The Inhibition of Human Multidrug and Toxin Extrusion 1 Is Involved in the Drug-Drug Interaction Caused by Cimetidine

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ABSTRACT:

Cimetidine is known to cause drug-drug interactions (DDIs) with organic cations in the kidney, and a previous clinical study showed that coadministration of cimetidine or probenecid with fexofenadine (FEX) decreased its renal clearance. FEX was taken up into human kidney by human organic anion transporter (hOAT) 3 (SLC22A8), but the mechanism of its luminal efflux has not been clarified. The present study examined the molecular mechanism of these DDIs. Saturable uptake of FEX was observed in human kidney slices, with $K_m$ and $V_{max}$ values of 157 ± 7 µM and 418 ± 16 nmol/15 min/g kidney, respectively. Cimetidine only slightly inhibited its uptake even at 100 µM, far greater than its clinically relevant concentration, whereas 10 µM probenecid markedly inhibited its uptake. As candidate transporters for the luminal efflux of FEX, we focused on human multidrug and toxin extrusions MATE1 (SLC47A1) and MATE2-K (SLC47A2). Saturable uptake of FEX could be observed in human embryonic kidney 293 cells expressing human MATE1 (hMATE1), whereas hMATE2-K-specific uptake of FEX was too small to conduct its further kinetic analysis. The hMATE1-mediated uptake clearance of FEX was inhibited by cimetidine in a concentration-dependent manner, and it was decreased to 60% of the control value in the presence of 3 µM cimetidine. Taken together, our results suggest that the DDI of FEX with probenecid can be explained by the inhibition of renal uptake mediated by hOAT3, whereas the DDI with cimetidine is mainly caused by the inhibition of hMATE1-mediated efflux of FEX rather than the inhibition of its renal uptake process.

Drug-drug interactions (DDIs) caused by the inhibition of metabolism and/or transport increase the plasma and tissue concentration of drugs and modify their pharmacological/adverse effects. Recent studies have revealed that many kinds of drug transporters play important roles in the tissue uptake and subsequent excretion of drugs in the liver and kidney. In general, drug transporters exhibit broad substrate specificities, and even the nonsubstrates of certain transporter can inhibit the transporter function, suggesting the possible transporter-mediated DDIs with many kinds of compounds (Shitara et al., 2005; Endres et al., 2006).

Cimetidine is well known to cause DDIs involving the inhibition of cytochrome P450-mediated metabolism in the liver. In addition, it also inhibits renal excretion of zwitterionic drugs, such as fexofenadine (FEX) (Yasui-Furukori et al., 2005) and cephalaxin (van Crugten et al., 1986), and cationic drugs, such as varenicline (Feng et al., 2008), procainamide (Somogyi et al., 1983), dofetilide (Abel et al., 2000), pindolol (Somogyi et al., 1992), pilsicainide (Shiga et al., 2000), and metformin (Somogyi et al., 1987), resulting in an increase in their area under the plasma concentration-time curves at the clinically relevant dose of cimetidine. Several drug uptake and efflux transporters are expressed in the proximal tubular cells and realize the directional transepithelial transport leading to the efficient secretion of drugs. Thus, these renal transporters can be potential target sites of DDIs with drugs mainly cleared in urine.

As for FEX, in addition to cimetidine, probenecid, a potent inhibitor for renal organic anion transporters (OATs), also decreases the renal clearance of FEX in healthy subjects (Yasui-Furukori et al., 2005). We have proposed that the inhibition of the renal uptake mediated by hOAT3 (SLC22A8) is a likely mechanism for the DDI with probenecid because 1) FEX is a substrate of hOAT3, but not other basolateral transporters, such as hOAT1 (SLC22A6), hOAT2 (SLC22A7), and human organic cation transporter (hOCT) 2 (SLC22A2) in the kidney; and 2) probenecid can inhibit hOAT3-mediated uptake at its clinical concentration (Tahara et al., 2006). In contrast, because the maximum plasma protein unbound concentration of cimetidine (5.2 µM) at a clinical dose (400 mg) (van Crugten et al., 1986) is far below its
reported $K_m$ (113 $\mu$M) and IC$_{50}$ (92 $\mu$M) values for hOAT3 (Khamdang et al., 2004; Tahara et al., 2006), and $K_m$ value for hOCT2 (67 $\mu$M) (Motohashi et al., 2004), its inhibitory effect on hOCT3- and hOCT2-mediated renal uptake should be negligible in the clinical situation. Therefore, cimetidine-mediated DDIs with FEX and other cationic drugs may be caused by the inhibition of their luminal efflux in the kidney.

