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Interaction Characteristics of Nucleoside Analogues with Human Organic Anion Transporter 1 and 3

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ABSTRACT– The present study aimed to investigate the interaction of nucleoside analogs with human organic anion transporter 1 and 3 (hOAT1 and hOAT3) that play a primary role in the tubular uptake of endogenous and exogenous organic anions in the kidney. The interactions of ddC, ara-C, ara-A and ara-U with hOAT1 and hOAT3 were examined using MDCK cells stably overexpressing hOAT1 or hOAT3. Among the tested drugs, ddC showed the highest affinity to hOAT1 with IC_{50} values of 5.2 mM, while ara-A, ara-C and ara-U weakly inhibited the cellular uptake of [^{3}H]-PAH in MDCK-hOAT1 cells at 1 mM. In contrast, all the tested drugs did not have any inhibition effect on the cellular uptake of [^{3}H]-estrone sulfate in MDCK-hOAT3 cells over the drug concentration of 0.01-2 mM, implying that they might not interact with hOAT3. Taken all together, the present study suggests that hOAT1 could weakly interact with nucleoside analogues such as ddC, ara-C, ara-A and ara-U but the interaction with hOAT3 during the urinary excretion of these nucleoside analogues may be negligible in the kidney.

Key words– Human organic anion transporter 1 (hOAT1), Human organic anion transporter 3 (hOAT3), Inhibition, Nucleosides, Cellular uptake

Clinically important drug interactions can be caused not only by a change in drug metabolism but also by the modulation of drug transporters. For example, several clinically significant drug interactions have been reported with digoxin after concomitant administration with P-gp inhibitors such as quinidine, verapamil, and talinolol.[1-3] In addition, cidofovir is used in conjunction with probenecid, a competitive inhibitor of the organic anion transporters, to reduce the dose-limiting nephrotoxicity in the treatment of cytomegalovirus retinitis in AIDS patients.[4] Therefore, it is important to evaluate the contribution of a carrier-mediated mechanism to the membrane transport of drugs and subsequently the potential drug interactions mediated by drug transporters.

In the kidney, organic anion transporters (OATs) are expressed in the apical and basolateral membranes of tubular epithelial cells and actively involved in the tubular secretion of organic anions including drugs, toxins and endogenous compounds.[5] Particularly, human organic anion transporter 1 (hOAT1) and 3 (hOAT3) are located in the basolateral side of renal tubular cells and have been shown to transport important therapeutics including β-lactam antibiotics, diuretics, nonsteroidal anti-inflammatory drugs (NSAIDs) and antiviral drugs.[6-9] Furthermore, Motohashi *et al.*[10] have reported that mRNA levels of hOAT1 and hOAT3 were much higher than those of other organic anion transporters in the human kidney cortex, suggesting that hOAT1 and hOAT3 play important roles in the tubular uptake of various drugs from the circulation. Therefore, it is important to characterize the substrate specificities of these transporters. In the present study, the interaction characteristics of hOAT1 and hOAT3 with nucleoside analogues were examined by using MDCK cells stably transfected with hOAT1 or hOAT3.

Materials and Methods

Materials

Adenine 9-β-D-arabinofuranoside (ara-A), cytosine-β-D-arabinofuranoside (ara-C), 1-β-D-arabinofuranosyluracil (ara-U), 2',3'-dideoxycytidine (ddC), [^{3}H]-para-aminohippurate (PAH), and [^{3}H]-estrone sulfate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Fetal Bovine Serum (FBS), cell culture media, antibiotics and all other reagents used in cell culture studies were purchased from Seolin Science Co. (Seoul, Korea). Madin-Darby canine kidney cells stably transfected with the human organic anion transporter 1 (MDCK-hOAT1 cells) were kindly provided by Dr. John B. Pritchard (NIEHS, North Carolina, USA). MDCK cells overexpressing hOAT3 (MDCK-hOAT3 cells) have been generated as described in the previous report[11] and the functional expression of hOAT3 was confirmed by the uptake of [H^3]-estrone

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sulfate. All other chemicals were reagent grade and all solvents were HPLC grade.

Cell cultures

Cells were routinely maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, 1% nonessential amino acids, penicillin (100 U/mL)/streptomycin (100 μg/mL) and 200 μg/ml G-418. All cells were maintained in an atmosphere of 5% CO_2 and 90% relative humidity at 37°C.

