**Stereoselective Interaction of Pantoprazole with ABCG2. I. Drug Accumulation in Rat Milk**

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**ABSTRACT:**

Active transport of drug into milk is a major concern in breastfeeding. Abcg2 plays a critical role in drug transfer into rat milk, which is consistent with evidence in humans. Although it is estimated that approximately half of all therapeutic agents are chiral, there have been few reports of stereoselective interactions with ABCG2. The purpose of this study was to investigate the interaction of pantoprazole (PAN) isomers with Abcg2 in vitro and in vivo experiments. Pantoprazole isomer flux was characterized using Abcg2-Madin-Darby canine kidney II (MDCKII) cells in Transwell plates. In a crossover design, Sprague-Dawley lactating rats were used to study PAN accumulation in milk after an intravenous infusion of pantoprazole mixture in the presence/absence of Abcg2 inhibitor [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 (GF120918)]. Samples were analyzed by high-performance liquid chromatography/liquid chromatography-mass spectrometry. The results indicated that pantoprazole isomers were transported in an identical fashion in vector-MDCKII cell lines, whereas a significant difference in flux was observed in Abcg2-MDCKII cell line. The administration of GF120918 slightly increased the concentration of both isomers in serum, but no statistical difference was observed. However, the systemic clearance of (+)-PAN (0.57 ± 0.1) was larger than (-)-PAN (0.44 ± 0.12) (P < 0.01). Milk to serum ratio (M/S) of (-)-PAN (1.36 ± 0.20) was 2.5-fold greater than that of (+)-PAN (0.54 ± 0.09) (P < 0.01). Administration of GF120918 decreased M/S of (-)-PAN to 0.50 ± 0.08 (P < 0.001) and (+)-PAN to 0.38 ± 0.07 (P > 0.05). In conclusion, Abcg2, which is responsible for differential accumulation in milk, interacts stereoselectively with PAN isomers. Stereoselective transport of ABCG2 may have broader consequences in drug disposition.

**Introduction**

ABCG2 (ATP binding cassette transporter isoform G member 2) exhibits a broad substrate specificity transporting hydrophobic, anionic, and cationic drugs (Mao and Unadkat, 2005) and widely distributes in such tissues as kidney, liver, blood-brain barrier, placenta, stem cells, and mammary gland and is reported to be of relevance in the absorption, distribution, elimination, and toxicity of drugs (Oostendorp et al., 2009). ABCG2 plays a significant role in the accumulation of xenobiotics in milk (Jonker et al., 2006; Merino et al., 2006). Some dietary carcinogens, toxins, and antibiotics that are substrates of ABCG2 are readily transported into milk and result in high milk to serum (M/S) ratios (Merino et al., 2006; van Herwaarden et al., 2006). Although other transporters may be present in lactating mammary epithelial cells (Alcorn et al., 2002), ABCG2 seems to be playing a prominent role in drug accumulation in milk. One hypothesis would hold that all viable ABCG2 substrates would exhibit high M/S ratio (greater than diffusion alone).

Pantoprazole (PAN) is a proton pump (H\(^{+}\)/K\(^{-}\)-ATPase) inhibitor that binds specifically and irreversibly to the proton pump to reduce gastric acid secretion (McDonagh et al., 2009). PAN has been used as an inhibitor and a substrate of murine Bcrp1 (Breedveld et al., 2004). A case report described an M/S value of 0.022 for racemic PAN. Although the clinical relevance of this finding is not definitive, PAN use in lactating women was regarded as safe given the low infant dose exposure (Plante et al., 2004). From a mechanistic perspective, the observation of such a low M/S ratio for an ABCG2 substrate is unclear and would seem to contradict the hypothesis that all such substrates would accumulate in milk. Perhaps PAN is not as good a substrate for human ABCG2 as was first thought, and hence, diffusion and PAN extensive serum protein binding dominate the milk to serum ratio. Another confounding factor is that PAN is marketed as a racemate, and the authors of the case report used did not measure individual isomers. PAN has been shown to undergo enantioselective hepatic metabolism in both humans and rats (Tanaka et al., 2001; Xie et al., 2005). Poor metabolizers of S-mephentoin (a CYP2C19 substrate) were less able to metabolize (+)-PAN than (-)-PAN (Tanaka et al., 2001). A marked difference in the interaction of PAN isomers with ABCG2 could be playing a role in its limited M/S ratio.

