A Species Difference in the Transport Activities of H₂ Receptor Antagonists by Rat and Human Renal Organic Anion and Cation Transporters

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ABSTRACT

A clinical drug-drug interaction between famotidine (a H₂ receptor antagonist) and probenecid has not been reproduced in rats. The present study hypothesized that the species-dependent probenecid sensitivity is due to a species difference in the contribution of renal organic anion and cation transporters. The transport activities of the H₂ receptor antagonists (cimetidine, famotidine, and ranitidine) by rat and human basolateral organic anion and cation transporters [human organic anion transporter (hOAT) 1, hOAT2, r/hOAT3, rat organic cation transporter (rOct) 1, and r/hOCT2] were compared using their cDNA transfectants. The transport activities (V_max/K_m) of famotidine (K_m, 345 μM) by rOat3 were 8- and 15-fold lower than those of cimetidine (K_m, 91 μM) and ranitidine (K_m, 155 μM), respectively, whereas the activity by hOAT3 (K_m, 124 μM) was 3-fold lower than that of cimetidine (K_m, 149 μM) but similar to that of ranitidine (K_m, 234 μM). Comparison of the relative transport activity with regard to that of cimetidine suggests that famotidine was more efficiently transported by hOAT3 than rOat3, and vice versa, for ranitidine. Only ranitidine was efficiently transported by hOAT2 (K_m, 396 μM), rOct1 accepts all of the H₂ receptor antagonists with a similar activity, whereas the transport activities of ranitidine and famotidine (K_m, 61/56 μM) by r/hOCT2 were markedly lower than that of cimetidine (K_m, 69/73 μM). Probenecid was a potent inhibitor of r/OAT3 (K_i, 2.6–5.8 μM), whereas it did not interact with OCTs. These results suggest that, in addition to the absence of OCT1 in human kidney, a species difference in the transport activity by hOAT3 and rOat3 accounts, at least in part, for the species difference in the drug-drug interaction between famotidine and probenecid.

The kidney plays important roles in the detoxification of xenobiotics and endogenous wastes as well as maintaining the correct balance of ions and nutrients in the body. Urinary excretion is the major detoxification mechanism in the kidney, and this is governed by glomerular filtration, tubular secretion across the proximal tubules, and reabsorption. The renal uptake of organic anions and cations on the basolateral membrane of the proximal tubules has been characterized by multispecific organic anion and cation transporters (OAT/SLC22 and OCT/SLC22), respectively (Lee and Kim, 2004; Wright and Dantzler, 2004; Shitara et al., 2005).

Molecular cloning of basolateral transporters from different species allows examination of differences in their substrate specificities and transport activities, leading to a better understanding of the molecular mechanisms of species differences in drug disposition. For OCTs, the isoform expressed in the kidney differs between rodents and humans. Both Oct1 (Slc22a1) and Oct2 (Slc22a2) are involved in the renal uptake of organic cations on the basolateral membrane of the proximal tubules in rodents, whereas OCT2 is the predominant isoform in the human kidney (Koepsell, 2004; Lee and Kim, 2004; Wright and Dantzler, 2004). As far as renal organic anion transporters are concerned, two isoforms (Oat1/Slc22a6 and Oat3/Slc22a8) in rodents and three iso-
forms (OAT1, OAT2/SLC22A7, and OAT3) in humans have been identified on the basolateral membrane of the proximal tubules (Hasegawa et al., 2002; Lee and Kim, 2004; Miyazaki et al., 2004; Wright and Dantzler, 2004). The mRNA expression level of hOAT3 quantified by real-time polymerase chain reaction was the highest followed by hOAT1, whereas that of hOAT2 was quite low (Motohashi et al., 2002). We have previously reported that the transport activities of nine substrates gave a poor correlation between rat and human OAT3 because of large species difference in the transport activities of estrone sulfate and indoxyl sulfate, whereas there was a minimal difference in the transport activities of 11 substrates between rat and human OAT1 (Tahara et al., 2005). Oat3/OAT3-mediated uptake may show a species difference, resulting in a difference in the contribution of transporters to the total uptake process between rats and humans.

