Title: P-glycoprotein plays a major role in the efflux of fexofenadine in the small intestine and blood-brain barrier, but only a limited role in its biliary excretion.

Authors:
Harunobu Tahara, Hiroyuki Kusuhara, Eiichi Fuse, and Yuichi Sugiyama

Graduate School of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo, Japan (H.T., H.K., and Y.S.)
Pharmacokinetic Research Laboratories, Pharmaceutical Research Institute, Kyowa Hakko Kogyo Co., LTD., Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka, Japan (H.T. and E.F.)
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Biliary excretion of fexofenadine

Corresponding author:
Yuichi Sugiyama, Ph.D., Professor
Graduate School of Pharmaceutical Sciences
University of Tokyo
Hongo, Bunkyo-ku,
Tokyo, 113-0033, Japan
Phone: +81-3-5841-4770
Fax: +81-3-5841-4766
Email: sugiyama@mol.f.u-tokyo.ac.jp

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Abbreviations: P-gp, P-glycoprotein; Bcrp, breast cancer resistance protein; Mrp2, multidrug resistance-associated protein 2; EHBR, Eisai hyperbilirubinemic rats; SDR, Sprague-Dawley rats; PhIP, 2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine
Abstract

Fexofenadine is a selective, non-sedating H1-receptor antagonist approved for symptoms of allergic conditions, which is mainly excreted into feces via biliary excretion. The purpose of this study is to investigate its pharmacokinetics in mice and rats to determine the role of P-glycoprotein in its biliary excretion. In mice, biliary excretion clearance (17 ml/min/kg) accounted for almost sixty-percent of the total body clearance (30 ml/min/kg). Comparing the pharmacokinetics after intravenous and oral administration indicated that the bioavailability of fexofenadine was at most 2% in mice. Knockout of Mdr1a/1b P-gp did not affect the biliary excretion clearance with regard to both plasma and liver concentrations, whereas the absence of P-gp caused a 6-fold increase in the plasma concentration after oral administration. In addition, the steady-state brain-to-plasma concentration ratio of fexofenadine was approximately 3-fold higher in Mdr1a/1b P-gp knockout mice than in wild-type mice. Taken together, these results show that P-glycoprotein plays an important role in efflux transport in the brain and small intestine, but only a limited role in biliary excretion in mice. In addition, there was no difference in the biliary excretion between normal and hereditarily Mrp2 deficient mutant rats (Eisai hyperbilirubinemic rats), and between wild-type and Bcrp knockout mice. These results suggest that the biliary excretion of fexofenadine is mediated by unknown transporters distinct from P-gp, Mrp2 and Bcrp.
Introduction

Fexofenadine is an orally-active non-sedating histamine H1-receptor antagonist that is prescribed for oral treatment of allergic rhinitis and chronic idiopathic urticaria. After oral administration of \[^{14}C\]fexofenadine, the radioactivity was recovered in the urine (2.5%), bile (28%) and feces (69%) of rats whose bile duct had been cannulated and, thus, its oral absorption is at most 30%, and biliary excretion likely plays a major role in its elimination (Common Technical Document for the Registration of Pharmaceuticals for Human Use).

