrations were not significantly different among the strains, regardless of administration with or without elacridar. The plasma metabolite-to-sunitinib ratios were not significantly different either (supplemental Fig. 3). In vehicle-treated mice, brain concentrations of N-desethyl sunitinib in wild-type, Abcb1a/1b(-/-) and Abcg2(-/-) mice were below the LLQ (~7.5 ng/g). However, brain concentrations of N-desethyl sunitinib in Abcb1a/1b(-/-)/Abcg2(-/-) mice were just above this limit. Elacridar treatment increased brain concentrations in wild-type mice to levels equal to those in Abcb1a/1b(-/-)/Abcg2(-/-) mice (Fig. 4B). The N-desethyl
sunitinib brain concentrations in Abcb1a/1b(-/-)/Abcg2(-/-) mice were not significantly affected by elacridar treatment. These data suggest that oral elacridar treatment could inhibit the activity of Abcb1 and Abcg2 in the BBB, leading to increased N-desethyl sunitinib concentrations in the brain. However, the size of this effect could not be assessed in the absence of quantifiable brain values for vehicle-treated wild-type mice.

Influence of elacridar on plasma and brain exposure of intravenous N-desethyl sunitinib
To circumvent these quantification problems, we injected N-desethyl sunitinib directly at an intravenous dose of 5 mg/kg to wild-type and Abcb1a/1b(-/-)/Abcg2(-/-) mice, 15 min after intravenous vehicle or elacridar administration, and we measured plasma and brain concentrations 1 h later. In the absence of elacridar, the plasma concentrations of N-desethyl sunitinib in Abcg2(-/-) and Abcb1a/1b(-/-)/Abcg2(-/-) mice, but not in Abcb1a/1b(-/-) mice, were 1.9-fold higher than in wild-type mice (Fig. 5A). Elacridar treatment increased the N-desethyl sunitinib plasma levels in wild-type mice to levels equal to those in Abcb1a/1b(-/-)/Abcg2(-/-) mice, and significantly higher than in vehicle-treated wild-type mice (Fig. 5B). Collectively, these data suggest that Abcg2, but not Abcb1a/1b, may contribute to plasma elimination of intravenous N-desethyl sunitinib. In the absence of elacridar, brain concentrations of N-desethyl sunitinib were not significantly different in wild-type, Abcb1a/1b(-/-), and Abcg2(-/-) mice, but they were 13.2-fold increased in Abcb1a/1b(-/-)/Abcg2(-/-) mice compared to wild-type mice (Fig. 5C). Intravenous elacridar increased N-desethyl sunitinib brain concentrations (3.3-fold) in wild-type mice as compared to wild-type mice without elacridar, but this level was still 4-fold lower than seen in Abcb1a/1b(-/-)/Abcg2(-/-) mice without elacridar (Fig. 5D). Surprisingly, the N-desethyl sunitinib brain levels found in Abcb1a/1b(-/-)/Abcg2(-/-) mice pretreated with elacridar were 35% lower than in these mice given N-desethyl sunitinib alone (Fig. 5D). This suggests some inhibition by elacridar of N-desethyl sunitinib brain uptake. In the absence of elacridar, brain-to-plasma ratios in Abcb1a/1b(-/-)/Abcg2(-/-) mice, but not Abcb1a/1b(-/-) and Abcg2(-/-) mice, were markedly increased as compared to wild-type mice (Fig. 5E). When looking at brain-to-plasma ratios, the difference between wild-type mice treated with elacridar or vehicle even lost statistical
significance (Fig. 5F, compare white bars). However, the brain-to-plasma ratios of *Abcb1a/b(-/-)/Abcg2(-/-) mice* either treated with elacridar or vehicle were still significantly increased as compared with wild-type mice without elacridar (Fig. 5F). The results indicate that the effect of intravenous elacridar in enhancing brain accumulation of *N*-desethyl sunitinib is quite limited, and that there may even be some inhibition of *N*-desethyl sunitinib uptake into the brain. This results in somewhat higher brain concentrations as compared with wild-type mice given *N*-desethyl sunitinib alone, but far less than the concentrations seen in mice genetically lacking both transporters.

