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Short Communication

Renal Transport of Adefovir, Cidofovir, and Tenofovir by SLC22A Family Members (hOAT1, hOAT3, and hOCT2)

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Purpose. The nephrotoxicity of the nucleotide antivirals adefovir, cidofovir and tenofovir is considered to depend on the renal tubular transport of them. Although it is known that the antivirals are substrates of the human renal organic anion transporter hOAT1 (SLC22A6), there is no information available on other organic ion transporters. The aim of the present study was to investigate whether the other renal organic anion transporter hOAT3 (SLC22A8) and organic cation transporter hOCT2 (SLC22A2) transport the antivirals.
Materials and Methods. Uptake experiments were performed using HEK293 cells transfected with cDNA of the organic ion transporters.
Results. The uptake of adefovir, cidofovir and tenofovir in monolayers stably expressing hOAT3 increased time-dependently, compared with control. Probenecid, a typical inhibitor of organic anion transporters, completely inhibited their transport. The amounts of the antivirals taken up by hOAT3 were much lower than those by hOAT1. The transient expression of hOCT2 did not increase uptake of the antivirals.
Conclusion. These results indicate that adefovir, cidofovir and tenofovir are substrates of hOAT3 as well as hOAT1, but that quantitatively hOAT1 is the major renal transporter for these drugs.

KEY WORDS: antivirals; nephrotoxicity; organic anion transporter; renal transport.

INTRODUCTION

The SLC22A superfamily comprises organic anion transporters and organic cation transporters, and is responsible for the tissue distribution and disposition of various organic compounds including endogenous metabolites, toxins, xenobiotics and drugs (1,2). So far, cDNAs of the human organic anion transporters 1–4 (hOAT1-4), the urate transporter (URAT) and the human organic cation transporters 1–3 (hOCT1-3), hOCTN1 and hOCTN2 have been isolated and characterized in terms of function and expression. The SLC22A members are known to transport clinically important drugs such as cephalosporins, diuretics, non-steroidal anti-inflammatory drugs, H_2 receptor antagonists, antivirals and antitumor agents, and the clinical relevance of each transporter has been assessed (3,4). In the members, hOAT1 (SLC22A6), hOAT3 (SLC22A8) and hOCT2 (SLC22A2) are highly expressed at the basolateral membrane in the renal proximal tubules, indicating that they play important roles in the tubular uptake of organic compounds from circulation (5).

hOAT1, hOAT3 and hOCT2 are determinants of the renal toxicity of cephaloridine, ochratoxin A, cisplatin and so on (6,7). In other words, the basolateral uptake of these compounds via the organic ion transporters in the renal proximal tubules is the first step in their nephrotoxicity. Similarly, the acyclic nucleotide analogues adefovir, cidofovir and tenofovir, which are currently used to treat infections of hepatitis B virus, cytomegalovirus and human immunodeficiency virus, respectively, induce renal impairment triggered by their renal tubular transport (8). It is shown that they are substrates of hOAT1 (9) and that the expression of hOAT1 is required for the cytotoxicity of adefovir and cidofovir (10), although there is no report on the transport of the antivirals by other SLC22A members.

Recently, we showed that mRNA expression levels of hOAT3 in the kidney cortex were higher than those of hOAT1 (5), and that mRNA levels of hOAT3 but not of hOAT1 correlated with the elimination rates of cefazolin and phenolsulfonphthalein in patients with renal diseases (11,12). Accordingly, to understand the renal basolateral transport mechanisms for the antivirals in detail, information on hOAT3 is required. The purpose of this study is to investigate the transport of adefovir, cidofovir and tenofovir by hOAT3. In addition, the contribution of hOCT2 was also examined.

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ABBREVIATIONS: hOAT, human organic anion transporter; hOCT, human organic cation transporter; MRP, multidrug resistance protein.

