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The inhibition of human multidrug and toxin extrusion 1 (hMATE1) is involved in the drug-drug interaction caused by cimetidine

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Abbreviations: DDI, drug-drug interaction; MATE, multidrug and toxin extrusion; FEX, fexofenadine; MDR, multidrug resistance; OAT, organic anion transporter; OCT, organic cation transporter; PAH, *p*-aminohippuate; PCG, benzylpenicillin; TEA, tetraethylammonium; AUC, area under the plasma concentration-time curve; P-gp, P-glycoprotein

Abstract

Cimetidine is known to cause drug-drug interactions (DDIs) with organic cations in the kidney and a previous clinical study showed that co-administration of cimetidine or probenecid with fexofenadine (FEX) decreased its renal clearance. FEX was taken up into human kidney by human organic anion transporter 3 (hOAT3; *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/15min/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 HEK293 cells expressing human MATE1 (hMATE1), while 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, while 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.

Introduction

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 non-substrates 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 CYP-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 cephalexin (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 (AUCs) 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 since (1) FEX is a substrate of hOAT3, but not other basolateral transporters, such as hOAT1 (*SLC22A6*), hOAT2 (*SLC22A7*), and human organic cation transporter 2 (hOCT2/*SLC22A2*) in the kidney, and (2) probenecid can inhibit hOAT3-mediated uptake at its clinical concentration (Tahara et al., 2006). In contrast, since 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 μM) and IC_{50} (92 μM) values for hOAT3 (Khamdang et al., 2004; Tahara et al., 2006), and K_m value for hOCT2 (67 μM) (Motohashi et al., 2004), its inhibitory effect on hOAT3- 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; Nozaki et al., 2007b). Then, the inhibitory effect of cimetidine on the luminal efflux transporters was examined. The present study especially focused on the multidrug and toxin compound extrusion (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^+ and a variety of organic cations, such as tetraethylammonium (TEA) and cimetidine, and some zwitter-ionic compounds, such as cephalexin (Tsuda et al., 2007). Since hMATE1 and hMATE2-K are

expressed on the brush border membrane of the proximal tubular cells in human kidney (Otsuka et al., 2005; Masuda et al., 2006; Ohta et al., 2006; Terada et al., 2006), they are thought to be candidate transporters for the efflux of cationic drugs in the kidney. Interestingly, Otsuka et al. (2005) have first shown that 10 μM cimetidine inhibited approximately half of the hMATE1-mediated transport of TEA and the K_m value of cimetidine for rMate1 is 3.0 μM (Ohta et al., 2006), which is comparable to 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.

Materials and Methods

Materials.

[^{3}H]*p*-Aminohippuate (PAH) (4.1 Ci/mmol) and [^{14}C]TEA (5 mCi/mmol) were purchased from Perkin-Elmer Life and Analytical Sciences (Boston, BA). [^{14}C]benzylpenicillin (PCG) (59 mCi/mmol) and [^{3}H]cimetidine (12.7 Ci/mmol) were purchased from GE Healthcare Bio Sciences (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, 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 2 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 (D-MEM) 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 previous reports (Nozaki et al., 2007a). Kidney slices (300 μm thick) from intact human cortical tissue were kept in ice-cold buffer before use. The buffer for the present study consisted of 120 mM NaCl, 16.2 mM KCl, 1 mM $CaCl_2$, 1.2 mM $MgSO_4$, and 10mM NaH_2PO_4/Na_2HPO_4 adjusted to pH 7.5. One slice, weighing 3 to 10 mg, was selected and incubated at 37 °C on a 12-well plate with 1 mL oxygenated buffer in each well after pre-incubation of slices for 5 min at 37 °C. After incubating for 15 min, slices were rapidly removed from the incubation buffer, washed twice in ice-cold buffer, blotted on filter paper, and weighed. The slices including FEX were solubilized in 100 μL 0.2 N NaOH, and kept overnight at room temperature. The solutions were neutralized by adding 50 μL 0.4 N HCl and used for LC/MS quantification (Shimadzu, Kyoto, Japan) as described below. The slices including radiolabeled compounds were dissolved in 1 mL Soluene-350 (Packard Instruments, Downers Grove, IL) at 55 °C for 12 hr. The radioactivity

in the specimens was then determined in scintillation cocktail (Hionic Flour; Packard Instruments).

Isolation of hMATE1 and hMATE2-K.