Histamine H₂ receptor antagonists have been widely and successfully used in the treatment of peptic ulcers and gastric acid hypertension. H₂ receptor antagonists are mainly eliminated by the kidney as the intact form by tubular secretion as well as glomerular filtration (Lin, 1991). Clinical drug-drug interaction studies in healthy subjects have reported that the renal secretion clearance of famotidine was considerably inhibited by oral coadministration of probenecid. Concomitantly, the plasma concentration of famotidine increased (Inotsume et al., 1990). However, this interaction has not been reproduced in rats, even at higher plasma concentrations of probenecid (Lin et al., 1988). Unlike famotidine, probenecid only slightly affects the renal secretion clearance of cimetidine both in humans and rats (ca. 20%) (Lin et al., 1988; Gisclon et al., 1989).

H₂ receptor antagonists are weak bases or organic cations at physiological pH and substrates of organic cation transporters (Grundemann et al., 1999). They have been referred to as bisubstrates that are recognized by both renal organic cation transporters (Grundemann et al., 1999). The vector constructs of hOAT2 and rOct3 were introduced into parental HEK293 cells (hOAT2-HEK and rOAT3-HEK) by FuGENE 6 (Roche Diagnostics, Indianapolis, IN) and Lipofectamine (Invitrogen) transfection reagent according to the manufacturers’ protocol, respectively. The stably transfected cells were selected by adding G418 sulfate (Invitrogen) to the culture medium. Two weeks after transfection, different clones were seeded on 12-well culture plates and the transport activity was tested for positive clones. The clone with the highest transport activity was used for the transport studies. hOAT1-, hOAT2-, hOAT3-, hOCT2-, rOat3-, rOct1-, or rOct2-expressing HEK293 cells were grown in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 400 μg/ml G418 at 37°C with 5% CO₂ and 95% humidity on the bottom of a dish, and hOAT1-, hOCT2-, hOAT3-, rOAT3-, and rOCT2-expressing cells were seeded in polystyrene-coated 12-well plates (BD Biosciences, Franklin Lakes, NJ) at a density of 1.2 x 10⁵ cells/well. Cell culture medium was replaced with culture medium supplemented with 5 mM sodium butyrate 24 h before transport studies to induce the expression of those proteins.

Transport Studies. Transport studies were carried out as described previously (Tahara et al., 2005). Uptake was initiated by adding medium containing a 10 μM concentration of the compounds after cells had been washed twice and preincubated with Krebs-Henseleit buffer at 37°C for 15 min. Probenecid was added to the uptake buffer simultaneously with the H₂ receptor antagonists. The Krebs-Henseleit buffer consisted of 118 mM NaCl, 23.8 mM NaHCO₃, 4.83 mM KCl, 0.96 mM KH₂PO₄, 1.20 mM MgSO₄, 12.5 mM HEPES, 5 mM glucose, and 1.53 mM CaCl₂ adjusted to pH 7.4. The uptake was terminated at a designated time by adding ice-cold Krebs-Henseleit buffer after removal of the incubation buffer. Then, cells were washed twice with 1 ml of ice-cold Krebs-Henseleit buffer. For determination of the uptake of cimetidine, ranitidine, and famotidine, cells were dissolved in 300 μl of 0.2 N NaOH and kept overnight. Aliquots (150 μl) were transferred to vials after adding 30 μl of 1 N HCl. Aliquots (100 μl) were used for LC-MS quantification as described below. The remaining 10 μl of the aliquots of cell lysate was used to determine the protein concentration by the method of Lowry with bovine serum albumin as a standard. Ligand uptake was given as the cell/medium concentration ratio determined as the amount of ligand associated with cells divided by the medium concentration.

Quantification of H₂ Receptor Antagonists by LC-MS. The quantification of cimetidine, ranitidine, and famotidine was performed by high-performance liquid chromatography (HPLC; Alliance 2690; Waters, Milford, MA) connected to a mass spectrometer (QZ; Micromass, Manchester, UK) (Nagata et al., 2004). Aliquots (100 μl) of samples containing H₂ receptor antagonists were precipitated by 100 μl of methanol containing an internal standard (famotidine for cimetidine and ranitidine; cimetidine for ranitidine), mixed, and centrifuged, and then 25 μl of the supernatants was injected into the LC-MS. HPLC analysis was performed on a C18 reverse phase column (3 μm, 4.6-mm i.d., 75 mm; Shimadzu, Tokyo, Japan) at room temperature. Elution was performed with a 0 to 90% linear gradient of 10 mM ammonium acetate-methanol over 4 min at 0.8 ml/min. A portion of the eluent (split ratio × 1:10) was introduced to the MS via an electrospray interface. Detection was performed by selected ionization monitoring in positive ion mode (m/z, 253, 315, and 338 for cimetidine, ranitidine, and famotidine, respectively).