It has been suggested that transporters play important roles in the disposition of fexofenadine. Fexofenadine has been shown to be a substrate of P-glycoprotein (P-gp) (Perloff et al., 2002; Cvetkovic et al., 1999). Vectorial transport of fexofenadine in the basal-to-apical direction was observed in Caco-2 cells, and this was inhibited by inhibitors of P-gp, such as ritonavir and verapamil (Perloff et al., 2002). Transfection of MDR1 P-gp cDNA into LLC-PK1 cells increased the vectorial transport of fexofenadine in the basal-to-apical direction (Cvetkovic et al., 1999). Furthermore, knockout of Mdr1a P-gp caused a significant increase in the plasma concentration of fexofenadine at 4 h after intravenous- and oral administration. In addition, among the tissues examined, the brain-to-plasma concentration ratio of \[^{14}C\]-fexofenadine compared with that in wild-type mice was also increased (Cvetkovic et al., 1999). Therefore, P-gp limits the bioavailability, and brain uptake of fexofenadine. It has been reported that non-sedating H1-receptor antagonists, such as loratadine, cetiridine, and desloratadine, are P-gp substrates, and their distribution in the brain is clearly increased in Mdr1a/1b P-gp knockout mice compared with wild-type, whereas there was no increases in the brain concentration of sedating H1-receptor antagonists (Chen et al., 2003). It is likely that P-gp acts as gatekeeper against non-sedating H1-receptor antagonists to prevent their adverse effects in the central nervous system (Chen et al., 2003). Fexofenadine is also transported by human and rat organic anion transporting polypeptides; OATP-A and OATP-B in humans, and Oatp1, Oatp2 and Oatp3 in rats (Cvetkovic et al., 1999; Dresser et al., 2002). These transporters have been proposed as candidate transporter involved in the hepatic and intestinal uptake of fexofenadine.
Especially, grapefruit juice reduced the bioavailability of fexofenadine, and this interaction has been proposed to be caused by an inhibition of intestinal uptake process mediated by OATP-B (Dresser et al., 2002, Nozawa et al., 2004).

Co-perfusion of erythromycin caused a reduction in the biliary excretion clearance (Milne et al., 2000). Since erythromycin did not affect the liver/perfusate concentration ratio of fexofenadine, but increased the bile/liver concentration ratio (Milne et al., 2000), the effect is ascribed to an inhibition of biliary excretion. Taking into consideration the fact that fexofenadine is a substrate of P-gp, it is possible that the biliary excretion of fexofenadine is mediated by P-gp. The primary purpose of the present study is to examine the involvement of P-gp in the biliary excretion of fexofenadine. In vivo pharmacokinetic studies were carried out under steady-state conditions using Mdr1a/1b double knockout mice. Export of xenobiotic compounds across the canalicular membrane is carried out by a number of transporters. In addition to P-gp, there are two other ABC transporters; multidrug resistance associated protein 2 (Mrp2/Abcc2) and breast cancer resistance protein (BCRP/ABCG2). It is generally accepted that Mrp2 plays a major role in the biliary excretion of various kinds of glutathione- and glucuronide conjugates, and non-conjugated amphipathic organic anions (Suzuki and Sugiyama, 2002). Bcrp/BCRP is an ATP-binding cassette half-transporter originally identified in mitoxantrone-resistant tumor cells (MCF-7) (Doyle et al., 1998; Ross et al., 1999). Recently, it was shown that murine Bcrp is involved in the hepatobiliary excretion of a food-derived carcinogen, PhIP, and its biliary excretion was markedly reduced in Bcrp knockout mice (van Herwaarden et al., 2003). These transporters may be involved in the biliary excretion of fexofenadine. Therefore, their involvement was also investigated using knockout mice, and Mrp2 deficient mutant rats (Eisai Hyper Biliurbinemic rats; EHBR).
Methods

Materials

Fexofenadine hydrochloride was purchased from Toronto Research Chemicals (North York, ON, Canada). All other chemicals and reagents were obtained from Kanto Kagaku (Tokyo, Japan) or Wako Pure Chemicals (Osaka, Japan) and were of the highest grade available.

Animals

Male FVB mice (wild-type) and Mdr1a/1b P-gp knockout mice were obtained from Taconic Farms (Germantown, NY). Male Bcrp knockout mice were previously established by Jonker et al (2002), and were a kind gift from Dr. A H Shinkel. Male SD rats and EHBR were purchased from SLC (Shizuoka, Japan) and maintained under standard conditions with a reverse dark-light cycle. Food and water were available ad libitum.

