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Interaction of human and rat organic anion transporter 2 with various cephalosporin antibiotics

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Abstract

Cephalosporin antibiotics are thought to be excreted into the urine via organic anion transporters (OATs) and OAT can mediate nephrotoxicity by cephalosporins, particularly by cephaloridine. The purpose of this study was to elucidate the interaction of human-OAT2 and rat-OAT2 with cephalosporin antibiotics using proximal tubule cells stably expressing human-OAT2 and rat-OAT2. Human-OAT2 is localized to the basolateral side of the proximal tubule, whereas rat-OAT2 is localized to the apical side of the proximal tubule. Cephalosporins tested were cephalothin, cefoperazone, cefazolin, ceftriaxone, cephaloridine, cefotaxime, cefadroxil and cefamandole. These cephalosporins dose-dependently inhibited organic anion uptake mediated by human-OAT2 and rat-OAT2. There was no species difference observed for the effects of OAT2 with cephalosporins between human and rat transporters. Kinetic analysis revealed that the inhibitory effects for human-OAT2 were competitive. Cephaloridine significantly decreased the viability of cells stably expressing human-OAT2, human-OAT1, human-OAT3 and human-OAT4. The decreased viability of cells stably expressing human-OAT1, human-OAT3 and human-OAT4 but not human-OAT2 was reversed by probenecid. In conclusion, human-OAT2 interacts with cephalosporins, and thus, human-OAT2 may mediate the uptake of cephalosporins on the basolateral side of the proximal tubule. The interaction of human-OAT2 with cephalosporins was the weakest among the basolateral human-OATs tested. In addition, it is suggested that human-OATs mediate cephaloridine-induced nephrotoxicity.

Keywords: Organic anion transporter; Proximal tubule; Cephalosporin antibiotic; Transport; Cell line

1. Introduction

The secretion of numerous organic anions, including endogenous metabolites, drugs and xenobiotics, is an important physiological function of renal proximal tubules. The process of secreting organic anions through these proximal tubular cells is achieved via unidirectional transcellular transport. This involves the uptake of organic anions into the cells from the blood across the basolateral membrane, followed by extrusion across the brush-border membrane into the tubular fluid (Pritchard and Miller, 1993). Recently, cDNAs encoding organic anion transporters (OATs) have been cloned, including OAT1 (Sekine et al., 1997; Reid et al., 1998; Hosoyamada et al., 1999), OAT2 (Sekine et al., 1998; Enomoto et al., 2002), OAT3 (Kusuhara et al., 1999; Cha et al., 2001) and OAT4 (Cha et al., 2000). Among these clones, human-OAT2, human-OAT1 and human-OAT3 were shown to be localized to the basolateral side of the proximal tubule (Enomoto et al., 2002; Hosoyamada et al., 1999; Cha et al., 2001), whereas human-OAT4 was localized to the apical side of the proximal tubule (Babu et al., 2002b).

The primary route of excretion of most of the cephalosporin antibiotics is renal. Cephalosporin antibiotics are thought to be not only filtered through the glomerulus but

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also actively secreted by the proximal tubules via the para-aminohippuric acid transporter system (Hori et al., 1982; Kasher et al., 1983; Moller and Sheikh, 1983; Takano et al., 1989; Ullrich et al., 1989). Cephaloridine is a first-generation cephalosporin antibiotic that induces acute renal failure in humans and animals (Tune, 1997). The nephrotoxicity induced by cephaloridine is characterized by acute proximal tubular necrosis and is mainly dependent on its accumulation and concentration in the renal cortex. It has been long assumed that cephaloridine is actively taken up by the proximal tubular cells from the blood via basolateral OAT. Consistent with these results, we have also observed that human/rat-OAT1, human/rat-OAT3 and human-OAT4 interact with various cephalosporin antibiotics (Jariyawat et al., 1999; Takeda et al., 1999, 2002a; Jung et al., 2002), and that rat-OAT1 and rat-OAT3 are involved in the induction of cephaloridine-induced nephrotoxicity (Jariyawat et al., 1999; Takeda et al., 1999; Jung et al., 2002). However, the interactions of human-OAT2 and rat-OAT2 with cephalosporin antibiotics, and the role of human-OATs in cephaloridine-induced nephrotoxicity have not been elucidated yet.