Materials and Methods

Materials. Famotidine and probenecid were purchased from Nacalai Tesque (Kyoto, Japan), and cimetidine and ranitidine were purchased from Sigma-Aldrich (St. Louis, MO). The purity of famotidine, cimetidine, and ranitidine was more than 98%, and that of probenecid was more than 95%. All other chemicals were of analytical grade and commercially available.

Establishment of Transfectants and Cell Culture. The stable transfectants expressing human OAT1 and OAT3 (Tahara et al., 2005) and OCT (Goralski et al., 2002; Schlatter et al., 2002) were established previously. Construction of hOAT2- and rOat3-stable transfectants was carried out as follows. The full coding region of hOAT2 was amplified from human liver cDNA by reverse transcription-polymerase chain reaction following the reported sequence given by accession number NM 006672. Full-length hOAT2 was cloned into mammalian expression vector pcDNA3.1(+) (Invitrogen, Carlsbad, CA). The construct of pcDNA3.1(+) containing rOAT3 was established previously (Tahara et al., 2005). The vector constructs of hOAT2 and rOCT3 were introduced into parental HEK293 cells (hOAT2-HEK and rOAT3-HEK) by FuGENE 6 (Roche Diagnostics, Indianapolis, IN) and Lipofectamine (Invitrogen) transfection reagent according to the manufacturers’ protocol, respectively. The stably transfected cells were selected by adding G418 sulfate (Invitrogen) to the culture medium. Two weeks after transfection, different clones were seeded on 12-well culture plates and the transport activity was tested for positive clones. The clone with the highest transport activity was used for the transport studies. hOAT1-, hOAT2-, hOAT3-, hOCT2-, rOat3-, rOct1-, or rOct2-expressing HEK293 cells were grown in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 400 μg/ml G418 at 37°C with 5% CO₂ and 95% humidity on the bottom of a dish, and hOAT1-, hOCT2-, hOAT3-, rOAT3-, and rOCT2-expressing cells were seeded in polystyrene-coated 12-well plates (BD Biosciences, Franklin Lakes, NJ) at a density of 1.2 x 10⁵ cells/well. Cell culture medium was replaced with culture medium supplemented with 5 mM sodium butyrate 24 h before transport studies to induce the expression of those proteins.
Kinetic Analyses. Kinetic parameters were obtained using the Michaelis-Menten equation as shown in eq. 1:

\[ v = \frac{V_{max} \times S}{K_m + S} \]  

where \( v \) is the uptake rate of the substrate (picomoles per minute per milligram of protein), \( S \) is the substrate concentration in the medium (micromolar), \( K_m \) is the Michaelis-Menten constant (micromolar), and \( V_{max} \) is the maximum uptake rate (picomoles per minute per milligram of protein). To obtain the kinetic parameters, the equation was fitted to the uptake velocity using a MULTI program (http://www.pharm.kyoto-u.ac.jp/byoyaku/index.html) (Yamaoka et al., 1981). The input data were weighted as the reciprocals of the observed values, and the Damping Gauss Newton Method algorithm was used for fitting.

Inhibition constants (\( K_i \)) were calculated assuming competitive inhibition using eq. 2 because the substrate concentration was sufficiently low compared with their \( K_m \) values,

\[ CL_{v_{inh}} = \frac{CL}{1 + I/K_i} \]  

where \( CL \) represents the uptake clearance and the subscript (+inh) represents the value in the presence of inhibitor and \( I \) represents the concentration of inhibitor (micromolar). Fitting was performed by the nonlinear least-square method using a MULTI program, and the Damping Gauss Newton Method algorithm was used for fitting.

