Regular Article

Rat Renal Organic Anion Transporter rOAT1 Mediates Transport of Urinary-Excreted Cephalosporins, but not of Biliary-Excreted Cefoperazone

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Summary: Most cephalosporin antibiotics are excreted into urine via glomerular filtration and active tubular secretion by renal organic anion transporters. In this study, we investigated the interaction of cephalosporins with rat organic anion transporter rOAT1, mainly expressed at the basolateral membrane of the renal proximal tubules, using Xenopus laevis oocytes, to assess the roles of rOAT1 in renal excretion of cephalosporin antibiotics. The expression of rOAT1 significantly stimulated the uptake of cefazolin, cefotiam and cephalexin into oocytes, but not of cefoperazone. The inhibition constants of these cephalosporins to rOAT1-mediated p-aminohippurate (PAH) uptake were 72 μM for cefazolin, 298 μM for cefoperazone, 718 μM for cefotiam and 6 mM for cephalexin. Eadie-Hofstee plot analysis revealed that cefoperazone as well as cefotiam inhibited rOAT1-mediated PAH uptake competitively. These results suggest that rOAT1 mediates basolateral uptake of cephalosporin antibiotics in the renal tubules. Furthermore, it is suggested that a minor contribution of the kidney to cefoperazone excretion could be related to the finding that cefoperazone is a poor substrate of rOAT1.

Key words: renal tubular secretion; organic anion transporter 1; cephalosporin antibiotics; Xenopus oocyte

Introduction

The kidney as well as liver is the main organ responsible for drug elimination. In the renal proximal tubules, various organic anion transporters mediate the secretion of anionic drugs into urine.1–3) Rat organic anion transporter 1 (rOAT1), expressed at the basolateral membranes of the renal proximal convoluted tubules, plays a major role for the active tubular uptake of a typical organic anion, p-aminohippurate (PAH) from blood.4–7) It has been reported that rOAT1 also mediates the transport of clinically important drugs such as acetazolamide, furosemide, indomethacin, methotrexate and salicylate, suggesting that rOAT1 contributes to the excretion of these drugs.8–10)

Most cephalosporin antibiotics are excreted into urine in the nonmetabolized forms, and the renal tubular secretion appears to be an important pathway for their renal clearance.11,12) Some cephalosporin antibiotics such as cephalexin have severe nephrotoxicity, and it is suggested that the toxic effect is related to the transport systems in the renal proximal tubules.13) Several in vivo and in vitro studies tried to elucidate the transport mechanisms of cephalosporins in the kidney.14–16) From these findings, it was suggested that rOAT1 would play a major role in the renal tubular uptake of cephalosporin antibiotics from blood. Jariyawat et al.17) actually showed that rOAT1 mediated the transport of cephalexin, and it is suggested that the rOAT1-mediated uptake of cephaloridine is related to its nephrotoxicity.18) But it has not yet been examined whether rOAT1 transports other cephalosporins.

In the present study, we performed transport experiments of various cephalosporins, using Xenopus laevis oocytes expressing rOAT1. The findings suggest that rOAT1 plays an important role for renal tubular uptake of cephalosporins.

Materials and Methods

Materials: p-[Glycyl-14C]aminohippurate (1.9 GBq/mmoll) was purchased from Du Pont-New England Nuclear Research Product (Boston, MA). Cefazolin (Fujisawa Pharmaceutical Co., Osaka, Japan), cefotiam (Takeda Chemical Industries, Osaka, Japan) and cephalexin (Shionogi Co., Osaka, Japan) were from the sources indicated. Cefoperazone was purchased from Sigma Co. (St. Louis, MO). Xenopus was ob-
tained from Hamamatsu Biological Research Service Inc. (Shizuoka, Japan). All other chemicals used were of the highest purity available.

**Functional Expression of rOAT1 in Xenopus Laevis Oocytes:** The capped cRNA of rOAT1 was transcribed in vitro from NotI-linearized pSPORT1 containing rOAT1 cDNA with T7 RNA polymerase, and injected into Xenopus oocytes as described previously. After 50 nl of water or rOAT1 cRNA (25 ng) was injected into each oocyte, they were maintained in modified Barth's medium (88 mM NaCl, 1 mM KCl, 0.33 mM Ca(NO₃)₂, 0.4 mM CaCl₂, 0.8 mM MgSO₄, 2.4 mM NaHCO₃ and 10 mM HEPES; pH 7.4) at 18°C.

**Uptake Experiments of [¹⁴C]PAH:** Two or three days after the injection, the oocytes were used for the uptake experiments. The uptake reaction was initiated in the 24-well plate by incubating the oocytes in 500 μl of uptake buffer (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂ and 5 mM HEPES; pH 7.4) containing [¹⁴C]PAH at 25°C in the absence or presence of an inhibitor. The uptake reaction was terminated by adding 2 ml of ice-cold uptake buffer to each well, and the oocytes were washed 5 times with 2 ml of the buffer. After washing, each oocyte was transferred to a scintillation counting vial and solubilized in 500 μl of 10% sodium lauryl sulfate. Five milliliters of ACSII (Amersham International, Buckinghamshire, UK) were added to each solubilized oocyte, and the radioactivity was determined in a liquid scintillation counter.

