Involvement of Human Multidrug and Toxin Extrusion 1 in the Drug Interaction between Cimetidine and Metformin in Renal Epithelial Cells

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ABSTRACT

In human proximal tubules, organic cations are taken up from blood into cells by human organic cation transporter 2 (hOCT2/SLC22A2) and then eliminated into the lumen by apical H⁺/organic cation antiporters, human multidrug and toxin extrusion 1 (hMATE1/SLC47A1) and hMATE2-K (SLC47A2). To evaluate drug interactions of cationic drugs in the secretion process, epithelial cells engineered to express both hOCT2 and hMATE transporters are required to simultaneously evaluate drug interactions with renal basolateral and apical organic cation transporters. In the present study, therefore, we assessed the drug interaction between cimetidine and metformin with double-transfected Madin-Darby canine kidney cells stably expressing both hOCT2 and hMATE1 as an in vitro model of the proximal tubular epithelial cells. The basolateral-to-apical transport and intracellular accumulation of [¹⁴C]metformin by a double transfectant were markedly inhibited by 1 mM cimetidine at the basolateral side. On the other hand, 1 μM cimetidine at the basolateral side moderately decreased the basolateral-to-apical transport of [¹⁴C]metformin and significantly increased the intracellular accumulation of [¹⁴C]metformin from the basolateral side, suggesting that cimetidine at a low concentration inhibits apical hMATE1, rather than basolateral hOCT2. Actually, in concentration-dependent inhibition studies by a single transporter expression system, such as human embryonic kidney 293 stably expressing hMATE1, hMATE2-K, or hOCT2, cimetidine showed higher affinity for hMATEs than for hOCT2. These results suggest that apical hMATE1 is involved in drug interactions between cimetidine and cationic compounds in the proximal tubular epithelial cells.

Drug interactions involving metabolism and/or excretion cause marked changes in plasma and intracellular concentrations of the affected drug. Such interactions can lead to severe adverse effects or unexpected pharmacological effects. Recent studies have shown that many transporters play important roles in the uptake and subsequent secretion of drugs in the liver and kidney, and several specific transporter proteins involved in drug interactions have been reported (Shitara et al., 2005; Endres et al., 2006; Li et al., 2006). However, most of these reports examined the involvement of either apical or basolateral transporters. To reproduce drug interactions via renal transporters in vivo, analyses by the epithelial cells with transcellular transport systems are needed.

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ABBREVIATIONS: h, human; OCT, organic cation transporter; SLC, solute carrier; MATE, multidrug and toxin extrusion; MDCK, Madin-Darby canine kidney; TEA, tetraethylammonium; HEK, human embryonic kidney; r, rat.
among hMATE1, hMATE2-K, and hOCT2, the affinity of various drugs for hMATE1 and hMATE2-K was elucidated. Then, based on a comparison of the affinity for the hMATE family and hOCT2, the involvement of hMATE1 in the drug interaction between cinemetine and metformin was evaluated by using double-transfected Madin-Darby canine kidney (MDCK) cells stably expressing both hOCT2 and hMATE1 (MDCK-hOCT2/hMATE1 cells). Recently, we have established this double transfectant, and demonstrated that MDCK-hOCT2/hMATE1 cells are an appropriate in vitro model of human tubular epithelial cells to evaluate the transepithelial transport of cationic drugs (Sato et al., 2008).

Materials and Methods

Materials. Pramipexole dihydrochlo-rohydrate and tali-peoxide hydrochloride were kindly provided by Nippon Boehringer Ingelheim (Tokyo, Japan). [14C]Tetraethylammonium (TEA) (2.035 GBq/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO). [14C]Metformin (962 MBq/mmol) and [3H]mannitol (2 GBq/mmol) were purchased from Moravek Biochemicals (Brea, CA). [N-Methyl-3H]cinemetine (470 GBq/mmol) was obtained from GE Healthcare (Chalfont St. Giles, UK). D-[1-3H(N)]mannitol (525.4 GBq/mmol) was acquired from PerkinElmer Life and Analytical Sciences (Boston, MA). Quinidine sulfate, verapamil hydrochloride,isopropylhydrochloride,diphenhydraminehydrochloride, (±)-chlorpheniramine maleate, and cinemetine were purchased from Nacalai Tesque (Kyoto, Japan). Metformin hydrochloride, diphenhydramine hydrochloride, procainamide hydrochloride, ranitidine hydrochloride, procainamide hydrochloride, ranitidine hydrochloride, and amantidine hydrochloride were obtained from Sigma-Aldrich (St. Louis, MO). Disopyramide, desipramine hydrochloride and famotidine were acquired from Wako Pure Chemicals (Osaka, Japan). Cetirizine was obtained from KLT Labs (St. Paul, MN). All other chemicals used were of the highest purity available.