The purposes of this study were (1) to elucidate the interaction of human-OAT2 with various cephalosporin antibiotics using cells derived from the second segment of the proximal tubule (S_2) that stably express human-OAT2 (S_2 human-OAT2); (2) to elucidate the species difference between human-OAT2 and rat-OAT2 with regard to their interactions with cephaloporin antibiotics; and (3) to determine whether human-OAT2, human-OAT1, human-OAT3 and human-OAT4 mediate cephaloridine-induced nephrotoxicity.

2. Materials and methods

2.1. Materials

Prostaglandin $F_{2\alpha}$ (7946 Ci/mmol) was purchased from Perkin-Elmer Life Sciences (Boston, MA, USA). Cephalosporin antibiotics and probenecid were obtained from Sigma (St. Louis MO, USA). Other materials used included fetal bovine serum, trypsin and geneticin from Gibco (Gaithersburg, MD, USA), recombinant epidermal growth factor from Wakunaga (Hiroshima, Japan), insulin from Shimizu (Shizuoka, Japan), RITC 80-7 culture medium from Iwaki (Tokyo, Japan) and TfX-50 from Promega (Madison, WI, USA). Pravastatin was a gift from Sankyo (Tokyo, Japan).

2.2. Cell culture and establishment of S_2 human-OAT2 and S_2 cells stably expressing rat-OAT2 (S_2 rat-OAT2)

S_2 cells, derived from transgenic mice harboring the simian virus 40 large T-antigen gene, were established as described previously (Hosoyamada et al., 1996). S_2 is the segment of the proximal tubule where human-OAT1 and human-OAT3 are localized (Hosoyamada et al., 1999; Cha et al., 2001). The establishment and characteristics of S_2 cells stably expressing human-OAT1, human-OAT3 and human-OAT4 (S_2 human-OAT1, S_2 human-OAT3 and S_2 human-OAT4) were previously described (Takeda et al., 2001, 2002a,b; Kimura et al., 2002; Babu et al., 2002a,b; Enomoto et al., 2002; Khamdang et al., 2002). S_2 human-OAT2 and S_2 rat-OAT2 were established as follows. The full-length cDNA of human-OAT2 was isolated by screening the human kidney cDNA library using rat-OAT2 cDNA (Sekine et al., 1998) as a probe. The full-length cDNAs of human-OAT2 and rat-OAT2 were subcloned into pcDNA 3.1 (Invitrogen, Carlsbad, CA), a mammalian expression vector. S_2 human-OAT2 and S_2 rat-OAT2 were obtained by transfecting S_2 cells with pcDNA3.1-human-OAT2 or pcDNA3.1-rat-OAT2 using TfX-50 according to the manufacturer's instructions. S_2 cells transfected with pcDNA3.1 lacking an insert were designated as S_2 pcDNA 3.1 and used as a control (mock). The cells were grown in a humidified incubator at 33 °C and 5% CO_2 atmosphere using RITC 80-7 medium containing 5% fetal bovine serum, 10 μg/ml transferrin, 0.08 U/ml insulin, 10 ng/ml recombinant epidermal growth factor and 400 μg/ml geneticin. The cells were subcultured in a medium containing 0.05% trypsin−EDTA solution (containing in mM: 137 NaCl, 5.4 KCl, 5.5 glucose, 4 $NaHCO_3$, 0.5 EDTA and 5 HEPES; pH 7.2) and used for 25−35 passages. Clonal cells were isolated using a cloning cylinder and screened by using the optimal substrate for human-OAT2 and rat-OAT2, i.e., [^{3}H]prostaglandin $F_{2\alpha}$. When the S_2 cells were cultured on the permeable support and [^{14}C]mannitol was loaded either from the basolateral side or the apical side, the basolateral to the apical transport of [^{14}C]mannitol was comparable to that from the apical to the basolateral side (unpublished observation). Based on this, S_2 monolayers were thought to be leaky. The reason for this leakiness may be due to a reduction in or loss of expression of tight junctions during the establishment of the cell line. In addition, vertical sections of S_2 human-OAT2 and S_2 rat-OAT2 were stained with polyclonal antibodies against human-OAT2 and rat-OAT2. The subcellular localization of the human-OAT2 and rat-OAT2 proteins was observed on the basolateral and apical portions of the cell membrane (unpublished observation). Therefore, the cells were cultured on a solid support for these experiments.

