Renal Secretion of Uric Acid by Organic Anion Transporter 2 (OAT2/SLC22A7) in Human

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The physiological function of organic anion transporter OAT2 (SLC22A7) remains unclear, but since OAT2 transports purine derivatives, it may be involved in renal handling of uric acid, the final metabolite of purine derivatives. In the present study, we studied uric acid transport in stably OAT2-expressing HEK293 cells (HEK293/OAT2). OAT2 mediated uptake, but not efflux, of [14C]uric acid. Uric acid transport was saturable with Km of 1168±335 μM (mean and S.E.M.) and Vmax of 2.57±0.350 nmol/min/mg protein. The [14C]uric acid uptake was sensitive to Cl− and was enhanced at acidic pH. In cis-inhibition assay, [14C]uric acid uptake was inhibited by several mono- or dicarboxylic acids, but it was not trans-stimulated by any of the compounds tested. The pattern of inhibition of OAT2-mediated uric acid transport by various drugs was different from that of OAT1- or OAT3-mediated transport. Furthermore, OAT2-mediated transport of uric acid was inhibited by an antiuricosuric drug, pyrazinecarboxylic acid. These results revealed distinct characteristics of uric acid transport via OAT2 compared with other uric acid transporters, suggesting that OAT2 plays a role in renal uric acid uptake from blood as a first step of tubular secretion. OAT2 may therefore be a potential target for regulating serum uric acid level.

Key words organic anion transporter 2; uric acid; transporter; uricosuric; antiuricosuric; serum uric acid level

Serum uric acid (SUA) level is controlled by the balance of uric acid synthesis and renal secretion.1,2 Increase in SUA (hyperuricemia) is associated with gout, hypertension, cardiovascular and renal diseases, while decrease in SUA (hypouricemia) is associated with multiple sclerosis, Parkinson’s disease and Alzheimer’s disease.3 More than four out of five cases of hyperuricemia involve decreased renal excretion of uric acid.4 Furthermore, several drugs are known to change urinary uric acid excretion and alter SUA level.5–8 It is of clinical importance to clarify the molecular mechanism of uric acid excretion from kidney.

Net renal excretion of uric acid is influenced by the rate of glomerular filtration, as well as reabsorption into and secretion from proximal tubular cells. Since uric acid is a hydrophilic compound, the latter two processes are likely mediated by transporter proteins. Reabsorption of uric acid from the glomerular ultrafiltrate at the proximal tubules may involve uric acid-anion exchangers, such as uric acid transporter URAT1 (SLC22A12), OAT4 (SLC22A11) and OAT10 (SLC22A13) at the apical membranes, followed by efflux across the basolateral membranes (to blood) by voltage-driven uric acid transporter URAT1v1 (SLC22A9). Previous studies have revealed that impairment of URAT1 and URAT1v1 function results in hypouricemia in humans.9,10 In the process of tubular secretion, uric acid is first translocated across the basolateral membranes (from blood) to proximal tubular cells by organic anion transporters, OAT1 (SLC22A6) and OAT3 (SLC22A8), which utilize an outwardly directed (cell to blood) dicarboxylate gradient sustained by sodium-dicarboxylate cotransporter NaDC3, and then secreted to the proximal tubular lumen by several transporters, including sodium phosphate transporter NPT1 (SLC17A1), and multidrug resistance-associated protein MRP4 (ABCC4).11–13 However, the analysis of urinary excretion of uric acid in Oat1 and Oat3 knockout mice identified a contribution of other molecular mechanisms to renal uptake of uric acid at proximal tubular cells, in addition to Oat1 and Oat3.14