Cell Culture. According to our previous report (Tanihara et al., 2007), HEK293 cells stably expressing hMATE1 (HEK-hMATE1 cells) or hMATE2-K (HEK-hMATE2-K cells) were cultured in complete medium consisting of Dulbecco’s modified Eagle’s medium (Sigma-Aldrich) with 10% fetal bovine serum (Invitrogen, Carlsbad, CA) and hygromycin B (0.2 mg/ml; Invitrogen) in an atmosphere of 5% CO2/95% air at 37°C. The double-transfected MDCK-hOCT2/hMATE1 cells were previously established in our laboratory (Sato et al., 2008). MDCK-hOCT2/hMATE1 cells were cultured in complete medium consisting of Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum, hygromycin B (0.2 mg/ml), and G418 (0.5 mg/ml; Nacalai Tesque) in an atmosphere of 5% CO2/95% air at 37°C. In the previous study, we demonstrated that hOCT2 and hMATE1 were localized on the basolateral and apical membrane, respectively, in MDCK-hOCT2/hMATE1 cells by immunofluorescence microscopy (Sato et al., 2008). HEK293 cells stably expressing hOCT2 (HEK-hOCT2 cells) were cultured according to a previous report (Urakami et al., 2004).

Preparation of Membrane Vesicles from HEK-hMATE1 and HEK-hMATE2-K Cells. HEK-hMATE1 or HEK-hMATE2-K cells were seeded on 100-mm plastic dishes (5 × 10⁶ cells/dish), with 25 dishes used in a single preparation. Then, plasma membrane vesicles were prepared according to our previous report (Tsuda et al., 2007).

Transport Experiments by Membrane Vesicles. The uptake of [14C]TEA by membrane vesicles from HEK-hMATE1 or HEK-hMATE2-K cells was measured using a rapid filtration technique according to our previous report (Tsuda et al., 2007). Nonspecific absorption was determined by the addition of [14C]TEA to 1 ml of ice-cold stop solution containing 20 μl of membrane vesicles. This value was subtracted from the total uptake data. The protein content was determined by the method of Bradford (1976) using a Bio-Rad Protein Assay Kit (Bio-Rad, Hercules, CA) with bovine γ-globulin as a standard.

Transport Experiments by Transfectants. The cellular uptake of radiolabeled compounds was performed as reported previously (Urakami et al., 2004; Tanihara et al., 2007). The transepithelial transport experiments with radiolabeled compounds were performed as described previously (Sato et al., 2008). In brief, MDCK-hOCT2/hMATE1 cells were seeded on microporous membrane filters (3.0-μm pores, 4.7-cm² growth area) inside a Transwell cell culture chamber (Corning Life Sciences, Lowell, MA) at a density of 18 × 10⁵ cells/well. On the 7th day after the seeding, the cells were used for transepithelial transport experiments. The composition of the incubation medium was as follows: 145 mM NaCl, 3 mM KCl, 1 mM CaCl₂, 0.5 mM MgCl₂, 5 mM d-glucose, and 5 mM HEPES, pH 7.4, or MES, pH 6.0. After the removal of the culture medium from both sides of the monolayers, the cell monolayers were preincubated with 2 ml of incubation medium, pH 7.4, on each side for 10 min at 37°C. Then, 2 ml of incubation medium, pH 7.4, containing both a radiolabeled compound and radiolabeled mannitol was added to the basolateral side, and 2 ml of nonradioactive incubation medium, pH 6.0, was added to the apical side. The monolayers were incubated for specified periods of time at 37°C. Radiolabeled mannitol, a compound that is not transported by the cells, was used to calculate paracellular flux and the extracellular trapping of substrates. For transport measurements, an aliquot (100 μl) of the incubation medium on the apical side was taken at specified times, and the radioactivity was determined in 5 ml of ACSII by liquid scintillation counting. For accumulation studies, the medium was removed by aspiration at the end of the incubation period, and the monolayers were rapidly washed twice with 2 ml of ice-cold incubation medium, pH 7.4, on each side. The filters were detached from the chambers, the cells on the filters were solubilized with 0.5 ml of 0.5 N NaOH, and the radioactivity in aliquots (300 μl) was determined in 3 ml of ACSII by liquid scintillation counting. The protein content of the solubilized cells was determined using a Bio-Rad Protein Assay Kit with bovine γ-globulin as a standard (Bradford, 1976).