2.3. Uptake experiments

Uptake experiments were performed as previously described (Takeda et al., 1999, 2001, 2002a,b; Kimura et al., 2002; Jung et al., 2002; Babu et al., 2002a,b; Enomoto et al., 2002; Khamdang et al., 2002). S_2 cells were seeded in 24-well tissue culture plates at a cell density of 1×10^5 cells/well. After the cells were cultured for 2 days, the cells were washed three times with Dulbecco's modified phosphate-

buffered saline (D-PBS) solution (containing in mM: 137 NaCl, 3 KCl, 8 Na_2HPO_4, 1 KH_2PO_4, 1 $CaCl_2$ and 0.5 $MgCl_2$; pH 7.4), and then preincubated in the same solution for 10 min in a water bath at 37 °C. Based on the time-course of prostaglandin $F_{2\alpha}$ uptake (Enomoto et al., 2002; Fig. 1A of this manuscript), S_2 human-OAT2 and S_2 rat-OAT2 were incubated in D-PBS containing 50 nM [^{3}H]prostaglandin $F_{2\alpha}$ in the absence or presence of various cephalosporin antibiotics at 37 °C for 20 s (human-OAT2) and 2 min (rat-OAT2), respectively. The uptake was stopped by adding ice-cold D-PBS, and the cells were washed three times with the same solution. The cells in each well were lysed with 0.5 ml of 0.1 N sodium hydroxide and 2.5 ml of aquasol-2, and radioactivity was determined using a β-scintillation counter (LSC-3100, Aloka, Tokyo, Japan).

2.4. Kinetic analysis

After preincubation as described above, S_2 human-OAT2 was incubated in D-PBS containing [^{3}H] prostaglandin $F_{2\alpha}$ at different concentrations in the absence or presence of various cephalosporin antibiotics at 37 °C for 20 s. Based on organic anion uptake under each condition, double reciprocal plot analyses were performed as previously described (Takeda et al., 2001, 2002a; Jung et al., 2002; Babu et al., 2002a,b; Enomoto et al., 2002). When the inhibition was competitive, K_i values were calculated based on the following equation, K_i = concentration of cephalosporin antibiotic/[(K_m prostaglandin $F_{2\alpha}$ with cephalosporin antibiotic/K_m of prostglandin $F_{2\alpha}$ without cephalosporin antibiotic) − 1].

2.5. Cell viability assay

Cell viability was assessed using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay (Mossmann, 1983; Takeda et al., 1999; Jung et al., 2002; Babu et al., 2002a,b). As described above, after S_2 human-OAT2, S_2 human-OAT1, S_2 human-OAT3, S_2 human-OAT4 and control were seeded at a cell density of 1×10^5 cells/well in 24-well plates and cultured for 2 days, the cells were treated with or without 5 mM cephaloridine in the absence or presence of organic anion transport inhibitors at 33 °C for 24 h. After culture, 50 μl of 5 mg/ml of MTT was added to the medium (0.5 ml), and the cells were further incubated at 33 °C for 4 h. After the cells were solubilized with isopropanol/HCl solution, optical density was measured at 570 nm with 630 nm as the reference (Beckman, Du640).

2.6. Statistical analysis

Data are expressed as means ±S.E. Statistical differences were determined using analysis of variance with Dunnett's post hoc test for the dose-dependent inhibition experiments or Student's unpaired *t*-test for the viability experiments. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Characterization of S_2 human-OAT2 and S_2 rat-OAT2

As previously described (Enomoto et al., 2002), S_2 human-OAT2 exhibited a time- and dose-dependent increase in the uptake of $PGF_{2\alpha}$ with a K_m value of 425 nM. As shown in Fig. 1A and B, S_2 rat-OAT2 exhibited a time- and dose-dependent increase in the uptake of prostaglandin $F_{2\alpha}$ with K_m values of 414 nM. Thus, human-OAT2 and rat-OAT2 mediate the transport of prostaglandin $F_{2\alpha}$ with a similar affinity.