It is estimated that approximately half of marketed therapeutic agents are chiral, with most available as 50:50 mixtures of their enantiomeric forms (Andersson and Weidolf, 2008). The stereoselective metabolism as well as genetic polymorphisms of cytochrome P450 and ABC transporter proteins can significantly affect drug disposition and pharmacodynamics in vivo. ABCG2 is responsible for stereoselective accumulation of drugs in milk, but its role in the interaction of enantiomers of drugs in vivo is not fully understood. To date, only a few reports have been available on the stereoselective interaction of drugs with ABCG2 in vivo, and the outcomes are not consistent with evidence in humans. Although it is estimated that approximately half of marketed therapeutic agents are chiral, there have been few reports of stereoselective interactions with ABCG2. The purpose of this study was to investigate the interaction of pantoprazole (PAN) isomers with Abcg2 in vitro and in vivo experiments. Pantoprazole isomer flux was characterized using Abcg2-Madin-Darby canine kidney II (MDCKII) cells in Transwell plates. In a crossover design, Sprague-Dawley lactating rats were used to study PAN accumulation in milk after an intravenous infusion of pantoprazole mixture in the presence/absence of Abcg2 inhibitor [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 (GF120918)]. Samples were analyzed by high-performance liquid chromatography/liquid chromatography-mass spectrometry. The results indicated that pantoprazole isomers were transported in an identical fashion in vector-MDCKII cell lines, whereas a significant difference in flux was observed in Abcg2-MDCKII cell line. The administration of GF120918 slightly increased the concentration of both isomers in serum, but no statistical difference was observed. However, the systemic clearance of (+)-PAN (0.57 ± 0.1) was larger than (-)-PAN (0.44 ± 0.12) (P < 0.01). Milk to serum ratio (M/S) of (-)-PAN (1.36 ± 0.20) was 2.5-fold greater than that of (+)-PAN (0.54 ± 0.09) (P < 0.01). Administration of GF120918 decreased M/S of (-)-PAN to 0.50 ± 0.08 (P < 0.001) and (+)-PAN to 0.38 ± 0.07 (P > 0.05). In conclusion, Abcg2, which is responsible for differential accumulation in milk, interacts stereoselectively with PAN isomers. Stereoselective transport of ABCG2 may have broader consequences in drug disposition.

**ABBREVIATIONS:** ABCG2, ATP binding cassette transporter isoform G member 2; PAN, pantoprazole; MDCK, Madin-Darby canine kidney; P-gp, P-glycoprotein; rAbcg2, rat Abcg2; 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; HPLC, high-performance liquid chromatography; LC-MS, liquid chromatography-mass spectrometry; DMSO, dimethyl sulfoxide; ER\(_{50}\), asymmetrical efflux ratio; M/S, milk to serum ratio; W/S, whole to skim milk ratio.
P450 and receptors can influence pharmacokinetics, pharmacodynamics, and toxicity (Katsuki et al., 2001; Tateishi et al., 2008; Miura and Uno, 2010). These differentiated interactions may be especially critical for those drugs that have a narrow therapeutic index (e.g., warfarin). It is remarkable that there are only limited reports of stereoselective interaction of drugs with transporters. Cetirizine exhibited moderate enantioselectivity in the absorptive and secretory flux across CaCo-2 monolayers, which was attributed to the interaction of the enantiomers with P-gp and multidrug resistance protein 2 (He et al., 2010). Several in vitro and in vivo studies suggest an enantioslective drug transport at the human blood-brain barrier and implicate P-gp (Choong et al., 2010). However, it should be noted that this is not a universal observation. No evidence was observed for stereoselective P-gp-mediated transport of S- and R-enantiomers of venlafaxine and its major metabolites into murine brain (Karlsson et al., 2010). There are no reports of individual PAN isomers interacting with ABCG2 or any other transporter. There is but a single report of stereoselective drug accumulation in milk. The M/S ratio for the R-enantiomers of fluoxetine (and its major metabolite, norfluoxetine) was 50% (40%) higher than the S-enantiomers in lactating women (Kim et al., 2006). The authors speculated this stereoselective distribution into milk was attributed to differences in the extent of protein binding of the enantiomers in milk compared with plasma (Kim et al., 2006).

