Expression Levels of Renal Organic Anion Transporters (OATs) and Their Correlation with Anionic Drug Excretion in Patients with Renal Diseases

Yuji Sakurai,[1] Hideyuki Motohashi,[1] Harumasa Ueo,[1] Satohiro Masuda,[1] Hideyuki Saito,[1] Masahiro Okuda,[1] Noriko Mori,[2] Motokazu Matsuura,[3] Toshio Doi,[3] Atsushi Fukatsu,[4] Osamu Ogawa,[5] and Ken-ichi Inui[1,6]

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Purpose. Because the urinary excretion of drugs is often decreased in renal diseases, dosage regimens are adjusted to avoid adverse drug reactions. The aim of present study was to clarify the alteration in the levels of renal drug transporters and their correlation with the urinary drug excretion in renal diseases patients.

Methods. We quantified the mRNA levels of human organic anion transporters (hOATs) by real-time polymerase chain reaction and examined the excretion of the anionic drug, cefazolin, in renal disease patients. Moreover, transport of cefazolin by hOAT1 and hOAT3 were examined using HEK293 transfectants.

Results. Among four hOATs, the level of hOAT1 mRNA was significantly lower in the kidney of patients with renal diseases than in the normal controls. The elimination constant of cefazolin showed a significant correlation with the values of phenolsulfonphthalein test and mRNA levels of hOAT3. The uptake study using HEK293 transfectants revealed that cefazolin and phenolsulfonphthalein were transported by hOAT3.

Conclusions. These results suggest that hOAT3 plays an important role for anionic drug secretion in patients with renal diseases and that the expression levels of drug transporters may be related to the alteration of renal drug secretion.

KEY WORDS: organic anion transporter; renal diseases; human kidney; renal tubular secretion; real-time PCR.

INTRODUCTION

Renal impairments often decrease the rate of drug excretion into urine, and optimal dosage regimens must be designed to avoid adverse effects. For many drugs which are eliminated by the kidney, dosage regimens are usually adjusted according to creatinine clearance with a simple equation (1). However, it is accepted that the creatinine clearance (CL_{cr}) reflects only the glomerular filtration rate of normal kidney, and there are some discrepancies between the true glomerular filtration rate and CL_{cr} in patients with renal insufficiency. In addition, numerous ionic drugs are secreted into urine by the transporters localized in the proximal tubules. Indeed, the dosage schedule based on CL_{cr} was inadequate for ampicillin and cephalexin dosing in some patients with renal insufficiency (2). The adjustment method of the cephalexin dosage regimen, in which CL_{cr} and phenolsulfonphthalein test were simultaneously considered, appeared to be more useful than the conventional CL_{cr} method (3). Most of the phenolsulfonphthalein excretion into urine depends on the tubular secretion mediated by the organic anion transport systems (4). Although the dosage adjustment method, considering the ability of renal tubular secretion, may be useful for patients with renal diseases, little information is available about the expression profiles of the organic ion transporters in renal diseases.

Both renal secretion and reabsorption across the tubular epithelium are mediated by various transporters, which are expressed in the apical and basolateral membranes of tubular epithelial cells (5–7). Human organic anion transporter hOAT1 was isolated from the kidney and was suggested to be the *p*-aminohippurate/dicarboxylate exchanger (8,9). We previously clarified that the expression level of hOAT1 was the second highest among the organic ion transporters (SLC22A) and that hOAT1 was located at the basolateral membrane of the proximal tubules (10). It was suggested that hOAT1 mediated the basolateral uptake into the epithelial cells from the blood circulation. Human (h)OAT2 was also expressed in the basolateral membrane of renal tubular epithelium (11). Human OAT3 was isolated from the kidney (12). The expression levels of hOAT3 mRNA was the highest among organic ion transporter family in the human kidney, and hOAT3 protein was detected in the basolateral membrane of the proximal tubules (10). Recently, Sweet *et al.* (13) established the Oat3 knockout mouse. Although the generated $Oat3^{-/-}$ mice were fertile and exhibited no obvious morphological defects, the uptake of taurocholate, estrone sulfate, and *p*-aminohippurate in renal slices prepared from $Oat3^{-/-}$ mice were greatly reduced in comparison with wild-type mice, suggesting OAT3 may play an important role for basolateral anion transport of the proximal tubules. Human OAT4 was also expressed in the kidney, whereas it was localized to the apical side of the proximal tubules (14). These hOATs mediate the transport of various drugs and contribute to the renal drug excretion (6).

The purpose of this study is to clarify the expression levels of renal hOATs in the patients with renal diseases and their correlation with the rate of the anionic drug elimination. We quantified the mRNA levels of renal organic anion transporters in the normal parts of kidney cortex from surgically nephrectomized patients and in the renal biopsy specimens from patients with renal diseases. In addition, elimination of the anionic drug, cefazolin was assessed to compare with the expression levels of renal transporters or the 15- or 120-min value of the phenolsulfonphthalein test (PSP15′ or PSP120′). The transport characteristics of phenolsulfonphthalein and cefazolin by HEK293 transfectants with hOAT1 or hOAT3 were also examined.