Intravenous infusion

Mice of both strains, weighing approximately 25-35 g, were used throughout the experiments. Under anesthesia with pentobarbital sodium (Nembutal, Dainippon Pharmaceutical, Osaka, Japan), the tail vein was cannulated with a needle catheter (30G) for the injection of fexofenadine. The bile duct was cannulated with a polyethylene catheter (SP-30) for bile collection after ligation of the gall bladder. The mice received a constant infusion of fexofenadine at a dose of 0.55-0.91 µmol/h/kg (approximately 100 µg/mL in saline) after an intravenous bolus injection at a dose of 1 mg/kg (Harvard Apparatus Syringe Infusion Pump, Harvard Apparatus, South Natick, MA). Since mice were anesthetized throughout the experiment, they were kept warm with a hot plate (Natsume Seisakusyo, Tokyo Japan). Bile was collected in preweighed test tubes at 30 min intervals throughout the experiment. The bile flow rate did not change throughout the experiment during the infusion of fexofenadine. Blood samples (approximately 50 µL) were collected from the fundus oculi at 75, 105, 135 and 150 min after starting the infusion. Plasma
was prepared by centrifuging the blood samples (3000 x g). The mice were sacrificed after 150 min and the entire liver and brain were excised immediately. The tissues were weighted and stored at –80 °C until required for assay.

SDR and EHBR, weighing approximately 300-350 g, were used throughout the experiments. Under anesthesia with pentobarbital sodium, the femoral vein was cannulated with a polyethylene catheter (PE-50) for the injection of fexofenadine. The bile duct was cannulated with a polyethylene catheter (PE-10) for bile collection. The rats received constant infusion of fexofenadine at a dose of 1.1-1.2 µmol/h/kg (approximately 100 µg/mL in saline) after an intravenous bolus injection at a dose of 1 mg/kg. Since rats were anesthetized throughout the experiment, they were kept warm with a hot plate. Bile was collected in preweighed test tubes at 30 min intervals throughout the experiment. The bile flow rate did not change throughout the experiment during the infusion of fexofenadine. Blood samples (approximately 100 µL) were collected from the tail vein at 105, 135, 165 and 215 min after starting the infusion. Plasma was prepared by centrifuging the blood samples (3000 x g). The rats were sacrificed after 215 min and the entire liver were excised immediately. The tissues were weighted and stored at –80 °C until required for assay. The protocols for the animal experiments were approved by Tokyo University Animal Care Committee.

**Oral administration**

Both wild-type and Mdr1a/1b P-gp knockout mice, weighing approximately 27-31 g were used throughout the experiments. The mice received a 10 mg/kg oral dose of fexofenadine (1 mg/mL in 0.5%vol Methylcellulose, Wako Pure Chemicals). Blood samples (approximately 30 µL) were collected from the tail vein at 0.5, 1, 2, 4, 6, and 8 h after oral administration under light ether anesthesia. Plasma was prepared by centrifuging the blood samples (3000 x g). The plasma was stored at –80 °C until required for assay.
Kinetic Analysis

The steady-state plasma concentration (C_{ss}) was assessed as the mean plasma concentration at 75, 105, 135 and 150 min for mice, and 105, 135, 165, 195 and 205 min for rats, while the steady–state liver (C_{liver}) and steady–state brain (C_{brain}) concentrations were determined at 150 min for mice and 205 min for rats, respectively. The total plasma clearance (CL_{plasma}) was obtained by dividing the infusion rate by C_{ss}. The biliary clearance (CL_{bile, plasma}) gave the mean clearance value calculated by dividing the biliary excretion rates (V_{bile}) by C_{plasma}. CL_{bile, liver} was the biliary clearance based on the liver concentration, which was obtained by dividing the biliary excretion rates by C_{liver}. The Kp_{liver} and Kp_{brain} represented the ratio of C_{liver} to C_{ss} and C_{brain} to C_{ss}, respectively. The area under the plasma concentration-time curve from time 0 to infinity (AUC_{0\text{–}inf}) of fexofenadine was calculated using the linear trapezoidal rule. The plasma AUC beyond the last time-point to infinity was estimated by dividing the predicted plasma concentration at the last time-point by the calculated terminal elimination rate constant (ke). The ke was obtained from a log-linear regression analysis of the last 3 data points of the plasma concentration–time curve. The terminal elimination half-life (t_{1/2}) was obtained from ln2/ke.