Measurement of Intracellular Volume. The equilibrium uptake of sulfanilamide was determined according to standard procedures for transepithelial transport experiments with a slight modification. In brief, after the preincubation, 2 ml of incubation medium, pH 7.4, containing both 10 mM sulfanilamide and [3H]mannitol was added to both sides. The intracellular volume was calculated from the equilibrium uptake (60 min) and external concentration of sulfanilamide. Sulfanilamide was diazotized and coupled with 2-DEAE-1-naphthylamine oxalate, and the amount of colored material was determined spectrophotometrically at 550 nm (Saito et al., 1986).

Data Analysis. Data were expressed as the means ± S.D. Two or three experiments were conducted, and representative results were shown. Apparent Kᵢ values were expressed as the means ± S.E. and analyzed statistically using the unpaired Student’s t test. Data from transport experiments were analyzed statistically with the one-way analysis of variance followed by Dunnett’s test.

Results

Determination of the Driving Force of hMATE1 and hMATE2-K with Membrane Vesicles from HEK-hMATE1 and HEK-hMATE2-K Cells. At first, to validate the driving force of hMATE1 and hMATE2-K, we carried out [14C]TEA uptake studies with membrane vesicles from HEK-hMATE1 or HEK-hMATE2-K cells. In the presence of an outwardly directed H⁺ gradient, a marked stimulation of [14C]TEA uptake (overshoot phenomenon) was observed in membrane vesicles from HEK-hMATE1 and HEK-hMATE2-K cells (Fig. 1, A and B). Furthermore, [14C]TEA uptake by hMATE1 and hMATE2-K was not altered by the presence of valinomycin with or without...
the H⁺ gradient (Fig. 1, C and D). These findings indicated that both transporters utilized an oppositely directed H⁺ gradient as a driving force. Subsequent experiments were carried out in the presence of an outwardly directed H⁺ gradient to evaluate the transport characteristics of the MATE family.

Comparison of Substrate Affinity for hMATE1, hMATE2-K, and hOCT2. To compare the substrate affinity for hMATE1 and hMATE2-K, the apparent $K_i$ values of various cationic drugs for the uptake of $[14C]$TEA were determined. Cationic drugs inhibited $[14C]$TEA uptake via hMATE1 and hMATE2-K in a dose-dependent manner. Figures 2 and 3 show representative inhibition curves of histamine H₂ receptor antagonists and antiparkinsonian agents, respectively. Table 1 provides a summary of the apparent $K_i$ values of various drugs for hMATE1 and hMATE2-K. Histamine H₂ receptor antagonists such as cimetidine and famotidine showed high affinity for hMATE1 and hMATE2-K as compared with other drugs. Although many drugs tended to have higher affinity for hMATE1 than for hMATE2-K, only pramipexole, an antiparkinsonian agent, exhibited higher affinity for hMATE2-K than for hMATE1. Table 2 shows the affinity for hMATE1, hMATE2-K, and OCT2 of various compounds excreted from the kidney. $K_m$ values of hOCT2 and rat (r) OCT2 were cited from previous papers (Urakami et al., 2001; Ishiguro et al., 2005; Koepsell et al., 2007). Most drugs showed higher affinity for OCT2 than for hMATEs.