**Partial saturation of Abcg2-mediated *N*-desethyl sunitinib transport in the BBB of mice following high-dose sunitinib**

We wanted to study the impact of *Abcb1* and *Abcg2* on brain accumulation of *N*-desethyl sunitinib following a high intravenous sunitinib administration (20 mg/kg) in knockout models. Plasma concentrations of *N*-desethyl sunitinib in *Abcg2(-/-) and Abcb1a/b(-/-)/Abcg2(-/-) mice, but not in *Abcb1a/b(-/-) mice*, were modestly but significantly higher compared to wild-type mice at *t* = 10 min (Fig. 6A). At 1 h, plasma levels of this metabolite had increased relative to 10 min in all strains and the plasma levels in *Abcg2(-/-) mice* were still significantly higher than those in wild-type mice (Fig. 6B). 10 min after intravenous sunitinib administration, brain levels of *N*-desethyl sunitinib were not detectable in wild-type, *Abcg2(-/-) and Abcb1a/b(-/-) mice*, but marginally detectable in *Abcb1a/b(-/-)/Abcg2(-/-) mice* (Fig 6C). At 1 h, *N*-desethyl sunitinib brain concentrations were detectable in all strains, and brain concentrations in *Abcb1a/b(-/-) and Abcb1a/b(-/-)/Abcg2(-/-) mice*, but not *Abcg2(-/-) mice*, were now significantly higher than those in wild-type mice (Fig. 6D). Brain-to-plasma ratios were also significantly increased in *Abcb1a/b(-/-)/Abcg2(-/-) mice*, but not in *Abcb1a/b(-/-) mice* (supplemental Fig. 4A). Upon high-dose intravenous sunitinib administration, the plasma *N*-desethyl sunitinib-to-sunitinib ratios were lower than after oral administration of sunitinib (10 mg/kg), but not significantly different among all strains except in *Abcg2(-/-) mice*, which displayed a 1.4-fold increase relative to wild-type mice (supplemental Fig. 4B).
Discussion

In this study, we show that N-desethyl sunitinib is actively transported \textit{in vitro} by human ABCB1, ABCG2 and murine Abcg2, but not by human ABCC2 or murine Abcc2. We also demonstrate in MDCKII cells stably coexpressing human ABCB1 and ABCG2 that the contribution of ABCG2 to N-desethyl sunitinib transport at 5 µM is nearly equal to that of ABCB1. Upon oral sunitinib administration, the plasma AUC\textsubscript{0-6h} of N-desethyl sunitinib did not differ among wild-type, \textit{Abcb1a/1b}\textsuperscript{-/-}, \textit{Abcg2}\textsuperscript{-/-}, and \textit{Abcb1a/1b}\textsuperscript{-/-}/\textit{Abcg2}\textsuperscript{-/-} strains. However, we found a profound effect (13.7-fold) on N-desethyl sunitinib brain accumulation when both transport systems were absent from the BBB. In addition, brain concentrations of N-desethyl sunitinib were increased to just above the LLQ by concomitant oral elacridar treatment in wild-type mice. After intravenous administration of N-desethyl sunitinib, Abcg2 deficiency was found to modestly enhance its systemic plasma levels, which may suggest a role in N-desethyl sunitinib elimination. In \textit{Abcb1a/1b}\textsuperscript{-/-}/\textit{Abcg2}\textsuperscript{-/-} mice, but not in \textit{Abcb1a/1b}\textsuperscript{-/-} and \textit{Abcg2}\textsuperscript{-/-} mice, the brain levels of intravenously administered N-desethyl sunitinib were highly increased as compared with wild-type mice. Thus, the brain accumulation of N-desethyl sunitinib, similar to that of its parent compound sunitinib, is critically dependent on the efflux activity of both Abcb1 and Abcg2. Intravenous elacridar increased N-desethyl sunitinib plasma and brain levels, but had no significant impact on the brain-to-plasma ratios of N-desethyl sunitinib in wild-type mice.