3.2. Effects of various cephalosporin antibiotics on organic anion uptake in S_2 human-OAT2 and S_2 rat-OAT2

We elucidated the effects of various cephalosporin antibiotics on organic anion uptake mediated by human-OAT2 and rat-OAT2. The cephalosporin antibiotics tested were cephalothin, cefoperazone, cefazolin, ceftriaxone, cephaloridine, cefotaxime, cefadroxil and cefamandole. As shown in Fig. 2, these cephalosporin antibiotics dose-

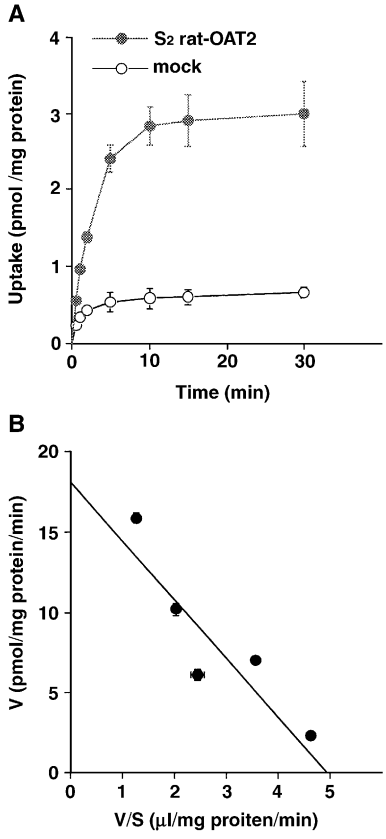

Fig. 1. Characterization of S_2 rat-OAT2. (A) Time-course of prostaglandin $F_{2\alpha}$ uptake in S_2 rat-OAT2. (B) Kinetic analysis of dose-dependent uptake of prostaglandin $F_{2\alpha}$ uptake in S_2 rat-OAT2. Each value represents the mean ± S.E. of four determinations from one typical experiment.

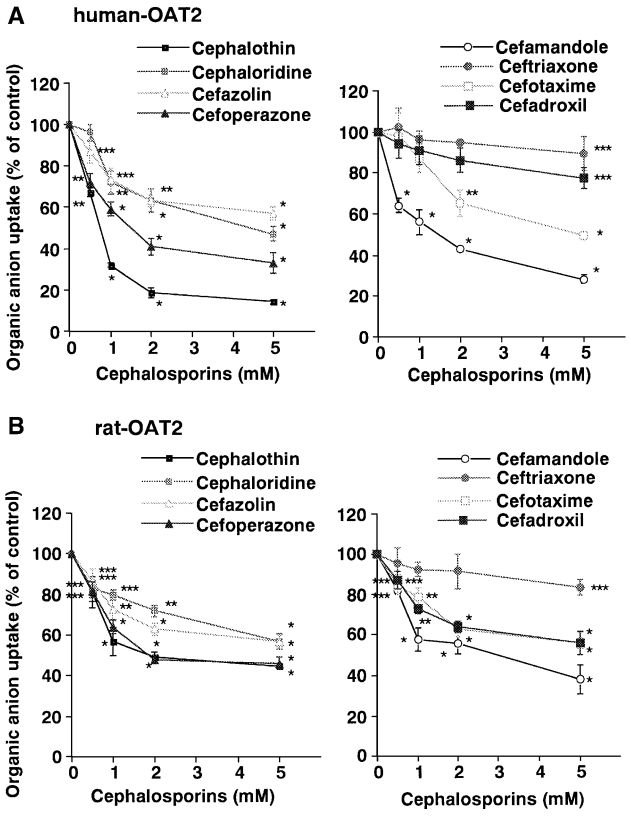

Fig. 2. Effects of various cephalosporin antibiotics on organic anion uptake in S_2 human-OAT2 (A) and S_2 rat-OAT2 (B). S_2 human-OAT2 and S_2 rat-OAT2 were incubated in medium containing 50 nM [^{3}H]prostaglandin $F_{2\alpha}$ for 20 s (human-OAT2) or 2 min (rat-OAT2) in the absence or presence of various concentrations of cephalothin, cephaloridine, cefazolin, cefoperazone, cefamandole, ceftriaxone, cefotaxime and cefadroxil. Each value represents the mean ± S.E. of four determinations from one typical experiment. *$P<0.001$, **$P<0.01$ and ***$P<0.05$ vs. control.