Quantitation of fexofenadine in Plasma, Bile, Liver and Brain

Sample Pretreatment

Plasma (5 µL) was mixed with 15 µL of 0.05vol% formic acid and precipitated with methanol (40 µL) containing midazolam (500 ng/mL) as the internal standard (I.S.). Bile (5 µL) was mixed with 15 µL of 0.05vol% formic acid and precipitated with methanol (200 µL) containing I.S. After centrifugation (15000 x g, 10 min) of the mixture, 50 µL of 0.05% formic acid was added to the 50 µL of supernatant. Liver and brain were added to a three-fold volume of 80vol% methanol containing I.S. and homogenized with a handy type homogenizer (Dremel Multipro 395, Racine, WI, USA). After centrifugation (15000 x g, 10 min) of the homogenate, the supernatant was diluted with an equal volume of 0.05vol% formic acid. In all cases, an aliquot of the supernatant was transferred to a new tube after centrifugation. The
resulting supernatant fractions from plasma (10 µL), bile (5 µL), liver (10 µL) and brain (40 µL) were subjected to LC/MS analysis to determine the concentration of fexofenadine.

**LC/MS instrumentation and operating conditions**

A sensitive method was developed to determine fexofenadine in plasma, bile and tissues by HPLC-electrospray ionization mass spectrometry with midazolam as the internal standard. The LC-MS consisted of an Alliance™ HT 2790 separation module with an autosampler (Waters, Milford, MA) and micromass ZQ mass spectrometer with an electro ion spray interface (Waters). The optimum operating conditions used were as follows: electrospray probe (capillary) voltage 2.7 kV, sample cone voltage 35 V and source temperature 100°C. The spectrometer was operated at a drying desolvation gas flow-rate of 300 L/h. The mass spectrometer was operated in the selected ion monitoring mode using the respective MH+ ions, m/z 502.3 for fexofenadine and m/z 326.3 for the internal standard. The mobile phase used for HPLC was: (A) methanol and (B) 0.05% formic acid. Chromatographic separation was achieved on a C18 column (Capcell pak C18, MG, 3 mm, 4.6 mm I.D. x 75 mm, particle size 3 µ, Shiseido, Tokyo, Japan) using a linear gradient from 55% A to 70% A over 5 min and returning to 55% A within 2 min. The quantification limits of this method were 2-500 ng/mL in plasma, 5-200 µg/mL in bile, 0.5-10 µg/g in liver and 5-100 ng/g in brain, respectively. Instrument control and data analysis were performed using Mass Lynx application software from Waters.
Results

Steady-State Pharmacokinetics of Fexofenadine in Mdr1a/1b P-gp knockout mice

Plasma concentrations and biliary excretion rates of fexofenadine at steady-state in mdra1/1b knockout mice are shown in Figure 1. The pharmacokinetic parameters are summarized in Table 1. The plasma concentrations of fexofenadine reached steady-state within the 75 min during the constant infusion of fexofenadine to both strains of mice (Figure 1A). The total plasma clearance (CL<sub>plasma</sub>) of fexofenadine was similar in wild-type and Mdr1a/1b P-gp knockout mice, 30.1±2.4 versus 30.7±5.4 ml/min/kg, respectively. The biliary excretion clearance (CL<sub>bile,plasma</sub>) accounts for 56 and 72% of the total body clearance in wild-type and Mdr1a/1b P-gp knockout mice (Table 1), and was slightly greater in Mdr1a/1b knockout mice. The intrinsic biliary excretion clearance of fexofenadine with respect to its liver concentration (CL<sub>bile,liver</sub>) was similar for wild-type and Mdr1a/1b P-gp knockout mice (Table 1). A significant increase was observed in the K<sub>P,brain</sub> between wild-type (0.0394±0.0033 ml/g brain) and Mdr1a/1b P-gp knockout (0.130±0.021 ml/g brain) mice (p<0.01, Figure 1C).