Our data suggest that there was no substantial role of Abcb1a/1b and Abcg2 in plasma pharmacokinetics of N-desethyl sunitinib following oral sunitinib administration. Accordingly, plasma levels of N-desethyl sunitinib were not substantially changed upon oral coadministration of elacridar and sunitinib (Fig. 4A). Upon direct intravenous administration, however, plasma levels of N-desethyl sunitinib were 1.9-fold increased in \textit{Abcg2}\textsuperscript{-/-} and \textit{Abcb1a/1b}\textsuperscript{-/-}/\textit{Abcg2}\textsuperscript{-/-} mice as compared with vehicle-treated wild-type mice, but not in \textit{Abcb1a/1b}\textsuperscript{-/-} mice (Fig. 6A). These results suggest that Abcg2 might play a role in the systemic elimination of N-desethyl sunitinib after intravenous administration. In the presence of elacridar, plasma concentrations of N-desethyl sunitinib in wild-type and \textit{Abcb1a/1b}\textsuperscript{-/-}/\textit{Abcg2}\textsuperscript{-/-} mice were not different anymore,
suggesting that intravenous elacridar reduces the plasma elimination of \(N\)-desethyl sunitinib in wild-type mice, presumably via Abcg2. Similarly, Lagas et al. (2009) demonstrated previously that elacridar can inhibit the systemic elimination of the TKI dasatinib via Abcb1 and/or Abcg2. Our data suggest that a possible systemic elimination contribution of Abcg2 only became apparent at higher plasma levels (which would occur especially shortly after intravenous \(N\)-desethyl sunitinib administration), but not at lower plasma levels (after oral sunitinib administration). One could speculate that at low plasma levels, alternative (non-Abcg2) \(N\)-desethyl sunitinib elimination processes predominate, but that these become saturated at higher plasma levels. If Abcg2 is not yet saturated at these levels, it will have a much more marked impact on \(N\)-desethyl sunitinib plasma concentrations than at lower plasma levels.

Like for many other shared Abcb1a/1b and Abcg2 substrates, the single disruption of Abcb1a/1b or Abcg2 in mice has only little effect on brain accumulation of \(N\)-desethyl sunitinib, whereas simultaneous disruption of the two transporters results in a dramatic increase in \(N\)-desethyl sunitinib brain accumulation. This disproportionate effect has led some researchers to envisage a compensatory change with upregulation of one transporter in the BBB of single knockout strains for the other transporter. However, extensive analyses of Abcg2 or Abcb1a expression in brain homogenates of, respectively, Abcb1a/1b or Abcg2 knockout mice as used by us revealed no significant change in the RNA and/or protein levels in either strain (Lagas et al., 2010; de Vries et al., 2007; Jonker et al., 2000). Moreover, Kodaira et al. (2010), using specific transport substrates for either transporter, found negligible shifts in brain accumulation of these substrates in the single knockout strains of the complementary transporter. This suggests that there is no substantial change in Abcb1a or Abcg2 activity in the BBB of the FVB background Abcg2 or Abcb1a/1b knockout mice, respectively. Kodaira et al. (2010) further showed that a (physiologically based) pharmacokinetic model of Abcb1a and Abcg2 activity in the BBB could readily describe the seemingly disproportionate effect of the combined disruption of these transporters as compared to the single disruptions, without invoking changes in activity of the remaining transporter.
Our data show that the brain accumulation of N-desethyl sunitinib was highly increased in $Abcb1a/1b^{(-/-)}/Abcg2^{(-/-)}$ mice 6 h after oral sunitinib administration. Therefore, we studied the brain accumulation of N-desethyl sunitinib following an oral coadministration schedule of elacridar and sunitinib that might also be feasible in a clinical setting. In this context it is important to note that the plasma exposure level of elacridar used in our study is also achievable in humans, as demonstrated by Kemper et al. (2001), who showed that a patient receiving 1000 mg of elacridar orally had almost the same elacridar plasma concentrations as mice treated with 100 mg/kg of elacridar orally. Somewhat disappointingly, brain levels of N-desethyl sunitinib were only just above the LLQ under this coadministration scheme (Fig. 4B). Direct intravenous administration of N-desethyl sunitinib at a dose of 5 mg/kg to wild-type mice resulted in higher brain levels of N-desethyl sunitinib, but still far lower than those in $Abcb1a/1b^{(-/-)}/Abcg2^{(-/-)}$ mice (Fig. 5A, C and E). The results of Fig. 5D and F suggest that elacridar could not fully inhibit the BBB efflux transporters with respect to N-desethyl sunitinib, resulting in lower brain accumulation as compared to genetic deletion of Abcb1a/1b and Abcg2. Unexpectedly, elacridar treatment in $Abcb1a/1b^{(-/-)}/Abcg2^{(-/-)}$ mice also lowered brain N-desethyl sunitinib levels as compared to vehicle-treated mice, whereas plasma levels were not significantly affected (Fig. 5B, D, F). One speculative explanation is that this reduction effect might be due to inhibition of putative uptake transporters for N-desethyl sunitinib into the brain. Note that such a reduction was not observed for the parent drug sunitinib (Tang et al., 2011).