dependently inhibited organic anion uptake mediated by human-OAT2 (A) and rat-OAT2 (B). The IC_{50} values of various cephalosporin antibiotics for organic anion uptake mediated by human-OAT2 and rat-OAT2 are listed in Table 1.

Table 1
IC_{50} values of various cephalosporin antibiotics for human-OAT2 and rat-OAT2-mediated prostaglandin $F_{2\alpha}$ uptake

Antibiotics	IC_{50} (mM)		IC_{50} (mM)[a]	
	Human-OAT2	Rat-OAT2	Rat-OAT1	Rat-OAT3
Cephalothin	1.41	1.50	0.57	0.08
Cefoperazone	1.33	1.62	0.78	1.05
Cefazolin	5>	5>	0.96	0.74
Ceftriaxone	5>	5>	0.84	2>
Cephaloridine	4.48	5>	1.58	1.26
Cefotaxime	4.68	5>	2>	0.80
Cefadroxil	5>	5>	2>	1.78
Cefamandole	1.57	3.18	0.45	0.09

S_2 human-OAT2 and S_2 rat-OAT2 were incubated in a solution containing 50 nM [^{3}H] prostaglandin $F_{2\alpha}$ in the absence or presence of various concentrations of cephalosporin antibiotics at 37 °C for 20 s.

[a] From the literature (Jung et al., 2002).

3.3. Kinetic analysis of the inhibitory effects of various cephalosporin antibiotics on organic anion uptake in S_2 human-OAT2

To further elucidate the inhibitory effects of various cephalosporin antibiotics on human-OAT2-mediated organic anion uptake, the inhibitory kinetics of these cephalosporin antibiotics on [^{3}H] prostaglandin $F_{2\alpha}$ in S_2 human-OAT2 were analyzed. The organic anion uptake mediated by human-OAT2 was determined in the absence or presence of various cephalosporin antibiotics at different concentrations. Analysis of the Lineweaver–Burke plot of the effects of cephalothin, cefoperazone, cefazolin, ceftriaxone, cephaloridine, cefotaxime, cefadroxil and cefamandole on human-OAT2-mediated organic anion uptake revealed that these drugs inhibited organic anion uptake by human-OAT2 in a competitive manner (data not shown). Table 2 shows the K_i values of the effects of cephalosporin antibiotics on human-OAT2-mediated organic anion uptake.

3.4. Effects of cephaloridine on the viability of S_2 human-OAT2, S_2 human-OAT1, S_2 human-OAT3 and S_2 human-OAT4 in the absence or presence of probenecid

In order to determine whether hOATs mediate cephaloridine-induced nephrotoxicity, S_2 human-OAT2, S_2 human-OAT1, S_2 human-OAT3, S_2 human-OAT4 and control were cultured in the absence or presence of 5 mM cephaloridine for 24 h. As shown in Fig. 3, cephaloridine significantly inhibited the viability of S_2 human-OAT2 (A), S_2 human-OAT1 (B), S_2 human-OAT3 (C) and S_2 human-OAT4 (D) compared with the control (E) ($N=4$, *$P<0.001$ vs. control). In order to further elucidate the role of human-OATs in cephaloridine-induced cell injury, we examined the effects of probenecid, an organic anion transport inhibitor, on the cephaloridine-induced decrease in viability. We have already shown that probenecid dose-dependently reverses the decreased viability of mouse proximal straight tubule

Table 2
K_i values of various cephalosporin antibiotics for human-OAT2-mediated prostaglandin $F_{2\alpha}$ uptake