Oral administration of Fexofenadine in Mdr1a/1b P-gp knockout mice

Plasma concentrations of fexofenadine after oral administration to Mdr1a/1b P-gp knockout and wild-type mice at a dose of 10 mg/kg are shown in Figure 2. The plasma concentration and AUC<sub>0-inf</sub> of fexofenadine were significantly increased in Mdr1a/1b P-gp knockout mice compared with wild-type mice after oral administration (p<0.01). The times to reach the peak plasma concentration were 1.50±0.71 and 4.0±0.0 h, and the peak serum concentrations (C<sub>max</sub>) were 73.6±5.9 and 303±71 nmol/L, for wild-type and Mdr1a/1b P-gp knockout mice, respectively. Knockout of Mdr1a/1b caused a 6-fold increase in the AUC<sub>0-inf</sub> from 243±37 to 1543±223 nmol·h/mL. The terminal half-lives in wild-type and Mdr1a/1b P-gp knockout mice were 1.49±0.21 h and 1.54±0.04 h, and this difference was not significant. The bioavailability calculated from the pharmacokinetic parameters obtained from in vivo studies was 2.38±0.37% and 15.5±2.2%, in wild-type and Mdr1a/1b P-gp knockout mice, respectively.
Steady-State Pharmacokinetics of Fexofenadine in EHBR

Plasma concentration and biliary excretion rates of fexofenadine at steady-state in EHBR are shown in Figure 3. The pharmacokinetic parameters are summarized in Table 2. The plasma concentrations of fexofenadine reached equilibrium at approximately 75 min during the constant infusion of fexofenadine to both normal rats and EHBRs (Figure 3A). The CL_{plasma} of fexofenadine in SDRs and EHBRs were 28.3±2.1 and 27.2±1.8 ml/min/kg, respectively, and the CL_{bile,liver} values were similar in SDRs and EHBRs (Table 2). Pravastatin was used as positive control, and its biliary clearance was markedly reduced in EHBRs compared with SDRs (data not shown).

Steady-State Pharmacokinetics of Fexofenadine in Bcrp knockout mice

Plasma concentrations and biliary excretion rate of fexofenadine at steady-state in Bcrp knockout mice are shown in Figure 4. The pharmacokinetic parameters are summarized in Table 3. The plasma concentration reached steady-state within approximately 75 min during the constant infusion of fexofenadine to both strains of mice (Figure 4A). The CL_{plasma} values of fexofenadine in wild-type and Bcrp knockout mice were 27.0±4.7 and 29.9±2.1 ml/min/kg, respectively. There was no statistical difference in the CL_{bile,plasma} or CL_{bile,liver} between wild-type and Bcrp knockout mice (Table 3). The Kp values in the liver and brain were also comparable in wild-type and Bcrp knockout mice (Figure 4C and D).
Discussion

In vivo steady-state pharmacokinetic analyses were carried out to determine quantitatively the importance of P-gp in the biliary excretion of fexofenadine at the canalicular membrane using Mdr1a/1b double knockout mice. An earlier study had been carried out in Mdr1a knockout mice (Cvetkovic et al., 1999). Knockout of Mdr1a causes absence of P-gp at the blood-brain barrier and small intestine, however it induces the expression of another isoform of Mdr1b P-gp in the liver and kidney (Schinkel et al., 1994). Therefore, the Mdr1a P-gp knockout mouse is not appropriate to investigate an involvement of P-gp in the biliary and urinary excretion.

The total body clearance and biliary clearance of fexofenadine with regard to the plasma concentration were determined in mice at steady-state conditions. The biliary excretion clearance of fexofenadine accounts for 50 to 70% of the total body clearance in both strains of mice, indicating the presence of another elimination pathways of fexofenadine in mice (Tables 1 and 3). According to the Common Technical Document for the Registration of Pharmaceuticals for Human Use, the recovery of the total radioactivity after intravenous administration of fexofenadine is 13% in rats. In addition, Kamath et al (2005) have reported that urinary recovery of intact fexofenadine was, at most, 15-20% after intravenous administration in rats. These report suggest that the urinary excretion accounts for limited part of the elimination pathway of fexofenadine from the body. Therefore, it is likely that the remained fraction will be accounted for by metabolic clearance in mice. Unexpectedly, the steady-state plasma concentration of fexofenadine was similar in wild-type and Mdr1a/1b P-gp knockout mice (Figure 1A). Since the total body clearance of fexofenadine is small enough compared with the hepatic blood-flow rate, the possibility that the hepatic clearance of fexofenadine is blood-flow limited can be excluded. The AUC after oral administration and $K_{p,\text{brain}}$ of fexofenadine was greater in Mdr1a/1b P-gp knockout mice (Figure 2). Furthermore, knockout of Mdr1a/1b did not affect the $K_{p,\text{liver}}$ or $CL_{\text{bile,liver}}$ of fexofenadine (Figure 1D and Table 1). Therefore, the increase in the AUC after oral administration caused by absence of P-gp is ascribed to an increase in intestinal absorption, but not to the reduction of hepatic clearance. These results suggest that P-gp plays an important role in the efflux transport of fexofenadine in the small intestine.
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The finding contradicts the previous report by Cvetkovic et al (1999) in which the plasma concentration of \[^{14}C\]fexofenadine was significantly greater in Mdr1a knockout mice after intravenous administration. There are two possibilities to account for this discrepancy. Enterohpetic circulation of fexofenadine is involved in the prolonged plasma elimination half-life due to increased oral absorption in P-gp knockout mice with intact biliary circulation. Since Cvetkovic et al determined the plasma concentration at 4 hr after administration, it may be possible that reabsorption of fexofenadine excreted into the intestine occurs. Alternatively, it is also possible that the total radioactivity in the plasma might include metabolites of fexofenadine as well as its intact form since Cvetkovic et al determined the plasma concentration by measuring the radioactivity without separation, and according to our kinetic study, the metabolic clearance accounts for a substantial fraction of the total body clearance (30~50%) at least, in mice (Table 1).