Inhibition studies in MDCK-hOAT1 and MDCK-hOAT3 cells

Cells were seeded in 12-well culture plates at a density of 10^5 cells/cm^2. At 4-5 days post-seeding, the cells were washed twice with pH 7.4 uptake buffer containing 1 mM $CaCl_2$, 1 mM $MgCl_2$, 150 mM NaCl, 3 mM KCl, 1 mM NaH_2PO_4, 5 mM D-glucose, and 5 mM HEPES. Each test solution (0.01-2 mM) containing [^{3}H]-PAH (0.1 μCi/mL) or [^{3}H]-estrone sulfate (0.1 μCi/mL) was added to each well and incubated for 5-10 min. At the end of incubation, drug solution was removed and the cells were washed three times with ice-cold phosphate buffer solution (pH 7.0). One milliliter of 1.5% ice-cold Triton X solution was added to each well. After 30 min incubation, cells were harvested and the radioactivity in each sample was determined by a scintillation counter. IC_{50} was determined from the nonlinear regression of a dose-response curve by using the SigmaPlot® 9.0 (Systat Software Inc., Point Richmond, CA, USA).

Statistical analysis

All the means are presented with their standard deviation. Student's t-test was used to determine the statistical significance of the difference in the parameters. A P value < 0.05 was considered statistically significant.

Results and Discussion

The multiple prescriptions of drugs are increasingly common in medical practice. It has been variously estimated that patients receive on average between 5 and 14 drugs during their stay in hospital.[12] Also, patients treated by general practitioners, at the time they are referred to hospital, are receiving, on average, 2 or 3 drugs.[13] Therefore, without doubt, there is enormous potential for drug interactions in current medical practice. Since the modulation of drug transporters has been considered as the cause of many clinically important drug interactions, it is important to evaluate the interaction characteristics of drugs with certain drug transporters.

To determine the affinity of nucleoside drugs for hOAT1 and hOAT3, the present study evaluated the inhibitory effects of ddC, ara-A, ara-C and ara-U on the uptake of [^{3}H]-PAH and [^{3}H]-estrone sulfate by MDCK-hOAT1 and MDCK-hOAT3, respectively. As illustrated in Figure 1 and 2, among the tested drugs, ddC showed the highest affinity to hOAT1 with IC_{50} values of 5.2 mM in MDCK-hOAT1 cells, while ara-A, ara-C and ara-U weakly inhibited the cellular uptake of [^{3}H]-PAH at 1 mM. In the comparison of previous inhibition studies against hOAT1, the affinity of these nucleoside drugs to hOAT1 appeared to be much less than other anions such as non-steroidal anti-inflammatory drugs, diuretics or cephalosporin

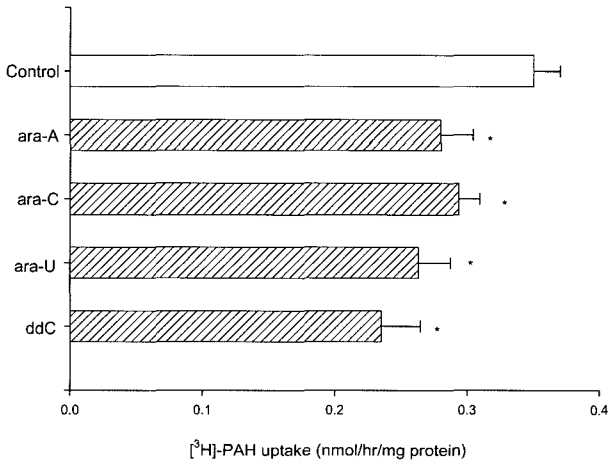

Figure 1–Inhibition effect of nucleoside analogues (1 mM) on the cellular uptake of [^{3}H]-PAH in MDCK-hOAT1 cells (Mean±S.D., n=6). *p<0.05.