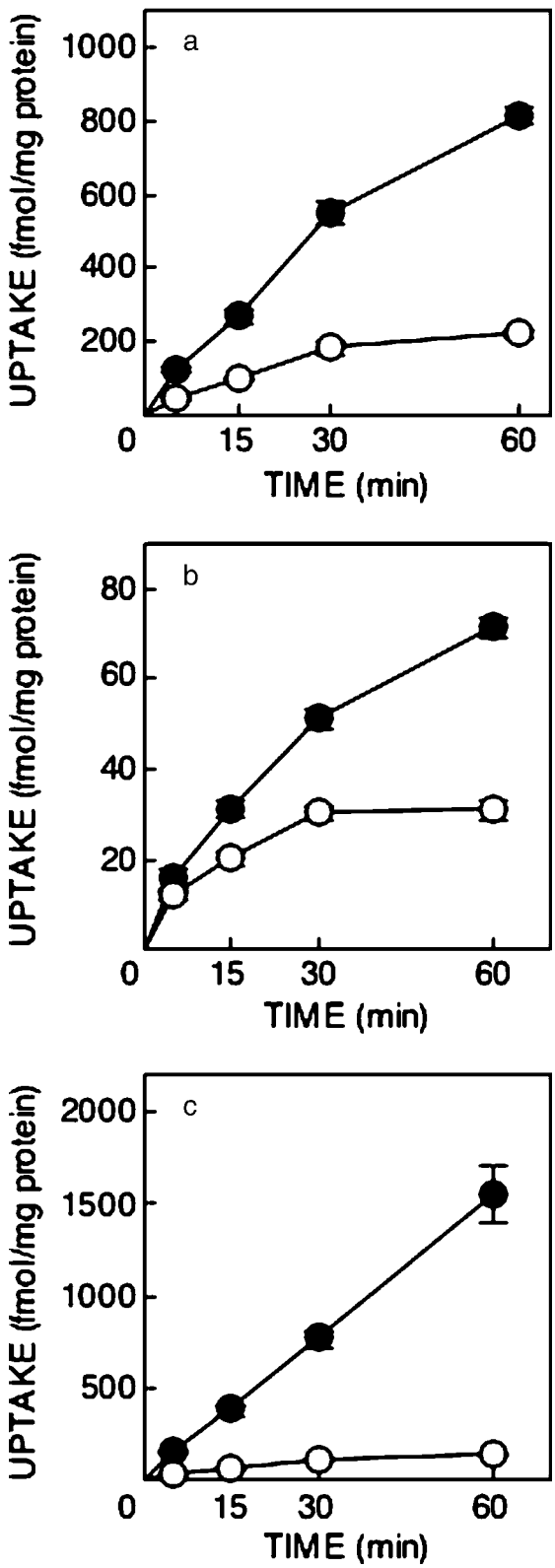

Fig. 1. Time-dependent uptake of adefovir (**a**), cidofovir (**b**) and tenofovir (**c**) by HEK-pBK and HEK-hOAT3. HEK-pBK (*open circle*) and HEK-hOAT3 (*closed circle*) were incubated with 111 nM [^{3}H]adefovir, 66.7 nM [^{3}H]cidofovir or 100 nM [^{3}H]tenofovir for the periods indicated. Each point represents the mean±S.E. of the uptake amounts of each antiviral in three monolayers. When the error bar is not shown, it is smaller than the symbol.