We previously have used Abcg2 expressed in a Madin-Darby canine kidney (MDCK) II cell line and a chemical knockout Abcg2 rat model (Abcg2 inhibited by GF120918) to examine the role of Abcg2 in drug accumulation in milk (Wang et al., 2008). The purpose of this article was to establish whether any stereoselective differences of PAN isomers with rat Abcg2 (rAbcg2) are manifested in the accumulation of PAN isomers in rat milk. The observations made in this article prompted further detailed in vitro characterization of PAN flux in the companion article (Wang et al., 2012).

**Materials and Methods**

**Chemicals.** Protonix I.V. (pantoprazole sodium, 50:50 stereo mixtures) was obtained from Wyeth Pharmaceuticals Inc. (Princeton, NJ), and zonisamide sodium was purchased from Sigma–Aldrich (St. Louis, MO). The isomers of PAN were obtained from Altana Pharma AG (Konstanz, Germany). GF120918 was a gift from GlaxoSmithKline (Research Triangle Park, NC). All of the organic solvents [high-performance liquid chromatography (HPLC) grade] were purchased from Thermo Fisher Scientific (Waltham, MA).

**Animals.** Five adult female lactating Sprague-Dawley rats (250–350 g) with 1- or 3-day-old pups were purchased from Harlan Laboratories (Indianapolis, IN). Animals were maintained under a 12-h light/dark cycle and had access to food and water during the experiments. The rats were acclimatized for at least 1 week before the experiment. All of the procedures were approved by the University of Kentucky Institutional Animal Care and Use Committee.

**Flux Study.** Rat Abcg2 and empty vector (pcDNA3.1) expressed in MDCKII cells has been established in our laboratory (Wang et al., 2008). Cells were seeded on microporous membrane filters [3-μm pore size, 24-mm diameter; Transwell 3414; Corning Life Sciences, Lowell, MA] at a density of 1.0 × 10⁴ cells/well. Cells were grown for 4 days to achieve transepithelial electrical resistance >200 Ω·cm², and medium was replaced every other day. Before the experiment, the medium at both the apical and basolateral side of the monolayer was replaced with 2 ml of Opti-MEM I medium (Invitrogen, Carlsbad, CA) without serum. The apical or basolateral side was loaded with 25 μM if either (+) or (−)PAN isomer containing 0.2 μCi/ml [3H]mannitol (PerkinElmer Life and Analytical Sciences, Waltham, MA). Cells were incubated at 37°C in 5% CO₂. To assess tight junctions of each monolayer, 50-μl aliquots were collected to assess the paracellular flux of [3H]mannitol into the opposite compartment. For PAN isomers transport, 140-μl aliquots were taken at 1, 2, 3, and 4 h. Samples were stored at −80°C until the time of analysis.

**In Vitro M/S.** The contribution of passive diffusion on the M/S of PAN was estimated in vitro using eq. 1 (Fleishaker et al., 1987; Fleishaker and McNamara, 1988):

\[
\frac{M}{S} = \frac{f^u}{f^s} \cdot \frac{f^s}{f^u} \cdot \frac{W}{S}
\]

where \( f^u \) is the un-ionized fraction of drug in serum or milk, \( f^s \) is the free fraction of drug in serum or milk, W/S is the ratio of the drug concentration in whole milk to the drug concentration in skim milk, and the subscripts s and m represent serum and milk, respectively.