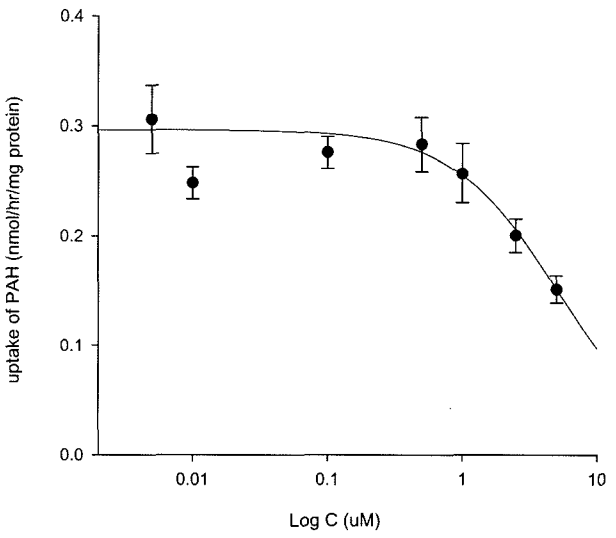

Figure 2–Inhibition effect of ddC on the cellular uptake of [^{3}H]-PAH in MDCK-hOAT1 cells (Mean±S.D., n=6).

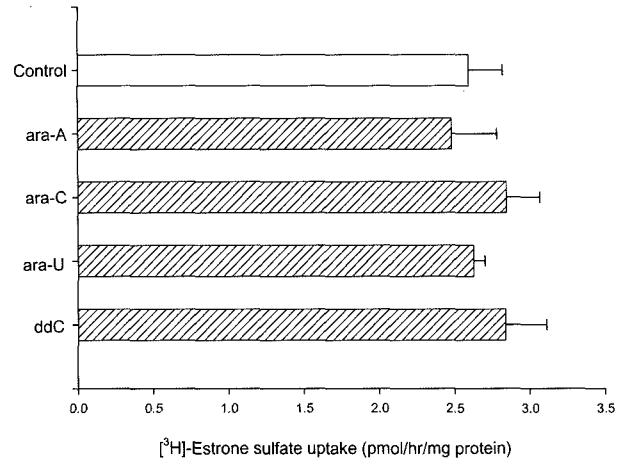

Figure 3–Inhibition effect of nucleoside analogues (2 mM) on the cellular uptake of [^{3}H]-estrone sulfate in MDCK-hOAT3 cells (Mean±S.D., n=6).

antibiotics.[7,8,14] The interaction of nucleoside analogues with hOAT3 was also evaluated in MDCK-hOAT3 cells. As shown in Figure 3, all the tested drugs did not have any inhibition effect on the cellular uptake of [^{3}H]-estrone sulfate in MDCK-hOAT3 cells at the drug concentration up to 2 mM, implying that they might not interact with hOAT3.

Ueo *et al.*[14] have reported that the IC_{50} values of cephalosporin antibiotics for the hOAT1-mediated uptake of [^{14}C]-PAH were within four-fold of those for the hOAT3-mediated uptake of [^{3}H]-estrone sulfate and suggested that hOAT3 plays a more important role than hOAT1 in the renal excretion of cephalosporin antibiotics. In contrast, the present study indicated that some of nucleoside drugs did not interact with hOAT3 while they exhibited weak inhibition against hOAT1. Therefore, there seems to be some differences in substrate recognition between hOAT1 and hOAT3. It has been also reported that hOAT1 and hOAT3 showed different Km values for the same substrates, suggesting that these transporters would have different contributions in tubular secretion of organic anions.[9,15] The Km values of AZT uptake by hOAT1 and hOAT3 were 45.9 μM and 145.1 μM, respectively.[9] The Km values of PAH uptake were 9 μM for hOAT1 and 87 μM for hOAT3.[15] Furthermore, time and concentration-dependent uptake of acyclovir was observed in hOAT1-expressing cells, while the uptake of acyclovir was not significant in hOAT3-expressing cells.[9,15] However, the uptake of valacyclovir, L-valyl ester of acyclovir, was observed only in hOAT3-expressing cells.[9] Therefore, there seems to be distinct differences in substrate recognition between hOAT1 and hOAT3. For more clarification on the structure-transporter affinity relationship, further studies should be performed with more structurally diverse drugs.

Taken all together, the present study suggests that hOAT1 could interact with nucleoside analogues such as ddC, ara-C, ara-A and ara-U but the interaction with hOAT3 during the urinary excretion of these nucleoside analogues may be negligible in the kidney.

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