[1] Department of Pharmacy, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606-8507, Japan.
[2] Department of Nephrology, Shizuoka Prefectural Hospital, Shizuoka, Japan.
[3] Department of Clinical Biology and Medicine, University of Tokushima, Tokushima, Japan.
[4] Division of Artificial Kidneys, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606-8507, Japan.
[5] Department of Urology, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606-8507, Japan.
[6] To whom correspondence should be addressed. (e-mail: inui@kuhp.kyoto-u.ac.jp)

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MATERIALS AND METHODS

Normal Parts of the Kidney Cortex and Renal Biopsy Samples

Normal parts of human kidney cortex were obtained from 35 surgically nephrectomized patients with renal cell carcinoma or transitional cell carcinoma at Kyoto University Hospital (24 males and 11 females; age, 64.1 ± 7.9 year [mean ± SD]). These patients did not have any diseases that affected the kidney other than the carcinoma. Human kidney biopsy samples for the diagnosis were from 42 patients with various renal diseases at Tokushima University Hospital and Shizuoka Prefectural Hospital (24 males and 18 females; age, 39.2 ± 19.3 year [mean ± SD]). The characteristics of these patients are summarized in Table I. The patients had various renal diseases that were histologically confirmed as lupus nephritis (n = 4), IgA nephropathy (n = 11), focal glomerular sclerosis (n = 4), membranoproliferative glomerulonephritis (n = 3), membranous glomerulonephropathy (n = 6), mesangial proliferative glomerulonephropathy (n = 8), and other nephropathies (n = 6). Kidney biopsy specimens were histologically confirmed to contain the cortical proximal tubules using the adjacent sections. The values of clinical tests, such as CL_{cr}, PSP15′, or PSP120′, were routinely measured in the hospital. After phenolsulfonphthalein (6 mg) was administered intravenously, urine samples were collected at 15 and 120 min. The amounts of phenolsulfonphthalein in urine samples were represented as % of the initial dose. This study was conducted in accordance with the Declaration of Helsinki and its amendments and was approved by Kyoto University Graduate School and Faculty of Medicine, Ethics Committee. All patients gave their written informed consent.

Quantification of Organic Anion Transporter mRNA Expression

The expression levels of the drug transporters were quantified as described previously (10). Briefly, total cellular RNA was isolated from specimens using a MagNA Pure LC RNA isolation Kit II (Roche Diagnostic GmbH, Mannheim, Germany) and was reverse-transcribed to yield cDNA. Real-time polymerase chain reaction (PCR) was performed using the ABI PRISM 7700 sequence detector system (Applied Biosystems, Foster, CA, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was also quantified as an internal control with GAPDH Control Reagent (Applied Biosystems).

Table I. Characteristics of the Patients

	Nephrectomized patients	Patients with renal diseases
Age (years)	64.1 ± 7.9	39.2 ± 19.3
Males/females	24/11	24/18
Aspartate aminotransferase (IU/l)	25.8 ± 16.3	19.4 ± 10.2
Alanine aminotransferase (IU/l)	25.8 ± 19.2	19.8 ± 21.5
Lactate dehydrogenase (IU/l)	190.4 ± 76.5	175.5 ± 44.8
Serum creatinine (mg/dl)	0.85 ± 0.22	1.3 ± 1.9
Uric acid (mg/dl)	5.4 ± 1.7	6.2 ± 1.7
Blood urea nitrogen (mg/dl)	15.2 ± 4.8	18.7 ± 13.9
Creatinine clearance (ml/min)	77.6 ± 25.8	58.8 ± 24.4

Calculation of the Apparent Elimination Constants and Renal Secretion of Cefazolin

After the renal biopsy, the patients received 1 g of cefazolin by 1 h intravenous infusion for the prophylaxis of infections. The blood samples of the patients were collected immediately and at 1 h after the infusion. To 100 μl of collected plasma, 200 μl of methanol and 50 μl of cefotiam (100 μg/ml), as an internal standard, were added. After standing for 1 h at room temperature, the mixtures were centrifuged and the supernatants were filtered through a Millipore filter (SJGVL, 0.45 μm, Bedford, MA, USA). The filtrate was analyzed using high-performance liquid chromatography (HPLC) as described below, and the apparent elimination constant (Ke_{cez}) was calculated using the plasma concentration at immediately and 1hr after cefazolin infusion. A high-performance liquid chromatography (LC-10AS, Shimadzu Co., Kyoto, Japan) equipped with a UV spectrophotometric detector (SPD-10AV, Shimadzu) was used for the analysis of cefazolin. The conditions were as follows: column, Zorbax ODS column 4.6 mm inside diameter × 150 mm (Du Pont, Wilmington, DE, USA); mobile phase, 30 mM phosphate buffer (pH 7.0):methanol = 80:20; flow rate, 1.0 ml/min; wavelength, 272 nm; injection volume, 50 μl; temperature, 40°C.

Uptake of *p*-Aminohippurate and Estrone Sulfate by HEK293 Cells Transfected with hOAT1 or hOAT3 cDNA, Respectively

Human OAT1 cDNA was isolated from TriplEX™ human kidney cDNA library (BD Biosciences Clontech, Palo Alto, CA, USA) and hOAT3 cDNA was from the Human Kidney Rapid-Screen™ cDNA Panel (OriGene Technologies, Rockville, MD, USA) according to the instruction manuals. Isolated cDNAs were sequenced using a fluorescence 373A DNA sequencer (Applied Biosystems), and were subcloned into pBK-CMV plasmid vector (Stratagene, La Jolla, CA, USA). Cell culture, transfection of cDNA, and uptake studies were performed as described previously (15) with some modifications. Briefly, the day before transfection, HEK293 cells were seeded on poly-D-lysine-coated 24-well plates at a density of 2×10^5 cells/well. The cells were transfected with plasmid cDNA using LipofectAMINE 2000 (Invitrogen, Carlsbad, CA, USA). At 48 h after transfection, the uptake of *p*-[^{14}C]aminohippurate or [^{3}H]estrone sulfate by the HEK293 cells was examined. The composition of the incubation medium was as follows (in mM): 145 NaCl, 3 KCl, 1 $CaCl_2$, 0.5 $MgCl_2$, 5 D-glucose, and 5 HEPES (pH 7.4). The cells were preincubated with 0.2 ml of incubation medium for 10 min at 37°C. After the preincubation, medium was replaced with 0.2 ml of the incubation medium containing 5 μM *p*-[^{14}C]aminohippurate or 18.8 nM [^{3}H]estrone sulfate. At the end of incubation period, the medium was aspirated, and then cells were washed two times with 1 ml of ice-cold incubation medium. The cells were lysed in 0.5 ml of 0.5 N NaOH solution, and the radioactivity in aliquots was determined in 5 ml of ACSII (Amersham International, Buckinghamshire, UK). The protein contents of the solubilized cells were determined by the method of Bradford (16) using the Bio-Rad Protein Assay kit (Bio-Rad, Hercules, CA, USA) with the bovine γ-globulin as a standard.