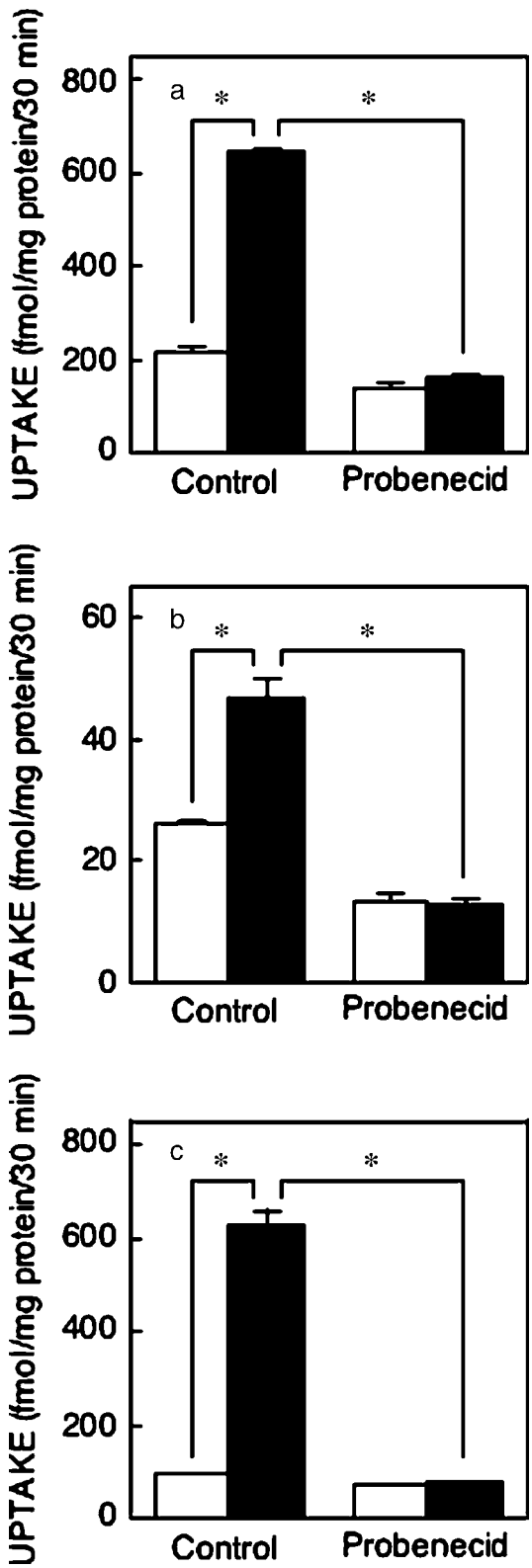

Fig. 2. Effect of probenecid on hOAT3-mediated uptake of adefovir (**a**), cidofovir (**b**) and tenofovir (**c**). HEK-pBK (*open column*) and HEK-hOAT3 (*closed column*) were incubated with 111 nM [^{3}H]adefovir, 66.7 nM [^{3}H]cidofovir or 100 nM [^{3}H]tenofovir in the absence (control) or presence of 1 mM probenecid for 30 min. Each column represents the mean±S. E. of the uptake amounts of each antiviral in three monolayers. *, $P<0.001$, significantly different.

Table I. Uptake of Adefovir, Cidofovir and Tenofovir by HEK293 Cells Stably Expressing hOAT1 or hOAT3

Compounds	Control	hOAT1	hOAT3
		μl/mg protein/5 min	
p-Aminohippurate	2.19±0.08	24.9±0.6**	3.38±0.17
Estrone sulfate	1.41±0.03	1.98±0.05*	14.4±0.1**
Adefovir	0.369±0.007	46.1±0.5**	1.38±0.08
Cidofovir	0.135±0.010	22.5±0.4**	0.295±0.024
Tenofovir	0.260±0.018	26.0±0.7**	1.22±0.07

HEK-pBK (control), HEK-hOAT1 and HEK-hOAT3 were incubated with 5 μM *p*-[^{14}C]aminohippurate, 17.5 nM [^{3}H]estrone sulfate, 111 nM [^{3}H]adefovir, 66.7 nM [^{3}H]cidofovir or 100 nM [^{3}H]tenofovir for 5 min. Each value represents the mean ± S. E. of the uptake amounts of each compound in three monolayers.
**, $P<0.001$, significantly different from control.
*, $P<0.01$, significantly different from control.