The cDNAs of hMATE1 and hMATE2-K were cloned from the human kidney total RNA (Clontech) by reverse transcription and subsequent PCR. Briefly, a reverse transcription reaction was carried out using 3 μg of the total RNA, an oligo (dT) primer and ReverTra Ace (TOYOBO) as a reverse transcriptase. The cDNAs of hMATE1 and hMATE2-K were isolated from the human kidney cDNA by PCR using KOD plus polymerase (TOYOBO) and the following primers: hMATE1-F (forward primer containing a SalI restriction site), 5'- GTC GAC GCC ACC ATG GAA GCT CCT GAG GAG CCC -3'; hMATE1-R (reverse primer containing a SmaI restriction site), 5'- ACC CGG GTT TCT TTC CTA CCA CGT CAC T -3'; hMATE2-K-F (forward primer containing an EcoRI restriction site), 5'- GAA TTC CGC CAC CAT GGA CAG CCT CCA GGA CAC AG -3'; hMATE2-K-R (reverse primer), 5'- GCT AGT GCC TGG TGG CTA GGA TC -3'. These primers were designed based on the sequence in GenBank™ (accession number, AAH10661 (hMATE1); AAH35288 (hMATE2-K)). PCR was

performed using the following conditions: 94°C for 2 min; 35 cycles of (i) 94°C for 20 sec, (ii) 60°C for 20 sec, and (iii) 72°C for 1.5 min. The amplified cDNA products were sub-cloned into pME18 vector, and digested with SalI and SmaI for hMATE1, and EcoRI and SalI, which is for a restriction site in pME18 vector, for hMATE2-K, and then transferred into a mammalian expression vector, pCI-neo (Promega, Madison, WI). Each sequence of the amplified cDNA product was determined with an automated sequencer (ABI PRISM 3100, Applied Biosystems, Foster City, CA) and confirmed to be identical to that in GenBank™.

Cell culture and stable transfection.

HEK293 cells were maintained at 37°C and 5% CO_2 in D-MEM with 10% fetal bovine serum (FBS), 100 units/ml penicillin and 100 µg/ml streptomycin. Cells were transfected with the hMATE1/pCI-neo or hNATE2-K/pCI-neo or pCI-neo (for mock cells) by the calcium phosphate co-precipitation method (Ohta et al., 2006) and cultured in D-MEM containing 10% FBS and 800 µg/ml geneticin for 2-3 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 x 10^5 cells per well and cultured for 24 to 36 hr. The transport study was carried out as described previously (Ohta et al., 2006). Before transport assays, cells were washed and preincubated for 15 min with uptake buffer (140 mM KCl, 0.4 mM KH_2PO_4, 0.8 mM $MgSO_4$, 1.0 mM $CaCl_2$, 25 mM glucose, and 10 mM HEPES, pH 7.4). For generating outwardly-directed proton gradient by intracellular acidification, cells were washed and preincubated with uptake buffer containing 20 mM NH_4Cl for 10 min, and then with uptake buffer without NH_4Cl for 5 min (Ohta et al., 2006). Uptake was initiated by adding the uptake buffer containing FEX or [^{3}H]cimetidine. All the procedures were conducted at 37 °C. The uptake was terminated at a designated time by adding ice-cold uptake buffer (2 mL). Then, cells were washed twice with 2 mL ice-cold buffer, solubilized in 500 μL 0.2 N NaOH, and kept overnight at room temperature. Aliquots (500 μL) were transferred to vials after adding 100 μL 1 N HCl. Regarding the measurement of FEX, aliquots (400 μL) were used for LC/MS quantification (Shimadzu) as described below.

Regarding the measurement of cimetidine, the radioactivity in the sample was measured in a liquid scintillation counting. The remaining 50 μL of the aliquots of cell lysate were used to determine the protein concentration by the Lowry method with bovine serum albumin as a standard.

LC/MS analyses.

The aliquots (150 μL) obtained from the uptake study using human kidney slices were precipitated with 300 μL methanol containing 50 nM midazolam as an internal standard. After centrifugation (15000 g, 10 min, 4 °C) of the mixture, 50 μL 0.05% formic acid was added to 50 μL supernatant. The aliquots (400 μL) obtained from the uptake study were precipitated with 800 μL methanol containing 5 nM midazolam as the internal standard. After centrifugation (15000 g, 10 min, 4 °C) of the mixture, the supernatant (750 μL) was evaporated using a centrifugal concentrator (CC-105, TOMY, Tokyo, Japan), and dissolved in 80 μL mobile phase. The obtained samples were subjected to the LC/MS analysis to determine the concentration of FEX.