Uptake of Cefazolin and Phenolsulfonphthalein by HEK293 Cells Transfected with hOAT1 or hOAT3 cDNA

For the uptake study of cefazolin and phenolsulfonphthalein, HEK293 cells were seeded on 6-cm poly-D-lysine-coated dish at a density of 2×10^6 cells/dish and then transfected with 8 μg hOAT1 or hOAT3 cDNA per dish at 24 h after seeding. At 48 h after transfection, the uptake studies of cefazolin and phenolsulfonphthalein were performed. The cells were preincubated with 2 ml of incubation medium for 10 min. After the preincubation, the medium was replaced with 2 ml of the incubation medium containing 200 μM cefazolin or 500 μM phenolsulfonphthalein. At the end of the 1-h incubation period, the medium was aspirated, and cells were washed once with 5 ml of ice-cold incubation medium containing 2% bovine serum albumin, and then four times with bovine serum albumin free ice-cold incubation medium. For measurement of the cefazolin accumulation, the cells were scraped and homogenized with 1 ml water. With 5 μl of the homogenate, protein contents were determined. To 0.98 ml of the homogenate, 20 μl of phosphoric acid was added and mixed for 30 s, then the samples were loaded onto an Oasis HLB cartridge (Waters Corporation, Milford, MA, USA) preconditioned with 1 ml each of methanol and water. The column was then washed with 1 ml of 5% methanol and then cefazolin was eluted from the column by 1 ml of methanol. The eluate was evaporated to bare dryness at 45–50°C and resuspended in 200 μl of mobile phase buffer, and the solution was filtered through a 0.45-μm polyvinylidene fluoride filter. The concentration of cefazolin was measured with HPLC under the following conditions: mobile phase, 30 mM phosphate buffer (pH 5.2):methanol = 88:12; flow rate, 1.0 ml/min; wavelength, 272 nm; temperature, 40°C. For measurement of the phenolsulfonphthalein accumulation, the cells were scraped with 1.5 ml of 75% ethanol, incubated for 1 h at room temperature, and centrifuged at 3000g for 10 min. After centrifugation, 1 ml of the supernatant was alkalized with 100 μl of 1 N NaOH and the concentration of phenolsulfonphthalein was determined spectrophotometrically at 546 nm. The pellet was solubilized with 1 ml of 1 N NaOH and protein contents were determined.

Statistical Analysis

Data were analyzed statistically using nonpaired *t* test or the one-way analysis of variance followed by Scheffe's test.

Materials

p-[Glycyl-^{14}C]aminohippurate (1.9 GBq/mmol) was purchased from Du Pont-New England Nuclear Research Product (Boston, MA, USA). [6,7-^{3}H(N)]Estrone sulfate ammonium salt (1.9 TBq/mmol) was obtained from Perkin Elmer Life Sciences (Boston, MA, USA). Cefazolin (Fujisawa Pharmaceutical Co., Osaka, Japan) was from the source. All other chemicals used were of the highest purity available.

RESULTS

Quantification of hOAT1, hOAT2, hOAT3, and hOAT4 mRNA Levels in the Normal Human Kidney Cortex and Renal Biopsy Samples

To investigate the expression levels of renal organic anion transporters, we performed quantitative real-time PCR. Fig. 1 shows the mRNA levels of organic anion transporters in normal parts of the renal cortex or the renal biopsy specimens from patients with kidney diseases. Only the level of hOAT1 mRNA in the renal biopsy specimens was significantly lower than that in the normal control ($p < 0.05$). The level of hOAT3 mRNA was slightly decreased, although the levels of hOAT2 and hOAT4 mRNA were increased in biopsy sections compared with each mRNA level in the normal kidney (differences not significant).

Correlation Between the Elimination Rate of Cefazolin and PSP15′, PSP120′, CL_{cr}, or Expression Levels of Organic Anion Transporters

Fig. 2 shows the linear regression of Ke_{cez} against PSP15′ (A), PSP120′ (B), or CL_{cr} (C) and the relation between CL_{cr} and PSP15′ (D) or PSP120′ (E). Although we detected a poor correlation between Ke_{cez} and CL_{cr} ($r = 0.40$, $p < 0.05$), there was a good linear correlation between Ke_{cez} and PSP15′ ($r = 0.75$, $p < 0.01$) or PSP120′ ($r = 0.67$, $p < 0.01$). However, there was a poor correlation between CL_{cr} and PSP15′ ($r = 0.48$, $p < 0.05$) or PSP120′ ($r = 0.56$, $p < 0.01$).

Fig. 3 shows the correlation between the Ke_{cez} and mRNA levels of organic anion transporters. The levels of hOAT3 mRNA was significantly correlated with the Ke_{cez} ($r = 0.44$, $p < 0.05$), although there was no correlation between hOAT1, hOAT2, or hOAT4 mRNA levels and Ke_{cez}.