The purpose of this study is to investigate the mechanism underlying the DDIs between FEX and cimetidine. The minor inhibitory effect of cimetidine on the basolateral uptake of FEX was confirmed by using human kidney slices, which had been used for the characterization of the contribution of each transporter to the renal uptake of drugs and the involvement of uptake transporters in the clinically relevant DDIs (Nozaki et al., 2007a,b). Then, the inhibitory effect of cimetidine on the luminal efflux transporters was examined. The present study especially focused on the multidrug and toxin exclusion (MATE) proteins as candidate target transporters of DDIs by cimetidine. Among MATE family proteins, human MATE1 (hMATE1/SLC47A1), hMATE2/2-K (SLC47A2), rat MATE1 (rMATE1), mouse MATE1 (mMATE1), and mMATE2 have been cloned in mammals (Otsuka et al., 2005; Masuda et al., 2006; Ohta et al., 2006; Terada et al., 2006). All MATE proteins function as an exchange of H$. Therefore, cimetidine-mediated DDIs with FEX and other cationic drugs may be caused by the inhibition of their luminal efflux in the kidney.

Materials and Methods

Materials. [3H]p-Aminophenylphosphate (PAH) (4.1 Ci/mmol) and [14C]TEA (5 mCi/mmol) were purchased from PerkinElmer Life and Analytical Sciences (Waltham, MA). [3H]Benzenesulfinic acid (PCG) (59 mCi/mmol) and [3H]cimetidine (12.7 Ci/mmol) were purchased from GE Healthcare Technologies (Waukesha, WI). FEX hydrochloride was purchased from Toronto Research Chemicals (North York, ON, Canada). All other chemicals and regents were of analytical grade and commercially available.

Preparation of Human Kidney Slices and Uptake of Test Compounds by Human Kidney Slices. The study protocol was approved by the Ethics Review Boards at both Graduate School of Pharmaceutical Sciences in The University of Tokyo (Tokyo, Japan) and Tokyo Women’s Medical University (Tokyo, Japan). All participants provided written informed consent.

Intact renal cortical tissues were obtained from two surgically nephrectomized patients with renal cell carcinoma at Tokyo Women’s Medical University in October 2006 and January 2007. Samples of human kidney from subjects were stored in Dulbecco’s modified Eagle’s medium (DMEM) low glucose (Invitrogen, Carlsbad, CA) on ice immediately after removal. After 30-min transportation, kidney slices were prepared as described below. Uptake studies were carried out as described in a previous report (Nozaki et al., 2007a). Kidney slices (300 $\mu$m in thickness) from intact human cortical tissue (Otsuka et al., 2005; Masuda et al., 2006; Ohta et al., 2006; Terada et al., 2006), are thought to be candidate transporters for the efflux of cationic drugs in the kidney. Interestingly, Otsuka et al. (2005) first showed that 10 $\mu$M cimetidine inhibited approximately half of the hMATE1-mediated transport of TEA, and the $K_m$ value of cimetidine for rMATE1 is 3.0 $\mu$M (Ohta et al., 2006), which is comparable with its clinical plasma protein unbound concentration. Accordingly, it is possible that cimetidine inhibits MATE proteins in the clinical situations and thereby decreases the renal clearance of substrates. In this study, the uptake of FEX was determined in HEK293 cells expressing hMATE1 and hMATE2-K, and the inhibitory effect of cimetidine on FEX uptake was also examined.

