EFFECT OF P-GLYCOPROTEIN ON INTESTINAL ABSORPTION AND BRAIN PENETRATION OF ANTIALLERGIC AGENT BEPOTASTINE BESILATE

Rikiya Ohashi, Yukari Kamikozawa, Mika Sugiura, Hajime Fukuda, Hikaru Yabuuchi, and Ikumi Tamai

Exploratory DMPK, Exploratory Toxicology and DMPK Research Laboratories, Tanabe Seiyaku Co., Ltd., Saitama, Japan (R.O., Y.K., M.S., H.F.); GenoMembrane, Inc., Yokohama, Japan (H.Y.); and Department of Molecular Biopharmaceutics, Faculty of Pharmaceutical Sciences, Tokyo University of Science, Noda, Japan (I.T.)

Received September 28, 2005; accepted January 31, 2006

ABSTRACT:
The antiallergic agent bepotastine besilate is a nonsedating, second-generation H1-antagonist with high oral absorption and negligible distribution into brain. To clarify the role of P-glycoprotein (P-gp) in the pharmacokinetics of bepotastine, intestinal absorption and brain penetration studies were performed. [14C]Bepotastine transport in P-gp-overexpressed LLC-PK1 cells indicated that bepotastine was a substrate of P-gp. The affinity of bepotastine to P-gp estimated by ATPase activity assay was low, with a Km value of 1.25 mM. After i.v. administration, the brain/plasma free concentration ratio in mdrl-knockout mice was 3 times higher than that in wild-type mice. The intestinal absorption studies of [14C]bepotastine in rats showed a clear regional difference, showing highest permeability at the upper part of small intestine with decreasing permeability in the descending part of small intestine. The apparent absorption rate constant (ka) of [14C]bepotastine in the small intestine was greatly increased by cyclosporin A and verapamil, especially in the distal portion, and the site-specific absorption of [14C]bepotastine disappeared. The concentration dependence of ka of [14C]bepotastine was observed with a higher ka at higher concentration (20 mM) compared with that at lower concentration (1 μM). In conclusion, bepotastine is a substrate for P-gp, and P-gp clearly limited the brain distribution of bepotastine, whereas the effect of P-gp on intestinal absorption of bepotastine was minimal, presumably because of high membrane permeability at the upper region of small intestine where P-gp is less expressed. Such intestinal absorption property of bepotastine is distinctly different from the low membrane-permeable P-gp substrate fexofenadine.

First-generation H1-receptor antagonists have been used for the treatment of allergic disorders. However, they are problematic because they induce sedation as they penetrate well to the brain (Nicholson 1983, Tagawa et al., 2001). Bepotastine besilate ((+)-(S)-4-[(4-chlorophenyl)(2-pyridyl) methoxy] piperidino) butyric acid monobenzenesulfonate, bepotastine besilate, TAU-284, Talion] was developed as a second-generation H1-antagonist. Bepotastine showed a high selectivity and potent antagonistic action to H1-receptor and exerted an excellent antiallergic action (Honda et al., 1997; Kato et al., 1997; Sakai et al., 1997; Yato et al., 1997). Nonclinical and clinical studies suggested that bepotastine does not exhibit sedation at the clinical therapeutic dose because of limited distribution into brain (Kadosaka et al., 1997; Ohashi et al., 1997). Bepotastine is metabolically stable in dogs and humans and is excreted into urine in an unchanged form >70% of dose after p.o. administration. Intestinal absorption of bepotastine was >85%, >70%, and >80% in rats, dogs, and humans, respectively, showing high absorption. The variation of drug concentration in plasma after p.o. administration to healthy volunteers was small, and plasma concentration was not significantly affected by food. The tissue distribution studies of [14C]bepotastine after p.o. administration to rats showed that [14C]bepotastine distributed widely in the whole body, whereas its brain distribution was lower than that of ketotifen, terfenadine, and its carboxylic metabolite (fexofenadine) (Kato et al., 1997).

The MDRI gene (multidrug resistance; ABCB1) product P-glycoprotein (P-gp) plays an important role in pharmacokinetics of drugs. P-gp is well known as an important factor to limit membrane permeability in several tissues and/or the elimination pathways into urine and bile. P-gp is highly expressed in the endothelial cells of brain capillaries and restricts the brain penetration of drugs (Tsujii et al., 1992; Schinkel et al., 1994). Although intestinal P-gp is probably a limiting factor of intestinal absorption of drugs, it is also true that substrates of P-gp exhibit good bioavailability (Varma et al., 2005). Therefore, the effect of P-gp on intestinal drug absorption is controversial compared with that on brain distribution of drugs (Lin and Yamazaki, 2003).

