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Interactions of human organic anion transporter 1 (hOAT1) with substances associated with forensic toxicology

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ABSTRACT

Renal excretion is an important elimination pathway for substances associated with forensic toxicology, such as medicines, agricultural chemicals, and industrial chemicals. This study aimed to elucidate the renal elimination pathway of substances using culture cells stably expressing the human organic anion transporter 1 (hOAT1) gene. Substances tested were diazepam, triazolam, haloperidol, amitriptyline, mianserin, bromovalerylurea, phenobarbital, acetaminophen, acetylsalicylic acid, lidocaine, aconitine, atropine, caffeine, nicotine, malathion, dichlorvos, fenitrothion, chlorpyrifosmethyl, paraquat, diquat, potassium cyanide, sodium arsenite, sodium azide, *o*-cresol, and probenecid (control, a representative inhibitor of hOAT1). Results demonstrated that diazepam, triazolam, amitriptyline, mianserin, malathion, fenitrothion, chlorpyrifosmethyl, and probenecid significantly inhibited representative substrates of hOAT1 and para-aminohippuric acid uptake by hOAT1. IC_{50} values of the aforementioned substances were 133.3, 185.2, 354.1, 312.6, 114.2, 26.6, 191.5, and 7.9 μM, respectively. Ki values were 83.5, 86.0, 573.9, 99.0, 134.0, 51.2, 324.6, and 9.1 μM, respectively. In conclusion, the current results suggest that fenitrothion and chlorpyrifosmethyl are transported with pharmacokinetics indicative of hOAT1 involvement in the human kidney.

1. Introduction

The kidneys and liver play a crucial role in excretion of numerous organic anions such as endogenous compounds, xenobiotics, and their metabolites [1–5]. In addition to glomerular filtration, renal excretion of these compounds greatly impacts physiological functioning of proximal tubule cells [2,6]. The process of excreting organic anions through proximal tubule cells is achieved via unidirectional transcellular transporters into the basolateral membrane [2]. Finally, proximal tubule cells actively excrete these compounds into urine. In particular, transporters are required to ensure the entry of hydrophilic compounds because hydrophilic compounds cannot penetrate the lipid bilayer of cell membranes. In a recent study, cDNAs encoding human organic anion transporter (hOAT) family members have been successively cloned, including hOAT1 [7–12], hOAT2 [13–15], hOAT3 [16–18], hOAT4 [19–21], hOAT5 [22], and hOAT6–hOAT9 [23]. The roles of hOATs in the pharmacokinetics of medicines have been gradually clarified in the kidneys. hOAT1 was shown to be localized on the basolateral side of proximal tubules [8]. hOAT1 is a para-aminohippuric acid (PAH)/dicarboxylate exchanger and mediates high-affinity transport of PAH in a sodium-independent manner [8]. hOAT1 can transport anionic medicines, such as β-lactam antibiotics, nonsteroidal anti-inflammatory drugs (NSAIDs), tetracycline, methotrexate, and antiviral drugs, as well as endogenous compounds. This multispecificity could cause drug–drug interactions when several medicines are taken concomitantly, and the risk of drug intoxication death may increase *in vivo*.

Our ultimate goal is to elucidate the interaction between illicit drugs, which is currently the most notable subject in forensic medicine, and generic drugs. To be more specific, the interaction between illicit drugs and generic drugs by concomitantly ingestion of them may lead to interrupt the excretion of illicit drugs into the urine. We suspect that ingested illicit drugs may not be detected by a screening test kits for drug of abuse such as Triage®DOA in forensic practice.

In this study, we conduct a screening test of hOAT1-mediated cellular uptake as the first step to clarify the molecular biological excretion mechanism of substances via transporters in the kidney.

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2. Materials and methods

2.1. Materials

[^{14}C]PAH (1.998 GBq/mmol) was purchased from Perkin Elmer Life Science (USA). The culture medium consisted of fetal bovine serum (FBS), *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), and penicillin/streptomycin (Invitrogen, USA); Dulbecco's modified Eagle's medium (DMEM), L-glutamine, and geneticin (Sigma, USA); and $NaHCO_3$ (Wako, Japan). Diazepam, triazolam, haloperidol, amitriptyline, mianserin, bromovalerylurea, phenobarbital, acetaminophen, lidocaine, atropine, caffeine, malathion, dichlorvos, fenitrothion, chlorpyrifosmethyl, diquat, *o*-cresol, potassium cyanide, sodium arsenite, sodium azide, and probenecid were purchased from Wako. Nicotine and aconitine were purchased from Sigma. Paraquat and acetylsalicylic acid were purchased from ICI (Japan) and Tsukishima (Japan), respectively.

2.2. Cell culture

S_2 cells were established by culturing microdissected S_2, the second segment of renal proximal tubules, derived from transgenic mice harboring temperature-sensitive simian virus 40 (SV40) large T-antigen [24]. S_2 cells transfected with pcDNA3.1 vectors containing an insert cDNA sequence of hOAT1 and pSV2neo, a neomycin resistance gene, were designated as S_2 hOAT1. Empty vector lacking the insert cDNA of hOAT1 were transfected into mock cells that were used as controls [8]. All cells were grown in a humidified incubator at 33 °C and under 10% CO_2 using DMEM containing 10% FBS, 100 U/mL penicillin/100 μg/mL streptomycin, 10 mM HEPES, 540 μg/mL geneticin, 2 mM L-glutamine, and 3.7 mg/mL $NaHCO_3$, pH adjust to 7.2 using 1 M HCl. The cells were subcultured in medium containing 0.05% trypsin–ethylenediaminetetraacetic acid (EDTA) solution (137 mM NaCl, 5.4 mM KCl, 5.5 mM glucose, 4 mM NaHCO3, 0.5 mM EDTA, and 5 mM HEPES; pH 7.2).