OAT2 (SLC22A7) was the second OAT family member to be identified,15 and mediates Na+-independent transport of acetysalicylate, prostaglandin E2 (PGE2), alpha-ketoglutarate, methotrexate, p-aminohippuric acid (PAH), and cephalosporin antibiotics. The substrate specificity of OAT2 is much narrower than those of OAT1 and OAT3.15–17 The driving force for OAT2-mediated transport remains controversial, although a recent study claimed that it is an organic anion/dicarboxylate exchanger.18 In human kidney, OAT2 is localized at the basolateral membrane of the proximal tubules.19 Therefore, we hypothesized that OAT2 mediates uric acid uptake from blood into cells in kidney independently of OAT1 and OAT3. Identification of OAT2 as a mediator of uric acid transport would improve our understanding of the mechanisms of alteration in SUA resulting from chronic renal failure or drug–drug interactions. Here, we present the first evidence that OAT2 transports uric acid in HEK293 cells expressing OAT2. Our results are consistent with the idea that OAT2 plays a role in uric acid secretion by proximal tubular cells.

MATERIALS AND METHODS

Chemicals [14C]Uric acid (1.96 TBq/mol) was purchased from Moravek Biochemicals, Inc. (Brea, CA, U.S.A.). All other reagents were purchased from Kanto Chemicals (Tokyo, Japan), Sigma-Aldrich (St. Louis, MO, U.S.A.), and Wako Pure Chemical Industries Ltd. (Osaka, Japan), respectively.

cDNA Cloning of Human OAT2 The OAT2 (SLC22A7) gene was isolated from human hepatocarcinoma

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HepG2 cells by reverse transcription-polymerase chain reaction (RT-PCR) as previously described, using Ex Taq™ DNA polymerase (Takara Bio INC., Shiga, Japan). The sequences of primers used were as follows: upstream primer, 5′-GGATTCCGAGCTGCTGGATACAGG-3′; downstream primer, 5′-TCTAGATAGCAGAGGTTCCGAT-3′ (both synthesized by Hokkaido SBI Science, Hokkaido, Japan). A 1.8-kb DNA fragment was cloned into pCR2.1-TOPO (Invitrogen, Carlsbad, CA, U.S.A.) and then the open reading frame of OAT2 was inserted into pcDNA3.1 (Invitrogen) or pGEMHE at the BamHI and XbaI sites. The obtained cDNA sequences were analyzed (Bio Matrix Research, Chiba, Japan) and confirmed to be identical with the reported sequence.19

Establishment of OAT2-Expressing HEK293 Cell Line
HEK293 cells were transfected with pcDNA3.1 plasmid DNA alone or containing OAT2 cDNA (pcDNA3.1/OAT2) using Lipofectamine 2000 (Invitrogen) as described previously.21 Stable transfectants were selected in the presence of 1 mg/ml G418 (Sigma-Aldrich) for about 2 weeks; the established cell lines were designated as HEK293/OAT2 and HEK293/pcDNA3.1, respectively. These cells were routinely grown in Dulbecco’s modified Eagle medium supplemented with 10% FBS, 100 units/ml penicillin, 100 μg/ml streptomycin, and 400 μg/ml G418 in a humidified incubator at 37 °C in an atmosphere of 5% CO2 in air.

Transport Study with Cultured Cells
OAT2-mediated uric acid transport was evaluated by means of uptake experiments using HEK293/OAT2 cells as described previously.22 In general, uptake reaction was initiated by placing the cells in transport buffer (136.7 mM NaCl, 5.4 mM KCl, 25 mM Na2HPO4, and 10 mM HEPES, pH 7.4) containing [14C]uric acid at 37 °C for 10 minutes, respectively, to obtain comparable amounts of [14C]uric acid in the two cell lines. Under these conditions, intracellular accumulation of [14C]uric acid was similar in the two cell lines. The cells were washed and incubated in [14C]uric acid-free transport buffer for 5 min at 37 °C. At the designated times, an aliquot of the medium was taken to measure radioactivity.