The W/S ratios, a measure of the partitioning of PAN isomers into milk fat, were determined following LC-MS analysis of PAN isomer concentrations in whole milk and skim milk into which isomer had been added. The un-ionized fraction in serum and skim milk, a measure of the pH partitioning of drug between serum and milk, was calculated from the pKₐ of each isomer and from the pH of serum, pH 7.45, and milk, pH 6.8 (Alcorn and McNamara, 2002).

The unbound fractions of PAN isomers in milk and serum were determined by equilibrium dialysis of these fluids against a 0.133 M phosphate buffer (milk, pH 6.8; serum, pH 7.45) in Plexiglas dialysis cells (single place, 0.5-mL cells). After 8 h equilibrium at 37°C, samples were taken from both donor and recipient sides. These concentrations were determined by LC-MS.

**In Vivo Studies.** The jugular and femoral veins of Sprague-Dawley lactating female rats were cannulated under ketamine/xylazine anesthesia on days 10 to 12 postpartum. A randomized crossover study design was used. Animals were randomized to receive a bolus pretreatment of GF120918 (10 mg/kg i.v. in DMSO) or with equivalent volume of DMSO (approximately 0.15 ml i.v.) administered 10 min before an intravenous infusion of PAN in saline (0.4 mg/ml per hour) for 8 h. All animals were crossed over on the 2nd day to complete both phases of pretreatment (Wang et al., 2008). One milliliter of normal saline was given hourly by intravenous infusion to prevent dehydration during infusion. The dams were separated from the pups 4 h before the infusion. Blood samples were drawn every 2 h during the infusion. The blood samples were protected from light in separator tubes (BD Microtainer; BD Biosciences, Franklin Lakes, NJ), centrifuged to harvest serum, and frozen at −80°C until analysis. These differentiated interactions may be especially critical for those drugs that have a narrow therapeutic index (e.g., warfarin). These observations made in this article prompted further detailed in vitro characterization of PAN flux in the companion article (Wang et al., 2012).
MS/MS spectra were obtained for selected precursor ions through incidental collision with neutral gas molecules (argon) at 2.0 mTorr in the collision cell of the mass spectrometer. The collision energy was set at 8.50 V. The parameters of the selected reaction monitoring transitions for the [M + H]$^+$ to selected product ions were optimized with the following typical values for the analytes and internal standard: PAN m/z 384 to 200 and the internal standard zonisamide m/z 213 to 132. The LC conditions were as follows. The column was a Lux 5-μm Amypure-2 150 × 4.60-mm Chiral column (Phenomenex). The initial mobile phase consisting of acetonitrile: water: formic acid (15:85: 0.02%) was delivered by dual ProStar 210 pumps (Varian, Inc.) at a flow rate of 0.3 ml/min. A gradient program was used; the acetonitrile phase was increased to 90% over 12 min and held constant for an additional 6 min. The acetonitrile phase was returned to 15% over 1 min and held constant for an additional 11 min for a total run time of 30 min. The retention times of (+)PAN, (-)PAN, and zonisamide were approximately 12.04, 13.05, and 12.01 min (Fig. 2). The standard curve was linear from 80 to 5000 ng/ml in serum, 10 to 5000 ng/ml in milk, and 25 to 1000 ng/ml in phosphate buffer. All of the standard curves showed an intraday and interday variability of <10% and $r^2 > 0.99$. Twenty microliters of the sample was injected onto the LC-MS.

Serum and milk concentrations of PAN were quantified according to standard procedures.

Pharmacokinetic Calculations. Systemic clearance was calculated using the following formula:

$$\text{Cl}_{s} = \frac{\text{Infusion rate}}{C_s}$$  \hspace{1cm} (2)

where $C_s$ is the average concentration of PAN in serum from 2 to 8 h. M/S was calculated by:

$$\frac{M}{S} = \frac{C_M}{C_s}$$  \hspace{1cm} (3)

where $C_M$ is concentration of PAN in milk at 8 h.