Even though elacridar is generally an effective inhibitor for ABCB1 and ABCG2 in vitro and in vivo, elacridar has been shown to be a better inhibitor of Abcb1 than Abcg2 in vitro (Allen et al., 2002; Matsson et al., 2009). We observed complete inhibition of ABCB1-mediated N-desethyl sunitinib transport, but only partial inhibition of human ABCG2- and especially murine Abcg2-mediated N-desethyl sunitinib transport (Fig. 1C, D, G and H). Note that, given the apparent difference in sensitivity to elacridar between mouse Abcg2 and human ABCG2, it could be that the effects of elacridar would be more pronounced in humans than in mice. In vivo, we were still able to observe a significant increase in brain concentration in wild-type mice treated with elacridar in combination with N-desethyl sunitinib, but the change in brain-to-plasma ratio
was not statistically significant. The increase in the brain concentrations of N-desethyl sunitinib in wild-type mice could therefore be partly due to the increased plasma concentrations in wild-type mice treated with elacridar (Fig. 5B). Our \textit{in vitro} finding of poor Abcg2 inhibition by elacridar thus seems to be in agreement with our \textit{in vivo} findings. Apparently, elacridar is overall not an efficient enough inhibitor of Abcg2-mediated transport of N-desethyl sunitinib. One could speculate that its affinity for Abcg2 is not high enough, as the high-affinity ABCG2/Abcg2 inhibitor Ko143 did completely inhibit transport \textit{in vitro}. Thus, higher concentrations of elacridar may be needed to fully inhibit Abcg2 than to inhibit Abcb1 in the BBB. For N-desethyl sunitinib, however, increasing elacridar dosage might be counterproductive as we cannot exclude that it might also inhibit brain uptake more strongly.

Our \textit{in vitro} data with ABCB1- and ABCG2-overexpressing MDCKII cells suggest that the transport contribution of ABCG2 in these cells is almost equal to that of ABCB1 at 5 µM of N-desethyl sunitinib. Possibly beginning saturation of ABCG2-mediated, but not of ABCB1-mediated N-desethyl sunitinib transport occurs at 20 µM. We have previously observed that plotting inverse transport ratios (AB/BA, or 1/r) obtained with this cell line yields good qualitative correlations with brain penetration data, especially for topotecan and sorafenib, but somewhat less for sunitinib (Poller et al., 2011). For its metabolite N-desethyl sunitinib, however, we again found a very good in vitro-in vivo correlation between brain concentration in the various wild-type and knockout strains and transcellular transport data obtained at 5 and 20 µM (supplemental Fig. 5, compare panels C, D, E and F).