Transport Study Using Xenopus laevis Oocytes
Complementary RNAs (cRNAs) of human OAT2 were prepared using a mMESSAGE mMACHINE kit according to manufacturer’s instruction (Ambion, Austin TX, U.S.A.). For the uptake study with OAT2, defolliculated oocytes were injected with a 25 ng of OAT2 or the same volume of water, and then cultured in modified Barth’s solution (88 mM NaCl, 1 mM KCl, 0.33 mM Ca(NO3)2, 0.41 mM CaCl2, 0.82 mM MgSO4, 2.4 mM NaHCO3, and 10 mM HEPES, pH 7.4) containing 50 μg/ml gentamycin at 18 °C for 3 d. For trans-stimulation study, oocytes were microinjected with a 50 nl of 100 mM drug solution or water as control. Immediately after the microinjection (within approximately 2 min), the oocytes were transferred to ND96 (96 mM NaCl, 2 mM KCl, 1 mM MgCl2, 1.8 mM CaCl2, and 5 mM HEPES, pH 7.4) buffer containing [14C]uric acid (20 μM) and incubated at 25 °C for 60 min. Uptake was terminated by washing the oocytes three times with ice-cold ND96 buffer. The oocytes were solubilized with 5% SDS solution.

Data Analysis
For the evaluation of the kinetic parameters, the uptake rates were fitted to the following Eq. 1 by means of nonlinear least-squares regression analysis using Kaleidagraph (Synergy Software, Reading, PA, U.S.A.),

\[ v = V_{\text{max}} \times s / (K_m + s) \]

Where, \( v \), \( s \), \( K_m \), and \( V_{\text{max}} \) are the uptake rate of substrate (pmol per indicated time per mg protein), the substrate concentration in the medium (micromol/liter), the apparent Michaelis–Menten constant (micromol/liter), and the maximal uptake rate (pmol per indicated time per mg protein), respectively.

Statistical significance of differences was determined with Student’s t test and a p-value of less than 0.05 was considered statistically significant.

RESULTS
Uric Acid Transport by OAT2
The time course of uric acid uptake was measured in OAT2-expressing HEK293 (HEK293/OAT2) cells and control cells (HEK293/pcDNA3.1). As shown in Fig. 1, the uptake of [14C]uric acid by HEK293/OAT2 cells was significantly higher than that by HEK293/pcDNA3.1 cells, and increased time-dependently during the first 10 min. Therefore, the intracellular accumulation of [14C]uric acid at 5 min was routinely measured to evaluate the initial rate of uric acid uptake in subsequent studies. As shown in Fig. 2, OAT2-mediated uric acid transport was measured in the concentration range from 20 to 2000 μM. OAT2-mediated uric acid transport was obtained

![Fig. 1. Time Course of [14C]Uric Acid Uptake by OAT2](image)
Uric acid uptake by HEK293/OAT2 and HEK293/pcDNA3.1 cells was measured over the concentration range of 20—2000 μM at 5 min, 37 °C and pH 7.4. OAT2-mediated uric acid uptake was obtained by subtracting the uptake of HEK293/pcDNA3.1 cells from that of HEK293/OAT2 cells. Inset, Eadie-Hofstee plot. Symbols and bars indicate the mean±S.E.M. (n=3).

Fig. 2. Concentration Dependence of Uric Acid Uptake by OAT2

Uric acid uptake by HEK293/OAT2 and HEK293/pcDNA3.1 cells was measured for 5 min immediately after preloading of the cells with organic anions. None of the organic anions examined influenced the effects on OAT2-mediated uptake of uric acid were tested by measuring the uptake by cells pre-loaded with organic anions. The uptake thus evaluated was saturable, and the mean±S.E.M. (n=3).