Nonsedative second-generation H1-antagonists such as fexofenadine, ebastine, and its metabolite (carebastine) are substrates of P-gp, and limited brain distribution of these drugs has been explained by the efflux transport by P-gp at the blood-brain barrier (BBB) (Cvetkovic et al., 1999; Tamai et al., 2000; Tahara et al., 2005). The oral absorption of fexofenadine and ebastine in rats was estimated using radiolabeled compounds to be about 30% and 50% of dose, respectively (Common

ABBREVIATIONS: P-gp, P-glycoprotein; BBB, blood-brain barrier; LC-MS, liquid chromatography mass spectrometry; Papp, apparent permeability coefficient; B-to-A, basal to apical; A-to-B, apical to basal; CFR, corrected flux ratio; KO, knockout; WT, wild type; CMC, carboxymethyl cellulose; P-gp KO, mdr1a/1b(−/−); ka, apparent first-order absorption rate constant; Kp,f, brain/plasma free concentration ratio.
Technical Document for the Registration of Pharmaceuticals for Human Use; (Fuji et al., 1994). Accordingly, the impact of P-gp on the drug absorption may not be high compared with that on the BBB, and this point has been discussed previously (Lin and Yamazaki, 2003).

In the present study, we examined the comparative effects of P-gp on the intestinal absorption and brain distribution of bepotastine, which was clarified to be a substrate of P-gp in the present study, by various in vitro and in vivo methods and by comparing the P-gp effect on bepotastine and fexofenadine. The structures of bepotastine and fexofenadine are shown in Fig. 1.

Materials and Methods

Chemicals. [14C]Bepotastine besilate ([14C](-)S)-4-((4-chlorophenyl)(2-pyridyl)methoxy)piperidino)butyric acid monobenzensulfonate (1.15 GBq/nmol) was synthesized by Amersham Biosciences UK, Ltd. (Little Chalfont, Buckinghamshire, UK). Unlabeled bepotastine besilate was supplied by Ube Industries, Ltd. (Yamaguchi, Japan). [3H]Digoxin and [14C]-mannitol (2.07 GBq/nmol) were obtained from American Radiolabeled Chemicals Inc. (St. Louis, MO), PerkinElmer (Boston, MA), and Moravek Biochemicals Inc. (Brea, CA), respectively. Fexofenadine hydrochloride, verapamil hydrochloride, and diphenhydramine hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO), Quimidine sulfate dihydrate and cyclosporin A were purchased from Wako Pure Chemical Industries (Osaka, Japan). P-gp-expressing membranes (High Five, BTI-TN5B1-4) were purchased from BD-Gentest (Woburn, MA), and ABC Transporter ATPase Assay Reagents Kit was purchased from Nacalai Tesque (Kyoto, Japan). All the other chemicals and reagents were commercial product of reagent grade.

LLC-PK1 and LLC-GA5-COL150. LLC-PK1 cells were obtained from Japan Health Science Research Resources Bank (Osaka, Japan). LLC-GA5-COL150 cells were purchased from Riken Gene Bank (Tsukuba, Japan). LLC-GA5-COL150 cells were established by transfection of human MDR1 (COL150) cells were purchased from Riken Gene Bank (Tsukuba, Japan). LLC-GA5-COL150 cells were purchased from Riken Gene Bank (Tsukuba, Japan). LLC-GA5-COL150 cells were purchased from Riken Gene Bank (Tsukuba, Japan). LLC-GA5-COL150 cells were purchased from Riken Gene Bank (Tsukuba, Japan). LLC-GA5-COL150 cells were purchased from Riken Gene Bank (Tsukuba, Japan). LLC-GA5-COL150 cells were purchased from Riken Gene Bank (Tsukuba, Japan). LLC-GA5-COL150 cells were purchased from Riken Gene Bank (Tsukuba, Japan).

Transcellular Transport Study in LLC-GA5-COL150 and LLC-PK1 Cells. LLC-PK1 and LLC-GA5-COL150 cells were seeded on microporous polycarbonate membrane Transwells (3-µm pore size, 6.5-mm diameter; Costar, Cambridge, MA) at a cell density of 1.0 x 10^6 and 6.5 x 10^5 cells/cm², respectively. Cells were cultured on the microporous membrane with 1.0 and 0.2 ml of complete medium without colchicine in the donor and receiver compartments 24 h before experiments, respectively. Cells were supplemented with fresh medium every 2 days and used for the transport studies on the sixth day after seeding. About 1 h before the initiation of the transport experiments, the medium in both the donor and receiver compartments was replaced with transport medium. The transport experiments were initiated by replacing the medium with medium containing or not containing a test compound. After 2 h, aliquots of medium were taken from the receiver compartment. The drug concentrations were measured in a liquid scintillation counter ([14C]bepotastine, [14C]digoxin, and [14C]-t-mannitol) or liquid chromatography mass spectrometry (LC-MS) system (fexofenadine). To examine the inhibitory effect of P-gp inhibitors such as cyclosporin A and verapamil on the P-gp-mediated transport in LLC-GA5-COL150 cells, they were added to the medium on the same sides with bepotastine in the cell monolayers at the same time with bepotastine. The paracellular leakage was monitored in terms of the permeability of [14C]-mannitol. The apparent permeability coefficient (Papp; cm/s) was calculated as described previously (Artursson 1990). The flux ratio was calculated by the following equation: Flux ratio = Papp (B-to-A)/Papp (A-to-B), where Papp (B-to-A) and Papp (A-to-B) represent the apparent permeability coefficients in the basal-to-apical direction and the apical-to-basal direction, respectively. In LLC-PK1/LLC-GA5-COL150 cells, the corrected flux ratio (CFR) was evaluated by the following equation (Adachi et al., 2001): CFR = (flux ratio in LLC-GA5-COL150) / (flux ratio in LLC-PK1).