To obtain a better understanding of the relative ability of sunitinib and N-desethyl sunitinib to penetrate the brain, we compared the data obtained in this study with our previous findings for sunitinib (compare Table 1 and supplemental Table 1). In wild-type mice, sunitinib has a 2.4-fold higher brain penetration than N-desethyl sunitinib, with a $P_{\text{brain}}$ of 1.6 and 0.67 observed for sunitinib and N-desethyl sunitinib, respectively. 6 h after oral sunitinib administration (10 mg/kg), sunitinib brain concentrations are 5.7-fold higher than N-desethyl sunitinib brain concentrations seen in wild-type mice. In addition, elacridar did not completely inhibit transport activity of human ABCG2 and murine Abcg2-mediated N-desethyl sunitinib transport \textit{in vitro}, suggesting that N-
desethyl sunitinib is a more avid human ABCG2 and murine Abcg2 substrate than sunitinib. Elacridar increased the brain-to-plasma ratio of sunitinib 12-fold (Tang et al., 2011), but that of its metabolite only 1.6-fold in wild-type mice (Fig. 5F). Therefore, elacridar had a much greater effect on the brain concentration of sunitinib than on that of N-desethyl sunitinib.

To the best of our knowledge, this is the first study demonstrating that Abcb1 and Abcg2 together restrict N-desethyl sunitinib brain accumulation, but not plasma pharmacokinetics of N-desethyl sunitinib upon oral sunitinib administration. Abcg2 may modestly enhance systemic elimination of N-desethyl sunitinib administered intravenously. Despite its effect on plasma and brain concentrations, intravenous elacridar had no significant impact on brain-to-plasma ratios of N-desethyl sunitinib in wild-type mice. Together with our previous findings on sunitinib, the effect of elacridar is more pronounced for the brain accumulation of sunitinib than for that of N-desethyl sunitinib. Therefore, coadministration of elacridar seems a more attractive strategy for sunitinib than for N-desethyl sunitinib in improving the clinical efficacy against brain metastases and brain tumors positioned behind a functionally intact BBB.

Acknowledgements. We thank Dilek Iusuf and Selvi Durmus for critical reading of the manuscript.

Authorship Contributions

*Participated in research design:* Tang, Lankheet, Poller, Wagenaar, Beijnen, Schinkel

*Conducted experiments:* Tang, Lankheet, Poller,

*Contributed new reagents or analytical tools:* Tang, Lankheet, Poller, Wagenaar, Beijnen

*Performed data analysis:* Tang, Lankheet, Poller, Schinkel

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


Tang SC, Lagas JS, Lankheet NA, Poller B, Hillebrand MJ, Rosing H, Beijnen JH, and Schinkel AH (2011) Brain accumulation of sunitinib is restricted by P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2) and can be enhanced by oral elacridar and sunitinib coadministration. *Int J Cancer* 10.1002/ijc.26000.

Footnotes

This work was supported by the Dutch Cancer Society [grant 2007-3764], an academic staff training scheme fellowship from the Malaysian Ministry of Science, Technology and Innovation to S.C.T. and a post-doctoral fellowship from the Swiss National Science Foundation to B.P. [grant PBBSP3-128567].

Reprint Requests

Alfred H. Schinkel, Division of Molecular Biology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands. Phone: +31-20-5122046; Fax: +31-20-6691383; Email: a.schinkel@nki.nl
Legends for figures

Figure 1. Transepithelial transport of \(N\)-desethyl sunitinib (5 µM) assessed using MDCKII parental cells (A and E) or MDCKII cells transduced with human ABCB1 (B and F), human ABCG2 (C and G), murine Abcg2 (D and H), human ABCC2 (I) or murine Abcc2 (J) cDNA. At \(t = 0\) h, \(N\)-desethyl sunitinib was applied in one compartment (apical or basolateral), and the amount of drug appearing in the opposite compartment at \(t = 2\) and 4 h was measured by LC-MS/MS and plotted as the percentage of the amount of initially applied drug (\(n = 3\)). Elacridar (5 µM) was applied to inhibit human and/or endogenous canine ABCB1 (E-J). ▲, translocation from basolateral to apical compartment; , translocation from apical to basolateral compartment. Points, mean; bars, SD. At \(t = 4\) h, 1% of transport is approximately equal to an apparent permeability coefficient \((P_{\text{app}})\) of \(0.30 \times 10^{-6}\) cm/s.

