INHIBITION OF OAT3-MEDIATED RENAL UPTAKE AS A MECHANISM FOR DRUG-DRUG INTERACTION BETWEEN FEXOFENADINE AND PROBENECID

Harunobu Tahara, Hiroyuki Kusuhara, Kazuya Maeda, Hermann Koepsell, Eiichi Fuse, and Yuichi Sugiyama

Graduate School of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo, Japan (H.T., H.Ku., K.M., Y.S.); Pharmacokinetic Research Laboratories, Pharmaceutical Research Institute, Kyowa Hokko Kogyo Co., Ltd., Shimotogari, Nagazumicho, Suntogun, Shizuoka, Japan (H.T., E.F.); and Institut für Anatomie und Zellbiologie, Universität Würzburg, Germany (H.Ko.)

Received November 14, 2005; accepted January 27, 2006

ABSTRACT:

Fexofenadine, a non-sedating antihistamine drug, is effective for the treatment of seasonal allergic rhinitis and chronic urticaria. Simultaneous administration of probenecid increases the plasma concentration of fexofenadine due to an inhibition of its renal elimination in healthy volunteers (Clin Pharmacol Ther 77:17–23, 2005). The purpose of the present study is to investigate the possibility that the drug-drug interaction between fexofenadine and probenecid involves the renal basolateral uptake process. The uptake of fexofenadine was compared between hOAT3 and organic anion-transporting peptide 1B3 (hOATP1B3), a transporter responsible for the hepatic uptake of fexofenadine (Drug Metab Dispos 33:1477–1481, 2005). The K_i values were determined to be 1.30 and 130 μM for hOAT3 and hOATP1B3, respectively, with Hill coefficients of 0.76 and 0.64, respectively. The K_i value of probenecid for hOAT3, but not for hOATP1B3, was significantly lower than the maximum unbound plasma concentration of probenecid at clinical dosages. These results suggest that the renal drug-drug interaction between fexofenadine and probenecid is probably explained by an inhibition of the renal uptake of fexofenadine via hOAT3, at least in part.

The kidney plays important roles in the detoxification of xenobiotics and endogenous wastes as well as maintaining stable levels of electrolytes and nutrients in the body. Urinary excretion consists of glomerular filtration in the glomeruli, tubular secretion across the proximal tubules, and reabsorption. Many studies have shown the importance of transporters in the tubular secretion of a large number of organic compounds, and a number of studies have described the role of multispecific organic anion and cation transporters (OAT/SLC22 and OCT/SLC22) in the renal uptake of drugs. OCT2 (SLC22A7) plays a predominant role in the renal uptake of organic cations in the human kidney, whereas OCT1 plays a predominant role in the hepatic uptake of organic cations in the human liver (Koepsell, 2004; Lee and Kim, 2004; Wright and Dantzler, 2004). Three isoforms of the organic anion transporter family (OAT1/SLC22A6, OAT2/SLC22A7, and OAT3/SLC22A8) have been identified on the basolateral membrane of the human proximal tubules (Lee and Kim, 2004; Miyazaki et al., 2004; Wright and Dantzler, 2004). It has been suggested that HOAT1 plays an important role in the renal uptake of hydrophilic organic anions with a low molecular weight, whereas HOAT3 plays an important role in the renal uptake of amphipathic organic anions as well as a basic drug, famotidine (Hasegawa et al., 2003; Tahara et al., 2005a). The mRNA of HOAT2 in the kidney is markedly lower than that of HOAT1 and HOAT3 (Motohashi et al., 2002), and its role in drug transport in the kidney remains unknown. Identification of the basolateral transporters provides a clue to understanding the molecular mechanisms of drug-drug interactions involving tubular secretion. Takeda et al. (2002) and Nozaki et al. (2004) have shown that rOat3/hOAT3-mediated renal uptake can be a potential drug-drug interaction site with some nonsteroidal anti-inflammatory drugs at clinical dosages by comparing their K_i values for rOat3/hOAT3 with the unbound plasma concentrations. In addition, we have reported that OAT3 could be the site of an interaction between famotidine and probenecid in humans (Tahara et al., 2005a).