Drug-Stimulated P-gp ATPase Activity Assay. The drug-stimulated P-gp ATPase activity was estimated using ABC Transporter ATPase Assay Reagents Kit (Nacalai Tesque). Human P-gp membranes (20 µg) were preincubated at 37°C for 5 min in 40 µl of reaction buffer and each test compound in the presence or absence of 50 µM sodium orthovanadate in 96-well plates. The reaction was initiated by the addition of 20 µl of 12 mM MgATP solution and was terminated 30 min later by the addition of 30 µl of stop solution (10 mM EDTA and LPS). Two hundred microliters of detection reagent (8% ascorbic acid, 0.8% ammonium molybdate, 3 mM zinc acetate) was added and incubated at 37°C for 20 min. The inorganic phosphate complex was detected by its absorbance at 750 nm and was quantitated by comparing the absorbance with a phosphate standard. The vanadate-sensitive ATP hydrolysis was determined by subtracting the value obtained with the vanadate-coincubated membrane fraction from vanadate-free membrane fraction. To estimate the kinetic parameters, ATP hydrolysis rate (v) was fitted to the following equation by means of nonlinear least-squares regression analysis using WinNonlin (Pharsight, Palo Alto, CA); v = Vmax × s/(Km + s), where v and s are ATP hydrolysis rate and concentration of P-gp substrate, respectively. The Km and Vmax are the half-saturation concentration (Michaelis constant) and maximum ATP hydrolysis rate, respectively.

Whole-Body Autoradiography of [14C]Bepotastine in P-gp Knockout and Wild-Type Mice. P-gp knockout (KO) and wild-type (WT) mice were sacrificed at 60 min after i.v. administration of [14C]bepotastine (0.8 mg/45 µCi/kg); the hair was rapidly clipped, and the nasal cavity and anus were filled with 5% carboxymethyl cellulose sodium (CMC-Na). The carcass was frozen in a dry ice/acetone mixture. The frozen carcass was embedded in 5% CMC-Na on a microtome stage, frozen again in a dry ice/acetone mixture, and held in a Cryomacrocult (Leica, Tokyo, Japan). Then, 40-µm-thick sections were cut, collected onto an adhesive tape (No. 810, Sumitomo 3M Ltd., Tokyo, Japan), and lyophilized. The sections were covered with protective membranes (4 µm, Dia Foil, Mitsubishi Polyester Film Co. Ltd., Tokyo, Japan) and placed in contact with imaging plates (TYPE BAS-III, Fuji Photo Film Co., Tokyo, Japan). The plates were exposed at room temperature for 24 h in lead shield boxes. After exposure, image of radioactivity on the imaging plates was analyzed using BAS2000 (Fuji Photo Film).

Plasma-Brain Disposition of [14C]Bepotastine in P-gp KO and WT Mice. FVB/NJ (WT) and mdr1a/1b gene-deficient (P-gp KO) mice were purchased from Jackson Laboratories (Bar Harbor, ME) and Taconic Farms, Inc. (Germantown, NY), respectively. [14C]Bepotastine (0.8 mg/45 µCi/kg) dissolved in saline solution in a total volume of 100 µl was administered by i.v. bolus injection. At 6 and 60 min after dosing, the mice were sacrificed under ether anesthesia. Mice were immediately dissected, and blood and brain samples were collected. Plasma samples were obtained by centrifuging the blood samples at 15,500 g for 3 min. Brain was rinsed with saline and was separated into cerebrum and cerebellum. The separated brain was blotted dried and weighed. Plasma unbound fraction was determined by ultrafiltration of plasma by using a Microcon YM-10 (Millipore Co., Bedford, MA). The samples associated with brain and plasma were solubilized in Soluene-350 at
60°C for 3 h, and the associated radioactivity was measured by liquid scintillation counting.

**In Situ Closed Loop Method.** Male Sprague-Dawley rats weighing 220 to 300 g (Charles River Japan, Kanagawa, Japan) were anesthetized with diethyl ether. An abdominal incision was carefully made to expose the intestinal tissue. The closed loop was made at the stomach, small intestine (proximal, mean, and distal regions), and colon, which was 8 cm in length. The intestinal contents were expelled from the respective segments, and the segments were flushed with prewarmed (37°C) isotonic phosphate-buffered saline. After this procedure, 0.25 ml of [14C]bepotastine (1 M) was introduced into a divided segment, and the dwell time was 0.58 s.

