THE SYSTEMIC EXPOSURE OF AN N-METHYL-D-ASPARTATE RECEPTOR ANTAGONIST IS LIMITED IN MICE BY THE P-GLYCOPEPTIDE AND BREAST CANCER RESISTANCE PROTEIN EFFLUX TRANSPORTERS

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Received January 30, 2004; accepted April 5, 2004

This article is available online at http://dmd.aspetjournals.org

ABSTRACT:

GV196771 [E-4,6-dichloro-3-(2-oxo-1-phenyl-pyrrolidin-3-glydenemethyl)-1H-indole-2-carboxylic acid] is a potent antagonist of the modulatory glycine site of the N-methyl-d-aspartate receptor. GV196771 has low oral bioavailability (<10%) and plasma clearance (~2 ml/min/kg) in rats. P-Glycoprotein (Pgp) and breast cancer resistance protein (Bcrp) are ATP-binding cassette (ABC) transporters that limit the oral absorption of drugs and dietary constituents. The objective of this work was to assess the involvement of Pgp and/or Bcrp on the systemic exposure of GV196771 in mice. In vitro, GV196771 was a Bcrp substrate (basolateral-to-apical/apical-to-basolateral (B→A/A→B ratio = 5.1) with high passive membrane permeability (P_app = 64-170 nm/s); however, GV196771 was not an in vitro Mdr1a substrate (B→A/A→B ratio = 1.5; no effect of GF120918 on efflux ratio). The role of Pgp and Bcrp on the systemic exposure of GV196771 was assessed by pretreatment of wild-type and Pgp-deficient mdr1a1b−/− mice with a single oral dose of GF120918 (50 mg/kg; a dual Pgp and Bcrp inhibitor) or vehicle (0.5% hydroxypropylmethylcellulose and 1% Tween 80) 2 h before administration of a single oral dose of GV196771 (2 mg/kg). Compared with wild-type animals, the GV196771 area under the plasma concentration-time curve [AUC(0-6h)] increased 6.2-fold in Pgp-deficient mice, 10.3-fold in GF120918-pretreated wild-type mice, and 16.4-fold in GF120918-pretreated Pgp-deficient mice. C_max values changed in parallel with the AUC(0-6h) values; however, t_max remained relatively unchanged. This study supports a role for Pgp and Bcrp in attenuating the systemic exposure of GV196771 in mice and demonstrates that two ABC efflux transporters can have nonredundant roles in attenuating the disposition of a compound.

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ABSTRACT:

GV196771 blocked hyperalgesia when administered prophylactically and was able to reverse established hyperalgesia (Quartaroli et al., 1999; Bordi and Quartaroli, 2000). Preliminary pharmacokinetic studies demonstrated that GV196771 had low oral bioavailability (<10%) and very low plasma clearance (~2 ml/min/kg) in the rat (R. J. Barnaby and G. Vitulli, personal communication). An excretion balance study in rats demonstrated that 94% of an oral dose of [14C]GV196771 was eliminated unchanged in the feces, with 5% and <1% eliminated in the bile and urine, respectively, as mainly unchanged GV196771. Finally, whole-body autoradiography studies demonstrated that the highest level of radiolabel was associated with the gastrointestinal tract (>485 μg Eq/g tissue), with liver and kidney levels more than 40-fold lower (<11 μg Eq/g tissue). These studies

ABBREVIATIONS: NMDA, N-methyl-d-aspartate; A→B, apical-to-basolateral; B→A, basolateral-to-apical; ABC, ATP-binding cassette; AUC, area under the plasma concentration-time curve; Bcrp, breast cancer resistance protein; Bcrp-MDCKII, Madin-Darby canine kidney cells transfected with the BCRP gene; CL, systemic plasma clearance; C_max, maximum plasma concentration; GF120918, N-(4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)[ethyl]-phenyl]-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide; GV150524, C_20H_{13}Cl_2N_2O_3; GV196771, E-4,6-dichloro-3-(2-oxo-1-phenyl-pyrrolidin-3-glydenemethyl)-1H-indole-2-carboxylic acid; LC-MS/MS, high-performance liquid chromatography with tandem mass spectrometry; MB, mass balance; MDR, multidrug resistance; mdr1a-LLC-PK1, porcine kidney cells transfected with the mouse mdr1a gene; P_app, apparent permeability; Pgp, P-glycoprotein.
suggest that the poor oral bioavailability of GV196771 was likely due to limited intestinal absorption.