Antibiotics	Concentrations	K_i (mM)	K_i (mM)[a]		
			OAT1	OAT3	OAT4
Cephalothin	2	1.04	0.22	0.04	0.20
Cefoperazone	2	1.14	0.21	1.89	2.80
Cefazolin	5	5.09	0.18	0.55	1.74
Ceftriaxone	5	6.76	0.23	4.39	2.38
Cephaloridine	5	2.09	0.74	2.46	3.63
Cefotaxime	5	5.21	3.13	0.29	6.15
Cefadroxil	5	6.41	6.14	8.62	not determined
Cefamandole	2	0.43	0.03	0.046	1.14

S_2 human-OAT2 were incubated in a solution containing various concentrations of prostaglandin $F_{2\alpha}$ in the absence or presence of various cephalosporin antibiotics at 37 °C for 20 s.

[a] From the literature (Takeda et al., 2002a).

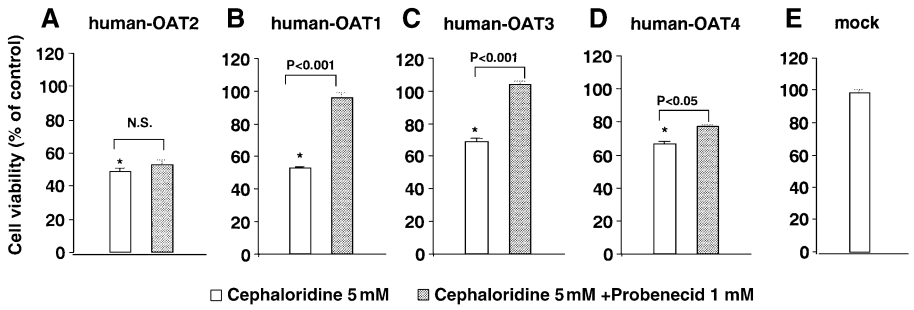

Fig. 3. Effects of cephaloridine on the viability of S_2 human-OAT2, S_2 human-OAT1, S_2 human-OAT3 and S_2 human-OAT4 in the absence or presence of probenecid. S_2 human-OAT2 (A), S_2 human-OAT1 (B), S_2 human-OAT3 (C), S_2 human-OAT4 (D) and control (E) were cultured in the medium with or without 5 mM cephaloridine in the absence or presence of 1 mM probenecid at 33 °C for 24 h. Each value represents the mean ± S.E. of four determinations from one typical experiment. *$P<0.001$ vs. control.

cells stably expressing rat-OAT1 and rat-OAT3 treated with cephaloridine at a concentration between 0.01 and 1 mM (Takeda et al., 1999; Jung et al., 2002). Probenecid (1 mM) alone did not significantly affect the viability of the cells (Takeda et al., 1999). As shown in Fig. 3, probenecid restored the decreased viability of S_2 human-OAT1 (B), S_2 human-OAT3 (C) and S_2 human-OAT4 (D) but not S_2 human-OAT2 (A) treated with 5 mM cephaloridine ($N=4$, $P<0.05$ or $P<0.001$ vs. cephaloridine). Thus, we undertook to elucidate the effects of probenecid at 2 and 3 mM, and pravastatin at 1, 2 and 3 mM on the viability of S_2 human-OAT2 treated with 5 mM cephaloridine for 24 h. We have already observed that pravastatin, a 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor, inhibits organic anion uptake mediated by human-OAT2 with an IC_{50} value of some hundred micromoles (unpublished observation). However, probenecid at 2 and 3 mM, and pravastatin at 2 and 3 mM significantly decreased the viability of S_2 human-OAT2 to 60–80% of that of the control (data not shown). In addition, probenecid at 2 and 3 mM, and pravastatin at 1, 2 and 3 mM could not change the viability of S_2 human-OAT2 treated with 5 mM cephaloridine for 24 h (data not shown).