Effects of Cimetidine on Transcellular Transport of $[14C]$Metformin in MDCK-hOCT2/hMATE1 Cells. It is interesting that histamine H₂ receptor antagonists such as cimetidine showed higher affinity for hMATEs than for OCT2 (Table 2). Furthermore, recent clinical pharmacokinetic and pharmacogenomic analyses of metformin have paid attention to the renal secretion process (Shikata et al., 2007; Song et al., 2008; Takane et al., 2008; Wang et al., 2008). Therefore, we examined the effect of different concentrations of cimetidine on the basolateral-to-apical transport and intracellular accumulation of $[14C]$metformin in MDCK-hOCT2/hMATE1 cells. We have demonstrated recently that MDCK-hOCT2/hMATE1 showed the vectorial transcellular transport of organic cations, such as TEA and metformin.
As shown in Fig. 4A, the transcellular transport of [14C]metformin was moderately inhibited by the presence of 1 mM cimetidine and almost completely inhibited by the presence of 1 mM cimetidine. The cellular accumulation of [14C]metformin was inhibited by 1 mM cimetidine but increased by 1 μM cimetidine (Fig. 4B). Furthermore, we examined the inhibitory effects of cimetidine on the uptake of [14C]metformin by hOCT2. Cimetidine had little effect at 1 μM but inhibited the uptake at 1 mM (Fig. 5). The apparent Ki value of cimetidine for hOCT2 was calculated to be 147.1 ± 11.0 μM. These results suggest that 1 mM cimetidine competitively inhibits the uptake of [14C]metformin by hOCT2, resulting in a drastic decrease in both the transcellular transport and the intracellular accumulation of [14C]metformin. On the other hand, 1 μM cimetidine inhibits the apical efflux of [14C]metformin via hMATE1, but not the uptake by hOCT2, causing a marked increase in its cellular accumulation.

**Intracellular Concentration of [3H]Cimetidine in MDCK-hOCT2/hMATE1 Cells.** To confirm that hMATE1 is specifically inhibited by intracellular cimetidine taken up from the basolateral side, the intracellular concentration of cimetidine was determined. The amount of intracellular cimetidine after 60 min of incubation was demonstrated to be 10.0 ± 0.5 pmol/mg protein. We then measured the intracellular volume of MDCK-hOCT2/hMATE1 cells with the equilibrium uptake of sulfanilamide and found that it was 2.2 ± 0.4 μl/mg protein. Based on these findings, the intracellular concentration of cimetidine was calculated as 4.5 ± 0.4 μM. Considering the Ki values of cimetidine for hMATE1 and hOCT2, this intracellular concentration suggests that the transport of cationic drugs via hMATE1 is specifically inhibited by intracellular cimetidine, but transport via hOCT2 is little inhibited by intracellular cimetidine (Fig. 6).

**Discussion**

We reported that hMATE1 and hMATE2-K have a similar substrate specificity for many endogenous organic cations and cationic drugs and that several substrates have higher affinity for hMATE1 than for hMATE2-K (Tanihara et al., 2007). In the present study, four cationic drugs had similar affinity for hMATE1 and for hMATE2-K, and 12 cationic drugs were found to have higher affinity for hMATE1 than for hMATE2-K (Table 1). This would suggest that hMATE1 and hMATE2-K have complementary roles in the renal se-
creatin of organic cations. It is interesting that only pramipexole showed markedly higher affinity for hMATE2-K than for hMATE1 (Fig. 3; Table 1). It has been reported that pramipexole is transported by OCT1 and OCT2 (Ishiguro et al., 2005), but it is unclear whether pramipexole is transported by the hMATE family and hOCT2. Pramipexole may be a good probe to distinguish the molecular mechanisms by which the hMATE family recognizes substrates.

In the present study, most drugs showed higher affinity for OCT2 than for hMATEs (Table 2). Histamine H₂ receptor antagonists such as cimetidine and famotidine, however, showed higher affinity for hMATEs than for hOCT2. In particular, the \( K_i \) values of cimetidine for hMATE1 and for hOCT2 were 1.1 and 8.6 to 73 \( \mu M \) (Koepsell et al., 2007), respectively. Taking into consideration the effective blood concentration of cimetidine (2.0–3.6 \( \mu M \)) (Benet et al., 1996), it is assumed that hOCT2 is little inhibited by cimetidine at clinical doses, but hMATE1 is inhibited by cimetidine taken up from blood into cells. To verify this hypothesis, we performed transcellular transport studies with a double transfectant composed of basolateral hOCT2 and apical hMATE1. We found that 1 \( \mu M \) cimetidine was taken up from the

