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Interaction of zalcitabine with human organic anion transporter 1

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The present study aimed to investigate the interaction of zalcitabine with human organic anion transporter 1 (hOATI) during renal excretion. Contribution of OAT1 to the renal transport of zalcitabine was examined using the transfected cell lines overexpressing the human organic anion transporter1 (CHO/hOAT1 cells). Zalcitabine exhibited the inhibition effect on the cellular uptake of [^{3}H]-PAH in CHO/hOAT1 cells with an IC_{50} value of 1.23 mM. Furthermore, the cellular uptake of zalcitabine increased threefold with the enhancement of hOATI activity in CHO/hOAT1 cells, while it was significantly reduced in the presence of OAT1 inhibitors such as ketoprofen, naproxen, PAH and 6-carboxyfluorescein. Those results suggest that hOATI contributes at least in part to the cellular uptake of zalcitabine across the basolateral membrane of proximal tubular cells.

Over the past decade, a tremendous amount of work has been done for the molecular characterization of transport proteins in animals and humans (Kim 2000; Ayrton and Morgan 2001). Subsequently, a number of studies have suggested that the degree of expression and functionality of transport proteins may directly affect the therapeutic effectiveness, safety and target specificity of substrate (Ayrton and Morgan 2001; Yamazaki et al. 1996). Furthermore, there is increasing evidence that clinically important drug interactions can be caused by the modulation of drug transporters in addition to a change in drug metabolism. For example, unexpected side-effects in the combination therapy of dofetilide and cimetidine resulted from the inhibition effect of cimetidine on the organic cation transporter-mediated renal excretion of dofetilide at the basolateral membrane of renal proximal tubules (Abel et al. 2000). Therefore, considering the clinical significance of drug-drug interactions mediated by drug transporters, it is important to evaluate the contribution of a carrier-mediated mechanism to the membrane transport of drugs.

Zalcitabine (2′,3′-Dideoxycytidine or ddC), a pyrimidine nucleoside, is highly active against human immunodeficiency virus (HIV) and also hepatitis B virus (HBV) (Dahlberg et al. 1987). Zalcitabine is rapidly and extensively absorbed after oral administration, and the primary route of elimination is renal excretion of unchanged drug with 60–70% of an oral dose recovered in urine within 24 h (Gustavson et al. 1990; Klecker et al. 1988). Renal clearance of zalcitabine exceeds glomerular filtration rate, implying that the drug undergoes the active tubular secretion in the kidney (Klecker et al. 1988). Massarella et al. (1996) has reported that tubular secretion of zalcitabine was inhibited by co-administration of probenecid in humans at least in part. Furthermore, brain perfusion experiments suggested that the removal of zalcitabine from the CNS by both the blood-brain barrier and choroid plexus was sensitive to probenecid (Gibbs and Thomas 2002). Therefore, the organic anion transporter family has been implicated in the distribution of zalcitabine to the CNS and the proximal tubular cells in the kidney. However, since previous studies mainly relied on the inhibition studies to demonstrate the interaction of zalcitabine with organic anion transporters, the cellular uptake characteristics of zalcitabine needs to be further clarified for more detail. Among organic anion transporters, organic anion transporter 1 (OAT1) localized in the basolateral membrane of renal proximal tubular cells plays a pivotal role in the renal excretion of a wide variety of chemically unrelated organic anions such as endogenous metabolites, hormones, second messengers, toxins, xenobiotics and fluorescent dyes (Burckhardt et al. 2003; Sekine et al. 1997). Therefore, the present study examined the contribution of OAT1 to the renal transport of zalcitabine using transfected cell lines overexpressing human organic anion transporter 1 (CHO/hOAT1). As illustrated in Fig. 1, zalcitabine exhibited the strong inhibition effect on the uptake of [^{3}H]-para-aminohippurate (PAH), a representative substrate of organic anion transporters, with an IC_{50} value of 1.23 ± 0.13 mM. In the comparison of other inhibition studies against hOAT1 (Mulato et al. 2000; Khamdang et al. 2002), zalcitabine appeared to be a less potent inhibitor of hOAT1 than nonsteroidal anti-inflammatory drugs or probenecid. To evaluate the potential contribution of hOATI to the translocation of zalcitabine across the cellular membrane, the cellular accumulation of zalcitabine was examined using CHO/hOAT1 cells as well as untransfected CHO cells (wild type). As shown in Fig. 2, the cellular accumulation of zalcitabine was threefold higher in CHO/hOAT1 cells than the uptake in the untransfected CHO cells, implying that the cellular uptake of zalcitabine was related to the enhancement of the hOATI activity in CHO/hOAT1 cells. Furthermore, the cellular uptake of zalcitabine decreased by approximately 35–50% in the presence of OAT1 inhibitors such as ketoprofen (0.5 mM), naproxen (0.5 mM), PAH (0.5 mM) and 6-carboxyfluorescein (0.1 mM). These results indicated that zalcitabine should be translocated by