Table 1. cis-Effects of Mono- and Dicarboxylic Acids on OAT2-Mediated Uptake of Uric Acid

<table>
<thead>
<tr>
<th>Drug</th>
<th>5 μM</th>
<th>100 μM</th>
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<tbody>
<tr>
<td>α-Ketoglutaric acid</td>
<td>92.4±0.1</td>
<td>82.6±0.1*</td>
</tr>
<tr>
<td>Glutaric acid</td>
<td>109.7±2.4</td>
<td>90.4±0.4</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>80.3±0.2*</td>
<td>82.5±0.5*</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>92.2±1.0</td>
<td>82.9±0.3*</td>
</tr>
<tr>
<td>t-Lactic acid</td>
<td>93.3±0.2</td>
<td>88.7±1.0*</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>88.9±0.4</td>
<td>35.0±0.7*</td>
</tr>
</tbody>
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Table 2. trans-Effects of Mono- and Dicarboxylic Acids on OAT2-Mediated Uptake of Uric Acid

<table>
<thead>
<tr>
<th>Drug</th>
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<tbody>
<tr>
<td>α-Ketoglutaric acid</td>
<td>94.9±12.1</td>
</tr>
<tr>
<td>Glutaric acid</td>
<td>117.3±2.4</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>115.3±12.4</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>121.9±21.7</td>
</tr>
<tr>
<td>t-Lactic acid</td>
<td>73.4±8.7</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>104.3±8.9</td>
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</tbody>
</table>

Effect of extracellular Cl⁻ on the uptake of [¹⁴C]uric acid by OAT2

Effects of extracellular Cl⁻ on the uptake of [¹⁴C]uric acid (20 μM) by HEK293/pcDNA3.1 (open column) and HEK293/OAT2 cells (closed column). Cl⁻ was replaced in the transport buffer with gluconic acid. [¹⁴C]Uric acid uptake by HEK293/OAT2 cells was measured at 5 min at 37 °C and pH 7.4. Columns and bars indicate the mean±S.E.M. (n=3). * p<0.05, significantly different from control.

Fig. 3. Efflux of [¹⁴C]Uric Acid Mediated by OAT2

Efflux of [¹⁴C]uric acid from HEK293/pcDNA3.1 (open circles) and HEK293/OAT2 cells (closed circles) was measured for 5 min immediately after preloading of the cells in transport buffer containing [¹⁴C]uric acid (33 μM for HEK293/pcDNA3.1 cells, 2 μM for HEK293/OAT2) at 37 °C for 10 min and pH 7.4. The values represent the remaining amount of radioactivity in the cells at each time. Symbols and bars indicate the mean±S.E.M. (n=3).

Table 2. trans-Effects of Mono- and Dicarboxylic Acids on OAT2-Mediated Uptake of Uric Acid

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Uptake of [¹⁴C]uric acid (20 μM) by OAT2-expressing or water-injected oocytes was measured in the absence (control) or presence of pre-injection of mono- and dicarboxylic acids (100 μM) for 60 min at 25 °C and pH 7.4. OAT2-mediated uptake was determined by subtracting the uptake by water-injected oocytes from that by OAT2-expressing oocytes, and the values obtained were divided by the control value in each assay. Each result represents the mean±S.E.M. (n=10). No statistic differences were observed.

Effect of extracellular Cl⁻ on the uptake of [¹⁴C]uric acid by OAT2

Effects of extracellular Cl⁻ on the uptake of [¹⁴C]uric acid (20 μM) by HEK293/pcDNA3.1 (open column) and HEK293/OAT2 cells (closed column). Cl⁻ was replaced in the transport buffer with gluconic acid. [¹⁴C]Uric acid uptake by HEK293/OAT2 cells was measured at 5 min at 37 °C and pH 7.4. Columns and bars indicate the mean±S.E.M. (n=3). * p<0.05, significantly different from control.