2.3. Uptake experiments

Cells were seeded in 24-well tissue plates at cell density of 1×10^5 cells/well. After the cells were cultured for two days, they were washed three times with Dulbecco's modified phosphate buffered saline (D-PBS: 137 mM NaCl, 3 mM KCl, 8 mM $NaHPO_4$, 1 mM KH_2PO_4, 1 mM $CaCl_2$, and 0.5 mM $MgCl_2$, pH 7.4) supplemented with 5.5 mM glucose. The cells were then preincubated in the same solution in a water bath at 37 °C for 10 min. The cells were then incubated in 0.25 mL D-PBS with 5 μM [^{14}C]PAH at 37 °C for 45 min. The uptake was stopped by the addition of ice-cold D-PBS, and the cells were washed three times with the same solution. The cells in each well were lysed with 0.5 mL 0.1 N NaOH, and 2.5 mL aquasol-2 (Perkin Elmer) was added. Radioactivity was determined using a liquid scintillation counter (LSC-5100; Aloka, Japan).

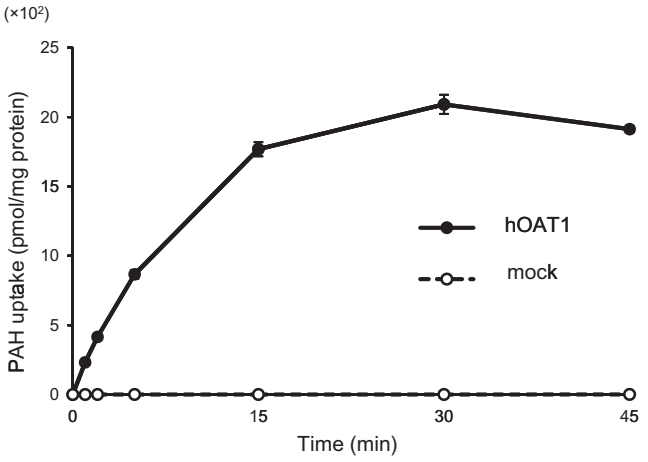

Fig. 1. Time course of PAH uptake by hOAT1. S_2 hOAT1 cells and S_2 mock cells are incubated in D-PBS containing 5 μM [^{14}C]PAH at 37 °C for 45 min. Each value represents the mean ± SEM of three determinations from one typical experiment.

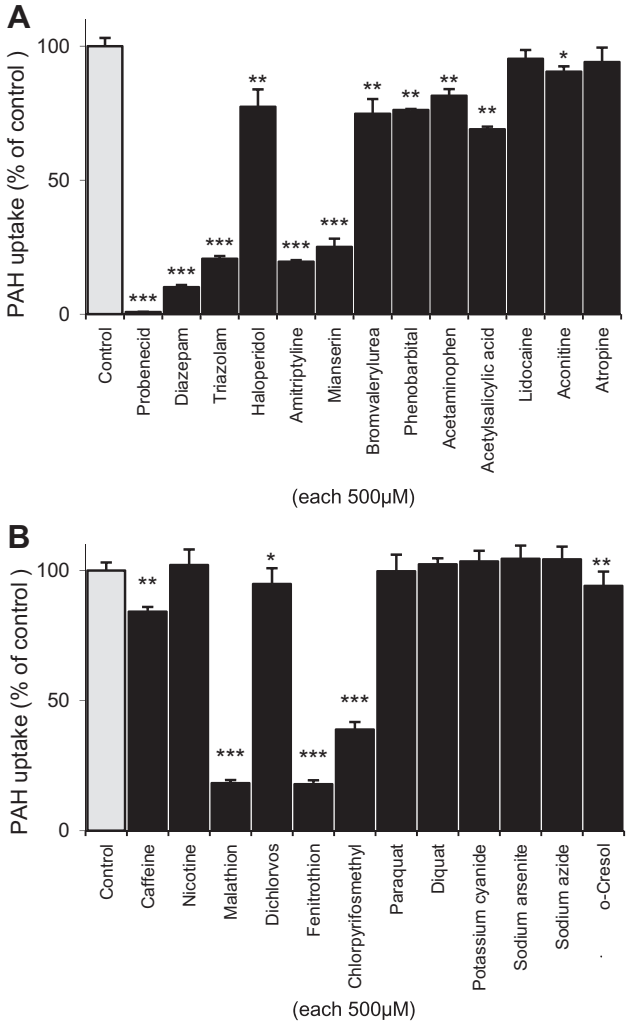

Fig. 2. Effects of various substances on PAH uptake mediated by hOAT1. (A and B) S_2 hOAT1 cells are incubated in D-PBS containing 5 μM [^{14}C]PAH in the presence or absence of various substances (500 μM) at 37 °C for 2 min. Each value represents the mean ± SEM of three determinations. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ vs. control.

Table 1
IC_{50} and Ki values of various substances on PAH uptake mediated by hOAT1.

Substances	IC_{50} (μM)	Ki (μM)
Diazepam	133.3	83.5[b]
Triazolam	185.2	86.0[b]
Amitriptyline	354.0	573.9[b]
Mianserin	312.6	99.0[b]
Malathion	114.2	134.0[b]
Fenitrothion	26.6	51.2[a]
Chlorpyrifosmethyl	191.5	324.6[a]

[a] Competitive inhibition,
[b] Noncompetitive inhibition.