Members of the ATP-binding cassette (ABC) superfamily of efflux transporters have been shown to influence the systemic exposure of a number of clinically important drugs (Ayrton and Morgan, 2001; Lin and Yamazaki, 2003; Mizuno et al., 2003). These primary active efflux transporters are expressed at the major barriers within the body (e.g., intestine, blood-brain barrier, placenta, kidney, and liver), where they reduce the uptake or enhance the clearance of drugs. P-Glycoprotein (Pgp, ABCB1) is the prototypic ABC transporter first identified through studies on multidrug resistance. Pgp is a single 170-kDa integral membrane protein composed of two similar halves, each consisting of six transmembrane domains and an ATP binding site. Besides its role in multidrug resistance, Pgp has been shown to reduce the intestinal absorption of digoxin, tamoxifen, fexofenadine, and vinblastine by transporting these drugs back to the apical surface of the intestinal enterocyte (Ayrton and Morgan, 2001; Lin and Yamazaki, 2003; Mizuno et al., 2003).

Pgp is only one of a family of ABC efflux transporters that can influence the intestinal absorption of drugs. Recently, the breast cancer resistance protein (Bcrp, ABCG1) has been identified to confer drug resistance to a variety of chemotherapeutic agents (Litman et al., 2000; Bates et al., 2001; Allen and Schinkel, 2002). The Bcrp gene was cloned and the protein product found to be a half-transporter, consisting of a single 70-kDa, six-transmembrane peptide that is half the size of Pgp. Bcrp is hypothesized to function as a homodimer and has been shown to limit the intestinal absorption of the anti-cancer compound topotecan and to protect mice from a chlorophyll-derived dietary phototoxin (Jonker et al., 2000, 2002).

Based on the oral bioavailability, results from excretion balance studies, and the plasma clearance, we hypothesized that ABC efflux transporters may play a role in the absorption and elimination of GV196771. Therefore, the objective of this study was to determine the role of Pgp and Bcrp on the systemic exposure of GV196771 through in vitro monolayer efflux studies and in vivo pharmacokinetic studies using wild-type and Pgp-deficient mdr1a/1b−/− mice dosed with the potent Pgp/Bcrp inhibitor GF120918. The results show that Pgp and Bcrp have nonredundant roles affecting the disposition of GV196771 in mice.

Materials and Methods

Materials. GV196771, GV150524, GF120918, and amprenavir were obtained from GlaxoSmithKline compound registry. Prazosin and Lucifer yellow were purchased from Sigma-Aldrich (St. Louis, MO). All other materials were of reagent grade. Transwells were purchased from Costar (Cambridge, MA).