Fexofenadine was concentrated in the bile even compared with the total concentration in the liver, about 10-fold, and a similar value has been reported by Milne et al (2000). Absence of the effect of P-gp knockout on the biliary excretion of fexofenadine suggests an involvement of another efflux transporter(s). Two ABC transporters, Mrp2 and Bcrp, are alternative candidates, and their involvement was investigated using Mrp2 deficient mutant rats and Bcrp knockout mice. The kinetic parameters for the biliary excretion clearance (CL\text{plasma}, CL\text{bile,plasma} and CL\text{bile, liver}) of fexofenadine in EHBRs were comparable with those in SDRs between SDR and EHBR (Table 2). Similar results were obtained for the comparison of the kinetic parameters between wild-type and Bcrp knockout mice (Figure 3 and Table 3). Absence of Mrp2 and Bcrp did not affect the biliary excretion of fexofenadine. Finally, we were able to exclude the involvement of P-gp, Mrp2 and Bcrp in the biliary excretion of fexofenadine. Multiplicity of canalicular transporters has been proposed for the biliary excretion of pravastatin, telmisartan, E3040-glucuronide, E3040-sulfate, grepafloxacin, the taurine conjugate of Z-335 (Z-335-tau) and the active metabolite of prurifloxacin (Takenaka et al., 1995; Yamazaki et al., 1996; Sasabe et al., 1998; Nishino et al., 2000; Kawabata et al., 2004). Of these compounds, the biliary excretion of Z-335-tau has been suggested to be
mediated by unknown ABC transporter(s) (Kawabata et al., 2004). ATP-dependent uptake of Z-335-tau was still observed in the membrane vesicles from EHBR, and inhibited by digoxin P-gp inhibitor, taurocholate, estrone sulfate estradiol 17β glucuronide and bromosulfophthalein, although their inhibition constants were greater than their own Km values. In addition to Z-335-tau, neither Mrp2 nor P-gp is a major transporter governing the biliary excretion of the active metabolite of prurifloxacin although the brain distribution is limited by P-gp (Yagi et al., 2003). Further studies are required to clarify the multiplicity of canalicular transport mechanisms for xenobiotics.

The present study provides evidence that P-gp restricts the brain penetration and intestinal absorption, but it plays only a minor role in the pharmacokinetics of fexofenadine in blood in mice. In contrast to the observations in mice, it is unlikely that P-gp plays an important role in limiting the oral absorption in humans under normal conditions since verapamil-treatment did not affect the intestinal absorption rate (Tannergren et al., 2003), and the C3435T polymorphism, which is associated with lower intestinal P-glycoprotein expression, has no effect on the disposition of fexofenadine (Drescher et al., 2002). However, treatment of rifampicin (Hamman et al., 2001) or St John's wort (Wang et al., 2002) caused an increase in oral clearance, which may be accounted for by an induction of intestinal P-gp through a nuclear receptor, PXR (Geick et al., 2001). Under such circumstances, P-gp is likely to become one of determinant factors limiting the oral absorption of fexofenadine as observed in mice. Whether P-gp limits the brain uptake of fexofenadine in human remains unknown. Sadeque et al have reported an interaction between quinidine and loperamide, which resulted in loperamide-respiratory depression (Sadeque et al., 2000). This interaction has been interpreted as the inhibition of P-gp-mediated efflux of loperamide by quinidine at the BBB. Taking into consideration the fact that non-sedating H1-receptor antagonists are substrates of P-gp, it presumably acts as a gate keeper for fexofenadine and other non-sedating H1-receptor antagonists at the BBB.