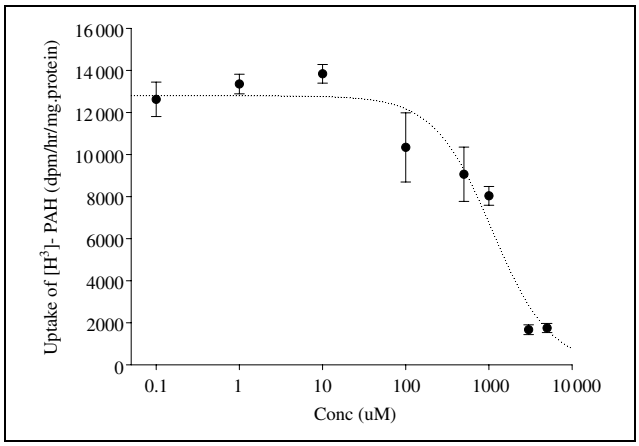

Fig. 1: Inhibition effect of zalcitabine on the uptake of [^{3}H]-PAH in CHO/hOAT1 cells (Mean ± SD, n = 6)

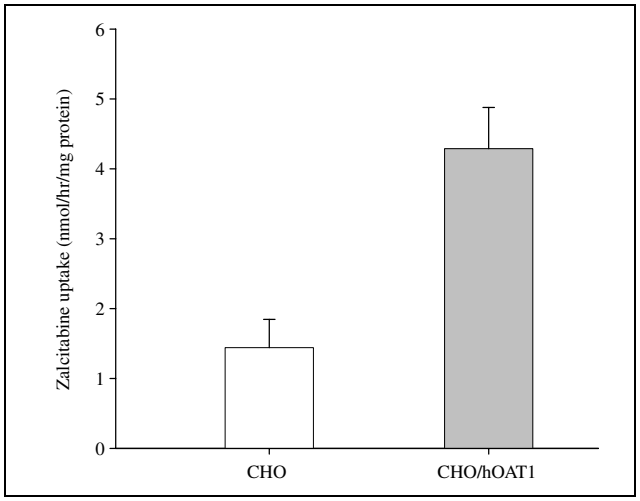

Fig. 2: Cellular accumulation of zalcitabine (0.25 mM) in CHO/hOAT1 cells as well as in the untransfected CHO cells (Mean ± SD, n = 6)

hOATI. Considering the short plasma half-life of zalcitabine (1–3 h), the inhibition of renal excretion of zalcitabine by the concomitant use of OAT1 inhibitors may provide a therapeutic benefit whereby it prolongs the duration of action of zalcitabine with longer plasma half-life, resulting in less frequent dosing of zalcitabine. Therefore, the clinical significance of a potential drug interaction of zalcitabine via hOATI transporters needs to be further evaluated at therapeutic dose levels.

In conclusion, the present study has demonstrated that zalcitabine could be translocated by hOATI across the cellular membrane.