Uptake Study Using MATE Expression Systems. Cells were seeded in 24-well plates coated with poly-L-lysine at a density of 1.5 $\times$ 10$^5$ cells per well and cultured in DMEM containing 10% fetal bovine serum and 800 $\mu$g/ml geneticin for 2 to 3 weeks. Antibiotic-resistant clones were selected and tested for the transport of cimetidine.

Cell Culture and Stable Transfection. HEK293 cells were maintained at 37°C and 5% CO2 in DMEM with 10% fetal bovine serum, 100 units/ml penicillin, and 100 $\mu$g/ml streptomycin. Cells were transfected with the hMATE1/pCI-neo or hMATE2-K/pCI-neo or pCI-neo (for control cells) by the calcium phosphate coprecipitation method (Ohta et al., 2006) and cultured in DMEM containing 10% fetal bovine serum and 800 $\mu$g/ml genetin for 2 to 3 weeks. Antibiotic-resistant clones were selected and tested for the transport of cimetidine.

Uptake Study Using MATE Expression Systems. Cells were seeded in 24-well plates coated with poly-L-lysine at a density of 1.5 $\times$ 10$^5$ cells per well and cultured in DMEM containing 10% fetal bovine serum and 800 $\mu$g/ml geneticin for 2 to 3 weeks. Antibiotic-resistant clones were selected and tested for the transport of cimetidine.

LCMS Analyses. The aliquots (150 $\mu$l) obtained from the uptake study using human kidney slices were precipitated with 300 $\mu$l of methanol containing 50 $\mu$l midazolam as an internal standard. After centrifugation (15,000g;
Transport Kinetics of the Uptake of FEX in Human Kidney Slices. Human kidney slices were prepared from two independent subjects for transport studies. To confirm whether the transport activity of hOAT1, hOAT3, and hOCT2 was maintained in human kidney slices, we used in the current study, the uptake of PAH, PCG, and TEA, which are typical substrates of hOAT1, hOAT3, and hOCT2, respectively, into human kidney slices was observed. The uptake clearance of PAH, PCG and TEA at the tracer concentration was 3.52 ± 0.44 (at 0.1 μM PAH), 3.42 ± 0.36 (at 10 μM PCG), and 9.14 ± 1.59 ml/15min/g kidney (at 10 μM TEA) (mean ± S.E.; n = 4), respectively, and the excess amount of each ligand decreased its uptake clearance [0.982 ± 0.028 (at 1 mM PAH), 0.953 ± 0.086 (at 1 mM PCG), and 1.57 ± 0.39 ml/15min/g kidney (at 1 mM TEA) (mean ± S.E.; n = 4)].

Saturable uptake of FEX was also clearly observed in two batches of human kidney slices, and the Km and Vmax values were determined to be 157 ± 7 μM and 418 ± 16 nmol/15min/g kidney (mean ± computer-calculated S.D.; n = 3 of two independent batches), respectively (Fig. 1).

Effects of Probencid and Cimetidine on the Uptake of FEX in Human Kidney Slices. The inhibitory effects of probenecid and cimetidine on the uptake of FEX (0.3 μM) in human kidney slices were examined. The uptake of FEX was significantly inhibited by 10 μM probenecid and above, whereas it was not significantly inhibited by up to 100 μM cimetidine, and only 1000 μM cimetidine could inhibit the FEX uptake (Fig. 2).

Uptake of FEX in hMATE1- and hMATE2-K-Expressing HEK293 Cells. The uptake of FEX (1 μM) in hMATE1- and hMATE2-K-expressing HEK293 cells was examined. The uptake of FEX in hMATE1-expressing cells was significantly greater than that in vector-transfected control cells (Fig. 3A), and its specific uptake clearance was decreased in the concentration-dependent manner (Fig. 3C). In contrast, the uptake of FEX in hMATE2-K-expressing cells was slightly larger than that in vector-transfected cells (Fig. 3B). Intracellular acidification by NH4Cl to generate the outward-directed proton gradient does not enhance the uptake of FEX in all kinds of transfectants (data not shown). As a positive control, the hMATE1- and hMATE2-K-specific uptake clearances of cimetidine (50 nM) were 55.0 ± 3.0 and 20.2 ± 0.6 μl/min/mg protein (mean ± S.E.; n = 4), respectively.