In Vitro Pgp Monolayer Efflux Studies. LLC-PK1 cells transfected with the murine mdr1a gene (mdr1a-LLC-PK1) and MDCK type II cells transfected with the murine Bcrp gene (Bcrp-MDCKII) cells were obtained from the Netherlands Cancer Institute (Amsterdam, Netherlands). Culturing of cells and transport studies were completed as previously described (Pollf et al., 2001). Briefly, cells were split twice weekly at a ratio of 1:10 and grown in the absence of any selection agent. For transport studies, cells were seeded onto polycarbonate Transwell filter membranes at a density of 300,000 cells/cm2, and monolayers were ready for studies 3 days later. Drugs were dissolved at 10 mM in 100% dimethyl sulfoxide and then diluted in transport buffer (Dulbecco’s modified Eagle’s medium with 25 mM HEPES and without phenol red). Drugs were tested at 3 µM, in two directions [apical-to-basolateral (A→B) and basolateral-to-apical (B→A)], at a single time point (90 min), and in triplicate. Monolayer efflux studies were conducted at 37°C in a humidified incubator with shaking (90 rpm). Transepithelial electrical resistance was measured with an Endohm Meter (World Precision Instruments Inc., Sarasota, FL). Reference drugs for paracellular transport (Lucifer yellow), Pgp efflux (amprenavir), or Bcrp efflux (prazosin) were included in each experiment. The concentrations of compounds in donor and receiver compartments were quantified by high-performance liquid chromatography with tandem mass spectrometry (LC-MS/MS). The calibration range was 1.0 nM to 1.5 µM (n = 5 calibration standards) for each drug. Dose and donor solutions were diluted in transport medium/acetone (1:1, v/v) as required to bring their concentrations into this range. Lucifer yellow concentrations were determined with a SpectraMax Gemini cytosensor fluorimeter ( Molecular Devices, Sunnyvale, CA) set to an excitation wavelength of 430 nm and an emission wavelength of 538 nm.

The apparent permeability (Papp) was calculated with the equation:

\[
P_{\text{app}} = \frac{dQ}{A \cdot dC} = \frac{\text{amount of drug transported per time}}{\text{membrane surface area}}
\]

where Papp = apparent permeability, A = membrane surface area, C0 = donor concentration at time 0, and dQ/dt = amount of drug transported per time. Data are presented as the average Papp (nm/s) ± standard deviation (S.D.) from three monolayers. A ratio of the B→A/A→B Papp values was calculated.

Involvement of a Pgp-mediated efflux mechanism was confirmed if the B→A/A→B ratio was >2.0. To confirm that drugs were Pgp or Bcrp substrates, drugs were also tested in the presence of GF120918 (2 µM for Pgp, 10 µM for Bcrp), a potent Pgp and Bcrp inhibitor. Inclusion of GF120918 reduces the B→A/A→B ratio to ~1 for Pgp and Bcrp substrates.

Mass balance (MB) is the percentage of original drug mass accounted for at the end of the experiment (sum of amount in the A and B chambers). MB is calculated with the following equation:

\[
\%\text{MB} = \left(\frac{(C_{A}\cdot V_{A}) + (C_{B}\cdot V_{B})}{C_{A}\cdot V_{A}}\right) \times 100
\]

where CA and CB are the drug concentrations in the apical (A) and basolateral (B) chambers at time (t), CA is the concentration of the donor at time 0, VA and VB are the volumes of the apical and basolateral chambers, and VA is the volume of the donor solution added to the appropriate chamber.

Pharmacokinetic Studies. The GlaxoSmithKline Institutional Animal Care and Use Committee approved the in vivo studies. Male wild-type FVB mdr1a/1b+/- and Pgp-deficient knockout FVB mdr1a/1b−/− mice (20–30 g) were purchased from Taconic Farms (Germantown, NY). Dose solutions of 0.2 mg/ml for GV196771 and 5.0 mg/ml for GF120918 were prepared fresh using 0.5% hydroxypropylmethylcellulose and 1% Tween 80 as a vehicle. Two hours before the administration of GV196771, the animals were divided into two groups. One group received a single oral dose (10 mg/kg) of vehicle and the second group received a single 50 mg/kg oral dose (10 mg/kg) of GF120918. Two hours later, all animals received a single 2 mg/kg oral dose (10 mg/kg) of GV196771. At scheduled time points, mice were anesthetized with CO2 and blood samples were obtained by cardiac puncture. Blood was centrifuged to yield plasma. The MDR genotype of each animal was confirmed by a polymerase chain reaction assay after study completion (data not shown). No behavioral changes were observed in the animals during the dosing portion of the study.

Calibration standards (1–3000 ng/ml, n = 8 standards) were prepared in untreated mouse plasma. Plasma proteins from samples and standards were precipitated by the addition of 4 volumes of acetonitrile containing 125 mg/ml GV150524 per volume of sample or standard. The samples and standards were centrifuged and drug concentrations of GV150524 and GF120918 were quantified by LC-MS/MS.