Results

Uptake of the H₂ Receptor Antagonists by hOAT1-, hOAT2-, hOAT3-HEK, and rOat3-HEK. Figures 1 and 2 show the time profiles and concentration dependence of the uptake of the H₂ receptor antagonists by hOAT1-, hOAT2-, hOAT3-, rOat3-3, and vector-HEK cells, respectively. As reported previously (Tahara et al., 2005), the uptake of cimetidine by hOAT1-HEK was slightly greater than that by mock cells (10.2 ± 0.8 versus 6.12 ± 1.71 μl/mg protein at 5 min). In addition, a slight increase was also observed in the uptake of ranitidine by hOAT1-HEK (12.7 ± 1.15 versus 10.2 ± 0.1 μl/mg protein at 5 min), but no specific uptake was observed for famotidine (Fig. 1A). Similar to hOAT1, the uptake of cimetidine and ranitidine by hOAT2-HEK was significantly greater than that in vector-HEK (1.65 and 6.93 μl/min/mg protein), but no specific uptake was observed for famotidine (Fig. 1B). In contrast, the uptake of cimetidine, famotidine, and ranitidine by hOAT3-HEK was significantly greater than in vector-HEK at all time points (Fig. 1C). The uptake of the H₂ receptor antagonists by rOat3-3-HEK was also significantly greater than in vector-HEK at all time points (Fig. 2A). Because the uptake of the H₂ receptor antagonists by hOAT3-3 and rOat3-3-HEK increased linearly up to 2 and 5 min of incubation, respectively, the uptakes of cimetidine and ranitidine by h/rOAT3 at 1 min, famotidine by hOAT3 at 2 min, and uptake by rOat3 at 5 min were used for further characterization.

The concentration dependence of the uptake of ranitidine by hOAT2-HEK, cimetidine, famotidine, and ranitidine by r/hOAT3-HEK was examined (Figs. 1B and C, and 2B). The kinetic parameters are summarized in Table 1. The \( K_m \) values of ranitidine for hOAT2 and hOAT3 were almost identical; however, the intrinsic transport activity (\( V_{max}/K_m \)) by hOAT2 was greater than that by hOAT3. The \( K_m \) value of famotidine was almost 3-fold greater for rOat3 than for hOAT3, and those of cimetidine and ranitidine for rat and human OATs were similar. The intrinsic transport activities of cimetidine by hOAT3 were three and four times greater than those of famotidine and ranitidine, whereas that of ranitidine by rOat3 was almost 14 and 2 times greater than that of famotidine and cimetidine, respectively.

Uptake of the H₂ Receptor Antagonists and TEA by hOCT2-, rOct1-, and rOct2-HEK. Figures 3 and 4 show the time profiles and concentration dependence of the uptake of the H₂ receptor antagonists by hOCT2-, rOct1-, and rOCT2-, and vector-HEK. The uptake of the H₂ receptor antagonists by hOCT2-, rOct1-, and rOCT2-HEK was significantly greater than in vector-HEK at all time points, although the specific uptake of ranitidine by rOCT2 was too low for further characterization (Figs. 3A and 4A). Because the uptake of the H₂ receptor antagonists by hOCT2-, rOCT2-, and rOCT2-HEK increased linearly up to an incubation time of 2 (hOCT2) and 3 (rOCTs) min, respectively, the uptake at 2 (hOCT2), 1 (rOCT1), and 3 (rOCT2) min was used for further characterization. In these OCTs-HEK, the uptake of TEA was significantly greater than in vector-HEK at all time points, and the transport activities by rOCT1-HEK (8.81 μl/min/mg protein) and hOCT2-HEK (4.74 μl/min/mg protein) were 3.2- and 1.7-fold greater than the activity by rOCT2-HEK (2.75).