MATERIALS AND METHODS

Materials

p-[^{14}C]Aminohippurate (1.9 GBq/mmol) was obtained from NEN™ Life Science Products Inc. (Boston, MA, USA). [^{3}H]Estrone sulfate, ammonium salt (2.1 TBq/mmol) and [^{14}C]tetraethylammonium bromide (88.8 MBq/mmol) were from Perkin-Elmer Life Sciences Inc. (Boston, MA, USA). [^{3}H]Adefovir (9 Ci/mmol), [^{3}H]cidofovir (15 Ci/mmol) and [^{3}H]tenofovir (10 Ci/mmol) were purchased from Moravek Biochemicals Inc. (Brea, CA, USA). Probenecid was obtained from Sigma (St. Louis, MO, USA). All other chemicals used were of the highest purity available.

Functional Analyses of hOAT1 and hOAT3

The functions of hOAT1 and hOAT3 were evaluated according to our former report (13), using HEK293 cells stably transfected with pBK-CMV vector containing hOAT1 cDNA, hOAT3 cDNA or no cDNA, named HEK-hOAT1, HEK-hOAT3 and HEK-pBK, respectively. In brief, 48 h after the cells were seeded on poly-D-lysine-coated 24-well plates at a density of 2×10^5 cells/well, the uptake of organic compounds by the cells was examined. The composition of the incubation medium was as follows: 145 mM NaCl, 3 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM D-glucose and 5 mM HEPES (pH 7.4). After the preincubation of the cells with 0.2 ml of the incubation medium at 37°C for 10 min, the medium was replaced with 0.2 ml of the incubation medium containing test compounds. At the end of the incubation, the medium was aspirated, and then the cells were washed twice with 1 ml of ice-cold incubation medium. The cells were lysed in 250 μl of 0.5 N NaOH solution, and the radioactivity in aliquots was determined in 3 ml of ACSII (Amersham International, Buckingham shire, UK). The protein contents of the solubilized cells were determined by the method of Bradford using the Bio-Rad protein assay kit (Bio-Rad, Hercules, CA, USA) with bovine γ-globulin as a standard.

Table II. Uptake of Adefovir, Cidofovir and Tenofovir by HEK293 Cells Transiently Expressing hOCT2

Compounds	Control	hOCT2
		μl/mg protein/30 min
Tetraethylammonium	12.8±0.8	97.7±9.0*
Adefovir	0.964±0.062	0.857±0.026
Cidofovir	0.189±0.023	0.161±0.007
Tenofovir	0.674±0.049	0.653±0.017

HEK293 cells transfected with empty pCMV6-XL4 (control) or hOCT2 cDNA were incubated with 5 μM [^{14}C]tetraethylammonium, 111 nM [^{3}H]adefovir, 66.7 nM [^{3}H]cidofovir or 100 nM [^{3}H]tenofovir for 30 min. Each value represents the mean±S. E. of the uptake amounts of each compound in three monolayers.
*, $P<0.001$, significantly different from control.

Functional Analysis of hOCT2

As previously reported (14), the transient expression system with HEK293 cells was used to examine whether hOCT2 transports the antivirals. Briefly, 1 day after 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). Forty-eight hours after the transfection, uptake experiments were performed as above described.

Statistical Analysis

Data were statistically analyzed with a one-way analysis of variance followed by Scheffe's test.

RESULTS AND DISCUSSION

First, we investigated the time-dependent uptake of adefovir, cidofovir and tenofovir by HEK-pBK and HEK-hOAT3. As shown in Fig. 1, the expression of hOAT3 enhanced the amounts of these antivirals taken up into the cells, and their uptake by hOAT3 was increased time-dependently. Fig. 2 depicts the effect of probenecid, a typical inhibitor of renal organic anion transporters, on the transport of the antivirals by hOAT3. Probenecid significantly inhibited the hOAT3-mediated uptake of the antivirals. These results indicate that hOAT3 recognizes adefovir, cidofovir and tenofovir as substrates.