Fexofenadine, an active metabolite of terfenadine, is a non-sedating histamine H1 receptor antagonist that is prescribed for the oral treatment of allergic rhinitis and chronic idiopathic urticaria. After oral administration of [14C]fexofenadine to healthy volunteers, 92% of the total dose was recovered, 12% in urine and 80% in feces, as the

ABBREVIATIONS: OAT, organic anion transporter; hOAT, human OAT; OCT, organic cation transporter; hOCT, human OCT; r, rat; hOATP, human organic anion-transporting peptide; AUC, area under the plasma concentration-time curve; HEK, human embryonic kidney; LC-MS, liquid chromatography-mass spectrometry.
unchanged form (Lippert et al., 1995). Since the average absolute oral bioavailability of fexofenadine was reported to be 33% (Dresser et al., 2005), about 36% of the bioavailable fexofenadine can be excreted into the urine during a 24-h period, and renal elimination makes a significant contribution to the total body clearance in addition to biliary excretion. Interactions of fexofenadine with drugs and food have been reported. The interactions with rifampicin (Hamman et al., 2001), St John’s wort (Wang et al., 2002), and fruit juice (Dresser et al., 2002) caused a reduction in the AUC of fexofenadine after oral administration, and these are hypothesized to include modulation of P-glycoprotein or inhibition of OATP2B1 in the small intestine (Cvetkovic et al., 1999; Nozawa et al., 2004). The interactions with verapamil (Yasui-Furukori et al., 2005) and ketoconazole (Simpson and Jarvis, 2000) increased the AUC of fexofenadine, probably because of an increase in the oral absorption produced by inhibition of intestinal P-glycoprotein. Probencid treatment caused a significant reduction in the unbound renal clearance of fexofenadine (Yasui-Furukori et al., 2005). Because probencid is a potent inhibitor of OATs (Tahara et al., 2005a), it is possible that this interaction involves renal transporters, such as OAT1, OAT2, and OAT3.

In the present study, to obtain an insight into the basolateral uptake mechanism of fexofenadine, the uptake was determined in cDNA-transfected cells expressing hOAT1, hOAT2, hOAT3, and hOCT2, and the effect of probenecid on the uptake was determined to examine whether it is inhibited by a clinically relevant concentration of probenecid.

Materials and Methods

Fexofenadine hydrochloride was purchased from Toronto Research Chemicals (North York, ON, Canada). Ranidine was purchased from Sigma-Aldrich (St. Louis, MO). [3H]p-Aminohippurate (151 GBq/mmol) was purchased from PerkinElmer Life and Analytical Sciences (Wellesley, MA). [3H]Benzylopenicillin (740 GBq/mmol) was purchased from GE Healthcare UK (Little Chalfont, Buckinghamshire, UK). 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.

The stable transfectants expressing hOAT1, hOAT2, hOAT3- (Tahara et al., 2005a), hOCT2- (Schlatter et al., 2002), and hOATP1B3-HEK (Shimizu et al., 2005) were established as described previously. These cells were grown in Dulbecco’s modified Eagle’s medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum, penicillin (100 U/ml), streptomycin (100 μg/ml), and G418 sulfate (400 μg/ml) at 37°C with 5% CO2 and 95% humidity on the bottom (Lippert et al., 1995). 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 high-performance liquid chromatography was: methanol (A) and 0.05% formic acid (B). Chromatographic separation was achieved on an C4, column (Capcell pak C4, MG, 4.6 mm i.d. × 75 mm, particle size 3 μm; Shimadzu, 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 limit of this method was 5 nM in the cell lysate. Instrument control and data analysis were performed using Mass Lynx application software from Waters.

Kinetic Analyses. In the present study, to obtain an insight into the basolateral uptake mechanism of fexofenadine, the uptake was determined in cDNA-transfected cells expressing hOAT1, hOAT2, hOAT3, and hOCT2, and the effect of probenecid on the uptake was determined to examine whether it is inhibited by a clinically relevant concentration of probenecid.