**Selective ion monitoring** was performed at 5-M mass range. Full-scan acquisitions were made over a mass range of 6894.76 Pa). The transport of [14C]bepotastine, fexofenadine, and [3H]digoxin across LLC-PK1 and LLC-GA5-COL150 monolayers in the presence or absence of P-gp inhibitor was evaluated, the amount transported to the receiver side in the A-to-B and B-to-A direction for 2 h after adding the drugs. Each value represents the mean ± S.E.M. of three to six experiments.

**TABLE 1**

<table>
<thead>
<tr>
<th>Condition</th>
<th>LLC-PK1 Papp (1 × 10⁻⁶ cm/s)</th>
<th>LLC-GA5-COL150 Papp (1 × 10⁻⁶ cm/s)</th>
<th>Flux Ratio</th>
<th>CFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>[14C]Bepotastine (5 μM)</td>
<td>5.87 ± 0.14</td>
<td>4.51 ± 0.31</td>
<td>0.77 ± 0.03</td>
<td>1.57 ± 0.04</td>
</tr>
<tr>
<td>[14C]Bepotastine (500 μM)</td>
<td>5.18 ± 0.13</td>
<td>3.98 ± 0.27</td>
<td>0.77 ± 0.03</td>
<td>1.81 ± 0.04</td>
</tr>
<tr>
<td>[14C]Bepotastine (5 μM) + cyclosporin A (10 μM)</td>
<td>4.78 ± 0.35</td>
<td>3.93 ± 0.23</td>
<td>0.82 ± 0.01</td>
<td>2.81 ± 0.07</td>
</tr>
<tr>
<td>[14C]Bepotastine (5 μM) + verapamil (100 μM)</td>
<td>4.65 ± 0.21</td>
<td>3.99 ± 0.18</td>
<td>0.86 ± 0.04</td>
<td>2.43 ± 0.14</td>
</tr>
<tr>
<td>Fexofenadine (5 μM)</td>
<td>0.77 ± 0.02</td>
<td>0.80 ± 0.04</td>
<td>1.04 ± 0.05</td>
<td>0.19 ± 0.03</td>
</tr>
<tr>
<td>[3H]Digoxin (20 nM)</td>
<td>3.66 ± 0.08</td>
<td>6.30 ± 0.35</td>
<td>1.74 ± 0.09</td>
<td>0.78 ± 0.02</td>
</tr>
</tbody>
</table>

**Notes:**

1. Statistical difference of the flux ratio between LLC-PK1 and LLC-GA5-COL150 cells was assessed by Student’s t test (P < 0.05).

2. Statistical difference of the corrected flux ratio (CFR) compared with that of [14C]bepotastine (5 μM) in the presence of excessive bepotastine, and P-gp inhibitor was assessed by Student’s t test (P < 0.05).

**Results**

**Permeability of [14C]Bepotastine and Fexofenadine in LLC-PK1 and LLC-GA5-COL150 Cells.** The role of P-gp in bepotastine transport was assessed using LLC-PK1 cells and LLC-GA5-COL150 cells that are stably transfected with human MDR1 gene. The results for the transcellular transport of [14C]bepotastine, fexofenadine, and [3H]digoxin in LLC-PK1 and LLC-GA5-COL150 monolayers, along with the flux ratios, are summarized in Table 1. The flux ratios of [14C]bepotastine (5 μM), fexofenadine (5 μM), and [3H]digoxin (20 nM) in LLC-GA5-COL150 cells were significantly greater than those in LLC-PK1, showing that the B-to-A flux exceeded those in the other direction in LLC-GA5-COL150 cells. The corrected flux ratio of [14C]bepotastine in the presence of excess bepotastine (500 μM) was lower than that of tracer concentration of [14C]bepotastine (5 μM). Furthermore, the corrected flux ratio of [14C]bepotastine declined in the presence of P-gp inhibitors such as cyclosporin A (10 μM) and verapamil (100 μM). These results indicated that bepotastine, as well as fexofenadine, is a substrate of human P-gp. However, in LLC-PK1 cells, the Papp of the A-to-B and the B-to-A of bepotastine were approximately 8 and 6 times higher than those of the A-to-B and B-to-A transport of fexofenadine, respectively. The apparent difference in transport in the absence of P-gp between bepotastine and fexofenadine may be ascribed to the difference in the intrinsic membrane permeability.