Inhibitory Effect of Cimetidine on the Uptake of FEX in hMATE1-Expressing HEK293 Cells. The inhibitory effect of cimetidine on the uptake of FEX (1 μM) in hMATE1-expressing HEK293 cells was examined. Cimetidine showed significant inhibitory effect on the specific uptake of FEX by hMATE1. Cimetidine inhibited the
hMATE1-mediated uptake of FEX by 40% at 3 µM and almost completely at 300 µM (Fig. 4).

Discussion

Despite the accumulated clinical evidences demonstrating that cimetidine inhibits the renal elimination of several cationic drugs, its underlying mechanism remains to be clarified. In the present study, we especially focused on the involvement of MATE family transporters in cimetidine-mediated DDIs using FEX as a test drug, whose renal uptake is mainly mediated by OAT3.

To confirm that clinically relevant concentration of cimetidine cannot significantly inhibit the OAT3-mediated renal uptake of FEX, we checked the inhibitory effect of cimetidine on FEX uptake in human kidney slices. The uptake of FEX into human kidney slices was saturable (Fig. 1), and its K_m value was almost comparable with that for human OAT3 (70 µM) (Tahara et al., 2006). Under such condition, its uptake clearance was not significantly reduced even in the presence of 100 µM cimetidine (Fig. 2B). Because the clinical maximum plasma unbound concentration of cimetidine (5.2 µM) at a dose of 400 mg (van Crugten et al., 1986) is far below the IC_{50} value in human kidney slices, inhibition of the basolateral uptake of FEX by cimetidine will not occur in the clinical situation. In contrast, probenecid significantly inhibited the uptake of FEX in human kidney slices even at 10 µM (Fig. 2A). Thus, as proposed previously by Tahara et al. (2006), the DDI between FEX and probenecid probably involves the inhibition of the renal uptake of FEX via hOAT3 by probenecid.

The inhibition of luminal efflux of FEX can be another potential mechanism of DDI by cimetidine because efficient transcellular transport of FEX across the renal proximal tubular cells is thought to be realized by the efflux transporters as well as the uptake transporters, but the transporters involved in its luminal efflux are still unknown. The fact that FEX is a substrate of human multidrug resistance (MDR) 1 and multidrug resistance-associated protein (MRP) 2 might suggest its involvement in the luminal efflux of FEX in human kidney (Cvetkovic et al., 1999; Matsushima et al., 2008a), although the deficient expression of Mdr1a/1b or Mrp2 in rodents did not affect the total and renal clearance of FEX (Tahara et al., 2005; Matsushima et al., 2008a). However, when considering the drug-drug interaction between cimetidine and FEX, the reported IC_{50} values of cimetidine for the transport mediated by human MDR1 and MRP2 are thought to be much higher (>50 µM) compared with its clinical protein unbound concentration (Schwab et al., 2003; Pedersen et al., 2008). This indicates that the inhibition of MDR1 and MRP2 by cimetidine should be negligible in the clinical drug-drug interaction. In a recent study, the MATE family proteins have been identified in mammals as efflux transporters for organic cations in the kidney in terms of their driving force and substrate specificity (Otsuka et al., 2005). Significant uptake of FEX was observed both in hMATE1- and hMATE2-K-expressing HEK293 cells compared with the control cells (Fig. 3, A and B). Furthermore, the hMATE1-mediated uptake of FEX was saturable (Fig. 3C) and also inhibited by cimetidine (Fig. 4). We performed the uptake assay under the K^+ -rich condition, which is the same situation as inside the cells. Thus, in vitro uptake via MATE is thought to correspond to the efflux transport in the kidney under the physiological situation. Oppositely directed proton gradient is originally thought to be a driving force of MATE family transporters (Otsuka et al., 2005, 2006; Tsuda et al., 2007). However, in our cases, the FEX transport was not sensitive to intracellular acidification because the preincubation with NHE1 did not significantly enhance its uptake. Very recently, Ohta et al. (2008) have demonstrated that norfloxacin uptake was also not sensitive to the intracellular acidification. Because both norfloxacin and FEX are zwitterionic drugs, they suggested that outwardly directed proton gradient is not necessary for the MATE-mediated transport of some compounds and the mode of rat Mate1-mediated transport might depend on the electrochemical characteristics of the substrates (Ohta et al., 2008).