The concentration dependence of the uptake of cimetidine, famotidine, and ranitidine by hOCT2-, rOCT1-, and rOCT2-HEK was examined (Figs. 3B and 4B), and the kinetic parameters were determined by nonlinear regression analysis (Table 2). In rat Octs, rOCT1 shows similar transport activities for all of the H₂ receptor antagonists examined, whereas rOCT2 preferentially transports cimetidine; the transport activities of famotidine and ranitidine by rOCT2 were fairly low compared with the activity of cimetidine. Ranitidine uptake by rOCT2 was 4-fold smaller than that of famotidine. The transport activities of the H₂ receptor antagonists by hOCT2 showed a similar trend, but the transport activity of ranitidine by hOCT2 was similar to that of famotidine. The \( K_m \) values of the H₂ receptor antagonists were similar between rat and human OCTs (Table 2). Because the specific uptake of ranitidine by hOCT2 was too low to allow determination of the kinetic parameters, ranitidine inhibition of the uptake of cimetidine and famotidine was investigated. Ranitidine inhibited the hOCT2-mediated transport of cimetidine and famotidine in a concentration-dependent manner with \( K_i \) values of 79.0 ± 8.6 and 30.5 ± 8.0 μM, respectively (Fig. 5A). Although ranitidine inhibited rOCT2-mediated famotidine uptake with a \( K_i \) value of 34.8 ± 7.2 μM, it had no effect on the rOCT2-mediated transport of cimetidine at the concentrations examined (0.1–1 mM) (Fig. 5A).

Effect of Probenecid on the Uptake of H₂ Receptor Antagonists (Cimetidine and Famotidine) by r/hOAT3, r/hOCT2, and rOCT1-HEK. The inhibitory effect of probenecid on the r/hOAT3-, r/hOCT2-, and rOCT1-mediated transport of cimetidine and famotidine was examined (Fig. 5, B and C). Probenecid strongly inhibited the r/hOAT3-mediated transport of cimetidine and famotidine in a concentration-dependent manner, whereas it had no inhibitory effect on the r/hOCT2- and rOCT1-mediated transport of cimetidine and famotidine at the concentrations examined (0.1–1 mM). The \( K_i \) values of probenecid for cimetidine and famotidine uptake by r/hOAT3-HEK were found to be 5.77 ± 0.97/3.37 ± 0.47 and 2.55 ± 0.31/4.17 ± 1.10 μM, respectively.
Fig. 1. Time profile and concentration dependence of the uptake of H₂ receptor antagonists by hOAT1-, hOAT2-, and hOAT3-HEK. The time-dependent uptake of cimetidine (CMD), famotidine (FMD), and ranitidine (RND) (10 μM) by hOAT1-, hOAT2-, and hOAT3-HEK was examined at 37°C. Closed and open circles represent the uptake by hOATs-HEK and vector-HEK, respectively (A). The concentration dependence of hOAT-mediated cimetidine, famotidine, and ranitidine uptake is shown as Eadie-Hofstee plots (B and C). The hOAT3-mediated uptake of cimetidine for 1 min, famotidine for 2 min, and ranitidine for 1 min and hOAT2-mediated uptake of ranitidine for 3 min were determined at various concentrations (3–1000 μM, range of concentration used). The hOAT-mediated transport was obtained by subtracting the transport velocity in vector-HEK from that in hOATs-HEK. Each point represents the mean ± S.E. (n = 3). Statistical differences in the uptake of h/rOAT3-HEK were compared with vector-HEK by a two-tailed 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).
To obtain an insight into the species-dependent effect of probenecid on the renal clearance of famotidine, the uptake of the H₂ receptor antagonists by basolateral organic ion transporters was examined. Transport studies using cDNA transfectants revealed that the H₂ receptor antagonists are substrates of r/hOAT3. The intrinsic transport activities (V_max/K_m) of the H₂ receptor antagonists by hOAT3 were similar, whereas famotidine showed a lower intrinsic transport activity by rOat3 than cimetidine and ranitidine (Figs. 1 and 2; Table 1). According to our previous characterization, the uptake of cimetidine normalized by benzylpenicillin transport was comparable between rat and human OAT3 (Tahara et al., 2005). In contrast, the relative transport activity of famotidine was 3-fold greater in hOAT3 than rOat3, whereas that of ranitidine was 8-fold greater in rOat3 than in hOAT3. Because the K_m value of famotidine for hOAT3 was 3-fold smaller than that for rOat3, the greater transport activity in hOAT3 is mainly accounted for by the smaller K_m value in hOAT3 (Table 1). For ranitidine, the increased V_max value in rOat3 will account for the greater transport activity by rOat3 than hOAT3 because the K_m values are similar (Table 1). A significant uptake of cimetidine and ranitidine was observed in hOAT2-HEK. In particular, the intrinsic transport activity of ranitidine by hOAT2 was greater than or similar to that of hOAT3 and hOCT2. Considering its low expression in the kidney compared with hOAT1, hOAT3, and hOCT2 (Motohashi et al., 2002), the contribution of hOAT2 to the total renal uptake may be limited. An inhibition study revealed that probenecid is a potent inhibitor of r/hOAT3 (Fig. 5). Because the unbound plasma concentrations of probenecid at clinical doses (0.5–2.0 g; range, 18–80 μM; Selen et al., 1982) were greater than its K_i value for hOAT3, inhibition of hOAT3 by probenecid could be clinically relevant.