**Stimulation of ATP Hydrolysis by Bepotastine and Verapamil.** The affinity of bepotastine (A) and verapamil (B) to P-gp was estimated by ATPase activity assay. Figure 2 shows the concentration-dependent stimulation of vanadate-sensitive P-gp ATPase activity. The Km, Vmax, and Vmax/Km values of P-gp-mediated ATP hydrolysis by bepotastine were 1.25 ± 0.02 mM, 108 ± 2.2 nmol/min/mg protein, and 0.087 ± 0.003 ml/min/mg protein, respectively. These data suggested that bepotastine has low affinity to P-gp. In contrast, verapamil strongly stimulated the ATP hydrolysis activity. The Km, Vmax, and Vmax/Km values of P-gp-mediated ATP hydrolysis by verapamil were 6.10 ± 0.32 μM, 93.4 ± 2.7 nmol/min/mg protein, and 15.3 ± 0.1 ml/min/mg protein, respectively. The estimated Km value of verapamil to P-gp showed good agreement with the previous report (4.06 μM) (Adachi et al., 2001).

**Brain Penetration of [14C]Bepotastine in P-gp KO and WT Mice.** To evaluate the involvement of P-gp in the in vivo brain penetration of bepotastine, the radioactivity of [14C]bepotastine at a dose of 0.8 mg/kg to WT and P-gp KO mice. The whole-body autoradiograms at 60 min after dosing are shown in Fig. 3A. The distribution of radioactivity was visually similar between WT and P-gp KO mice, excluding the central nervous system tissues. The radioactivity in central nervous system tissues of P-gp KO mice was high compared with that of WT mice. The plasma total concentrations of [14C]bepotastine was measured after i.v. bolus administration of [14C]bepotastine at a dose of 0.8 mg/kg to WT and P-gp KO mice. The whole-body autoradiograms at 60 min after dosing are shown in Fig. 3A. The distribution of radioactivity was visually similar between WT and P-gp KO mice, excluding the central nervous system tissues. The radioactivity in central nervous system tissues of P-gp KO mice was high compared with that of WT mice. The plasma total concentrations of [14C]bepotastine in WT and P-gp KO mice at 6 min after dosing were 580 ± 54.6 ng/ml and 467 ± 9.3 ng/ml, and 280 ± 9.4 ng/ml, respectively. The plasma protein binding of WT and P-gp KO mice were 41.1 ± 0.8% and 45.9 ± 1.2%, respectively. The estimated plasma free concentrations of [14C]bepotastine in WT mice at 6 and 60 min after dosing were 341 ± 32.1 ng/ml and 153 ± 9.3 ng/ml.
results indicated that bepotastine exhibited regional difference in the intestinal absorption from the gastrointestinal tract and the proximal region is a major site for intestinal absorption of bepotastine. The effects of verapamil (10 mM) on \[^{14}C\]bepotastine absorption at the proximal and distal regions in small intestine are shown in Fig. 4B. The absorption of \[^{14}C\]bepotastine from the proximal region in the presence and absence of verapamil was 63.0 ± 2.4% and 72.4 ± 1.1%, respectively, and that from the distal region was 10.9 ± 1.2% and 62.7 ± 2.8%, respectively. Interestingly, the regional difference of \[^{14}C\]bepotastine absorption at the small intestine disappeared in the presence of P-gp inhibitor verapamil.

Comparison of Intestinal Absorption at the Proximal and Distal Regions between Bepotastine and Fexofenadine. Figure 5 compares the intestinal absorption of \[^{14}C\]bepotastine, fexofenadine, and \[^{14}C\]mannitol (paracellular marker) from the proximal and distal regions of small intestine by in situ loop method for 30 min. The absorption rates of \[^{14}C\]bepotastine from the proximal and distal regions were 65.6 ± 3.3% and 10.0 ± 2.2%, respectively. On the contrary, the absorption rates of fexofenadine from the proximal and distal regions were 8.5 ± 0.3% and 4.2 ± 1.2%, respectively, and were similar to those of \[^{14}C\]mannitol. The fractional absorptions of \[^{14}C\]bepotastine in the proximal and distal regions were 7.7 and 2.4 times larger than those of fexofenadine.

Effect of P-gp Inhibitors on Intestinal Absorption of \[^{14}C\]Bepotastine and Fexofenadine at Proximal and Distal Small Intestinal Regions. The impact of P-gp-mediated efflux transport on intestinal absorption of bepotastine was examined by comparing the effects of P-gp inhibitors on fexofenadine. The \(k_a\) value of \[^{14}C\]bepotastine (1 \(\mu M\)) at the proximal region was approximately 8 times larger than that at the distal region. The absorption of \[^{14}C\]bepotastine was not affected by the first-generation H1-antagonist diphenhydramine (20 \(\mu M\)), which induces the sedation, whereas the same concentration of bepotastine significantly increased the absorption rate constant of \[^{14}C\]bepotastine from 1.98 to 2.84 h\(^{-1}\). In contrast, the \(k_a\) value of \[^{14}C\]bepotastine increased 1.7 and 1.3 times at the proximal region in the presence of P-gp inhibitors such as cyclosporin A and verapamil, respectively. At the distal region, the \(k_a\) values of \[^{14}C\]bepotastine were increased 7.4, 7.8, and 4.9 times in the presence of P-gp inhibitors cyclosporin A, verapamil, and quinidine, respectively. In the case of fexofenadine, the \(k_a\) values at the proximal and distal regions were increased approximately 4 times in the presence of P-gp inhibitors with a comparative effect in the proximal and distal regions, whereas the effects of P-gp inhibitors were more significant in the distal region in the case of bepotastine.