Figure 2. Transepithelial transport of \(N\)-desethyl sunitinib (5 µM) assessed using MDCKII parental cells (A) or MDCKII cells transduced with human ABCG2 (B and D) or murine Abcg2 (C and E) cDNA. \(N\)-desethyl sunitinib application and measurement was as described in the legend of Figure 1. The ABCB1 inhibitor zosuquidar (5 µM) was applied to specifically measure ABCG2/Abcg2-mediated transport (B and C). The ABCG2 inhibitor Ko143 (1 µM) was additionally applied to verify ABCG2/Abcg2-mediated transport (D and E). ▲, translocation from basolateral to apical compartment; , translocation from apical to basolateral compartment. Points, mean; bars, SD. At \(t = 4\) h, 1% of transport is approximately equal to an apparent permeability coefficient \((P_{\text{app}})\) of \(0.30 \times 10^{-6}\) cm/s.

Figure 3. Plasma concentration-time curves (A) and relative brain accumulation at \(t = 6\) h (B) of \(N\)-desethyl sunitinib in male wild-type, \(Abcb1a/1b(-/-), Abcg2(-/-),\) and \(Abcb1a/1b(-/-)/Abcg2(-/-)\) mice following oral administration of 10 mg/kg sunitinib malate. Multiple blood samples (~50 µl) were collected from the tail vein at 15 and 30 min, and 1, 2, and 4 h, using lithium-heparinized capillary tubes. Relative brain accumulation was calculated by dividing brain concentration at \(t =\)
6 h by the plasma AUC_{0-6}. Columns, means (n = 3-7); bars, SD. *p < 0.05, compared to wild-type mice (one-way ANOVA).

Figure 4. Plasma (A) and brain (B) concentrations of N-desethyl sunitinib in male wild-type, \textit{Abcb1a/1b(-/-)}, \textit{Abcg2(-/-)}, and \textit{Abcb1a/1b(-/-)/Abcg2(-/-)} mice 1 h following oral administration of 10 mg/kg sunitinib malate with or without elacridar administration. Sunitinib malate was administered 2 h after the oral administration of either elacridar (100 mg/kg) or vehicle. Columns, means (n = 5); bars, SD.

Figure 5. Effect of genetic deletion on plasma (A) and brain (C) concentrations and brain-to-plasma ratios (E) of N-desethyl sunitinib in male wild-type, \textit{Abcb1a/1b(-/-)}, \textit{Abcg2(-/-)}, and \textit{Abcb1a/1b(-/-)/Abcg2(-/-)} mice 1 h after intravenous injection of 5 mg/kg of N-desethyl sunitinib. Effect of elacridar on plasma (B) and brain (D) concentrations and brain-to-plasma ratios (F) in wild-type and \textit{Abcb1a/1b(-/-)/Abcg2(-/-)} mice. N-desethyl sunitinib was administered 15 min after intravenous administration of either elacridar (10 mg/kg) or vehicle. Columns, means (n = 3-4); bars, SD. *p < 0.05, ***p < 0.001, compared with wild-type mice receiving vehicle (one-way ANOVA). †p < 0.05, ††p < 0.01, †††p < 0.001 compared with wild-type mice receiving vehicle (Student’s t-test). ‡p < 0.05, ‡‡p < 0.01 compared with \textit{Abcb1a/1b(-/-)/Abcg2(-/-)} receiving vehicle (Student’s t-test).

Figure 6. Plasma concentrations (A, B) and brain concentrations (C, D) of N-desethyl sunitinib in male wild-type, \textit{Abcb1a/1b(-/-)}, \textit{Abcg2(-/-)}, and \textit{Abcb1a/1b(-/-)/Abcg2(-/-)} mice 10 min (A, C) or 1 h (B, D) after intravenous injection of 20 mg/kg sunitinib malate. Columns, means (n = 5); bars, SD. *p < 0.05, **p < 0.01, ***p < 0.001, compared with wild-type mice (one-way ANOVA).
Table 1. Pharmacokinetic parameters, brain concentrations (t = 6 h), and relative brain accumulation of N-desethyl sunitinib after oral administration of 10 mg/kg sunitinib malate to various mouse strains