Characterization of Organic Anion Uptake in HEK293 Cells Transfected with hOAT1 or hOAT3 cDNA

Good linear correlation between Ke_{cez} and PSP15′ or PSP120′ suggested that both cefazolin and phenolsulfonphthalein were excreted via the same organic anion transporters in the renal tubules. Therefore, we investigated the effects of cefazolin or phenolsulfonphthalein on the hOAT1 and hOAT3 transport activities. The transport function of hOAT1 and hOAT3 was assessed by the uptake of *p*-[^{14}C]aminohippurate and [^{3}H]estrone sulfate in HEK293 cells, respectively. Fig. 4 shows the time course of *p*-aminohippurate or estrone sulfate uptake by hOAT1- or hOAT3-expressing HEK293 cells. The accumulations of *p*-aminohippurate and estrone

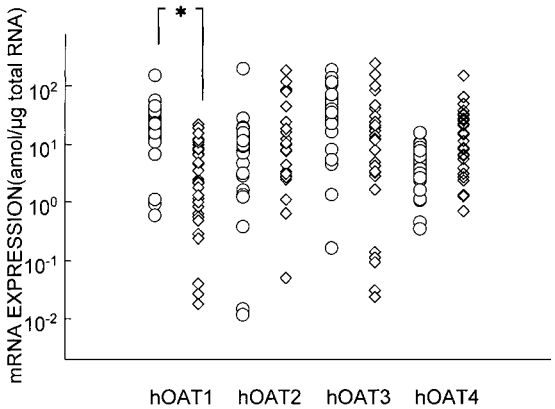

Fig. 1. Expression levels of hOAT1, hOAT2, hOAT3, and hOAT4 mRNA in the human kidney. Total cellular RNA was extracted from normal human kidney cortex (○) and renal biopsy specimens of patients with renal diseases (◇). The mRNA levels of these transporters were determined by real-time PCR. *$p < 0.05$, significant difference.

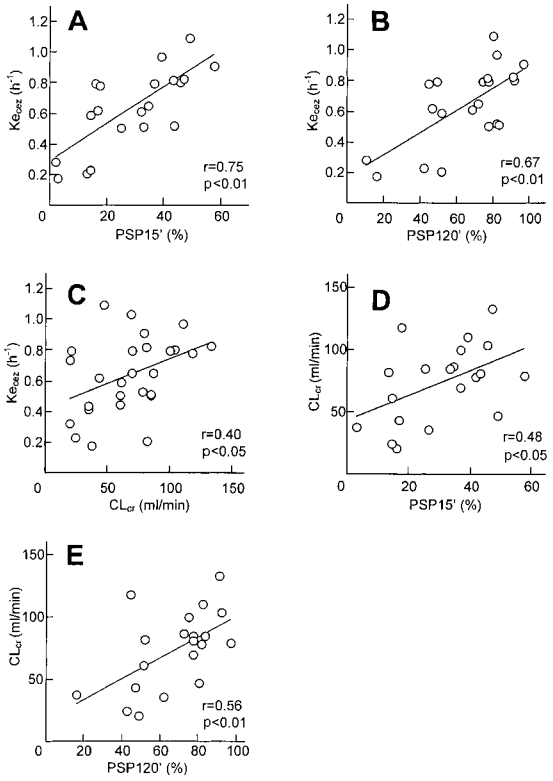

Fig. 2. The linear regression of the elimination constant of cefazolin against the 15- (A) or 120-min (B) values of the phenolsulfonphthalein test or creatinine clearance (C), and the relationship between creatinine clearance and 15- (D) or 120-min (E) values of the phenolsulfonphthalein test. The plasma concentration of cefazolin was measured by HPLC, and elimination constant of cefazolin (Ke_{cez}) was calculated.

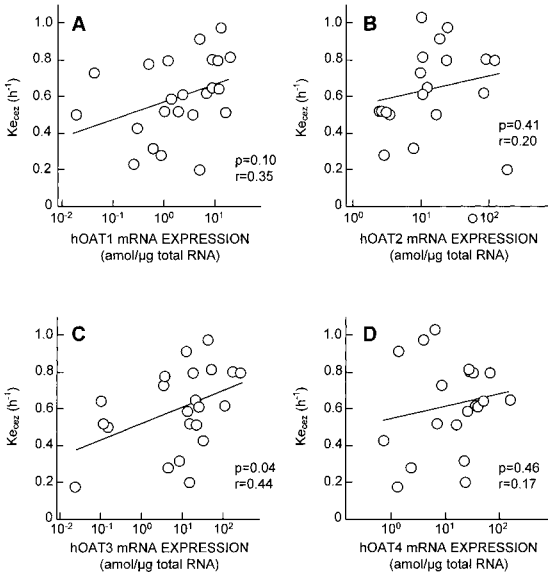

Fig. 3. The linear regression of Ke_{cez} against hOAT1 (A), hOAT2 (B), hOAT3 (C), and hOAT4 (D) mRNA levels. The plasma concentration of cefazolin was measured by HPLC, and Ke_{cez} was calculated. Total cellular RNA was extracted from the kidney biopsy specimens. The mRNA levels of hOAT1, hOAT2, hOAT3, and hOAT4 were quantified by real-time PCR.

sulfate were increased in a time-dependent manner, although the accumulations of these substrates in HEK293 cells, transfected with the blank vector alone, exhibited a negligible increase.