An LC/MS-2010 EV equipped with a Prominence LC system (Shimadzu) was used for the analysis. The samples were separated on a CAPCELL PAK

C18 MG column (3 μm, 4.6 mm ID, 75 mm, Shiseido, Tokyo, Japan) in binary gradient mode. For the mobile phase, 0.05% formic acid and methanol were used. The methanol concentration was initially 48%, then linearly increased to 61.5% over 4.5 min. Finally, the column was re-equilibrated at a methanol concentration of 48% for 3 min. The total run time was 7.5 min. FEX and midazolam were eluted at 4.1 min and 2.8 min, respectively. In the mass analysis, FEX and midazolam were detected at a mass-to-charge ratio of 502.3 and 326.1 under positive ionization conditions, respectively. The interface voltage was 3.5 kV, and the nebulizer gas (N_2) flow was 1.5 L/min. The heat block and curved desolvation line temperatures were 200 and 150 °C, respectively.

Kinetic analysis for the uptake by human kidney slices.

Ligand uptake was expressed as an uptake volume (μL/g kidney), given as the amount of the kidney slices (pmol/g kidney) divided by the substrate concentration in the incubation medium (μM). Kinetic parameters were obtained using the following equation :

$$v = \frac{V_{max} \times S}{K_m + S} \qquad \text{(Eq.1)}$$

where *v* is the uptake velocity of the substrate (pmol/g kidney), S is the substrate concentration in the medium (μM), K_m is the Michaelis constant (μM), and V_{max} is the maximum uptake rate (pmol/g kidney). Fitting was performed by the nonlinear least squares method using a MULTI program and the Damping Gauss-Newton Method algorithm (Yamaoka et al., 1981).

Statistical Analyses

Statistical differences were analyzed by using Student's *t* test to identify significant differences between two sets of data and by one-way analysis of variance with Dunnett's test for multiple pair-wise comparisons. Significant differences were considered to be present at $P < 0.05$.

Results

Transport kinetics of the uptake of FEX in human kidney slices

Human kidney slices were prepared from 2 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 1mM PAH), 0.953 ± 0.086 (at 1mM PCG), and 1.57 ± 0.39 mL/15min/g kidney (at 1mM TEA) (mean ± S.E., n=4)).

Saturable uptake of FEX was also clearly observed in two batches of human kidney slices, and the K_m, and V_{max} 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 (Figure 1).

Effects of probenecid 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, while it was not significantly inhibited by up to 100 μM cimetidine and only 1000 μM of cimetidine could inhibit the FEX uptake (Figure 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 (Figure 3A), and its specific uptake clearance was decreased in the concentration-dependent manner (Figure 3C). On the other hand, the uptake of FEX in hMATE2-K-expressing cells was slightly larger than that in vector-transfected cells (Figure 3B). Intracellular acidification by NH_4Cl to generate the outwardly-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 (Figure 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 (Figure 1) and its K_m value was almost comparable to 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 (Figure 2B). Since 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 (Figure 2A). Thus, as

previously proposed by Tahara et al (2006), the DDI between FEX and probenecid likely 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 since 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 1 (MDR1) and multidrug resistance-associated protein 2 (MRP2) might suggest its involvement in the luminal efflux of FEX in human kidney (Cvetkovic et al., 1999; Matsushima et al., 2008b), though 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. Recently, 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 (Figure 3A). Furthermore, the hMATE1-mediated uptake of FEX was saturable (Figure 3C) and also inhibited by cimetidine (Figure 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; Ohta et al., 2006; Tsuda et al., 2007). However, in our cases, the FEX transport was not sensitive to intracellular acidification because the preincubation with NH_4Cl 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 one for rMate1 (Hiasa et al., 2006), and was much higher than that for renal uptake. In addition, since 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 K_i or IC_{50} 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 cephalexin, 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. On the other hand, Yasui-Furukori et al. (2005) have shown that coadministration of cimetidine did not affect the systemic exposure of FEX, though 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, though 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, while the DDI between FEX and probenecid can be explained by the inhibition of its uptake via hOAT3.

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Footnotes

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Legends for Figures

Figure 1 Concentration-dependent uptake of FEX into human kidney slices.

The concentration-dependent uptake of FEX into human kidney slices was determined using two different batches of human kidney slices and the results were shown as an Eadie-Hofstee plot. The solid line represents the fitted curves calculated by non-linear regression analysis. The uptake of FEX was measured for 15 min at 37 °C. Three slices were used in each batch of human kidney. Each point and vertical/horizontal bar represents the mean ± S.E. (n=6).

Figure 2. Effects of probenecid and cimetidine on the uptake of FEX by human kidney slices.