LC-MS/MS Analysis of in Vitro and in Vivo Samples. High-performance liquid chromatography was conducted on a Hewlett Packard 1100 (Hewlett Packard, Palo Alto, CA) equipped with a column-switching valve. A 50-µl sample was loaded onto the column by means of a Gilson 215 autosampler (Gilson Medical Electronics, Middleton, WI). Chromatography was performed at room temperature on a 30 × 2.1 mm (i.d.), 3.5-µm Waters Symmetry C18 column (Waters, Milford, MA) at a flow rate of 0.6 ml/min. The mobile phase consisted of two solvents: A, 0.1% formic acid in water; and B, 0.1% formic acid in acetonitrile. The gradient profile was 0 to 1.0 min, 5% (v/v) B; 1.0 to 1.1 min, linear gradient to 95% (v/v) B; 1.1 to 2.3 min, 95% (v/v) B; 2.3 to 2.4 min, linear gradient to 5% (v/v) B; 2.4 to 3.5 min, 5% (v/v) B. Mass spectrometry was performed on a PerkinElmerSciex API4000 (PerkinElmer-Sciex Instruments, Boston, MA) equipped with a turbo ion spray source for electrospray ionization. Concentration of drug in the samples was calculated determined.
from the chromatographic peak area with Analyst 1.3 software (Applied Biosystems, Toronto, ON, Canada).

Pharmacokinetic Analysis of Destructive Sampling Data. Group means and standard deviations of plasma concentrations were calculated at each sampling time point (n = 3 samples/time point; individual animals used for each time point) using Microsoft Excel 97 (Microsoft, Redmond, WA). Pharmacokinetic parameters were determined by noncompartmental analysis of the mean plasma concentrations (WinNonlin Enterprise v3.1; Pharsight, Mountain View, CA). Area under the curve (AUC) and associated variance was determined by the method of Bailar (1988) for pharmacokinetic studies involving destructive sampling. Statistical significance was determined using Student’s t test or, in the case of AUC, by using the z-statistic for the normal distribution table (Bailer, 1988).

Results

In vitro Efflux Studies. The chemical structure of GV196771, an NMDA receptor antagonist, is shown in Fig. 1. To determine whether GV196771 was a substrate for the murine Mdr1a and Bcrp transporters, in vitro monolayer efflux studies were completed using the mdr1a-LLC-PK1 and Bcrp-MDCKII cell lines. In the mdr1a-LLC-PK1 cell line, the Pgp efflux ratio (B→A/A→B) of 3 μM GV196771 was 1.9, and the ratio was 2.7 in the presence of GF120918, a potent Pgp and Bcrp inhibitor (Table 1). In contrast, amiprenavir, a known Pgp substrate (Polli et al., 2001), exhibited a Pgp efflux ratio of 20.1, which reduced to 1.0 in the presence of GF120918, demonstrating the functional activity of Pgp in the mdr1a-LLC-PK1 cell line. These findings suggest that GV196771 was not an in vitro Pgp substrate. GV196771 had a passive membrane permeability value of 64 to 170 nm/s (Table 1, P_app + GF120918), indicating good intestinal absorption potential (Artursson et al., 2001).