**Uptake Experiments of Cephalosporin Antibiotics:** The uptake experiments of cephalosporins were performed as previously reported with slight modifications. Briefly, the uptake reaction was initiated in a 1.5-ml tube by incubating the oocytes in 100 μl of uptake buffer containing each cephalosporin. The uptake reaction was terminated by adding 1 ml of ice-cold uptake buffer to each tube, and the oocytes were washed 5 times with 1.5 ml of the buffer. After the wash, 50 μl per oocytes of extraction solution (10 mM sodium acetate/methanol at 1:1 for cefazolin; 30 mM phosphate buffer (pH 7.0)/methanol at 1:1 for cefoperazone, cefotiam and cephalexin) was added into the each tube, and homogenized. The homogenate was centrifuged at 13,000 rpm for 30 min and the supernatant obtained was filtrated through a Millipore filter (SJGVL, 0.45 μm). Cephalosporins taken up by oocytes were determined by use of high performance liquid chromatograph, LC-10AS (Shimadzu Co., Kyoto, Japan), equipped with a UV spectrophotometric detector (SPD-6A, Shimadzu Co.) and an integrator (Chromatopac C-R1A, Shimadzu Co.) under the following conditions: column, TSK-gel ODS 80TM, 4.6-mm inside diameter 150 mm (Tosoh, Tokyo, Japan); mobile phase, 10 mM sodium acetate in methanol at 8:2 for cefazolin, 30 mM phosphate buffer (pH 7.0) in methanol at 7:3 for cefoperazone, cefotiam and cephalexin; flow rate, 0.8 ml/min; wavelength, 272 nm for cefazolin, 240 nm for cefoperazone and 262 nm for cefotiam and cephalexin; temperature, 40°C. The detection limit of cefazolin, cefoperazone, cefotiam and cephalexin was 100, 100, 100 and 200 nM, respectively.

**Statistical Analysis:** Data were analyzed statistically with analysis of variance, followed by Fisher's t test for multiple comparisons.

**Results**

**Transport of Cephalosporin Antibiotics by rOAT1:** We measured the uptake of cephalosporins by rOAT1-expressing oocytes. As shown in Fig. 1, the expression of rOAT1 significantly stimulated uptake of cefazolin, cefotiam and cephalexin into the oocytes. In contrast, rOAT1-mediated cefoperazone transport was not observed.

**Inhibitory Effect of Cephalosporin Antibiotics on rOAT1:** To compare the affinity of cefazolin, cefoperazone, cefotiam and cephalexin for rOAT1, we examined the dose-dependent inhibitory effects of these cephalosporins on rOAT1-mediated [¹⁴C]PAH uptake. Triplicate experiments for each cephalosporin were performed, and representative plots are shown in Fig. 2. The IC₅₀ values were estimated by nonlinear regression...
Fig. 2. Dose-dependent inhibition of \(^{14}\text{C}\)PAH uptake by cefazolin, cefoperazone, cefotiam and cephalixin with rOAT1-expressing oocytes. rOAT1-mediated \(^{14}\text{C}\)PAH uptake was determined by incubating oocytes with 25 \(\mu\text{M}\) \(^{14}\text{C}\)PAH in the absence or presence of cefazolin (open circle), cefoperazone (closed circle), cefotiam (open triangle) and cephalixin (closed triangle) at various concentrations for 1 hr. Uptake amounts of \(^{14}\text{C}\)PAH in each oocyte were determined. Each plot represents the mean ± S.E. of 6 to 10 oocytes.

Fig. 3. Dose-dependent uptake of \(^{14}\text{C}\)PAH by rOAT1-expressing oocytes in the presence of cefoperazone and cefotiam. rOAT1-expressing oocytes were incubated with \(^{14}\text{C}\)PAH at various concentrations in the absence (open circle) or presence of 300 \(\mu\text{M}\) cefoperazone (open triangle) or 1 mM cefotiam (closed triangle) for 1 hr. Uptake amounts of \(^{14}\text{C}\)PAH in each oocyte were determined. The figure was drawn after subtraction of the uptake amounts in water-injected oocytes from those in rOAT1-expressing oocytes. Inset shows the Eadie-Hofstee plots of the data: \(V\), initial uptake rate (pmol/oocyte/hr); \(S\), PAH concentration (\(\mu\text{M}\)). Each plot represents the mean ± S.E. of 6 to 10 oocytes.

Table 1. IC\(_{50}\) values of cefazolin, cefoperazone, cefotiam and cephalixin for rOAT1-mediated \(^{14}\text{C}\)PAH uptake. The values represent the means±S.E. of 3 experiments.