### TABLE 1

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Drug</th>
<th>Affinity for hMATE1</th>
<th>Affinity for hMATE2-K</th>
<th>Comparison of Affinity</th>
<th>Effective Plasma Concentration</th>
<th>( K_i ) Values (( K_i ) for hMATE1/( K_i ) for hMATE2-K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histamine H₂ receptor antagonists</td>
<td>Cimetidine</td>
<td>1.1 ± 0.3</td>
<td>7.3 ± 0.7</td>
<td>1.5</td>
<td>0.15</td>
<td>MATE &lt; OCT</td>
</tr>
<tr>
<td></td>
<td>Famotidine</td>
<td>0.6 ± 0.2</td>
<td>9.7 ± 0.4</td>
<td>0.06</td>
<td>0.10</td>
<td>MATE &lt; OCT</td>
</tr>
<tr>
<td></td>
<td>Ranitidine</td>
<td>25.4 ± 2.1</td>
<td>25.0 ± 0.8</td>
<td>1.02</td>
<td>0.33</td>
<td>MATE &lt; OCT</td>
</tr>
<tr>
<td>Antiparkinsonian agents</td>
<td>Amantadine</td>
<td>111.8 ± 5.9</td>
<td>1167.0 ± 100.5</td>
<td>0.10</td>
<td>0.55</td>
<td>MATE &lt; OCT</td>
</tr>
<tr>
<td></td>
<td>Pramipexole</td>
<td>141.4 ± 24.2</td>
<td>24.1 ± 3.4</td>
<td>5.86</td>
<td>0.05</td>
<td>MATE &lt; OCT</td>
</tr>
<tr>
<td></td>
<td>Talipexole</td>
<td>66.0 ± 3.7</td>
<td>119.5 ± 15.3</td>
<td>0.55</td>
<td>0.06</td>
<td>MATE &lt; OCT</td>
</tr>
</tbody>
</table>

\( K_i \) values of various drugs for \([^{14}C]TEA\) uptake by hMATE1 and hMATE2-K. Each value represents the mean ± S.E. for three independent experiments.

**Notes:**
- \( K_i \) values obtained from previous work (Tanihara et al., 2007).
- The effective plasma concentration obtained from the data of Benet et al. (1996).
basolateral side without an inhibitory effect on metformin transport via hOCT2, and intracellular cimetidine inhibited the transport of metformin via hMATE1 (Figs. 4–6). These results suggest that hMATE1 is responsible for the drug interaction between cimetidine and metformin. In fact, it has been reported that cimetidine significantly increased the area under the curve of metformin by an average of 50% and reduced its renal clearance over 24 h by 27% in human subjects (Somogyi et al., 1987). Cimetidine has been shown to be an inhibitor of the active tubular secretion of many cationic drugs, including procainamide (Somogyi et al., 1983; Christian et al., 1984), N-acetylpromacainamide (Somogyi et al., 1983), ranitidine (van Crugten et al., 1986), triamterene (Muirhead et al., 1986), pilsicainide (Shiga et al., 2000), and varenicline (Feng et al., 2008). These drug interactions could be partially mediated by hMATE1 at the apical membrane in the proximal tubules.

Recently, it was reported that the 808G>T polymorphism of hOCT2 is associated with a reduced tubular secretion clearance of metformin, and the inhibition by cimetidine also seemed to be dependent on this mutation (Wang et al., 2008). These results suggested that the drug interaction between cimetidine and metformin was affected by accumulation of metformin in the renal epithelial cells. This report would support our notion that the hMATE family plays an important role in the cimetidine-metformin interaction in vivo.
Yokoo et al., 2007). The present findings suggested that coadministration of cimetidine may increase the tubular accumulation of oxaliplatin by inhibiting hMATE2-K. Double-transfected MDCK-hOCT2/hMATE1 and MDCK-hOCT2/hMATE2-K cells should be very useful in vitro tools for the screening of renal drug interactions with cationic drugs and nephrotoxicity.

In this study, we clearly demonstrated the driving force of hMATE1 and hMATE2-K to be an oppositely directed H⁺ gradient, not inside-negative membrane potential (Fig. 1). These results suggested that [14C]TEA transport via hMATE1 and hMATE2-K is the electroneutral antipporter of H⁺ and it. The results showed that there was no difference in the driving force between hMATE1 and hMATE2-K and are consistent with a report on transport study with human renal brush-border membrane vesicles (Ott et al., 1990). We also demonstrated previously that [14C]TEA transport by rMATE1 is driven by an oppositely directed H⁺ gradient and is electroneutral (Tsuda et al., 2007), suggesting the driving force for the MATE family to be conserved among human and rodents.

In conclusion, we demonstrated for the first time that apical hMATE1 is involved in the drug interaction between cimetidine and metformin in renal epithelial cells. These results provide important information on the physiological and pharmacokinetic roles of the MATE family and may help to avoid the unexpected adverse effects of drug interactions with cationic drugs.

References


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