Table I represents the amounts of adefovir, cidofovir and tenofovir taken up by HEK-pBK, HEK-hOAT1 and

HEK-hOAT3, including those of *p*-aminohippurate and estrone sulfate, the representative substrates of hOAT1 and hOAT3, respectively. As previously reported (9), hOAT1-mediated transport of adefovir, cidofovir and tenofovir was observed. The uptake amounts of the antivirals via hOAT1 were much greater than those via hOAT3. It is important to compare the kinetic parameters of the hOAT1-mediated versus hOAT3-mediated transport of adefovir, cidofovir and tenofovir. However, we could not perform the experiments on the concentration-dependent uptake, because their unlabeled compounds were not commercially available. Information on the expression levels of hOAT1 and hOAT3 in HEK293 cells would make the data in Table I more significant. We previously quantified mRNA levels of each transporter in HEK-hOAT1 and HEK-hOAT3 to be 64.9 and 225.6 amol/μg total RNA, respectively (13). Because the transport activities of the typical substrates by each transfectant in the present study tended to be similar to those in our previous report (13), it was considered that the mRNA levels of hOAT3 in HEK-hOAT3 used in this study could be higher than those of hOAT1 in HEK-hOAT1. Taking these findings into account, it is suggested that hOAT1 is a potent transporter of adefovir, cidofovir and tenofovir, compared with hOAT3, under the conditions tested in this study. Furthermore, the facts that the blood levels of adefovir and tenofovir in patients are comparable to the concentrations examined in the present study could indicate that hOAT1 is a key transporter mediating the entry of adefovir and tenofovir into the renal tubules from blood.

As described in the Introduction, we recently quantified mRNA levels of drug transporters in normal parts of the kidney cortex from nephrectomized patients and in the renal biopsy specimens of patients with renal diseases. Two important findings were made. First, mRNA levels of hOAT3 were about 3-time higher than those of hOAT1 in the normal kidney cortex (5). Second, mRNA levels of only hOAT1 among hOAT1-4 were significantly lower in the biopsy specimens, compared in the normal segments of the kidney cortex, suggesting that hOAT1 expression is readily influenced by renal diseases (11). Therefore, there is a possibility that the contribution of hOAT3 to tubular uptake of the antivirals could be greater in patients with renal failure.

Now, multidrug resistance protein 4 (MRP4) is thought to be a candidate which transports these antivirals from the proximal epithelial cells into the lumen, because MRP4-mediated efflux of adefovir from cells was observed (15,16) and the functional expression of Mrp4 in the brush-border membrane of the renal proximal tubule was recognized using Mrp4-knockout mice (17). Our previous study using serial sections showed that the localization of hOAT1 and hOAT3 was not completely identical in the proximal tubules (5). An investigation of the exact distribution of MRP4 in the renal proximal tubules and a comparison with hOAT1 and hOAT3 would facilitate elucidation of the mechanisms behind the nephrotoxicity of adefovir, cidofovir and tenofovir.

The transport of adefovir, cidofovir and tenofovir by hOCT2 was also examined in this study. As represented in Table II, no uptake of these antivirals via hOCT2 was observed. Previously, we reported that cimetidine was transported by hOAT1 and hOAT3 as well as hOCT2, and that hOAT3 but not hOAT1 or hOCT2 transported famotidine (18). Because famotidine and cimetidine exist partly in the cationic forms at the experimental pH, the results of the study were surprising, and the substrate recognition of the SLC22A family might be complicated. Although our previous report indicated that preconceived ideas on the substrate recognition of SLC22A members should be reconsidered, the findings of the present study were consistent with the preconception that hOCT2 would not transport the anionic antivirals. hOCT2 is not likely to contribute to the elimination of adefovir, cidifovir and tenofovir.

In conclusion, this is the first report representing that adefovir, cidofovir and tenofovir are substrates of hOAT3 as well as hOAT1. Furthermore, it is suggested that hOAT1 rather than hOAT3 plays a crucial role in the basolateral uptake of the antivirals into the renal proximal tubules. These findings provide useful information for the elucidation of the molecular mechanisms of disposition and nephrotoxicity of adefovir, cidofovir and tenofovir.

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