Site-Specific Absorption of \[^{14}C\]Bepotastine in Rat Gastrointestinal Tract. The intestinal absorption of bepotastine was evaluated in terms of disappearance of \[^{14}C\]bepotastine from in situ closed loops of stomach, small intestine, and colon in 30 min. The absorption of \[^{14}C\]bepotastine in the stomach and colon was relatively small, and it seems to be absorbed predominantly from the small intestine (Fig. 4A). The absorption from the proximal region of small intestine (83.4 ± 3.1% of dose) was higher than those from the middle and distal regions. These
The sedation of H1-antagonist is well known as an adverse reaction in the central nervous system, and the seriousness of the sedation is a result of the brain penetration, the affinity, and/or the selectivity to H1-receptor and receptor occupation (Yanai et al., 1999; Tagawa et al., 2001). Among the factors that determine the brain concentration are physicochemical properties of drugs and the transporters at the BBB. The first-generation H1-antagonist mepyramine is taken up via carrier-mediated transport system, resulting in a severe central nervous system side effect (Yamazaki et al., 1994a, 1994b). However, second-generation H1-antagonists such as fexofenadine, ebastine, epinastine, and cetirizine exhibit limited distribution into brain because of a P-gp-mediated efflux transport (Tamai et al., 2000; Polli et al., 2003; Ishiguro et al., 2004; Tahara et al., 2005). The influence of P-gp-mediated efflux transport on intestinal absorption process of therapeutic agents is also important because P-gp is highly expressed in the small intestine, whereas the impact of P-gp-mediated efflux transport is variable among agents (Varma at al., 2005). Bepotastine has a high selectivity for the histamine H1-receptor in the presence of P-gp inhibitors such as cyclosporin A and verapamil. The whole-body autoradiograms showed that the radioactivity level of [14C]bepotastine in the brain of WT mice was lower than that in P-gp KO mice. From the pharmacokinetic analysis, the Kp,f in P-gp KO mice was 2.2 and 3.1 times higher than that in WT mice at 6 and 60 min after dosing, respectively. The plasma free concentration of [14C]bepotastine in WT mice was lower than the Km value of bepotastine to P-gp. Accordingly, the P-gp function was not saturated in the endothelial cells of brain capillaries by bepotastine in plasma. Polli et al. (2003) previously reported similar results for cetirizine and showed that the values of Kp,f of cetirizine in P-gp KO mice were approximately 2.3 and 4.6 times greater compared with those in WT mice at 5 and 60 min after dosing, respectively. These results clearly showed that bepotastine is a substrate of P-gp. It was previously reported that bepotastine has a high selectivity for the histamine H1-receptor in the central nervous system, and the brain/plasma concentration ratio of bepotastine in rats and mice is lower than that of the other H1-antagonists (Kato et al., 1997; Tamai et al., 2000; Polli et al., 2003, Ishiguro et al., 2004). These observations suggested that bepotastine has a low permeability and/or binding properties to central nervous system tissues, and these characteristics are explained by the involvement of P-gp, resulting in a low sedative effect.

Although the low brain distribution of bepotastine is explained by P-gp, it is apparent controversial with high bioavailability after p.o. administration because the intestinal P-gp limits the intestinal membrane permeability. Accordingly, the intestinal absorption of bepotastine was examined. The absorption of bepotastine in rat gastrointestinal tract showed clear regional difference, with high absorption in the proximal part of small intestine (Fig. 4A). The similar phenomenon has been reported in the absorption of P-gp substrates cyclosporin A and cilostazol (Tamura et al., 2003; Tsubobuku et al., 2003). The regional difference of [14C]bepotastine absorption disappeared in the presence of P-gp inhibitors and excess bepotastine (Fig. 4B; Tables 2 and 3). Furthermore, the alteration of intestinal absorption of [14C]bepotastine in the presence of P-gp inhibitors was more significant at the distal region than the proximal region, whereas that of fexofenadine in the presence of P-gp inhibitors at the distal region was comparable with that at the proximal region (Fig. 4B; Table 2 and 3). Because the apparent difference between
bepadotin and fexofenadine is in the influx membrane permeability at the proximal region of the small intestine (Fig. 5), the influx permeability would affect the oral bioavailability and cause the apparent difference of the effects of P-gp inhibitors.