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Wild-type</th>
<th>Abcb1a/1b(-/-)</th>
<th>Abcg2(-/-)</th>
<th>Abcb1a/1b(-/-)/Abcg2(-/-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma AUC(0-6), ng.h/ml</td>
<td>152 ± 12.9</td>
<td>170 ± 49.6</td>
<td>125 ± 66.4</td>
<td>95.8 ± 41.1</td>
</tr>
<tr>
<td>Fold change AUC(0-6)</td>
<td>1.00</td>
<td>1.12</td>
<td>0.82</td>
<td>0.63</td>
</tr>
<tr>
<td>Sunitinib AUC(0-6) a, ng.h /ml</td>
<td>445 ± 203</td>
<td>514 ± 145</td>
<td>311 ± 172</td>
<td>288 ± 88</td>
</tr>
<tr>
<td>N-desethyl-sunitinib-to-sunitinib AUC ratio</td>
<td>0.34</td>
<td>0.33</td>
<td>0.40</td>
<td>0.33</td>
</tr>
<tr>
<td>Cmax, ng/ml</td>
<td>36.3 ± 4.00</td>
<td>33.6 ± 7.00</td>
<td>24.9 ± 12.4</td>
<td>24.8 ± 15.2</td>
</tr>
<tr>
<td>Cbrain, ng/g</td>
<td>10.1 ± 0.94</td>
<td>11.8 ± 5.20</td>
<td>13.2 ± 2.20</td>
<td>80.7 ± 40.0**</td>
</tr>
<tr>
<td>Fold change Cbrain</td>
<td>1.00</td>
<td>1.20</td>
<td>1.30</td>
<td>8.00</td>
</tr>
<tr>
<td>Pbrain (x10⁻¹ h⁻¹)</td>
<td>0.67 ± 0.12</td>
<td>0.73 ± 0.34</td>
<td>1.27 ± 0.60</td>
<td>9.20 ± 5.90*</td>
</tr>
<tr>
<td>Fold change Pbrain</td>
<td>1.00</td>
<td>1.10</td>
<td>1.90</td>
<td>13.7</td>
</tr>
</tbody>
</table>
Data are means ± SD (n = 3-7). Sunitinib AUC data for comparison were taken from Tang et al. (2011). *p < 0.05, **p < 0.01, compared with wild-type mice (one-way ANOVA). $P_{\text{brain}}$: relative brain accumulation at 6 h after oral administration, calculated by determining the $N$-desethyl sunitinib brain concentration relative to the $\text{AUC}_{(0-6)}$. 
Figure 1

Graphs showing transport (%) over time (h) for different conditions:

A. Parental
B. ABCB1
C. ABCG2
D. Abcg2
E. Parental + elacridar
F. ABCB1 + elacridar
G. ABCG2 + elacridar
H. Abcg2 + elacridar
I. ABCC2 + elacridar
J. Abcc2 + elacridar

The graphs illustrate the influence of various conditions on transport efficiency, with correlation coefficients (r) indicated for each condition.
Figure 2

A. Parental + zosuquidar

B. ABCG2 + zosuquidar

C. Abcg2 + zosuquidar

D. ABCG2 + zosuquidar + Ko143

E. Abcg2 + zosuquidar + Ko143
Figure 3

A

N-desethyl sunitinib plasma concentration (ng/ml)

- Wild-type
- Abcb1a/1b (-/-)
- Abcg2 (-/-)
- Abcb1a/1b (-/-)/Abcg2 (-/-)

Time (h)

0 1 2 3 4 5 6

B

N-desethyl sunitinib accumulation (×10^-3 l/h)

Wild-type  Abcb1a/1b (-/-)  Abcg2 (-/-)  Abcb1a/1b (-/-)/Abcg2 (-/-)

*
Figure 4

A  Plasma

N-desethyl sumitinib plasma concentration (ng/ml)

Vehicle  Elacridar

Wild-type  Abcb1a/1b(-/-)  Abcg2(-/-)  Abcb1a/1b(-/-)/Abcg2(-/-)  Wild-type

B  Brain

N-desethyl sumitinib brain concentration (ng/g)

Vehicle  Elacridar

LLQ = 7.5 ng/g

Wild-type  Abcb1a/1b(-/-)  Abcg2(-/-)  Abcb1a/1b(-/-)/Abcg2(-/-)  Wild-type  Abcg2(-/-)