In conclusion, P-gp are involved in the efflux transport of fexofenadine in the small intestine and BBB in mice, however, other unknown efflux transporters will be a determinant factor of the biliary
excretion.
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ATP-dependent transport of a novel thromboxane A2 receptor antagonist,


Legends for Figures

Figure 1. Plasma concentration-time profile (A), biliary excretion rate (B), brain to plasma (Kp\textsubscript{brain}, C) and liver to plasma (Kp\textsubscript{liver}, D) concentrations ratios of fexofenadine in Mdr1a/1b P-gp knockout and wild-type mice during the constant infusion of fexofenadine.

The infusion rates of fexofenadine in Mdr1a/1b P-gp knockout (open circle) and wild-type (closed circle) mice were 0.694 ± 0.01 and 0.805 ± 0.09 µmol/h/kg, respectively. Statistical differences in Mdr1a/1b P-gp knockout mice and wild-type mice were calculated by a two-sided unpaired Student’s \(t\)-test with \(p<0.05\) as the limit of significance (\(*p<0.05; **p<0.01\)). Each point represents the mean ± S.E. (n=3).

Figure 2. Plasma concentration-time profiles of fexofenadine after oral administration to Mdr1a/1b P-gp knockout (open circle) and wild-type mice (closed circle) at a dose of 10 mg/kg. Each point represents the mean±S.E. (n=3).

Figure 3. Plasma concentration-time profile (A) and biliary excretion rate (B) of fexofenadine during constant infusion to SDR and EHBR during the constant infusion of fexofenadine.

The infusion rates of fexofenadine in SDR (open circle) and EHBR (closed circle) were 1.08±0.02 and 1.14±0.07 µmol/h/kg, respectively. Each point represents the mean ± S.E. (n=4).

Figure 4. Plasma concentration-time profile (A), biliary excretion rate (B), brain to plasma (Kp\textsubscript{brain}, C) and liver to plasma (Kp\textsubscript{liver}, D) concentrations ratios of fexofenadine in Bcrp knockout and wild-type mice during the constant infusion of fexofenadine.

The infusion rates of fexofenadine in Bcrp knockout (open circle) and wild-type (closed circle) mice were 0.614±0.037 and 0.554±0.038 µmol/h/kg, respectively. Statistical differences between Bcrp knockout mice and wild-type mice were calculated by a two-sided unpaired Student’s \(t\)-test with \(p<0.05\) as the limit of significance (\(*p<0.05; **p<0.01\)). Each point represents the mean ± S.E. (n=3).
Table 1  Pharmacokinetic parameters of fexofenadine during constant infusion to Mdr1a/1b P-gp knockout and wild-type mice

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<td>Mdr1a/1b(+/+)</td>
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<td>10.8 ±1.1</td>
<td>30.1 ±2.4</td>
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<td>30.7 ±5.4</td>
<td>22.2* ±2.1</td>
<td>0.714 ±0.053</td>
<td>8.28 ±1.13</td>
<td>2.33</td>
</tr>
</tbody>
</table>

Data are expressed as mean±S.E.  (*Mdr1a/1b P-gp knockout mouse, wild-type mouse: n=3).  Statistical differences in Mdr1a/1b P-gp knockout mice and wild-type mice were calculated by a two-sided unpaired Student’s t-test with p<0.05 as the limit of significance (*p<0.05; **p<0.01).
Table 2  Pharmacokinetic parameters of fexofenadine during constant infusion to EHBR and SDR

