Interactions of Human- and Rat-Organic Anion Transporters With Pravastatin and Cimetidine

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Abstract. We have elucidated the interactions of human and rat organic anion transporters (hOATs and rOATs) with pravastatin and cimetidine. Pravastatin inhibited hOAT1/rOAT1, hOAT2/rOAT2, hOAT3/rOAT3, and hOAT4. The mode of inhibition was noncompetitive for hOAT1 and hOAT2, whereas it was competitive for hOAT3 and hOAT4. Cimetidine also inhibited hOAT1/rOAT1, hOAT3/rOAT3, and hOAT4. The mode of inhibition was a combination of competitive and noncompetitive manners for hOAT1, whereas it was competitive for hOAT3. The effects of OAT inhibitors on OAT1, OAT2, and OAT3 exhibited some but not so remarkable interspecies differences between humans and rats. In conclusion, we have characterized pravastatin and cimetidine as OAT inhibitors.

Keywords: organic anion transporter, pravastatin, cimetidine

The secretion and reabsorption of numerous organic anions, including endogenous metabolites, drugs, and xenobiotics, are important physiological functions of the renal proximal tubule. The process of secreting organic anions through proximal tubule cells is achieved via unidirectional transcellular transport involving the uptake of organic anions into the cells from blood across the basolateral membrane, followed by extrusion across the brush-border membrane into the proximal tubule fluid. Various organic anion transport inhibitors are used to elucidate the mechanisms of renal organic anion transport experimentally and clinically.

We have already demonstrated that organic anion transport inhibitors including probenecid, betamipron, cilastatin, and 8-(noradamantan-3-yl)-1,3-dipropylxanthine, inhibit human organic anion transporter (hOAT1) 1, hOAT2, hOAT3, and hOAT4 (1, 2). Probenecid is the conventional and standard organic anion transport inhibitor used in experiments, and it is used as a uricosuric drug clinically. On the other hand, betamipron and cilastatin are administered in combination with carbapenem antibiotics, panipenem and imipenem, respectively (3, 4), in order to prevent the nephrotoxicities of carbapenem antibiotics.

It was recently demonstrated that pravastatin is a relatively specific inhibitor of rat OAT (rOAT) 3 rather than of rOAT1 (5). Pravastatin, a structural analog of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA), is a competitive inhibitor of HMG-CoA reductase and therefore blocks the synthesis of cholesterol in the liver (6). Cimetidine, a histamine H₂-receptor antagonist, is thought to be an inhibitor for organic anion/cation transport (7, 8).

The purpose of this study was firstly to characterize pravastatin and cimetidine in terms of the interactions with OATs and secondarily to elucidate the interspecies difference for the interactions of OATs with various OAT inhibitors between humans and rats, using the cell lines stably expressing OATs.

[¹⁴C]para-Aminohippuric acid (PAH) (1.86 GBq/mmol), [³H]prostaglandin F₂α (PGF₂α) (6808 GBq/mmol), and [³H]estrone sulfate (ES) (1961 GBq/mmol)
were purchased from Perkin Elmer Life Sciences (Boston, MA, USA). Probenecid and cimetidine were from Sigma Chemicals (St. Louis, MO, USA). Betamipron and pravastatin were kind gifts from Sankyo Pharmaceutical Co. (Tokyo). Cilastatin was from Banyu Pharmaceutical Co. (Tokyo).

We have already established and characterized the mouse second portion of the proximal tubule (S2) cells stably expressing hOAT1, hOAT2, hOAT3, hOAT4, rOAT1, rOAT2, and rOAT3 (S2; hOAT1, S2; hOAT2, S2; hOAT3, S2; hOAT4, S2; rOAT1, S2; rOAT2, and S2; rOAT3) (9 – 11). These cells were grown in a humidified culture plate at a cell density of 1 × 10^6 cells/well. After culture for two days, they were preincubated in a solution containing either 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. Uptake experiments were performed as described previously (1). S2 cells were seeded in 24-well tissue culture plates at a cell density of 1 × 10^6 cells/well. After culture for two days, they were preincubated in a Dulbecco’s modified phosphate-buffered saline (D-PBS) solution (137 mM NaCl, 3 mM KCl, 8 mM Na₂HPO₄, 1 mM KH₂PO₄, 1 mM CaCl₂, and 0.5 mM MgCl₂, pH 7.4) at 37°C for 10 min. After that, S2 hOATs/rOATs cells were incubated at 37°C in a solution containing either 5 μM [³¹C]PAH for 2 min (hOAT1/rOAT1), 50 nM [³¹H]PGF₂α for 20 s (hOAT2) and for 2 min (rOAT2), or 50 nM [³¹H]ES for 2 min (hOAT3/rOAT3 and hOAT4) in the absence or presence of various inhibitors. Probenecid, cilastatin, and pravastatin were dissolved in dimethylsulfoxide. The final concentration of dimethylsulfoxide in the medium was adjusted to be less than 0.2%. After incubation, 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). For the kinetic analysis, S2 hOATs cells were incubated in a solution containing various concentrations of either [³¹C]PAH (hOAT1), [³¹H]PGF₂α (hOAT2), or [³¹H]ES (hOAT3 and hOAT4) at 37°C in the absence or presence of pravastatin or cimetidine. Based on the organic anion uptake under each condition, double reciprocal plot analyses were performed as described previously (1). Statistical differences were determined using Student’s t-test.