The inhibition potency of cimetidine for hMATE1 was similar to the previously reported potency for ratMate1 (Hiasa et al., 2006), and was much higher than that for renal uptake. In addition, because cimetidine is a substrate of basolateral uptake transporters (human OAT1, OAT3, and OCT2) in the kidney, it is actively taken up from the blood into the kidney, which results in the higher protein unbound concentration in the proximal tubular cells than that in the blood circulation. Therefore, cimetidine may show greater inhibitory effect on the luminal efflux mediated by hMATE1 than our estimation calculated by the plasma protein unbound concentration. Taken together, it is possible that the DDI between FEX and cimetidine is mainly caused by the inhibition of hMATE1-mediated luminal efflux of FEX by cimetidine in the kidney. The hMATE2-K-specific uptake of FEX was not high enough to examine the inhibitory effect of cimetidine. Currently, the relative contribution of hMATE1 and hMATE2-K to the overall luminal efflux of drugs remains to be
elucidated. Further studies are needed to demonstrate the importance of each MATE isoform in the luminal efflux of FEX and the impact of its inhibition by cimetidine on the change in the clinical pharmacokinetics of FEX.

In addition to FEX, the inhibition of MATE1 by cimetidine may account for other DDIs of cationic drugs. As noted previously, the Ki or IC50 values of cimetidine for hOCT2 are also much higher than the clinical plasma protein unbound concentration (Khamdang et al., 2004; Motohashi et al., 2004), suggesting the inhibition of hOCT2 by cimetidine never cause the clinical DDIs. Alternatively, it is possible that these DDIs can be ascribed to the inhibition of MATE-mediated efflux across the brush-border membrane of the kidney rather than the inhibition of renal uptake. Indeed, metformin, procainamide, and cephalaxin, which interact with cimetidine in the clinical situation, have been identified as substrates of rMate1, hMATE1 and hMATE2-K (Terada et al., 2006; Tsuda et al., 2007).

Otsuka et al. (2005) showed that MATE1 is expressed not only in the kidney but also in the liver and skeletal muscle. We and other groups demonstrated that transporters other than Mdr1, Mrp2, and Bcrp are involved in the biliary excretion of FEX at least in mice (Matsushima et al., 2008a; Tian et al., 2008). Because hMATE1 is located on the bile canalicular membrane in the liver (Otsuka et al., 2005), hMATE1 might be one of the candidate transporters involved in the biliary excretion of FEX and cimetidine may also inhibit its hepatobiliary transport. In contrast, Yasui-Furukori et al. (2005) showed that coadministration of cimetidine did not affect the systemic exposure of FEX, although it significantly reduced its renal clearance in human clinical study. From our estimation, two thirds of the bioavailable FEX is excreted into bile in humans (Matsushima et al., 2008b). Thus, if any, the possible inhibition of hMATE1 in the liver by cimetidine does not have an impact on the systemic exposure of FEX, although we never deny the possible involvement of human MATE1 in its hepatic distribution.

In conclusion, our results suggest that the DDI between FEX and cimetidine is mainly caused by the inhibition of the luminal efflux mediated by MATE1 rather than the inhibition of renal uptake, whereas the DDI between FEX and probenecid can be explained by the inhibition of its uptake via hOAT3.

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Tahara H, Kusuhara 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.


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