The intrinsic transport activities of the H₂ receptor antagonists were compared between rOct1 and r/hOCT2. The K_m

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**Discussion**

To obtain an insight into the species-dependent effect of probenecid on the renal clearance of famotidine, the uptake of the H₂ receptor antagonists by basolateral organic ion transporters was examined. Transport studies using cDNA transfectants revealed that the H₂ receptor antagonists are substrates of r/hOAT3. The intrinsic transport activities (V_max/K_m) of the H₂ receptor antagonists by hOAT3 were similar, whereas famotidine showed a lower intrinsic transport activity by rOat3 than cimetidine and ranitidine (Figs. 1 and 2; Table 1). According to our previous characterization, the uptake of cimetidine normalized by benzylpenicillin transport was comparable between rat and human OAT3 (Tahara et al., 2005). In contrast, the relative transport activity of famotidine was 3-fold greater in hOAT3 than rOat3, whereas that of ranitidine was 8-fold greater in rOat3 than in hOAT3. Because the K_m value of famotidine for hOAT3 was 3-fold smaller than that for rOat3, the greater transport activity in hOAT3 is mainly accounted for by the smaller K_m value in hOAT3 (Table 1). For ranitidine, the increased V_max value in rOat3 will account for the greater transport activity by rOat3 than hOAT3 because the K_m values are similar (Table 1). A significant uptake of cimetidine and ranitidine was observed in hOAT2-HEK. In particular, the intrinsic transport activity of ranitidine by hOAT2 was greater than or similar to that of hOAT3 and hOCT2. Considering its low expression in the kidney compared with hOAT1, hOAT3, and hOCT2 (Motohashi et al., 2002), the contribution of hOAT2 to the total renal uptake may be limited. An inhibition study revealed that probenecid is a potent inhibitor of r/hOAT3 (Fig. 5). Because the unbound plasma concentrations of probenecid at clinical doses (0.5–2.0 g; range, 18–80 μM; Selen et al., 1982) were greater than its K_i value for hOAT3, inhibition of hOAT3 by probenecid could be clinically relevant.