In contrast to the Pgp efflux studies, GV196771 was a substrate of the murine Bcrp transporter. The Bcrp efflux ratio (B→A/A→B) for GV196771 was 5.1 and reduced to 1.7 in the presence of the Pgp/Bcrp inhibitor GF120918 (Table 1). Prazosin, a prototypical Bcrp substrate (Litman et al., 2000), exhibited an efflux ratio of 54.9 and reduced to 1.7 in the presence of the Pgp/Bcrp inhibitor GF120918 (Table 1). Pretreatment of wild-type animals with GF120918, an inhibitor of both Pgp and Bcrp, yielded a 10.3- and 13.8-fold increase in the GV196771 AUC(0–6 h) and C_max; there was no change in t_max. These results demonstrate that GF120918-inhibited efflux transporters limit the systemic exposure of GV196771. To quantify the impact of Pgp-mediated efflux, GV196771 was dosed to wild-type and Pgp-deficient mice. The AUC(0–6 h) and C_max of GV196771 in Pgp-deficient mice were 6.2- and 6.0-fold greater compared with wild-type mice, demonstrating that Pgp attenuates GV196771 disposition in wild-type mice. The final dosing arm was completed to demonstrate the importance of Bcrp in the systemic exposure of GV196771. Bcrp-mediated efflux was differentiated from Pgp efflux by using Pgp-deficient mice and treatment of these animals with GF120918. Pretreatment of Pgp-deficient mice with GF120918 resulted in a 2.6- and 2.8-fold increase in the AUC(0–6 h) and C_max of GV196771 over vehicle-treated Pgp-deficient mice, and an overall 16.4- and 16.9-fold increase in the AUC(0–6 h) and C_max over vehicle-treated wild-type mice. This demonstrates that Bcrp also limits the disposition of GV196771.

Discussion

The importance of ABC membrane transporters influencing the disposition and efficacy of drugs is well recognized. GV196771 is an NMDA receptor antagonist with low oral bioavailability in rats and mice. This study investigated whether the ABC efflux transporters Pgp and Bcrp influence the disposition of GV196771. Results of the in vivo studies are summarized in Table 2 and Fig. 3. Compared with wild-type mice, the largest increases in GV196771 AUC(0–6 h) and C_max were seen for Pgp-deficient mice pretreated with GF120918 (>16-fold higher than wild-type mice), followed by wild-type mice pretreated with GF120918 (>10-fold) and Pgp-deficient mice treated with vehicle (>6-fold). These findings suggest that both Pgp and Bcrp contribute to limit the systemic exposure of GV196771. It will be of interest to confirm these findings in bcrp knockout mice when they become commercially available.

One unexpected finding in this study was the importance of Pgp-mediated efflux in vivo but the lack of Pgp-mediated efflux in vitro. Currently, there is no clear explanation for the lack of correlation between the in vitro and in vivo studies. However, the negative findings in vitro are unlikely to be concentration dependence or species differences, since these two possibilities were explored in a number of other studies. First, a separate monolayer efflux study testing GV196771 at concentrations of 0.3, 1, and 3 μM using the mdr1a-LLC-PK1 cell line failed to show transport of GV196771 by Pgp. Second, efflux of GV196771 was negative using the MDR1-MDCKII cell line expressing human Pgp, eliminating the possibility of a species difference between mouse and human. This observation was also consistent with the findings that GV196771 (concentration range of 0.2–50 μM, n = 4 concentrations) failed to stimulate ATPase activity in Sf9 membranes expressing recombinant human Pgp, whereas verapamil yielded a 10-fold stimulation. Third, GV196771 at test concentrations from 0.3 to 100 μM (n = 10) did not inhibit the transport of digoxin, a prototypical Pgp substrate, in the human MDR1- and murine mdr1a-expressing cell lines, further supporting...
the lack of interaction between GV196771 and Pgp in vitro. Finally, GV196771 had a passive membrane permeability value of 64 to 170 nm/s (Table 1, Papp), a passive permeability rate that has a low likelihood to short-circuit Pgp efflux (Polli et al., 2001). In contrast, GV196771 was a Bcrp substrate in vitro, providing a correlation between the in vitro and in vivo studies. This result demonstrates that efflux of GV196771 by an ABC transporter can be detected in vitro. An explanation for the inability to detect Pgp-mediated efflux in vitro remains to be elucidated. One further possibility is that GV196771 is not a Pgp substrate and that the in vivo results in Pgp-deficient mice reflect changes in another mechanism(s) important in the disposition of GV196771.