The inhibitory effects of probenecid (A) and cimetidine (B) were determined using two different batches of human kidney slices. The uptake of FEX was measured for 15 min at 37 °C. Three slices were used in each batch of human kidney. Each point and bar represents the mean ± S.E. (n=6)

*: P<0.05, **: P<0.01

Figure. 3 Uptake study of FEX using MATE-expressing HEK293 cells.

Time profiles of the uptake of 1μM FEX in hMATE1- (A and C) and hMATE2-K- (B) expressing HEK293 cells were investigated. Closed and open circles represent the uptake in MATE-expressing and vector-transfected control cells, respectively. Concentration-dependent uptake of FEX in hMATE1-expressing cells for 5 min was determined (C). Each point and bar represents the mean ± S.E. (n=3). Where no vertical bars are shown, the S.E. values are within the limits of the symbols. *: P<0.05, **: P<0.01

Figure. 4 Inhibitory effect of cimetidine on hMATE1-mediated uptake of FEX.

The inhibitory effect of cimetidine on the hMATE1-mediated uptake of 1μM FEX was observed. Each point and bar represents the mean ± S.E. (n=3). **: P<0.01

Figure 1

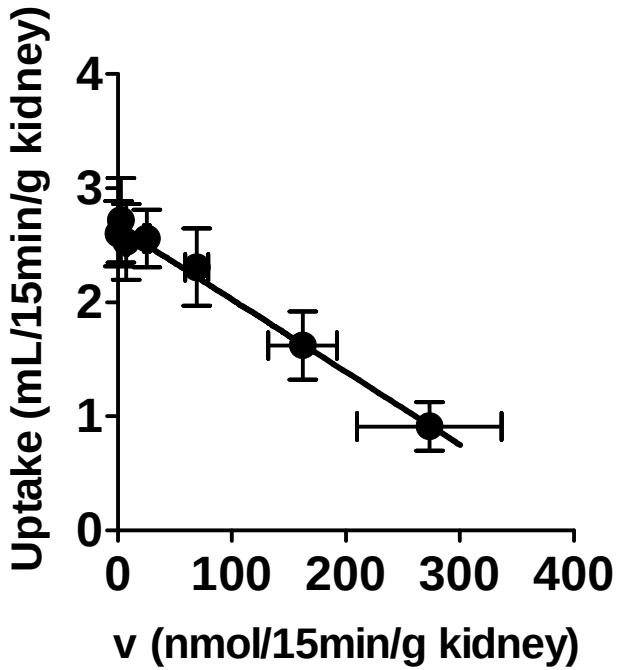

Figure 2

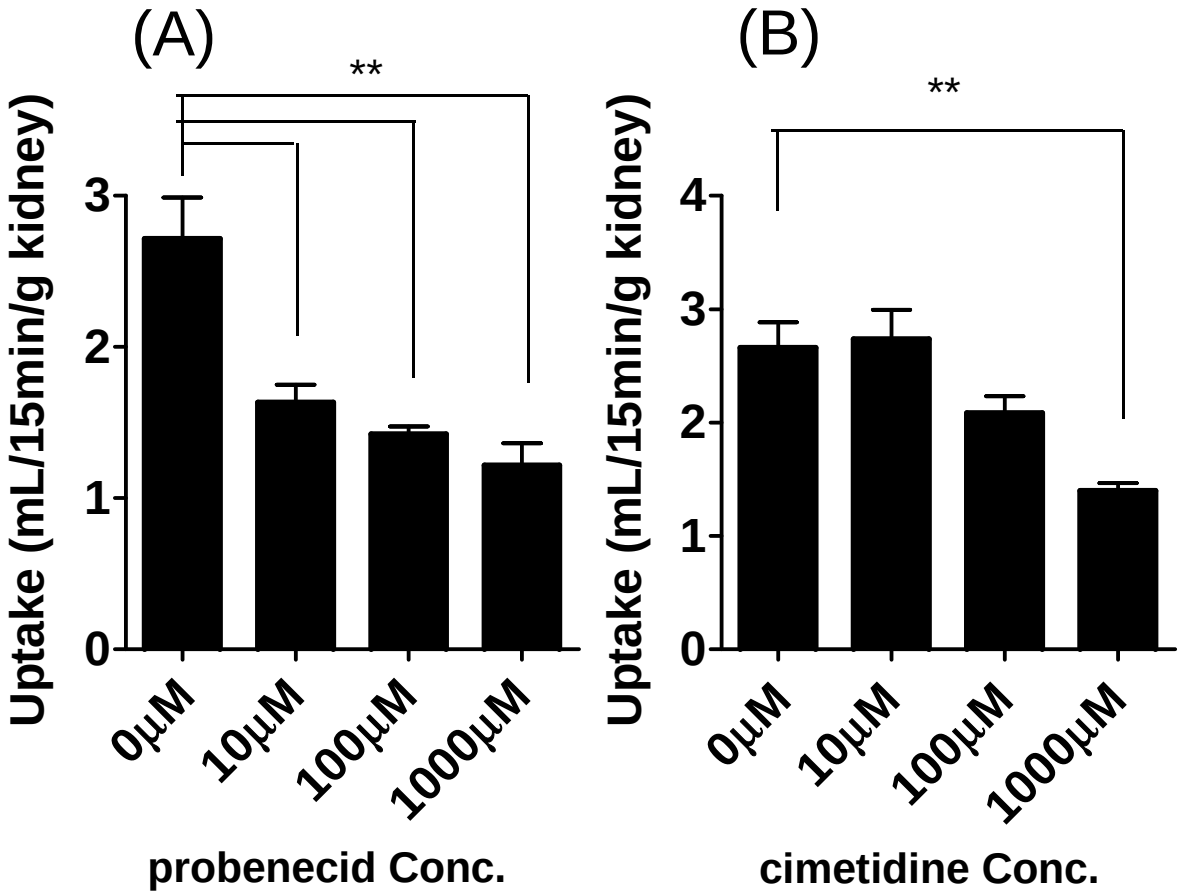

Figure 3

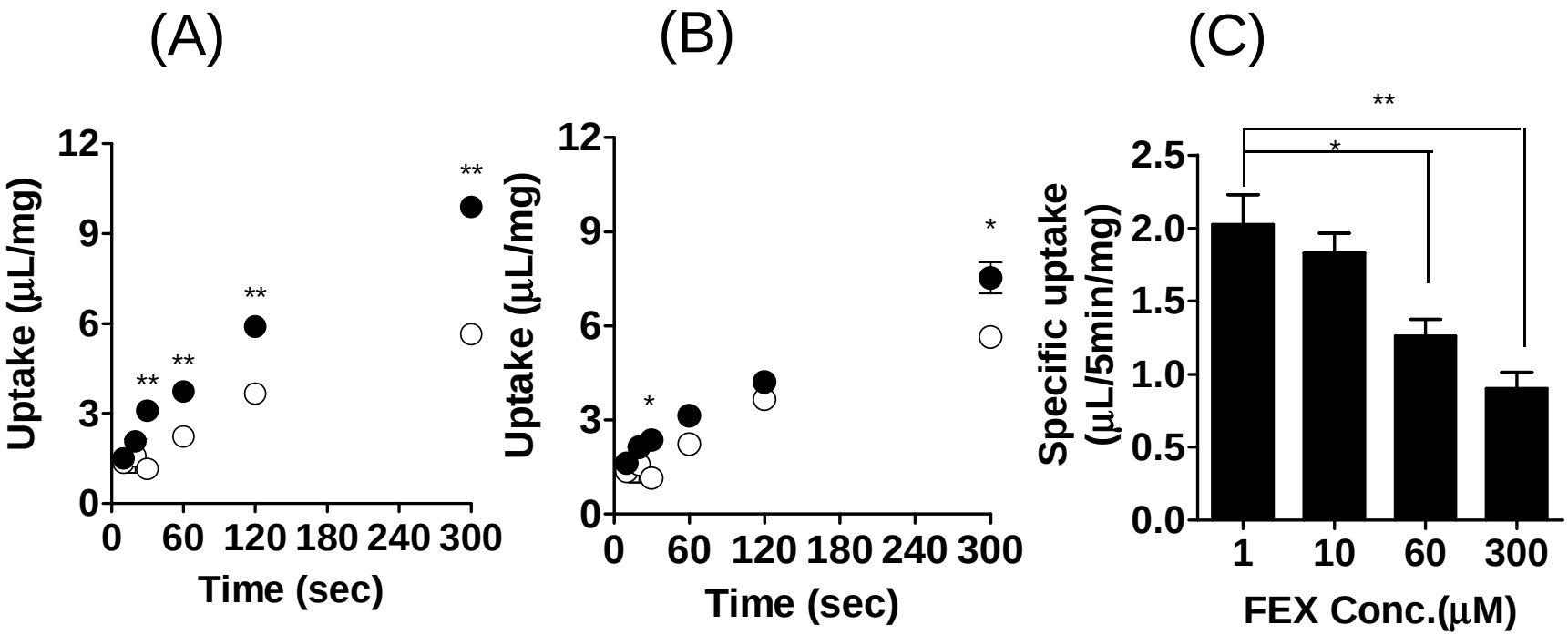

Figure 4