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**Table 1**

<table>
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<tr>
<th>Isoform</th>
<th>Cimetidine</th>
<th>Famotidine</th>
<th>Ranitidine</th>
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<td></td>
<td>K_m μM</td>
<td>V_max pmol/min/mg protein</td>
<td>V_max/K_m</td>
</tr>
<tr>
<td>hOAT2</td>
<td>N.D.</td>
<td>1.65 (1)</td>
<td></td>
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<tr>
<td>hOAT3</td>
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<td>9.86 (1)</td>
<td>124 ± 4</td>
</tr>
<tr>
<td>rOat3</td>
<td>90.7 ± 4.8</td>
<td>5.64 (1)</td>
<td>345 ± 22</td>
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N.D., not determined; N.T., not transported.
values of the H₂ receptor antagonists were similar between rOct1 and rOct2; however, it was found that there was a difference in the intrinsic transport activities. The intrinsic transport activities by rOct1 are similar among the H₂ receptor antagonists, whereas rOct2 preferentially transports cimetidine (Table 2). The same trend was observed for hOCT2, although the absolute value of the intrinsic transport activity of ranitidine was greater than that by rOct2 (Table 2). Because the Kᵢ value of ranitidine for the uptake of cimetidine by rOct2 was very close to its Kᵢm value for hOCT2 (Table 2), this is likely accounted for by the difference in the Vₘₐₓ values. It is noteworthy that ranitidine had no effect on the uptake of cimetidine by rOct2 (Fig. 5). This observation may be explained by a recent model involving the structure of the substrate binding region of the poly-specific organic cation transporters (Popp et al., 2005). This model suggests that the organic cation transporters contain large binding regions with different binding sites for structurally different cations and explains why two transported cations may not inhibit each other. Mмотоhashi et al. (2004) could not detect any specific uptake of famotidine by hOCT2 in eRNA-injected Xenopus laevis oocytes. Furthermore, the IC₅₀ value of famotidine for the uptake of TEA by hOCT2 was reported to be 1.8 mM (Mмотоhashi et al., 2004), greater than its own Kᵢm value for hOCT2 determined in this study. This partly may be due to the difference in the method of detection and/or host cells between X. laevis oocytes and HEK293 cells. Because of the difference in the detection limit between LC-MS (1 nM) and HPLC-UV (200 nM) analysis, the substrate concentration employed in drug-drug interaction studies (Emanuelsson and Paalzow, 1988; Lin et al., 1988). Indeed, probenecid had a weak effect against OCTs. Probenecid was only a weak inhibitor with an IC₅₀ value (700 μM) greater than the plasma unbound concentration employed in drug-drug interaction studies (Emanuelsson and Paalzow, 1988; Lin et al., 1988). Indeed, probenecid had a weak effect against OCTs. Therefore, the absence of an inhibitory effect of probenecid on the renal elimination of cimetidine in rodents is rational. Both Oct1 and Oct2 can mediate the renal uptake of the H₂ receptor antagonists in rodent kidney. Their contribution was estimated based on the relative transport activities of the H₂ receptor antagonists with regard to the uptake of TEA to the renal uptake to which Oct1 and Oct2 apparently make identical contributions (Jonker et al., 2003). The normalized uptake data for TEA transport indicated that the Vₘₐₓ/Kᵢm of famotidine for hOCT2 determined in this study into consideration, it is possible that saturation of hOCT2-mediated transport makes it difficult to detect the specific transport of famotidine by hOCT2. However, the discrepancy involving the Kᵢm and IC₅₀ values remains to be resolved. Ciarimboli et al. (2004) reported that the IC₅₀ value of TEA for hOCT2 was different in CHO and HEK293 cells. Because the IC₅₀ value was modified by protein kinase activation (Mehrens et al., 2000; Ciarimboli et al., 2004), the host-dependent IC₅₀ value might be explained by the difference in the basal regulation of hOCT1. It is possible that such a difference in basal regulation of hOCT2 in X. laevis oocytes and HEK293 cells accounts for the discrepancy in the uptake of famotidine by hOCT2 and its Kᵢm and IC₅₀ values.

Fig. 3. Time profile and concentration dependence of the uptake of H₂ receptor antagonists by hOCT2-HEK. The time-dependent uptake of cimetidine (CMD), famotidine (FMD), and ranitidine (RND) (10 μM) by hOCT2-HEK was examined at 37°C. Closed and open circles represent the uptake by hOCT2-HEK and vector-HEK, respectively (A). The concentration dependence of hOCT2-mediated cimetidine, famotidine, and ranitidine uptake is shown as Eadie-Hofstee plots (B). The hOCT2-mediated uptake of cimetidine for 1 min, famotidine for 2 min, and ranitidine for 1 min was determined at various concentrations (3–1000 μM, range of concentration used). The hOCT2-mediated transport was obtained by subtracting the transport velocity in vector-HEK from that in rOAT3-HEK. Each point represents the mean ± S.E. (n = 3). Statistical differences in the uptake of hOCT2-HEK were compared with vector-HEK by a two-tailed 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).
ratio (rOct1/rOct2) is 4 for famotidine and 0.4 for cimetidine, suggesting that rOct1 plays a relatively more important role in famotidine uptake than rOct2 and vice versa as far as cimetidine uptake is concerned. This estimation is in good agreement with a previous report using Oct1 knockout mice in which the knockout of mOct1 resulted in only a small reduction in cimetidine accumulation in the kidney (Jonker et al., 2001). Lin et al. (1988) found that quinine inhibited the renal elimination of famotidine but did not affect the renal clearance of cimetidine. Because quinine is a more potent inhibitor of rOct1 than rOct2 (6-fold) (Arndt et al., 2001), the difference in the inhibition potency also supports our speculation.