As shown in Fig. 5, a concentration-dependence of *p*-[^{14}C]aminohippurate and [^{3}H]estrone sulfate uptake was observed in hOAT1- and hOAT3-transfected cells. Using a nonlinear least squares regression analysis, kinetic parameters were calculated according to the Michaelis-Menten equation from three separate experiments. Apparent Michaelis-Menten constants (K_m) for *p*-[^{14}C]aminohippurate transport via hOAT1 and for [^{3}H]estrone sulfate transport via hOAT3 were 47.8 ± 19.5 and 6.6 ± 3.0 μM (mean ± SE), respectively, which were consistent with previous report (17). Maximal uptake rate (V_{max}) values for hOAT1 and hOAT3 were 305.7 ± 95.4 and 44.2 ± 8.1 pmol/mg protein/min (mean ± SE), respectively.

Fig. 6 shows that the effects of cefazolin or phenolsulfonphthalein on the hOAT1 or hOAT3 transport activity. Both cefazolin and phenolsulfonphthalein inhibited the organic anion uptake by hOAT1- and hOAT3-transfected cells, respectively, in a dose-dependent manner. The IC_{50} values were estimated by nonlinear regression analysis of the competition curves with one compartment model with the following equation: $V = 100 \times IC_{50} / (IC_{50} + [I]) + A$, where V is the uptake amount (% of control), $[I]$ is the concentration of cefazolin or phenolsulfonphthalein, and A is the nonspecific organic anion uptake (% of control). The IC_{50} values for cefazolin and phenolsulfonphthalein on hOAT1-mediated *p*-aminohippurate uptake were 100.6 ± 25.3 μM and 8.1 ± 1.3 μM, respectively, and the IC_{50} values for cefazolin and phenolsulfonphthalein on hOAT3-mediated estrone sulfate uptake were 116.6 ± 13.0 μM and 66.0 ± 16.5 μM, respectively.

Uptake of Cefazolin and Phenolsulfonphthalein by hOAT1- and hOAT3-Expressing HEK293 Cells

To investigate whether cefazolin and phenolsulfonphthalein are the substrates for hOAT1 and hOAT3, we measured the accumulation of cefazolin and phenolsulfonphthalein in hOAT1- and hOAT3-expressing HEK293 cells. The cefazolin accumulation in hOAT3-expressing HEK293 cells was significantly higher than that in control cells and the uptake of

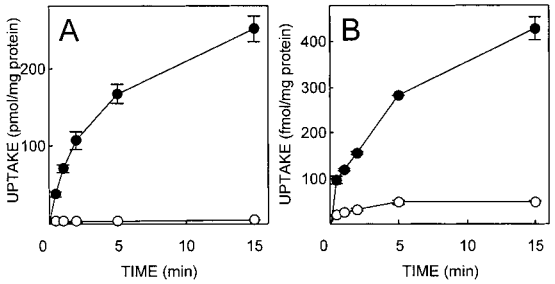

Fig. 4. Time course of *p*-[^{14}C]aminohippurate and [^{3}H]estrone sulfate accumulation in HEK293 cells. (A) *p*-[^{14}C]aminohippurate accumulation in HEK293 cells transfected with pBK-CMV vector (○) or hOAT1 (●) cDNA. The cells were incubated with 5 μM *p*-[^{14}C] aminohippurate at 37°C for specified periods. (B) [^{3}H] estrone sulfate accumulation in HEK293 cells transfected with pBK-CMV vector (○) or hOAT3 (●) cDNA. The cells were incubated with 18.8 nM [^{3}H]estrone sulfate at 37°C for specified periods. Each point represents the mean ± SE of three monolayers

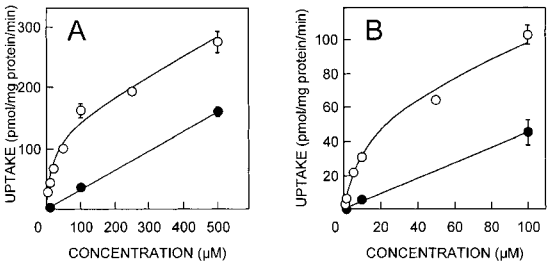

Fig. 5. Concentration dependence of *p*-[^{14}C]aminohippurate (A) and [^{3}H]estrone sulfate (B) accumulation in HEK293 cells transfected with hOAT1 or hOAT3 cDNA, respectively. The cells transfected with hOAT1 or hOAT3 cDNA were incubated with various concentrations of *p*-[^{14}C]aminohippurate (A) or [^{3}H]estrone sulfate (B) in the absence (○) or presence (●) of unlabeled 5 mM *p*-aminohippurate (A) or 1 mM estrone sulfate (B) at 37°C for 1 min. After incubation, radioactivity of solubilized cells was measured. Each point represents the mean ± SE of nine monolayers from three separate experiments.

cefazolin by hOAT3 was inhibited by phenolsulfonphthalein similar to the level of controls (Fig. 7A). In contrast, hOAT1-mediated cefazolin transport was not detected. We confirmed hOAT1-mediated *p*-aminohippurate uptake and hOAT3-mediated estrone sulfate uptake in the same transfected cells (Figs. 7B and C). As shown in Fig. 8, furthermore, the phenolsulfonphthalein accumulations in both hOAT1- and hOAT3-expressing HEK293 cells were significantly higher than those in control cells, and cefazolin inhibited the phenolsulfonphthalein uptake by both cells to the control level.

DISCUSSION

Renal secretion of various drugs is mediated by the drug transporters expressed in the tubular epithelial cells, and the alteration of these transporter levels may affect the drug elimination by the kidney. In the present study, the expression levels of organic anion transporters in kidney diseases were quantified to compare with those in normal controls, and then correlations between the mRNA levels of these transporters and anionic drug excretion were analyzed.