The influence of Pgp on the intestinal absorption of small molecules is well studied, and a number of clinically important drugs have been identified for which Pgp significantly influences their intestinal absorption (Ayton and Morgan, 2001; Lin and Yamazaki, 2003; Mizuno et al., 2003). In contrast, Bcrp is a relatively new ABC transporter with only two published examples in which Bcrp limits the intestinal absorption of a compound (Jonker et al., 2000, 2002). The intestinal absorption of topotecan, a camptothecin topoisomerase I inhibitor used as an anti-cancer agent, is greatly influenced by Bcrp in rodents and humans. Compared with wild-type mice, the largest increases in topotecan AUC were seen for Pgp-deficient mice pretreated with vehicle or GF120918 (50 mg/kg). Two hours before the administration of GV196771, the animals were divided into two groups. One group received a single oral dose (10 mg/kg) of vehicle and the second group received a single 50 mg/kg oral dose (10 mg/kg) of GF120918. Two hours later, all animals received a single 2 mg/kg oral dose (10 mg/kg) of GV196771. At scheduled time points, mice (n = 3 per time point) were anesthetized with CO2 and blood samples were obtained by cardiac puncture.

![Dosing Arm](image1)

**Fig. 2.** The in vivo experimental design used to differentiate Pgp and Bcrp efflux components affecting the intestinal absorption of GV196771 in mice.

**TABLE 1**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Papp A→B&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mass Balance</th>
<th>Papp B→A</th>
<th>Mass Balance</th>
<th>Efflux Ratio&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>GF120918</td>
<td>309 ± 3</td>
<td>69.0 ± 3</td>
<td>154 ± 3</td>
<td>81.5 ± 1</td>
<td>0.9</td>
</tr>
</tbody>
</table>

<sup>a</sup> Papp = apparent permeability, A = apical, B = basolateral. Data are reported as the average ± standard deviation from n = 3 monolayers. The Papp values were determined at a test concentration of 3 μM and a single time point of 90 min.

<sup>b</sup> Drugs are classified as substrates if the efflux ratio is ≥2 and the ratio reduces to 1.0 in the presence of GF120918. The efflux ratio is calculated by Papp B→A/Papp A→B.

GV196771 is a substrate, and a concentration of 3 μM/s (Table 1, GV196771 had a passive membrane permeability value of 64 to 170 nm/s (Table 1, GV196771 + GF120918) had a passive membrane permeability value of 64 to 170 nm/s (Table 1, GV196771 + GF120918). In contrast, Bcrp is a relatively new ABC transporter with only two published examples in which Bcrp limits the intestinal absorption of a compound (Jonker et al., 2000, 2002). The intestinal absorption of topotecan, a camptothecin topoisomerase I inhibitor used as an anti-cancer agent, is greatly influenced by Bcrp in rodents and humans. Compared with wild-type mice, the largest increases in topotecan AUC were seen for Pgp-deficient mice pretreated with vehicle or GF120918 (50 mg/kg). Two hours before the administration of GV196771, the animals were divided into two groups. One group received a single oral dose (10 mg/kg) of vehicle and the second group received a single 50 mg/kg oral dose (10 mg/kg) of GF120918. Two hours later, all animals received a single 2 mg/kg oral dose (10 mg/kg) of GV196771. At scheduled time points, mice (n = 3 per time point) were anesthetized with CO2 and blood samples were obtained by cardiac puncture.