<table>
<thead>
<tr>
<th>Cephalosporin</th>
<th>IC(_{50})</th>
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<tr>
<td>Cefazolin</td>
<td>72 ± 8</td>
</tr>
<tr>
<td>Cefoperazone</td>
<td>298 ± 97</td>
</tr>
<tr>
<td>Cefotiam</td>
<td>718 ± 72</td>
</tr>
<tr>
<td>Cephalixin</td>
<td>6,010 ± 520</td>
</tr>
</tbody>
</table>

Table 1. IC\(_{50}\) values of cefazolin, cefoperazone, cefotiam and cephalixin for rOAT1-mediated \(^{14}\text{C}\)PAH uptake. The values represent the means±S.E. of 3 experiments.

Transport of cephalosporins by organic anion transporter analysis of the competition curves with one compartment, the following equation:

\[
V = \frac{100 \times IC_{50}}{IC_{50} + [I]} + A
\]

where \(V\) is uptake amount (% of control), \([I]\) is the concentration of each cephalosporin and A is non-specific PAH uptake (% of control). The findings are summarized in Table 1. The rOAT1-mediated PAH uptake was inhibited by cefazolin > cefoperazone > cefotiam > cephalixin, in the order of inhibitory potency.

Next, the inhibition manner of cefoperazone and cefotiam was investigated by Eadie-Hofstee plot analysis. Although the \(K_m\) values of rOAT1-mediated PAH uptake were increased from 12 \(\mu\text{M}\) to 34 \(\mu\text{M}\) and 31 \(\mu\text{M}\) in the presence of cefoperazone and cefotiam, respectively, the \(V_{\text{max}}\) values were not affected by both cephalosporins (Fig. 3). These findings indicate that cefoperazone as well as cefotiam inhibited PAH uptake by rOAT1 in a competitive manner.

**Discussion**

Most cephalosporin antibiotics are mainly excreted into urine, and the administration of probenecid, a representative inhibitor of the renal organic anion transport system, delayed the elimination of various cephalosporins.\(^{11}\) Therefore, it is suggested that the renal organic anion transporters mediate tubular secretion of cephalosporin antibiotics. In animal studies, it was reported that the administration of PAH to rabbits decreased the renal clearance of cephalosporins such as cefmenoxime, cefazolin and cefotiam.\(^{14}\) In addition, Takano et al.\(^{15}\) reported that various cephalosporins interact with the PAH transport system but not with the organic cation transport system in rat renal basolateral membrane vesicles. Furthermore, Nagai et al.\(^{16}\) showed that PAH and probenecid significantly inhibited the uptake of cefazolin and cefotiam via the basolateral membrane in opossum kidney cells which possess a PAH up-
take system similar to rOAT1. These findings suggest that rOAT1 should be the major transporter responsible for renal tubular uptake of cephalosporins as well as PAH from blood. The present study clearly revealed that rOAT1 mediates the transport of cefazolin, cefotiam and cephalexin (Fig. 1).

Exceptionally, cefoperazone is mainly excreted into bile.²⁰ To clarify why cefoperazone is subjected to bile excretion, it is important to clarify whether the renal organic anion transport system secretes cefoperazone like urinary-excreted cephalosporins, as well as to examine the hepatic handling of cefoperazone. To our knowledge, there have been no studies investigating the mechanisms of renal tubular secretion of cefoperazone.

Renal clearance of cefazolin and cefoperazone is reported to be 53 and 14 ml/min, respectively,²⁰ and the clearance of non-binding drugs is estimated to be 530 ml/min and 140 ml/min because the protein binding ratio of the cephalosporins is 90%. These data suggest that renal tubular secretion of cefoperazone would be negligible although urinary secretion of cefazolin plays an important role for its renal excretion. The findings of the present study showed that rOAT1 transported urinary-excreted cefazolin, cefotiam and cephalexin, but not cefoperazone (Fig. 1). Therefore, it is implied that the poor-recognition of cefoperazone by rOAT1 as a transport substrate should be responsible for the minor contribution of the kidney for cefoperazone excretion.

In conclusion, the present study showed that rOAT1 transports cefazolin, cefotiam and cephalexin, but not cefoperazone. Taken together with previous findings,¹⁴-¹⁶ it is suggested that rOAT1 plays an important role for renal tubular uptake of cephalosporin antibiotics. Furthermore, the minor contribution of the kidney for cefoperazone excretion could be explained by the finding that cefoperazone is a poor substrate of rOAT1. These findings provide useful information for optimal use of cephalosporins with regarding to disease states, and for drug delivery systems.

Acknowledgements: This work was supported by a Grant-in-Aid for Research on Human Genome, Tissue Engineering and Food Biotechnology from Ministry of Health, Labor and Welfare of Japan (H12-Genome-019) and a Grant-in-Aid for Scientific Research from Ministry of Education, Culture, Sport, Science and Technology of Japan. Y. Uwai is a research fellow supported by the Japan Society for the Promotion of Science.

References


Transport of cephalosporins by organic anion transporter