Pravastatin dose-dependently inhibited organic anion uptake by hOAT1/rOAT1, hOAT2/rOAT2, hOAT3/rOAT3, and hOAT4, with the IC₅₀ values shown in Table 1. As shown in Fig. 1, analysis of the Lineweaver-Burk plot revealed that the inhibitory mode of pravastatin for hOAT1 (A) and hOAT2 (B) was noncompetitive, whereas that for hOAT3 (C) and hOAT4 (D) was competitive. On the other hand, cimetidine dose-dependently inhibited organic anion uptake mediated by hOAT1 and hOAT3/rOAT3, whereas it weakly inhibited that by rOAT1 and hOAT4, with the IC₅₀ values shown in Table 1. In contrast, cilastatin did not inhibit organic anion uptake by hOAT2/rOAT2. As shown in Fig. 1, the inhibitory mode of cilastatin for hOAT1 was a combination of competitive and noncompetitive manners (E), whereas that for hOAT3 (F) was competitive. In addition, probenecid, betamipron, and cilastatin dose-dependently inhibited organic anion uptake mediated by rOAT1 and rOAT3, whereas probenecid but not betamipron and cilastatin dose-dependently inhibited organic anion uptake mediated by rOAT2. The IC₅₀ values for these inhibitory effects are presented in Table 1.

### Table 1. IC₅₀ values (μM) of various organic anion transport inhibitors for hOATs and rOATs

<table>
<thead>
<tr>
<th></th>
<th>Pravastatin</th>
<th>Cimetidine</th>
<th>Probenecid</th>
<th>Betamipron</th>
<th>Cilastatin</th>
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<tbody>
<tr>
<td>hOAT1</td>
<td>408 ± 144</td>
<td>492 ± 152</td>
<td>12.3 ± 5.17</td>
<td>39.0 ± 16.0</td>
<td>2550 ± 622</td>
</tr>
<tr>
<td>rOAT1</td>
<td>1620 ± 287***</td>
<td>2000***</td>
<td>15.3 ± 7.08</td>
<td>12.5 ± 2.13*</td>
<td>2387 ± 1021</td>
</tr>
<tr>
<td>hOAT2</td>
<td>352 ± 153</td>
<td>N.I.</td>
<td>668 ± 206a</td>
<td>N.I.</td>
<td>N.I.</td>
</tr>
<tr>
<td>rOAT2</td>
<td>449 ± 105</td>
<td>N.I.</td>
<td>977 ± 265</td>
<td>N.I.</td>
<td>N.I.</td>
</tr>
<tr>
<td>hOAT3</td>
<td>13.7 ± 1.49</td>
<td>92.4 ± 10.9</td>
<td>4.93 ± 1.37</td>
<td>9.92 ± 3.55</td>
<td>249 ± 98.9</td>
</tr>
<tr>
<td>rOAT3</td>
<td>15.6 ± 7.50</td>
<td>166 ± 58.3</td>
<td>6.03 ± 0.27</td>
<td>47.5 ± 9.02**</td>
<td>742 ± 100*</td>
</tr>
<tr>
<td>hOAT4</td>
<td>591 ± 177</td>
<td>2000-</td>
<td>25.4 ± 7.86</td>
<td>483 ± 83.5</td>
<td>N.I.</td>
</tr>
</tbody>
</table>

S2; hOAT1, S2; hOAT2, S2; hOAT3, S2; hOAT4, S2; rOAT1, S2; rOAT2, and S2; rOAT3 cells were incubated in a solution containing 5 μM [³¹C]PAH for 2 min (OAT1), 50 nM [³¹H]PGF₂α for 20 s (hOAT2) and for 2 min (rOAT2), or 50 nM [³¹H]ES for 2 min (OAT3 and hOAT4) in the absence or presence of various OAT inhibitors at 37°C. Each value represents the mean ± S.D. from six monolayers of two separate experiments. N.I. indicates not inhibitory. *from Ref. 1 and †from Ref. 2. A significant interaspecies difference was observed between humans and rats concerning OAT1 interactions with pravastatin (***P<0.001), cimetidine (**P<0.001), and betamipron (*P<0.05) as well as OAT3 interactions with betamipron (**P<0.01) and cilastatin (*P<0.05).
Fig. 1. Kinetic analysis of the inhibitory effects of pravastatin and cimetidine on organic anion uptake mediated by hOATs. The cells were incubated in a solution containing various concentrations of $^{[14]}\text{C}PAH$ for 2 min (hOAT1), $^{[3]}\text{H}PGE_{2\alpha}$ for 20 s (hOAT2), or $^{[3]}\text{H}ES$ for 2 min (hOAT3 and hOAT4) at 37°C in the presence or absence of pravastatin at 1000 μM (hOAT1), 1000 μM (hOAT2), 50 μM (hOAT3), and 1000 μM (hOAT4) or cimetidine at 1000 μM (hOAT1) and 200 μM (hOAT3) at 37°C. Analyses of Lineweaver-Burke plots were performed. A: hOAT1-pravastatin, B: hOAT2-pravastatin, C: hOAT3-pravastatin, D: hOAT4-pravastatin, E: hOAT1-cimetidine, and F: hOAT3-cimetidine. Each value represents the mean ± S.D. from six monolayers from two separate experiments.
Fig. 2. Chemical structures of PGF$_{2\alpha}$, paravastatin, cilastatin, and betamipron (A) and their overlapping (B).
Recently, cDNAs encoding proteins of the OAT family have been successively cloned, including hOAT1/rOAT1, hOAT2/rOAT2, hOAT3/rOAT3, and OAT4 (12). In the kidney, hOAT1/rOAT1, hOAT2, and hOAT3/rOAT3 are localized to the basolateral side of the proximal tubule. In contrast to hOAT2, rOAT2 is localized on the apical side of the medullary thick ascending limb of Henle’s loop (13). On the other hand, hOAT4 is located on the apical side of the proximal tubule (14). Using cell lines stably expressing OATs, we have elucidated the interactions of OATs with inhibitors.