The present findings highlight the importance of OAT3 as the site of drug-drug interactions involving probenecid. It is suggested that the species-dependent effect of probenecid is attributed to the following two factors. 1) The intrinsic transport activity of famotidine by OAT3 is greater in humans...
than in rats, and 2) unlike rodents, the renal uptake of organic cations in humans is solely accounted for by hOCT2, which preferentially transports cimetidine. These factors increase the contribution of OAT3 to the renal uptake of famotidine in humans. Although there are no clinical implications at present, it is possible that the renal clearance of ranitidine is also probenecid-inhibited in humans. Transport studies using cortical slices as well as isolated proximal tubules from human kidney will support this speculation. Because of the limited availability of human materials for transport studies, animals whose transporters have similar transport properties to human orthologs will be helpful. Rats or mice are not appropriate animal models for this purpose because of the inevitable species difference. Recently, we found that monkey OATs show similar transport properties to human OATs (Tahara et al., 2005). Therefore, the monkey will be a better animal model than rodents for predicting the incidence of drug-drug interactions in humans as far as basolateral up-

### TABLE 2

Kinetic parameters of the uptake of H₂ receptor antagonists by organic cation transporters

$K_m$ and $V_{max}$ were determined by nonlinear regression analysis as described under Materials and Methods. Data are taken from Figs. 3 and 4. Each value represents the mean ± computer-calculated S.D. The value in parentheses represents the relative transport activity with regard to cimetidine transport.

<table>
<thead>
<tr>
<th>Isoform</th>
<th>Cimetidine</th>
<th>Famotidine</th>
<th>Ranitidine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_m$ (μM)</td>
<td>$V_{max}$/pmol/min/mg protein</td>
<td>$V_{max}$/μl/min/mg protein</td>
</tr>
<tr>
<td>hOCT2</td>
<td>72.6 ± 13.9</td>
<td>2170 ± 240</td>
<td>29.9 (1)</td>
</tr>
<tr>
<td>rOct1</td>
<td>71.5 ± 10.7</td>
<td>2210 ± 190</td>
<td>30.9 (1)</td>
</tr>
<tr>
<td>rOct2</td>
<td>68.8 ± 8.0</td>
<td>1490 ± 100</td>
<td>21.7 (1)</td>
</tr>
</tbody>
</table>

N.D., not determined.
take is concerned. This should be further confirmed by comparing in vivo pharmacokinetics in monkeys and humans.

In addition to fumidzin, inhibition of renal elimination by probenicid has been reported for benzylpenicillin (Overbosch et al., 1988), cephalosporins (Shitara et al., 2005), oseltamivir (its active metabolite Ro 64-0802) (Hill et al., 2002), furosemide (Vree et al., 1995), bumetanide (Lau et al., 1983), cipronoxacin (Jaehe et al., 1995), enalaprilat/enalaprilat (Noormohamed et al., 1990), and fexofenadine (Yasui-Furukori et al., 2005). Among these drugs, benzylpenicillin (Tahara et al., 2005), cephalosporins (Jung et al., 2002), Uwai et al. (2002), oseltamivir (its active metabolite Ro 64-0802) (Hill et al., 2002), furosemide, and bumetanide (Hasanen et al., 2004) have been reported to be substrates of OAT1 and/or OAT3. Thus, these interactions are likely to involve inhibition of basolateral uptake in the kidney. Further studies are necessary to determine the importance of OAT1 and/or OAT3 in these drug-drug interactions. In addition to the kidney, Nagata et al. (2004) found that probenicid, given by an i.v. constant infusion, increased the cerebrospinal fluid concentrations of H₂ receptor antagonists (also given by an i.v. constant infusion) by inhibiting OAT3-mediated efflux at the choroid plexus (Nagata et al., 2004). In humans, probenicid will synergistically increase the accumulation of OAT3 substrates in the cerebrospinal fluid by inhibiting renal elimination as well as efflux transport at the choroid plexus.

In conclusion, the results of the present study suggest that the species difference in the drug-drug interaction between famotidine and probenicid may be ascribed to the difference in transport activity of fumidzin between rat and human OAT3 and the absence of OCT1 in the human kidney.

References


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