The mRNA level of hOAT1 in biopsy samples of patients with renal diseases was lower than that in the normal kidney cortex (Fig. 1). Although the level of hOAT3 mRNA also tended to decrease, the levels of hOAT2 and hOAT4 mRNA were apt to increase. Recently, we reported the alteration of the renal transporter expression in 5/6 nephrectomized rats, which have been widely used to study the progression of renal damage resulting from reduction of nephron mass (18–20). Rat organic cation transporter OCT2 protein was markedly decreased and H^+/peptide cotransporter PEPT2 protein was significantly increased in the kidney of these rats. Moreover, other transporters were not changed

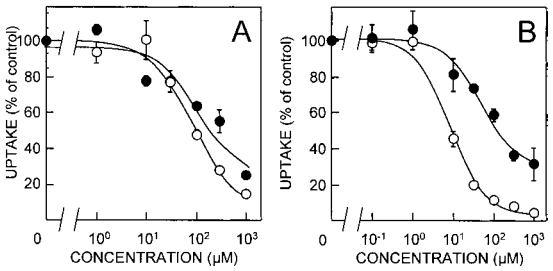

Fig. 6. Effects of cefazolin (A) or phenolsulfonphthalein (B) on *p*-[^{14}C]aminohippurate and [^{3}H]estrone sulfate accumulation in HEK293 cells transfected with hOAT1 or hOAT3 cDNA, respectively. Monolayers of hOAT1 (○)- or hOAT3 (●)-expressing HEK293 cells were incubated for 1 min at 37°C with 5 μM *p*-[^{14}C]aminohippurate (○) or 18.8 nM [^{3}H]estrone sulfate (●) in the presence of various concentrations of cefazolin or phenolsulfonphthalein. After incubation, the radioactivity of solubilized cells was measured. Each point represents the mean ± SE of nine monolayers from three separate experiments.

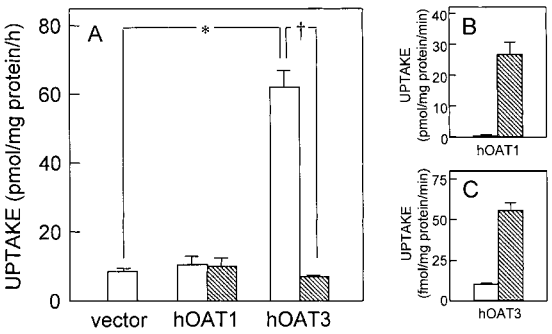

Fig. 7. Uptake of cefazolin in hOAT1 or hOAT3 transfected HEK293 cells. (A) Monolayers of hOAT1- or hOAT3-expressing HEK293 cells were incubated for 1 h at 37°C with 200 μM cefazolin in the absence (open columns) or presence (hatched columns) of 3 mM phenolsulfonphthalein. After incubation, the accumulation of cefazolin in the cells was measured by HPLC. (B) *p*-[^{14}C]aminohippurate accumulation in HEK293 cells transfected with pBK-CMV vector (open column) or hOAT1 (hatched column) cDNA. (C) [^{3}H]estrone sulfate accumulation in HEK293 cells transfected with pBK-CMV vector (open column) or hOAT3 (hatched column) cDNA. The cells were incubated with 5 μM *p*-[^{14}C]aminohippurate or 18.8 nM [^{3}H]estrone sulfate at 37°C for 1 min. Each column represents the mean ± SE of three monolayers. *$p < 0.01$, †$p < 0.01$, significant differences.

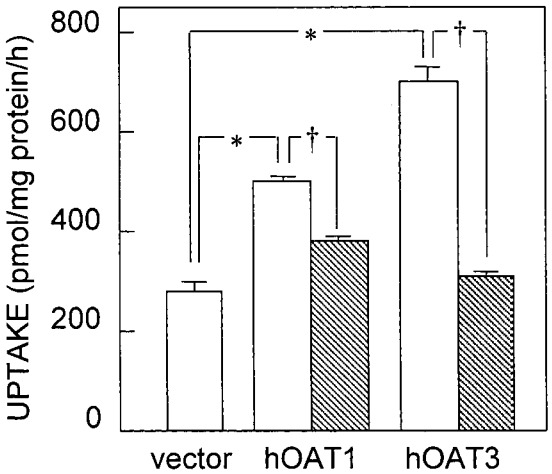

Fig. 8. Uptake of phenolsulfonphthalein in hOAT1 or hOAT3 transfected HEK293 cells. Monolayers of hOAT1- or hOAT3-expressing HEK293 cells were incubated for 1 h at 37°C with 500 μM phenolsulfonphthalein in the absence (open columns) or presence (hatched columns) of 10 mM cefazolin. After incubation, the accumulation of phenolsulfonphthalein in the cells was measured spectrophotometrically. Each column represents the mean ± SE of three monolayers. *$p < 0.01$, †$p < 0.01$, significant differences.

significantly. It was suggested that each transporter underwent a different effect in the impaired kidney.

In our previous study, we demonstrated that hOAT1 and hOAT3 could be major transporters in the human kidney cortex and localized at the basolateral membrane of the proximal tubular cells (10). Because hOAT1 and hOAT3 may play important roles in renal anion secretion, it was assumed that the changes in these expression levels affected renal drug secretion. In this study, cefazolin was selected to evaluate renal drug secretion, because it was reported that tubular secretion amounted to 50-80% of total excreted cefazolin in the patients with a glomerular filtration rate above 25 ml/min (21). In addition, the cefazolin elimination rate was significantly decreased by co-administration of probenecid to about 60% (22), suggesting that cefazolin was secreted by anion transport systems. Indeed, the Ke_{cez} varied among the patients. While no correlation could be found between the elimination rate of cefazolin (Ke_{cez}) and hOAT1, hOAT2 or hOAT4 expression levels, there is a significant correlation between Ke_{cez} and hOAT3 mRNA levels (Fig. 3). Although renal drug excretion is affected by many factors, such as glomerular filtration rate, renal blood flow rate, protein binding and transport abilities of tubular epithelial cells, the expression levels of transporters may be concerned mainly with transport capacities of epithelial cells. In addition, the patients in this study had various renal diseases and stages. Further investigation is needed to clarify more precise correlation between expression levels of transporters and renal drug secretion. So far, present results suggested that the renal excretion of the cefazolin was partly affected by hOAT3 expression levels.