Fig. 4. Effects of Extracellular Cl⁻ on the Uptake of [¹⁴C]Urmetric Acid by OAT2

Effects of extracellular Cl⁻ on the uptake of [¹⁴C]uric acid (20 μM) by HEK293/pcDNA3.1 (open column) and HEK293/OAT2 cells (closed column). Cl⁻ was replaced in the transport buffer with gluconic acid. [¹⁴C]Uric acid uptake by HEK293/OAT2 cells was measured at 5 min at 37 °C and pH 7.4. Columns and bars indicate the mean±S.E.M. (n=3). * p<0.05, significantly different from HEK293/OAT2 cells with NaCl.
Some SLC22A transporters, such as OAT4 and OAT10, which are uric acid transporters in human kidney, show pH dependence, while others do not.\(^{11,25,29}\) Hence, to test whether the transport activity of OAT2 is dependent on the pH of the extracellular fluid, transport buffers at various pH values were employed. As shown in Fig. 5, \(^{14}\)C]uric acid uptake by HEK293/OAT2 cells decreased as the pH was increased. Strikingly, the uptake was highest at pH 5.0, and the intracellular concentration of \(^{14}\)C]uric acid was nearly 20-fold greater in HEK293/OAT2 cells than in HEK293/pcDNA3.1 cells. These results suggest that the driving force for uric acid transport by OAT2 is an inwardly directed H\(^+\) and/or Cl\(^-\) gradient.

**Effects of Drugs on Uric Acid Transport by OAT2** We tested the cis-inhibitory effects of some uricosuric and antiuricosuric drugs on \(^{14}\)C]uric acid uptake by HEK293/OAT2 cells in order to examine whether therapeutic drugs might interfere with OAT2-mediated uric acid transport. OAT2-mediated uric acid transport was decreased to 84 and 50% of the control in the presence of 100 \(\mu\)M and 1 mM pyrazinecarboxylic acid, respectively (Fig. 6a). Uric acid uptake by OAT2 was not significantly reduced by 10 \(\mu\)M benzbro- marone or 100 \(\mu\)M probenecid, although 10-fold higher concentrations of these drugs decreased OAT2-mediated uptake of uric acid to 20% and 40% of the control, respectively. Furthermore, several angiotensin receptor blockers (ARBs) and diuretics hydrochlorothiazide and furosemide were similarly tested for their inhibitory effects on OAT2-mediated transport of uric acid, since these drugs have been reported to alter SUA levels.\(^{6—8,27,28}\) Losartan (10 \(\mu\)M), telmisartan (10 \(\mu\)M), hydrochlorothiazide (100 \(\mu\)M) and furosemide (100 \(\mu\)M) significantly reduced OAT2-mediated uptake of uric acid (Fig. 6b) to 85, 58, 65 and 18% of the control, respectively. Because OAT2 transports hypoxanthine,\(^{29}\) the inhibitory effects of purine derivatives and purine-related drugs on the uptake of \(^{14}\)C]uric acid by OAT2 were also tested (Fig. 6b). Oxypurinol and xanthine significantly reduced \(^{14}\)C]uric acid uptake.

**DISCUSSION**

It is important to clarify the mechanism of renal uric acid handling mediated by OAT2, because altered serum uric acid levels are associated with various diseases.\(^{3,30}\) Many SLC22A family members, including URAT1, OAT1, OAT3, OAT4 and OAT10, are known to transport uric acid and to be involved in uric acid handling in kidney.\(^{8,11,25,26,31,32}\) So far, it has been unclear whether OAT2 expressed at the basolateral membrane of proximal tubular cells is involved in uric acid transport. Our present data represent the first evidence that OAT2 can transport uric acid. When OAT2 was expressed in HEK293 cells, there was a significant increase in uptake of \(^{14}\)C]uric acid, whereas its efflux was unchanged (Figs. 1—3). Accordingly, it is likely that OAT2, as well as OAT1 and OAT3, contributes to uric acid uptake from blood into cells as a first step of tubular secretion at the basolateral membrane of human proximal tubular cells.