Recently, the heterogeneous expression of P-gp mRNA and protein in intestine of rats and humans was reported with a higher expression of P-gp at the lower site compared with an upper site of small intestine (Mouly and Paine, 2003; Takara et al., 2003; Valenzuela et al., 2004; Zimmermann et al., 2005). This regional difference of expression levels of P-gp may contribute to the variation of impact of P-gp on the intestinal absorption among P-gp substrates. Fricker et al. (1996) previously reported that the decrease of the intestinal absorption of cyclosporin A markedly correlated to the expression of mRNA for P-gp over the gastrointestinal tract. The site-specific P-gp-mediated efflux transport for tacrolimus in rat small intestine was investigated, and the activity of P-gp-mediated efflux transport for tacrolimus showed good agreement with the site-specific expression of P-gp (Tamura et al., 2002, 2003). The drug concentration at the proximal region is highest compared with the distal part after oral administration, and P-gp-mediated efflux transport could have more chance to be saturated at the proximal region. In the case of bepotastine, the saturation of P-gp may not explain the high absorption at the proximal site of small intestine because the initial concentration in the intestinal proximal site at a therapeutic dose (10 mg, b.i.d.) with 200 ml of water is approximately 90 μM, and bepotastine has a low affinity to P-gp with K_m value of 1.25 mM. Accordingly, the regional difference in the intestinal absorption of bepotastine can be explained by a gradual increase of P-gp expression level from the proximal to distal region. In other words, the variation of intrinsic influx permeability and the gradual increase of P-gp-mediated efflux activity in the small intestine may explain the variable effect of P-gp on oral bioavailability of P-gp substrates. From these observations, the following points on the effects of P-gp could be elucidated. First, P-gp substrates with high solubility and high membrane permeability are well absorbed because of the limited efflux transport by low abundance of P-gp expression at the proximal small intestine. Second, P-gp substrates with good membrane permeability yet poor solubility are affected by P-gp because they are not absorbed well at the proximal site of small intestine. Third, formulating the P-gp substrates as a slow-sustained release preparation may not improve the overall absorption because of extensive efflux along the middle to distal region in small intestine as a result of the low drug concentration and the higher expression of P-gp.

The involvement of the influx transporter for intestinal absorption of H1-antagonist has been suggested. The uptake of diphenhydramine in Caco-2 cells was mediated by pH-dependent transport system (Mizuuchi et al., 1999). Furthermore, fexofenadine was transported not only by P-gp but also by an organic anion transporting polypeptide family (Cvetkovic et al., 1999; Nozawa et al., 2004). Involvement of organic anion transporting polypeptide transporters in fexofenadine absorption was also suggested by the reduction of intestinal absorption by the fruit juices in humans and rats (Dresser et al., 2002, 2005; Kamath et al., 2005). However, it is unclear why bepotastine has high

---

### TABLE 2

<table>
<thead>
<tr>
<th>Condition</th>
<th>Absorption Rate</th>
<th>ka</th>
</tr>
</thead>
<tbody>
<tr>
<td>[14C]Bepotastine (1 μM)</td>
<td>10.9 ± 1.2</td>
<td>0.26 ± 0.03 (1.00)</td>
</tr>
<tr>
<td>[14C]Bepotastine (20 mM)</td>
<td>38.3 ± 2.0*a</td>
<td>0.97 ± 0.07* (3.79)</td>
</tr>
<tr>
<td>[14C]Bepotastine (1 μM) + cyclosporin A (50 μM)</td>
<td>59.7 ± 1.4</td>
<td>1.89 ± 0.07* (7.41)</td>
</tr>
<tr>
<td>[14C]Bepotastine (1 μM) + verapamil (10 mM)</td>
<td>62.7 ± 2.6*a</td>
<td>1.98 ± 0.15* (7.77)</td>
</tr>
<tr>
<td>[14C]Bepotastine (1 μM) + quinidine (10 mM)</td>
<td>46.0 ± 2.5*a</td>
<td>1.24 ± 0.09* (4.85)</td>
</tr>
<tr>
<td>Fexofenadine (370 μM)</td>
<td>4.2 ± 1.2</td>
<td>0.09 ± 0.03 (1.00)</td>
</tr>
<tr>
<td>Fexofenadine (370 μM) + cyclosporin A (50 μM)</td>
<td>17.9 ± 0.9*a</td>
<td>0.40 ± 0.02* (4.58)</td>
</tr>
<tr>
<td>Fexofenadine (370 μM) + verapamil (10 mM)</td>
<td>13.0 ± 3.2</td>
<td>0.28 ± 0.08* (3.27)</td>
</tr>
<tr>
<td>[14C]Mannitol (1 μM)</td>
<td>3.5 ± 1.1</td>
<td>0.07 ± 0.03</td>
</tr>
</tbody>
</table>

*a Statistical difference of the absorption rate (% of dose) and the apparent absorption rate constant, ka, in the presence of excessive bepotastine and several drugs including P-gp inhibitors was assessed by Student’s t test (P < 0.05).