The elimination rate of cefazolin was correlated with the urinary excretion of phenolsulfonphthalein (Fig. 2), suggesting that these compounds were excreted through common transporters. However, it was not certain which transporter mediated the secretion of these drugs in the human kidney. Phenolsulfonphthalein is mainly secreted from the kidney, and is often used for the diagnosis of renal function. It was reported that the accumulation of phenolsulfonphthalein into the renal tubules was inhibited by anionic compounds such as probenecid or 2,4-dinitrophenol (23). Therefore, tubular secretion of phenolsulfonphthalein should be mediated by the anion transport system (4). Hosoyamada *et al.* reported that phenolsulfonphthalein inhibited p-aminohippurate transport by hOAT1 (8). In this study, we confirmed that phenolsulfonphthalein inhibits the hOAT1-mediated *p*-aminohippurate transport in a dose-dependent manner. Moreover, it was shown that the phenolsulfonphthalein was transported by hOAT1 and hOAT3 (Fig. 8). Therefore, it is suggested that renal secretion of phenolsulfonphthalein is mediated by these transporters in the normal kidney.

It was reported that cefazolin inhibited the hOAT1- and hOAT3-mediated transport (24–26), and was transported by rOAT1 (27). In the present study, cefazolin inhibited the hOAT1- and hOAT3-mediated uptake, supporting previous finding. However, only hOAT3-mediated cefazolin transport could be detected in the present study (Fig. 7). Since this experiment was performed with higher concentration of cefazolin than IC_{50} value for cefazolin on hOAT1-mediated p-aminohippurate uptake, it could not be denied that cefazolin transport by hOAT1 should be detected at lower concentration. However, it is suggested that cefazolin is more effectively transported by hOAT3 than by hOAT1 and that hOAT3, rather than hOAT1, is the common transport pathway of tubular secretion of phenolsulfonphthalein and cefazolin.

The serum concentration of cefazolin at immediately after the infusion was 101.5 to 431.0 μM (data not shown). Because about 80% of cefazolin bound to the protein (21), the concentration of free serum cefazolin is comparable to IC_{50} value for cefazolin on hOAT3 transport. It is possible that cefazolin is efficiently transported by hOAT3 from blood circulation to tubular epithelial cells.

Patients with chronic renal insufficiency or nephritic syndrome frequently manifest diuretic resistance. It was suggested that the carrier-mediated tubular secretion of diuretics is important for its efficacy (28,29). In a previous study, we suggested that rOAT1 contributes, at least in part, to renal tubular secretion of acetazolamide, thiazides, and loop diuretics (30). In the present study, the hOAT1 mRNA level in the renal diseases was lower than that of normal parts of the kidney. It is possible that diuretic resistance may be partly due to the reduction of tubular secretion by the decreased hOAT1 expression.

This is the first report showing the expression profile of renal drug transporters in patients with kidney diseases and provided novel information as follows. Firstly, the hOAT1 mRNA levels in the kidney of patients with renal diseases were lower than in the normal kidney cortex. Secondly, renal excretion of the anionic drug cefazolin was significantly correlated with hOAT3 mRNA levels in patients with renal diseases. Thirdly, both phenolsulfonphthalein and cefazolin were transported by hOAT3. These results suggested that hOAT3 should play an important role on the secretion of these anionic drugs in patients with renal diseases. Although further investigation is needed to apply the expression levels of drug transporters to dosage adjustment, it is possible that the expression profiles of drug transporters may be useful information for understanding the alteration of renal drug secretion.

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REFERENCES

1. L. Dettli. Drug dosage in renal disease. *Clin. Pharmacokinet.* **1**:126–134 (1976).
2. R. Hori, K. Okumura, A. Kamiya, H. Nihira, and H. Nakano. Ampicillin and cephalexin in renal insufficiency. *Clin. Pharmacol. Ther.* **34**:792–798 (1983).
3. R. Hori, K. Okumura, H. Nihira, H. Nakano, K. Akagi, and A. Kamiya. A new dosing regimen in renal insufficiency: application to cephalexin. *Clin. Pharmacol. Ther.* **38**:290–295 (1985).
4. J. V. Moller and M. I. Sheikh. Renal organic anion transport system: pharmacological, physiological, and biochemical aspects. *Pharmacol. Rev.* **34**:315–358 (1982).
5. G. Burckhardt and N. A. Wolff. Structure of renal organic anion and cation transporters. *Am. J. Physiol. Renal Physiol.* **278**:F853–F866 (2000).