Concentration Dependence of the Uptake of Fexofenadine by hOAT1, hOAT2, hOAT3, and hOCT2-HEK. Figure 1 shows the time profiles of the uptake of the typical substrates and fexofenadine by hOAT1-, hOAT2-, hOAT3-, and hOCT2- vector-HEK. Cells were exposed to test compounds for 5 min. The uptake of fexofenadine by hOAT3-HEK was determined to be 1.30 ± 0.30 pmol/min/mg protein with a Hill coefficient of 0.76.

Results

Time Profile of the Uptake of Fexofenadine by hOAT1, hOAT2, hOAT3, and hOCT2-HEK. Figure 1 shows the time profiles of the uptake of the typical substrates and fexofenadine by hOAT1-, hOAT2-, hOAT3-, and hOCT2- vector-HEK. Cells were exposed to test compounds for 5 min. The uptake of fexofenadine by hOAT3-HEK was determined to be 1.30 ± 0.30 pmol/min/mg protein with a Hill coefficient of 0.76.

Concentration Dependence of the Uptake of Fexofenadine by hOAT3-HEK, and the Effect of Probenecid. The concentration dependence of the uptake of fexofenadine by hOAT3-HEK was examined (Fig. 2). The uptake was saturable, and the Km and Vmax values, determined by nonlinear regression analysis, were 70.2 ± 2.7 μM and 120 ± 3 pmol/min/mg protein, respectively.

The inhibitory effect of probenecid on hOAT3-mediated uptake of fexofenadine was examined (Fig. 3). The Km value of probenecid for the uptake of fexofenadine by hOAT3-HEK was determined to be 130 ± 40 μM with a Hill coefficient of 0.64 (Fig.
Fig. 1. Time profile of the uptake of typical substrates and fexofenadine by hOAT1-, hOAT2-, hOAT3-, and hOCT2-HEK. The time-dependent uptake of the typical substrates and fexofenadine (10 μM) by hOAT1-, hOAT2-, hOAT3-, and hOCT2-HEK was examined at 37°C. Closed and open circles represent the uptake by hOATs/OCTs-HEK and vector-HEK, respectively. Statistical differences in the uptake of hOATs/OCTs-HEK were compared with vector-HEK by a two-tailed unpaired t test with the limit of significance (*, p < 0.05; **, p < 0.01). Each point represents the mean ± S.E. (n = 3).}

**Discussion**

Fexofenadine is an orally active nonselective histamine H1 receptor antagonist. Only a small amount of the orally administered [14C]fexofenadine was recovered in the urine of healthy volunteers (12%), and urinary excretion has been considered to be a minor elimination pathway. However, the fact that the absolute oral bioavailability of fexofenadine is, on average, 33% means that a considerable amount of fexofenadine is excreted into the urine over a 24-h period (36% of the amount absorbed into the circulating blood) and suggests that renal elimination makes a significant contribution to the total clearance. The renal clearance of fexofenadine is greater than the glomerular filtration rate, indicating that tubular secretion accounts for the major part of the renal clearance (Table 1). Simultaneously administered probenecid caused an approximately 50% increase in the AUC of fexofenadine in healthy subjects, and this is largely explained by a 73% inhibition of the renal clearance of fexofenadine (Table 1) (Yasui-Furukori et al., 2005). In the present study, we examined the possible role of renal organic anion and cation transporters in the drug-drug interaction between fexofenadine and probenecid.