---

### TABLE 3

Influence of P-gp inhibitor on intestinal absorption of [14C]bepotastine and fexofenadine at distal region in rats

The absorption at the distal region of [14C]bepotastin and fexofenadine in the presence or absence of P-gp inhibitor was evaluated by the closed loop method. The data correspond to the intestinal absorption at the distal region within 30 min after injection to intestinal loop (8 cm). Each value represents the mean ± S.E.M. of three animals. The values in parentheses represent the ratio of the apparent absorption rate constant (ka) by [14C]bepotastin (1 μM), fexofenadine (370 μM), and [14C]mannitol (2 μM).

<table>
<thead>
<tr>
<th>Condition</th>
<th>Absorption Rate</th>
<th>ka</th>
</tr>
</thead>
<tbody>
<tr>
<td>[14C]Bepotastine (1 μM)</td>
<td>10.9 ± 1.2</td>
<td>0.26 ± 0.03 (1.00)</td>
</tr>
<tr>
<td>[14C]Bepotastine (20 mM)</td>
<td>38.3 ± 2.0*a</td>
<td>0.97 ± 0.07* (3.79)</td>
</tr>
<tr>
<td>[14C]Bepotastine (1 μM) + cyclosporin A (50 μM)</td>
<td>59.7 ± 1.4</td>
<td>1.89 ± 0.07* (7.41)</td>
</tr>
<tr>
<td>[14C]Bepotastine (1 μM) + verapamil (10 mM)</td>
<td>62.7 ± 2.6*a</td>
<td>1.98 ± 0.15* (7.77)</td>
</tr>
<tr>
<td>[14C]Bepotastine (1 μM) + quinidine (10 mM)</td>
<td>46.0 ± 2.5*a</td>
<td>1.24 ± 0.09* (4.85)</td>
</tr>
<tr>
<td>Fexofenadine (370 μM)</td>
<td>4.2 ± 1.2</td>
<td>0.09 ± 0.03 (1.00)</td>
</tr>
<tr>
<td>Fexofenadine (370 μM) + cyclosporin A (50 μM)</td>
<td>17.9 ± 0.9*a</td>
<td>0.40 ± 0.02* (4.58)</td>
</tr>
<tr>
<td>Fexofenadine (370 μM) + verapamil (10 mM)</td>
<td>13.0 ± 3.2</td>
<td>0.28 ± 0.08* (3.27)</td>
</tr>
<tr>
<td>[14C]Mannitol (1 μM)</td>
<td>3.5 ± 1.1</td>
<td>0.07 ± 0.03</td>
</tr>
</tbody>
</table>

*a Statistical difference of the absorption rate (% of dose) and the apparent absorption rate constant, ka, in the presence of excessive bepotastine and several drugs including P-gp inhibitors was assessed by Student’s t test (P < 0.05).
membrane permeability at the proximal region in the small intestine compared with fexofenadine because there is no experimental evidence of involvement of the influx transporter to intestinal absorption of bepotastine until now. Further study is needed to clarify the underlying molecular mechanism of influx transport of bepotastine in small intestine.

In conclusion, the antiallergic agent bepotastine is a substrate of P-gp as much as fexofenadine, and P-gp clearly limits the brain distribution of bepotastine, whereas P-gp effect on the bioavailability of bepotastine after p.o. administration is negligible by showing almost complete absorption. The lack of the effect of P-gp on bepotastine absorption may be explained by its high membrane permeability at the proximal region of small intestine, where the P-gp expression is minimal at the proximal region of small intestine, whereas the effect of P-gp is extensive at the distal region of small intestine, where most of bepotastine has been already absorbed at the proximal region of small intestine. Such effect of P-gp on the bioavailability of bepotastine is distinct from the low membrane-permeable P-gp substrate fexofenadine. At present, the mechanism of the high permeability of bepotastine at the proximal site of the small intestine has not been well clarified, and the influx permeability at the upper small intestine of p.o. administered drugs is an important factor to determine the apparent effect of P-gp on bioavailability.

Acknowledgments. We thank to Dr. Yusuke Tanigawa at Keio University Hospital and Dr. Kazumitsu Ueda at Kyoto University for providing the LLC-GAS-COL150 cells; Masakazu Takemura at GenoMembrane, Inc., for fruitful advice; and Yoko Togo, Masao Yamanouchi, and Masakatsu Takahashi for their helpful animal experiment and excellent technical assistance of construction of the whole-body autoradiograms.

References


Tanigawa T, Koshiba H, Fuse E, and Sugiyama Y (2005) 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. Drug Metab Dispos 33:963–968.


Address correspondence to: Dr. Rikiya Ohashi, Exploratory DMPK, Exploratory Toxicology and DMPK Research Laboratories, Tanabe Sankyo Co., Ltd., 2-2-50, Kawagishi, Toda, Saitama 333-8505, Japan. E-mail: r-ohashi@tanabe.co.jp

799

ROLE OF P-gp IN PHARMACOKINETICS OF BEPOTASTINE