Uric acid uptake mediated by OAT1, OAT3, OAT4 and OAT10 is efficiently enhanced by an outwardly directed gradient of dicarboxylate, whereas uric acid uptake mediated by URAT1 and OAT10 is increased by intracellular monocarboxylic acids.\(^{25,26,33}\) It was reported that estrone-3-sulfate uptake by OAT2 was trans-stimulated by dicarboxylic acids, such as succinic acid.\(^{118}\) In addition, monocarboxylic short chain fatty acids are substrates of mouse Oat2.\(^{119}\) Hence, the effects of dicarboxylic acids and monocarboxylic acids on
OAT2-mediated uptake of uric acid were examined. As shown in Table 1, nicotinic acid inhibited most of the OAT2-mediated uric acid uptake, but none of the dicarboxylic and monocarboxylic acids tested exhibited trans-stimulatory effects on uric acid transport activity by OAT2 (Table 2). Considering the previous report, there might be a difference in the effects on uric acid transport activity by OAT2 (Table 2). Consequently, OAT2 may not be identical to that of OAT1 and OAT3. Therefore, it is possible to find a novel compound to modulate transport activity of OAT2 without interfering with OAT1 and OAT3. Accordingly, OAT2 appears to be a candidate for therapeutic drugs to control SUA level.

As shown in Fig. 4, in medium in which Cl− had been replaced with gluconic acid, [14C]uric acid uptake by OAT2 was greatly decreased. As the pH was increased, [14C]uric acid uptake by OAT2 declined (Fig. 5). Cl− is the most abundant inorganic anion in the blood and is involved in various physiological processes, including the regulation of cell volume, regulation of intracellular pH, and maintenance of blood osmolarity. Physiologically, the Cl− concentration in blood ranges from 95 to 105 mM in healthy individuals. However, in patients with diseases such as acidosis and alkalosis, the range is broader: 78 to 128 mM. Previously, it was reported that patients with diseases such as acidosis and alkalosis do show changes in their SUA level. A possible explanation for the alteration in SUA level could be due to change of OAT2-mediated uric acid transport dependent upon H+ or Cl− concentration varied in the patients, although it is remained unclear that how much degree OAT2 contributes to net urinary secretion of uric acid. Therefore, further studies should be warranted to clarify the Cl− and/or pH effects on OAT2 transport for a better understanding the role of OAT2 in controlling SUA level in patients with diseases deregulating ion homeostasis.

Pyrazinamide (PZA) is used in the treatment of tuberculous meningitis and the WHO recommends its use in pregnancy. Long-term treatment with PZA may cause hyperuricemia, arthralgia, or symptoms of gout. It has been suggested that the hyperuricemic effect of PZA is caused by a trans-stimulation of reabsorption of uric acid via URAT1 at the apical membrane of proximal tubular cells. In patients treated with PZA, its plasma concentration reaches about 500 μM. Since protein binding of PZA is around 10 to 20% (manufacturer’s product information), unbound fraction in plasma is estimated around 400 to 450 μM. At this concentration of PZA, OAT2-mediated uric acid transport may be inhibited, based on our data in Fig. 6a. This is consistent with the idea that OAT2 is a potential target of the antiuricosuric effect of PZA, as well as URAT1.

Since it is reported that OAT2 transports hypoxanthine and theophylline, which are purine derivatives, we carried out a cis-inhibitory interaction study with other endogenous purine-derivative drugs, such as allantoin and xanthine, and purine-derivative drugs, such as allopurinol and oxypurinol. Our data clearly show that oxypurinol and xanthine significantly reduced [14C]uric acid uptake (Fig. 6b), and provide the evidence that purine-derivative drugs can interact with OAT2. OAT2 may play a role in regulating intracellular and/or extracellular levels of hypoxanthine and xanthine, as well as uric acid.

In conclusion, OAT2 should be considered as a physiologically important transporter functioning in uric acid uptake from blood to proximal tubular cells, and thus playing a role in the first step of tubular secretion of uric acid. In addition, OAT2 may function to control urinary uric acid excretion in a different manner from OAT1 and OAT3. Interactions of uricosuric and antiuricosuric drugs with OAT2 should be further studied to elucidate the contribution of OAT2 to the alteration of SUA by clinically used drugs.

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