Statistical Analysis. Unless otherwise noted, all of the data are expressed as mean ± S.D. Asymmetrical efflux ratio (ER$_a$) and steady-state concentrations for in vitro flux studies as well as the protein binding and whole to skim milk ratio (W/S) data were analyzed by unpaired $t$ test. A $P$ value of <0.05 was considered statistically significant. Analysis of CIs and M/S in this structured repeated measurements design (isomer, treatment, and the interaction) was performed using SAS 9.2 PROC MIXED (SAS Institute, Cary, NC).

**Results**

**Pantoprazole Isomers Transport in Rat Abcg2 Expressed in MDCKII Cell Line.** The expression level and function of rat Abcg2-MDCKII were confirmed by Western blot, flow-cytometry, and nitrofuranoin transport in previous studies (Wang et al., 2008). At 25 μM, the flux for both isomers and in both directions approached steady-state after 3 h in empty vector and Abcg2-MDCKII cells. Initial rates were calculated during the 1st h. The flux for both PAN isomers in both directions was identical in empty MDCKII cells (Fig. 1A). The ER$_a$ of (+) and (-)PAN in the parent cell line at the 1-h time point were 0.90 and 0.95, respectively. In the Abcg2- MDCKII cell line, the initial flux rate for basolateral to apical flux was considerably greater than the flux in the opposite direction. The mass transport of (+)PAN from basolateral to apical side across monolayer seemed greater than (-)PAN (Fig. 1B), whereas the flux from apical to basolateral was greater for the (-)PAN relative to the (+)PAN. The ER$_a$ values as defined (Kalvass and Pollack, 2007) for (+) and (-)PAN at the 1-h time point were different ($P < 0.01$) and were 5.15 and 1.51, respectively, in Abcg2-MDCKII cell line. These stereoselective differences in initial rates were maintained as the profiles approached steady state of (+) and (-)PAN were 3.82 and 1.77 ($P < 0.01$), respectively.

Predicted M/S Ratios of Pantoprazole Isomers. Table 1 contains individual parameter estimates that contribute to the diffusion model prediction of M/S of the PAN isomers (Fleishaker et al., 1987). The un-ionized fraction approximated to 1 for both PAN isomers because their $pK_a$ (3.9) was considerably lower than the pH of both rat milk and serum. The free fractions of PAN isomers were determined by equilibrium dialysis using serum concentrations obtained in rat infusion study. The serum-free fraction of (+)PAN, (-)PAN, and zonisamide were approximately 12.04, 13.05, and 12.01 min (Fig. 2). The standard curve was linear from 80 to 5000 ng/ml in serum, 10 to 5000 ng/ml in milk, and 25 to 1000 ng/ml in phosphate buffer. All of the standard curves showed an intraday and interday variability of <10% and $r^2 > 0.99$. Twenty microliters of the sample was injected onto the LC-MS. Serum and milk concentrations of PAN were quantified according to standard procedures.

**Table 1**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(+)PAN</th>
<th>(-)PAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction unbound serum, %$^a$</td>
<td>2.33 ± 0.51</td>
<td>2.38 ± 0.74</td>
</tr>
<tr>
<td>Fraction unbound milk, %$^a$</td>
<td>26.0 ± 2.7</td>
<td>27.1 ± 7.3</td>
</tr>
<tr>
<td>Whole to skim milk ratio$^b$</td>
<td>1.35</td>
<td>1.3</td>
</tr>
<tr>
<td>Predicted M/S (eq. 1)</td>
<td>0.12</td>
<td>0.11</td>
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$^a$ Mean ± S.D. (n = 4).

$^b$ Single determination from a pooled sample.
than did \((-\)PAN \((0.44 \pm 0.12)\). The two-way analysis of variance indicated a significant isomer and treatment effect but no interaction.