In cDNA-transfected cells, fexofenadine is efficiently transported only by hOAT3, whereas specific uptake by hOAT1, hOAT2, and hOCT2 was below the limit of detection, suggesting that hOAT3 plays a major role in the renal uptake of fexofenadine (Fig. 1). The transport activity of fexofenadine by hOAT3 was much lower than that of benzylpenicillin (1.71 versus 10.7 μl/min/mg protein). This was in good agreement with clinical data showing that the renal tubular secretion clearance of benzylpenicillin in healthy volunteers was 983 ml/min (Bins and Mattie, 1988), at least 9-fold higher than that of fexofenadine (113 ml/min, Table 1). Probenecid is a potent inhibitor of fexofenadine transport by hOAT3 or hOATP1B3-HEK in the presence of inhibitors versus that in the absence of inhibitors. The Hill coefficient value was 0.763 ± 0.047. Each point represents the mean ± S.E. (n = 3).
of hOAT3, and the unbound plasma concentration of probenecid at clinical doses (0.5–20 g), ranging from 12 to 52 μM (Selen et al., 1982), is greater than its $K_i$ value for hOAT3 (Table 1; Fig. 3). Therefore, probenecid will produce almost complete inhibition of hOAT3 in clinical situations, consistent with clinical reports, 73% inhibition of the renal clearance of fexofenadine by probenecid (Yasui-Furukori et al., 2005). Therefore, inhibition of basolateral uptake can be one of the sites of interaction between fexofenadine and probenecid. Cimetidine inhibits the renal clearance of fexofenadine by 39% on average in healthy subjects (Table 1). Since the clinical unbound plasma concentration of inhibitor (Selen et al., 1982; van Crgten et al., 1985).

|$R$ value was calculated according to the following equation: $R = 1/1 + L_\text{max}/K_i$.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Cimetidine</th>
<th>Probenecid</th>
</tr>
</thead>
<tbody>
<tr>
<td>$AUC_{0-\text{h}}$ (ng · h/ml)</td>
<td>3637 ± 1199</td>
<td>4124 ± 2019</td>
<td>6510 ± 3972</td>
</tr>
<tr>
<td>Ratio to control</td>
<td>1</td>
<td>1.08</td>
<td>1.53</td>
</tr>
<tr>
<td>$CL_{\text{renal}}$ (ml/min)$^a$</td>
<td>230 ± 78</td>
<td>152 ± 70</td>
<td>74 ± 52</td>
</tr>
<tr>
<td>Ratio to control</td>
<td>1</td>
<td>0.610</td>
<td>0.270</td>
</tr>
<tr>
<td>$CL_{\text{fex}}$ (ml/min)$^a$</td>
<td>133</td>
<td>55.0</td>
<td>0 (almost)</td>
</tr>
<tr>
<td>Ratio to control</td>
<td>1</td>
<td>0.414</td>
<td>0.100</td>
</tr>
<tr>
<td>$L_\text{max}$, maximum unbound plasma concentration of inhibitor (Selen et al., 1982; van Crgten et al., 1985).</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### TABLE 1

Effects of cimetidine and probenecid treatments on pharmacokinetic parameters of fexofenadine

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Cimetidine</th>
<th>Probenecid</th>
</tr>
</thead>
<tbody>
<tr>
<td>$AUC_{0-\text{h}}$ (ng · h/ml)</td>
<td>3637 ± 1199</td>
<td>4124 ± 2019</td>
<td>6510 ± 3972</td>
</tr>
<tr>
<td>Ratio to control</td>
<td>1</td>
<td>1.08</td>
<td>1.53</td>
</tr>
<tr>
<td>$CL_{\text{renal}}$ (ml/min)$^a$</td>
<td>230 ± 78</td>
<td>152 ± 70</td>
<td>74 ± 52</td>
</tr>
<tr>
<td>Ratio to control</td>
<td>1</td>
<td>0.610</td>
<td>0.270</td>
</tr>
<tr>
<td>$CL_{\text{fex}}$ (ml/min)$^a$</td>
<td>133</td>
<td>55.0</td>
<td>0 (almost)</td>
</tr>
<tr>
<td>Ratio to control</td>
<td>1</td>
<td>0.414</td>
<td>0.100</td>
</tr>
<tr>
<td>$L_\text{max}$, maximum unbound plasma concentration of inhibitor (Selen et al., 1982; van Crgten et al., 1985).</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### References


Tahara H, Kusuhara H, Fuse E, and Sugiyama Y (2005b) 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. Yuichi Sugiyama, Professor, Graduate School of Pharmaceutical Sciences University of Tokyo Hongo, Bunkyo-ku, Tokyo, 113-0033, Japan, E-mail: sugiyama@mol.f.u-tokyo.ac.jp