6. K. Inui, S. Masuda, and H. Saito. Cellular and molecular aspects of drug transport in the kidney. *Kidney Int.* **58**:944–958 (2000).
7. T. Sekine, S. H. Cha, and H. Endou. The multispecific organic anion transporter (OAT) family. *Pflugers Arch.* **440**:337–350 (2000).
8. M. Hosoyamada, T. Sekine, Y. Kanai, and H. Endou. Molecular cloning and functional expression of a multispecific organic anion transporter from human kidney. *Am. J. Physiol.* **276**:F122–F128 (1999).
9. R. Lu, B. S. Chan, and V. L. Schuster. Cloning of the human kidney PAH transporter: narrow substrate specificity and regulation by protein kinase C. *Am. J. Physiol.* **276**:F295–F303 (1999).
10. H. Motohashi, Y. Sakurai, H. Saito, S. Masuda, Y. Urakami, M. Goto, A. Fukatsu, O. Ogawa, and K. Inui. Gene expression levels and immunolocalization of organic ion transporters in the human kidney. *J. Am. Soc. Nephrol.* **13**:866–874 (2002).
11. A. Enomoto, M. Takeda, M. Shimoda, S. Narikawa, Y. Kobayashi, T. Yamamoto, T. Sekine, S. H. Cha, T. Niwa, and H. Endou. Interaction of human organic anion transporters 2 and 4 with organic anion transport inhibitors. *J. Pharmacol. Exp. Ther.* **301**: 797–802 (2002).
12. S. H. Cha, T. Sekine, J. Fukushima, Y. Kanai, Y. Kobayashi, T. Goya, and H. Endou. Identification and characterization of human organic anion transporter 3 expressing predominantly in the kidney. *Mol. Pharmacol.* **59**:1277–1286 (2001).
13. D. H. Sweet, D. S. Miller, J. B. Pritchard, Y. Fujiwara, D. R. Beier, and S. K. Nigam. Impaired organic anion transport in kidney and choroid plexus of organic anion transporter 3 (Oat3 (Slc22a8)) knockout mice. *J. Biol. Chem.* **277**:26934–26943 (2002).
14. E. Babu, M. Takeda, S. Narikawa, Y. Kobayashi, A. Enomoto, A. Tojo, S. H. Cha, T. Sekine, D. Sakthisekaran, and H. Endou. Role of human organic anion transporter 4 in the transport of ochratoxin A. *Biochim. Biophys. Acta* **1590**:64–75 (2002).
15. Y. Urakami, M. Akazawa, H. Saito, M. Okuda, and K. Inui. cDNA cloning, functional characterization, and tissue distribution of an alternatively spliced variant of organic cation transporter hOCT2 predominantly expressed in the human kidney. *J. Am. Soc. Nephrol.* **13**:1703–1710 (2002).
16. M. M. Bradford. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**:248–254 (1976).
17. M. Takeda, S. Narikawa, M. Hosoyamada, S. H. Cha, T. Sekine, and H. Endou. Characterization of organic anion transport inhibitors using cells stably expressing human organic anion transporters. *Eur. J. Pharmacol.* **419**:113–120 (2001).
18. K. Takahashi, S. Masuda, N. Nakamura, H. Saito, T. Futami, T. Doi, and K. Inui. Upregulation of H^+-peptide cotransporter PEPT2 in rat remnant kidney. *Am. J. Physiol. Renal Physiol.* **281**:F1109–F1116 (2001).
19. A. Takeuchi, S. Masuda, H. Saito, T. Doi, and K. Inui. Role of kidney-specific organic anion transporters in the urinary excretion of methotrexate. *Kidney Int.* **60**:1058–1068 (2001).
20. L. Ji, S. Masuda, H. Saito, and K. Inui. Down-regulation of rat organic cation transporter rOCT2 by 5/6 nephrectomy. *Kidney Int.* **62**:514–524 (2002).
21. E. K. Brodwall, T. Bergan, and O. Ørjavik. Kidney transport of cefazolin in normal and impaired renal function. *J. Antimicrob. Chemother.* **3**:585–592 (1977).
22. G. R. Brown. Cephalosporin-probenecid drug interactions. *Clin. Pharmacokinet.* **24**:289–300 (1993).
23. M. I. Sheikh. Renal handling of phenol red. I. A comparative study on the accumulation of phenol red and p-aminohippurate in rabbit kidney tubules in vitro. *J. Physiol.* **227**:565–590 (1972).
24. S. Jariyawat, T. Sekine, M. Takeda, N. Apiwattanakul, Y. Kanai, S. Sophasan, and H. Endou. The interaction and transport of β-lactam antibiotics with the cloned rat renal organic anion transporter 1. *J. Pharmacol. Exp. Ther.* **290**:672–677 (1999).
25. K. Y. Jung, M. Takeda, M. Shimoda, S. Narikawa, A. Tojo, K. Kim do, A. Chairoungdua, B. K. Choi, H. Kusuhara, Y. Sugiyama, T. Sekine, and H. Endou. Involvement of rat organic anion transporter 3 (rOAT3) in cephaloridine-induced nephrotoxicity: in comparison with rOAT1. *Life Sci.* **70**:1861–1874 (2002).
26. M. Takeda, E. Babu, S. Narikawa, and H. Endou. Interaction of human organic anion transporters with various cephalosporin antibiotics. *Eur. J. Pharmacol.* **438**:137–142 (2002).
27. Y. Uwai, H. Saito, and K. Inui. Rat renal organic anion transporter rOAT1 mediates transport of urinary-extreted cephalosporins, but not of biliary-extracted cefoperazon. *Drug. Metabol. Pharmacokin.* **17**:125–129 (2002).
28. M. Burg, L. Stoner, J. Cardinal, and N. Green. Furosemide effect on isolated perfused tubules. *Am. J. Physiol.* **225**:119–124 (1973).
29. J. F. Seely and J. H. Dirks. Site of action of diuretic drugs. *Kidney Int.* **11**:1–8 (1977).
30. Y. Uwai, H. Saito, Y. Hashimoto, and K. Inui. Interaction and transport of thiazide diuretics, loop diuretics, and acetazolamide via rat renal organic anion transporter rOAT1. *J. Pharmacol. Exp. Ther.* **295**:261–265 (2000).