At 8 h, PAN isomers were assumed to be at steady state and were distributed differently into milk in the rat. The milk concentration of \((-\)PAN \((2.26 \pm 0.84 \mu g/ml)\) was considerably higher than \((+)PAN \((0.64 \pm 0.16 \mu g/ml)\). The milk concentrations were lower for both isomers, and the difference between the isomers diminished \([-\)PAN \((1.10 \pm 0.33 \mu g/ml)\) and \((+)PAN \((0.53 \pm 0.16 \mu g/ml)\)] after GF120918 administration. The M/S ratios for PAN isomers are shown in Fig. 4, C and D. In control (DMSO) group, M/S ratio of \((-\)PAN was 2.5 times that of \((+)PAN. Administration of GF120918 decreased M/S ratios of \((+)PAN from 0.54 \pm 0.09 to 0.38 \pm 0.07 and \((-\)PAN from 1.36 \pm 0.20 to 0.50 \pm 0.08. The two-way analysis of variance indicated a significant isomer and treatment effect, as well as a significant interaction between isomer and treatment.

\[\text{Fig. 2. Enantiomeric separation of PAN (156 ng/ml) and internal standard zonisamide (4 \mu g/ml) using a triple quadrupole mass spectrometer with an electrospray ionization source and a chiral column while monitoring selected product ions for PAN m/z 384 to 200 and the internal standard zonisamide m/z 213 to 132. A and C, chromatograms depicting the internal standard in PAN serum standard (156 ng/ml) and blank serum, respectively. B and D, chromatograms of PAN isomers in a PAN serum standard (156 ng/ml) and blank serum (lower attenuation), respectively.}\]

\[\text{Fig. 3. PAN isomer concentrations (mean \pm S.D., n = 5) in serum in lactating Sprague-Dawley rats at 2-, 4-, 6-, and 8-h infusions (0.4 mg/ml per hour infusion of racemic pantoprazole) 10 min after administration of dosing vehicle alone (DMSO) or GF120918 (10 mg/kg). Linear regression of serum concentration versus time showed that the slope was not significantly different from zero \((P > 0.05)\), indicating that steady state was achieved (2–8 h). Milk concentrations (mean \pm S.D., n = 5) at the end of the infusion are also shown (offset in time by 0.3 h for better visualization).}\]
The current article demonstrated that Abcg2 interacts stereoselectively with PAN isomers in both in vitro and in vivo studies, which resulted in a differential accumulation of substrate isomers in milk. The M/S of (−)PAN was almost three times that of (+)PAN in control groups, and the M/S of both isomers converged to a similar value following the administration of GF120918. Because P-glycoprotein is not expressed in lactating rat epithelium (Wang et al., 2008), this observation would indicate that Abcg2 contributes to PAN distribution in rat milk, with Abcg2 serving as a major determinant for (−)PAN accumulation.

ABCG2 is a widely distributed efflux transporter and plays an important role in absorption, distribution, metabolism, and excretion of drugs, and there is a growing concern regarding the role of ABCG2 in drug-drug interactions as well (Giacomini et al., 2010; Huang and Woodcock, 2010). ABCG2 has been found on the apical surface of lactating mammary epithelial cells (Jonker et al., 2005), and a number of investigators have demonstrated the importance of this transporter in the accumulation of drugs in milk (Jonker et al., 2005; Perez et al., 2009). An overarching hypothesis would hold that ABCG2 substrates accumulate in milk, resulting in relatively high M/S ratios, greater than that predicted by simple diffusion.

A clinical case report of a low M/S for PAN (Plante et al., 2004) would seem to counter this hypothesis given that PAN is thought of a good ABCG2 substrate (Breedveld et al., 2004). The reason for the low M/S is unclear. PAN is highly protein bound (>98%) in human serum (Andersson and Weidolf, 2008) and rat serum (Xie et al., 2005). Data in the current work confirm that the protein binding of both PAN isomers in rat serum is extensive and may play some role in mitigating the contribution of rAbcg2-related accumulation in milk. PAN has a modest octanol to water partitioning, and hence, the milk fat partitioning is higher (reflected in a higher W/S value) than many drugs, which should result in a higher M/S. The un-ionized fractions for the two isomers were assumed to be identical and estimated to be very low and similar for skim milk and serum, hence, no ion trapping influence on M/S. Therefore, the overall M/S ratios predicted by diffusion for both isomers were low and identical. The observed M/S in rat were 4.4- ([+]PAN] and 12.5-fold [−]PAN] higher than predicted by diffusion, clearly suggesting a role of rAbcg2 in PAN accumulation in milk. The observed M/S ratios of (+)PAN and (−)PAN in the rat were 20 to 60 times higher than the M/S value reported for one lactating woman (Plante et al., 2004). The explanation for the low human M/S remains unclear.