4. Discussion

We have recently isolated human-OAT2 cDNA and found that human-OAT2 is also localized to the basolateral side of the proximal tubule (Enomoto et al., 2002). hOAT2 mediates the transport of organic anions including prostaglandin E_2, prostaglandin $F_{2\alpha}$, zidovudine, tetracycline and salicylate (Takeda et al., 2002b; Kimura et al., 2002; Babu et al., 2002a; Khamdang et al., 2002). Human-OAT1 and human-OAT3, also localized to the basolateral side of the proximal tubule (Hosoyamada et al., 1999; Cha et al., 2001), have been shown to mediate the basolateral uptake of various drugs and endogenous substances such as nonsteroidal anti-inflammatory drugs, anti-tumor drugs, histamine H_2 receptor antagonists, prostaglandins, diuretics, angiotensin-converting enzyme inhibitors and beta-lactam antibiotics. On the other hand, human-OAT4, localized to the apical side of the proximal tubule, mediates not only the uptake, but also the efflux of anionic drugs such as estrone sulfate ochratoxin A and tetracycline (Takeda et al., 2002a; Babu et al., 2002a,b); however, this transporter exhibits a relatively narrow substrate profile compared with human-OAT1 and human-OAT3 (Cha et al., 2000).

The urinary excretion rates of unchanged cephalosporin antibiotics including cephalothin, cefoperazone, cefazolin, ceftriaxone, cephaloridine, cefotaxime, cefadroxil and cefamandole are reported to be 52%, 29%, 80%, 49%, 60 ~ 80%, 55%, 93% and 96%, respectively (Hardman and Limbird, 2001). In addition, since the average weight of a normal subject is 70 kg, the renal clearance rates of cephalothin, cefoperazone, cefazolin, ceftriaxone, cefotaxime, cefadroxil and cefamandole are thought to be 469, 84, 66.5, 16.8, 259, 203 and 196 ml/min, respectively (Hardman and Limbird, 2001). Considering that the creatinine clearance rate in normal subjects is 104–139 ml/min (Gabbai and Blantz, 2001), it was suggested that cephalothin, cefotaxime, cefadroxil and cefamandole are mainly excreted into the urine by tubular secretion. In addition, it is possible that cefoperazone, cefazolin and ceftriaxone, the renal clearance of which is less than that of creatinine, could also be recognized and transported via basolateral OATs, whereas the reabsorption of these drugs may overwhelm the tubular secretion.

The cephalosporin antibiotics tested dose-dependently inhibited organic anion uptake mediated by human-OAT2. Thus, human-OAT2 interacts with various cephalosporin antibiotics. In addition, human-OAT2 is localized to the basolateral side of the proximal tubule. Furthermore, the cephalosporin antibiotics tested are excreted into the urine via tubular excretion. Based on these findings, it is suggested that human-OAT2 mediates the uptake of these cephalosporin antibiotics on the basolateral side of the proximal tubule. Table 2 gives the K_i values of various cephalosporin antibiotics for human-OAT2-mediated organic anion uptake and the reported K_i values of various

cephalosporin antibiotics for human-OAT1- and human-OAT3-mediated organic anion uptake (Takeda et al., 2002a). The K_i values of cephalothin, cefazolin and cefamandole for human-OAT2-mediated organic anion uptake are much higher than those for human-OAT1- and human-OAT3-mediated organic anion uptake (more than threefold) (Zhang et al., 1999). Those of cefoperazone and ceftriaxone are much higher than those for human-OAT1-mediated organic anion uptake, while that of cefotaxime is much higher than that for human-OAT3-mediated organic anion uptake. The K_i values of cephaloridine and cefadroxil are comparable with those for human-OAT1 and human-OAT3-mediated organic anion uptake (within threefold). In general, among basolateral human-OATs, human-OAT2 appears to interact the weakest with various cephalosporin antibiotics.