Experimental

1. Inhibition studies in CHO/hOAT1 cells

Chinese hamster ovary cells stably expressing hOAT1 (CHO/hOAT1 cells) have been generated and characterized as described by Ho et al. (2000). Cells were seeded in 12-well culture plates at a density of 10^5 cells/cm^2. At 3–4 days post-seeding, the inhibition effects of zalcitabine on the cellular uptake of [^{3}H]-PAH (20 μM, 0.1 μCi/mL) were examined as described by Mulato et al. (2000). 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).

2. Uptake studies

Cells were seeded in 6-well culture plates at a density of 10^5 cells/cm^2. At 4 days post-seeding, the uptake studies of zalcitabine (0.25 mM) were performed as described by Mulato et al. (2000). The cellular concentrations of zalcitabine were determined by a HPLC assay reported by Ibrahim and Boudinot et al. (1989) with minor modification.

3. 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.

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References

Abel S, Nichols DJ, Brearley CJ, Eve MD (2000) Effect of cimetidine and ranitidine on pharmacokinetics and pharmacodynamics of a single dose of dofetilide. Br J Clin Pharmacol 49: 64–71.

Ayrton A, Morgan P (2001) Role of transport proteins in drug absorption, distribution and excretion. Xenobiotica 31: 469–497.

Burckhardt BC, Burckhardt G (2003) Transport of organic anions across the basolateral membrane of proximal tubule cells. Rev Physiol Biochem Pharmacol 146: 95–158.

Dahlberg JE, Mitsuya H, Blam SB, Broder S, Aaronson SA (1987) Broad spectrum antiretroviral activity of 2′,3′-dideoxynucleosides. Proc Natl Acad Sci USA 84: 2469–2473.

Gibbs JE, Thomas SA (2002) The distribution of the anti-HIV drug, 2′,3′-dideoxycytidine (ddC), across the blood-brain and blood-cerebrospinal fluid barriers and the influence of organic anion transport inhibitors. J Neurochem 80: 392–404.

Ho ES, Lin DC, Mendel DB, Cihlar T (2000) Cytotoxicity of antiviral nucleotides adefovir and cidofovir is induced by the expression of human renal organic anion transporter 1. J Am Soc Nephrol 11: 383–393.

Ibrahim SS, Boudinot FD (1989) Pharmacokinetics of 2′,3′-dideoxycytidine in rats: Application to interspecies scale-up. J Pharm Pharmacol 41: 829–834.

Khamdang S, Takeda M, Noshiro R, Narikawa S, Enomoto A, Anzei A, Piyachaturawat P, Endou H (2002) Interactions of human organic anion transporters and human organic cation transporters with nonsteroidal anti-inflammatory drugs. J Pharmacol Exp Ther 303: 534–539.

Kim RB (2000) Transporters in drug disposition. Curr Opin Drug Discov Devel 3 : 94–101.

Klecker RW, Collins JM, Yarchoan RC, Thomas R, McAtee N, Broder S, Myers CE (1988) Pharmacokinetics of 2′,3′-dideoxycytidine in patients with AIDS and related disorders. J Clin Pharmacol 28: 837–842.

Massarella JW, Nazareno LA, Passe S, Min B (1996) The effect of probenecid on the pharmacokinetics of zalcitabine in HIV-positive patients. Pharm Res 13: 449–452.

Mulato AS, Ho ES, Cihlar T (2000) Nonsteroidal anti-inflammatory drugs efficiently reduce the transport and cytotoxicity of adefovir mediated by the human renal organic anion transporter 1. J Pharmacol Exp Ther 295: 10–15.

Sekine T, Cha SH, Endou H (2000) The multispecific organic anion transporter (OAT) family. Pflügers Arch-Eur J Physiol 440: 337–350.

Yamazaki M, Suzuki H, Sugiyama Y (1996) Recent advances in carrier-mediated hepatic uptake and biliary excretion of xenobiotics. Pharm Res 13: 497–513.