**Fig. 3.** Pharmacokinetics of GV196771 after an oral 2 mg/kg dose to male wild-type and Pgp-deficient mice pretreated with vehicle or GF120918 (50 mg/kg). Two hours before the administration of GV196771, the animals were divided into two groups. One group received a single oral dose (10 mg/kg) of vehicle and the second group received a single 50 mg/kg oral dose (10 mg/kg) of GF120918. Two hours later, all animals received a single 2 mg/kg oral dose (10 mg/kg) of GV196771. At scheduled time points, mice (n = 3 per time point) were anesthetized with CO2 and blood samples were obtained by cardiac puncture. Data are reported as the average ± standard deviation from n = 3 monolayers.
In conclusion, the disposition of GV196771 in mice is limited by Pgp and Bcrp. In vitro, GV196771 was a substrate for Bcrp but not Pgp. In vivo, elimination of Pgp-mediated efflux by genetic disruption led to a 6-fold increase in the AUC\(_{0-\text{max}}\) and \(C_{\text{max}}\), demonstrating that Pgp influences the systemic exposure of GV196771. Administration of GF120918, a dual Pgp and Bcrp inhibitor to Pgp-deficient animals, resulted in a 16-fold increase in the AUC\(_{0-6\text{ h}}\) and \(C_{\text{max}}\) of GV196771, suggesting that Bcrp also limits the systemic exposure of GV196771. This study demonstrates that two ABC transporters can have similar, nonredundant roles in limiting the disposition of a compound.

Acknowledgments. We thank Christopher Matheny for assistance with the statistical analysis of the pharmacokinetic studies involving destructive sampling.

References


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**Table 2**

<table>
<thead>
<tr>
<th>PK Parameter</th>
<th>Wild-Type</th>
<th>Wild-Type + GF120918</th>
<th>Fold Change</th>
<th>mdr1a/1b(^{-/-})</th>
<th>Fold Change</th>
<th>mdr1a/1b(^{-/-}) + GF120918</th>
<th>Fold Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC(_{0-\text{max}}) (ng·h/ml)</td>
<td>184</td>
<td>1886(^{a})</td>
<td>10.3</td>
<td>1142(^{b})</td>
<td>6.2</td>
<td>3014(^{c})</td>
<td>16.4</td>
</tr>
<tr>
<td>(C_{\text{max}}) (ng/ml)</td>
<td>106</td>
<td>1463</td>
<td>13.8</td>
<td>632</td>
<td>6.0</td>
<td>1793</td>
<td>16.9</td>
</tr>
<tr>
<td>(t_{\text{max}}) (h)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.0</td>
<td>0.5</td>
<td>1.0</td>
<td>1.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

* Blood samples were collected from three individual animals per time point. Plasma concentrations of GV196771 and GF120918 were determined by LC-MS/MS analysis. Group means and standard deviations of plasma concentrations were calculated at each sampling time point and pharmacokinetic parameters were determined by destructive noncompartmental analysis of the mean plasma concentrations.

\(^{a}\) The GF120918 plasma concentrations ranged from 2340 to 815 ng/ml during the 0- to 6-h time period after dosing GV196771. The mean (± standard deviation) GF120918 concentrations over this 6-h time period used for GV196771 blood sampling were 1309 ± 475 ng/ml in wild-type animals and 1602 ± 485 ng/ml in Pgp-deficient animals (\(P > 0.05\)). The GF120918 AUC\(_{0-6\text{ h}}\) values were 7775 and 8707 ng·h/ml in the wild-type and Pgp-deficient animals, demonstrating that there was no difference (\(P > 0.05\)) in the plasma concentrations of GF196771 between these groups.

\(^{b}\) The fold change values are calculated by comparison with wild-type mice.

\(^{c}\) The fold change values were calculated by comparison with wild-type mice. AUC and associated variance was determined by the method of Bailer (1988) for pharmacokinetic studies involving destructive sampling. Statistical significance was determined using Student’s \(t\) test or, in the case of AUC, by using the \(z\)-statistic for the normal distribution table (Bailer, 1988).

\(^{*}\) Even though the fold change was notable (1.60-fold), the results were not statistically significant (\(P > 0.05\)) for comparisons of the AUC for wild-type mice treated with GF120918 and mdr1a/1b\(^{-/-}\) (\(P = 0.099\)).

\(^{*}\) \(P < 0.001\) compared with the area under the curve (AUC) for wild-type mice. AUC and associated variance was determined by the method of Bailer (1988) for pharmacokinetic studies involving destructive sampling. Statistical significance was determined using Student’s \(t\) test or, in the case of AUC, by using the \(z\)-statistic for the normal distribution table (Bailer, 1988).