Based on the dose-dependent inhibition curves and the IC_{50} values, there appear to be no interspecies differences in the interaction of OAT2 with cephalosporin antibiotics between human and rat transporters. Consistent with this, as described before, rat-OAT2 was shown to mediate the transport of prostaglandin $F_{2\alpha}$ with an affinity similar to that of human-OAT2. In addition, human-OAT2 was shown to mediate zidovudine transport with an affinity similar to that of rat-OAT2, i.e., 26.8 vs. 26.0 μM (Takeda et al., 2002b; Morita et al., 2001). However, in contrast to the fact that human-OAT2 is localized on the basolateral side of the proximal tubule, rat-OAT2 was shown to be localized on the apical side of the medullary thick ascending limbs of Henle's loop, and cortical and medullary collecting ducts (Kojima et al., 2002). Thus, the interaction of human-OAT2 with cephalosporin antibiotics may be associated with the basolateral uptake of these drugs, and the interaction of rat-OAT2 with cephalosporin antibiotics may be associated with the apical reabsorption of these drugs. In addition, an interspecies difference in the interactions of OATs with various drugs between humans and rats was also observed for OAT2 and prostaglandin E_2 (Kimura et al., 2002; Morita et al., 2001) and nonsteroidal anti-inflammatory drugs including ketoprofen, diclofenac and ibuprofen (Khamdang et al., 2002), and for OAT1 and nonsteroidal anti-inflammatory drugs including acetylsalicylate, acetaminophen and ibuprofen (Khamdang et al., 2002), and cefazolin (Takeda et al., 2002a,b). Thus, there appears to be some interspecies difference in interactions of OATs with drugs between human and rat transporters, depending on the drugs as substrates for transporters.

Cephaloridine significantly decreased the viability of S_2 human-OAT2 compared with the control, suggesting that human-OAT2 mediates the induction of cephaloridine-induced nephrotoxicity. However, the decreased viability of S_2 human-OAT2 treated with cephaloridine was not reversed by 1 mM probenecid. This may be because the K_i value of probenecid for human-OAT2-mediated organic anion uptake is 766 μM (Enomoto et al., 2002). In this regard, a high-affinity inhibitor for human-OAT2 should be determined and the role of human-OAT2 in cephaloridine-induced nephrotoxicity should be further elucidated. In contrast, cephaloridine significantly decreased the viability of S_2 human-OAT1 and S_2 human-OAT3 compared with the control, and these decreases in viability were reversed to almost 100% by 1 mM probenecid. The K_i values of probenecid for human-OAT1- and human-OAT3-mediated organic anion uptake were 12.1 and 9.0 μM, respectively (Takeda et al., 2001). These results suggest that human-OAT1 and human-OAT3 mediate the uptake of cephaloridine into the proximal tubule from the peritubular capillary and the induction of its nephrotoxicity. In contrast to S_2 human-OAT1 and S_2 human-OAT3, probenecid did not restore the viability of S_2 human-OAT4 treated with cephaloridine to 100%. The K_i value of probenecid for human-OAT4-mediated organic anion uptake was 54.9 μM (Enomoto et al., 2002). Such an incomplete recovery of cell viability by probenecid was also observed in mouse proximal tubule cells stably expressing rat-OAT1 and rat-OAT3 treated with cephaloridine (Takeda et al., 1999; Jung et al., 2002). Since there was no interspecies difference in the affinity of OAT1 or OAT3 for cephaloridine and probenecid between human and rat transporters (Table 1; unpublished observation), there may exist some other factors that determine the effects of probenecid on cephaloridine-induced cytotoxicity other than the affinity of drugs for transporters.

Among the various cephalosporin antibiotics tested, cefoperazone and ceftriaxone are known to be metabolized in the liver and excreted into the bile duct. In addition to the proximal tubule of the kidney, human-OAT2 and rat-OAT2 were shown to be localized to the basolateral membrane of the liver (unpublished observation). In this regard, human-OAT2 and rat-OAT2 may contribute to the basolateral uptake of cefoperazone and ceftriaxone in the liver.

In conclusion, human-OAT2 and rat-OAT2 interact with various cephalosporin antibiotics; thus, human-OAT2 may mediate the basolateral uptake of these drugs in the proximal tubule. The interaction of human-OAT2 with cephalosporins was the weakest among basolateral human-OATs. In addition, there was no interspecies difference in the interaction of OAT2 with cephalosporin antibiotics. Furthermore, it is suggested that not only human-OAT2 but also human-OAT1, human-OAT3 and human-OAT4 mediate the uptake of cephaloridine and the induction of nephrotoxicity.

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