Pravastatin inhibited the organic anion uptake mediated by hOAT1/rOAT1, hOAT2/rOAT2, hOAT3/rOAT3, and hOAT4. The IC_{50} values of pravastatin for rOAT1 and rOAT3 obtained in the current study are comparable to the K_i values of pravastatin for rOAT1 and rOAT3 reported by Hasegawa et al. (5), that is, 1150 μM and 13.4 μM, respectively, whereas the substrate for rOAT3 was pravastatin in the previous study. In addition, the IC_{50} value of pravastatin for hOAT3 was approximately 30-, 25-, and 40-fold lower than those for hOAT1, hOAT2, and hOAT4, respectively. Similarly, the IC_{50} value of pravastatin for rOAT3 was approximately 100- and 30-fold lower than those for rOAT1 and rOAT2, respectively. Thus, these results suggest that pravastatin is a relatively specific inhibitor of OAT3 not only in rats but also in humans. In addition, the results would provide useful information regarding the safer use of pravastatin by avoiding the drug interactions between pravastatin and hOAT3-transporting drugs.

Pravastatin, but not betamipron and cilastatin, inhibited the transport of PGF_{2α} mediated by rOAT2 as well as hOAT2. We speculated on the reason behind such a differential inhibitory effect on the structural basis as follows: as shown in Fig. 2A, pravastatin has a bulky cyclic moiety, carbon chain (C_{6}), and anionic carboxyl group. When the chemical structure of pravastatin is overlaid with that of PGF_{2α} (Fig. 2B), there is a better overlapping of their chemical structures than that of betamipron and cilastatin structures. This may be the reason behind such a differential inhibitory effect.

Although cimetidine has been utilized as an organic cation transport inhibitor (7), we have recently demonstrated that cimetidine also inhibits hOAT3-mediated organic anion transport (8). In the current study, in addition to hOAT3, we have also observed that cimetidine, an organic cation, inhibits organic anion uptake mediated by hOAT1 and hOAT4 but not by hOAT2. This could occur based on the assumption that the structures of the binding sites of OATs and organic cation transporters (OCTs) are quite similar, except for the charge recognition sites (15). Consistent with this, we already demonstrated that hOATs and hOCTs interact with PGE_2 and PGF_{2α}, which possess anionic moieties (10). The K_i value of cimetidine for organic cation uptake in isolated human proximal tubules was reported to be 11 μM (7), where hOAT1, hOAT3, and hOCT2 are colocalized (16). Thus, the results of studies using cimetidine as an OCT inhibitor have to be carefully interpreted considering the effect of this substrate on the OAT system.

Burkhardt et al. (2003) have already demonstrated that hOAT1 mediated the uptake of cimetidine (17). However, we noted that cimetidine inhibited the PAH uptake mediated by hOAT1 in a combination of competitive and noncompetitive manners. The possible reason for this discrepancy is that there may be several substrate recognition sites in the hOAT1 molecule.

Various OAT inhibitors inhibited organic anion uptake by rOATs. Comparing the IC_{50} values of OAT inhibitors for organic anion uptake mediated by rOAT1, rOAT2, and rOAT3 between humans and rats in the current study and in the literature (1, 2), there exist some interspecies difference between humans and rats, that is, OAT1 interactions with pravastatin, cimetidine, and betamipron and OAT3 interactions with betamipron and cilastatin. The significant difference in K_i value or IC_{50} value was thought to be more than threefold (15). These results would be useful for the extrapolation of results of the functional analysis of rat organic anion transport using OAT inhibitors in humans.

In conclusion, we have characterized pravastatin and cimetidine as OAT inhibitors. In addition, there was some but not so remarkable interspecies difference for the interactions of OATs with inhibitors.

Acknowledgments

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References