<table>
<thead>
<tr>
<th></th>
<th>C_{ss}</th>
<th>C_{liver}</th>
<th>CL_{total}</th>
<th>CL_{bile,plasma}</th>
<th>CL_{bile,liver}</th>
<th>V_{bile}</th>
<th>Bile flow</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmol/L</td>
<td>nmol/L</td>
<td>mL/min/kg</td>
<td>mL/min/kg</td>
<td>mL/min/kg</td>
<td>nmol/min/kg</td>
<td>µL/min</td>
</tr>
<tr>
<td>SDR</td>
<td>680</td>
<td>13.5</td>
<td>28.3</td>
<td>11.4</td>
<td>0.537</td>
<td>7.73</td>
<td>22.6</td>
</tr>
<tr>
<td></td>
<td>±51</td>
<td>±1.4</td>
<td>±2.1</td>
<td>±1.6</td>
<td>±0.033</td>
<td>±0.78</td>
<td>±0.7</td>
</tr>
<tr>
<td>EHBR</td>
<td>675</td>
<td>13.2</td>
<td>27.2</td>
<td>10.7</td>
<td>0.496</td>
<td>7.27</td>
<td>14.5**</td>
</tr>
<tr>
<td></td>
<td>±56</td>
<td>±0.8</td>
<td>±1.8</td>
<td>±1.9</td>
<td>±0.027</td>
<td>±0.69</td>
<td>±0.6</td>
</tr>
</tbody>
</table>

Data are expressed as mean±S.E. (EHBR, SDR, n=4). Statistical differences between EHBRs and SDRs were calculated by a two-sided unpaired Student’s t-test with p<0.05 as the limit of significance (*p<0.05; **p<0.01).
Table 3  Pharmacokinetic parameters of fexofenadine during constant infusion to Bcrp knockout and wild-type mice

<table>
<thead>
<tr>
<th></th>
<th>C&lt;sub&gt;ss&lt;/sub&gt;</th>
<th>C&lt;sub&gt;liver&lt;/sub&gt;</th>
<th>CL&lt;sub&gt;total&lt;/sub&gt;</th>
<th>CL&lt;sub&gt;bile,plasma&lt;/sub&gt;</th>
<th>CL&lt;sub&gt;bile,liver&lt;/sub&gt;</th>
<th>V&lt;sub&gt;bile&lt;/sub&gt;</th>
<th>Bile flow</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmol/L</td>
<td>nmol/L</td>
<td>mL/min/kg</td>
<td>mL/min/kg</td>
<td>mL/min/kg</td>
<td>nmol/min/kg</td>
<td>µL/min</td>
</tr>
<tr>
<td>bcrp(+/+)</td>
<td>354 ±55</td>
<td>8.59 ±0.99</td>
<td>27.0 ±4.7</td>
<td>12.0 ±0.8</td>
<td>0.444 ±0.051</td>
<td>4.08 ±1.02</td>
<td>2.54 ±0.16</td>
</tr>
<tr>
<td>bcrp(-/-)</td>
<td>348 ±41</td>
<td>8.49 ±1.45</td>
<td>29.9 ±2.1</td>
<td>14.5 ±1.0</td>
<td>0.550 ±0.060</td>
<td>4.19 ±1.20</td>
<td>2.19 ±0.19</td>
</tr>
</tbody>
</table>

Data are expressed as mean±S.E. (Bcrp knockout mouse, wild-type mouse: n=3). Statistical differences between Bcrp knockout mice and wild-type mice were calculated by a two-sided unpaired Student’s-<i>t</i> test with <i>p</i>&lt;0.05 as the limit of significance (*<i>p</i>&lt;0.05; **<i>p</i>&lt;0.01).
Figure 1

A. Plasma concentration (nM) over time (min).

B. Biliary excretion rate (nmol/min/kg) over time (min).

C. Kp,brain comparison between wild and Mdr1a/1b KO.

D. Kp,liver comparison between wild and Mdr1a/1b KO.
Figure 2
Figure 3
Figure 4

A. Plasma concentration (nM) over time (min) for wild and Bcrp KO mice.

B. Biliary excretion rate (nmol/min/kg) over time (min) for wild and Bcrp KO mice.

C. Kp,brain for wild and Bcrp KO mice.

D. Kp,liver for wild and Bcrp KO mice.