Our in vitro experiment demonstrated a rAbcg2-mediated, apically directed transport of PAN and indicated that PAN is a substrate of rAbcg2, which is consistent with the reports for human and mouse ABCG2/Abcg2 (Breedveld et al., 2004, 2005). The flux of the (+)PAN was greater than the flux of the (−)PAN isomer at 25 μM (Fig. 1B). Hence, one might conclude that the (+)PAN is a better substrate than (−)PAN which, would contradict the in vivo M/S result. To rationalize this apparent contradiction, a more detailed in vitro experiment of PAN transport using a range of concentrations was performed (Wang et al., 2012). These result suggests that the (−)PAN isomer is, in fact, the better ABCG2 substrate, possessing a greater affinity and overall apical efflux permeability for rAbcg2. The greater flux of (+)PAN (Fig. 1B) arises due to the interplay between the two membranes (basolateral and apical) in determining the overall flux. At lower concentrations, PAN (both isomers) flux across the apical membrane (rAbcg2 mediated) is so rapid that the overall rate-limiting step is diffusion across the basolateral membrane. At 25 μM concentration, (−)PAN isomer saturates rAbcg2 at the apical membrane, slowing its overall flux across the monolayer, leading to the appearance of a more rapid flux for (+)PAN (Fig. 1). A more comprehensive analysis is presented in the companion article (Wang et al., 2012).

In the chemical Abcg2 knockout rat model, coadministration of GF120918 decreased the systemic clearance of nitrofurantoin (Wang et al., 2008). In the present study, (−)PAN had a lower systemic clearance via the rAbcg2 route than (+)PAN.
clearance compared with (+)PAN and was consistent with the literature (Masubuchi et al., 1998). The administration of GF120918 had a modest effect on PAN isomer systemic clearance, which would indicate that neither Abcg2 nor P-glycoprotein (also inhibited by GF1209018) plays a major role in the systemic clearance of PAN. The systemic clearance of PAN in humans is largely determined by hepatic metabolism of PAN (Huber et al., 1996).

Stereochemistry is an important aspect of biology and plays a role in many aspects of drug disposition (Bhatia et al., 2008); yet, there are few studies examining stereoselective transport of drugs (He et al., 2010; Choong et al., 2010). PAN has been used as a competitive ABCG2 inhibitor to identify other ABCG2 substrates or to study drug-drug interaction. PAN is marketed as a racemic mixture whose stereoselective disposition has been characterized (Masubuchi et al., 1998; Tanaka et al., 2001). However, the interaction of its isomers with transporters (e.g., ABCG2) has not been described. The current work clearly indicates stereoselectivity in the flux of PAN. The complexity of interpreting the in vitro flux data (Wang et al., 2012) might suggest that stereoselectivity may be overlooked in other transporter studies.

In conclusion, the present work has demonstrated a clear difference in the in vivo transport of the isomers of PAN by rAbcg2 responsible for a stereoselective difference in the accumulation of (+)PAN in milk. The current work also supports the hypothesis that ABCG2 substrates will accumulate in milk but does not explain the previous clinical observation of a low M/S ratio. Additional studies will be needed to clarify this apparent species difference.

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Authorship Contributions

Participated in research design: Wang and McNamara. Conducted experiments: Wang. Contributed new reagents or analytic tools: Wang. Performed data analysis: Wang and McNamara. Wrote or contributed to the writing of the manuscript: Wang and McNamara.

References


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