

Transport of the Dipeptidyl Peptidase-4 Inhibitor Sitagliptin by Human Organic Anion Transporter 3, Organic Anion Transporting Polypeptide 4C1, and Multidrug Resistance P-glycoprotein

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ABSTRACT

Sitagliptin, a selective dipeptidyl peptidase 4 inhibitor recently approved for the treatment of type 2 diabetes, is excreted into the urine via active tubular secretion and glomerular filtration in humans. In this report, we demonstrate that sitagliptin is transported by human organic anion transporter hOAT3 ($K_m = 162 \mu\text{M}$), organic anion transporting polypeptide OATP4C1, and multidrug resistance (MDR) P-glycoprotein (Pgp), but not by human organic cation transporter 2 hOCT2, hOAT1, oligopeptide transporter hPEPT1, OATP2B1, and the multidrug resistance proteins MRP2 and MRP4. Our studies suggested that hOAT3, OATP4C1, and MDR1 Pgp might play a role in transporting sitagliptin into and out of renal proximal tubule cells, respectively. Sitagliptin did not inhibit hOAT1-mediated cidofovir uptake, but it showed weak inhibition of hOAT3-mediated cimetidine uptake ($\text{IC}_{50} = 160 \mu\text{M}$). hOAT3-mediated sitagliptin

uptake was inhibited by probenecid, ibuprofen, furosemide, fenofibric acid, quinapril, indapamide, and cimetidine with IC_{50} values of 5.6, 3.7, 1.7, 2.2, 6.2, 11, and $79 \mu\text{M}$, respectively. Sitagliptin did not inhibit Pgp-mediated transport of digoxin, verapamil, ritonavir, quinidine, and vinblastine. Cyclosporine A significantly inhibited Pgp-mediated transport of sitagliptin ($\text{IC}_{50} = 1 \mu\text{M}$). Our data indicate that sitagliptin is unlikely to be a perpetrator of drug-drug interactions with Pgp, hOAT1, or hOAT3 substrates at clinically relevant concentrations. Renal secretion of sitagliptin could be inhibited if coadministered with OAT3 inhibitors such as probenecid. However, the magnitude of interactions should be low, and the effects may not be clinically meaningful, due to the high safety margin of sitagliptin.

Sitagliptin [also known as MK-0431 (Fig. 1)], is a selective, reversible inhibitor of dipeptidyl-peptidase 4 recently approved by the Food and Drug Administration for the treatment of type 2 diabetes (Deacon, 2005; Kim et al., 2005). Dipeptidyl-peptidase 4 inhibitors have a glucose-lowering effect by inhibiting the inactivation of incretin peptides, including glucagon-like peptide-1 and glucose-dependent insulinotropic peptide, which are released upon nutrient ingestion, stimulate meal-induced insulin secretion, and

contribute to glucose homeostasis (Kieffer and Habener, 1999). The pharmacokinetics, metabolism, and excretion of sitagliptin have been investigated in humans and animals (Bergman et al., 2005, 2006; Herman et al., 2005, 2006; Beconi et al., 2007). After a single oral dose of sitagliptin (1.5–600 mg) in healthy male volunteers, sitagliptin was well absorbed with an apparent terminal half-life ranging from 8 to 14 h. Sitagliptin was primarily renally eliminated as unchanged drug (Herman et al., 2005), with metabolism playing only a minor role (Vincent et al., 2007). Renal clearance of sitagliptin averaged approximately 388 ml/min in humans (Herman et al., 2005). Given that sitagliptin is approximately

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ABBREVIATIONS: MK-0431, (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*A*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine; OAT, organic anion transporter; OCT, organic cation transporter; OATP4C1, organic anion transporting polypeptide 4C1; PEPT1, oligopeptide transporter 1; h, human; MDR, multidrug resistance; Pgp, P-glycoprotein; MRP, multidrug resistance protein; TEA, tetraethylammonium; Gly-Sar, glycylsarcosine; PAH, *p*-aminohippuric acid; CsA, cyclosporin A; PCR, polymerase chain reaction; CHO, Chinese hamster ovary; HEK, human embryonic kidney; HBSS, Hanks' balanced salt solution; A, apical; B, basolateral; LC-MS/MS, liquid chromatography-tandem mass spectrometry.

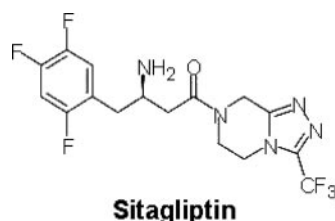


Fig. 1. Chemical structure of sitagliptin.

38% bound to plasma proteins (Beconi et al., 2007) and that the typical glomerular filtration rate for young healthy males is approximately 125 ml/min, active renal secretion seems to be involved in renal elimination of sitagliptin in humans.

Carrier-mediated processes play a key role in the secretion and reabsorption of many endogenous and xenobiotic compounds by the kidney (Lee and Kim, 2004; Wright and Dantzer, 2004; Launay-Vacher et al., 2006). Recent studies have revealed that multiple transporters are expressed in the renal proximal tubule of animals and humans and that they transport a variety of substrates, including organic anions and cations, peptides, and nucleosides (Lee and Kim, 2004; Robertson and Rankin, 2006). Among the transporters identified in humans, organic anion transporters OAT1 (*SLC22A6*), OAT3 (*SLC22A8*), organic cation transporter OCT2 (*SLC22A2*), and the organic anion transporting polypeptide OATP4C1 (*SLCO4C1*) are localized in the basolateral membrane of the renal proximal tubule cells, and they can transport organic anions and cations and some amphipathic compounds from the blood into cells (Cha et al., 2001; Mikkaichi et al., 2004; Fujita et al., 2006; Robertson and Rankin, 2006), whereas MDR1 P-glycoprotein (Pgp) (*ABCB1*), the multidrug resistance protein MRP2 (*ABCC2*) and MRP4 (*ABCC4*) as well as the recently identified multidrug and toxin extrusion transporters 1/2 are efflux pumps localized in the brush-border membrane transporting compounds into the urine (Tanigawara, 2000; Launay-Vacher et al., 2006; Omote et al., 2006). The urate-anion transporter 1 (*SLC22A12*) and the oligopeptide transporters PEPT1 (*SLC15A1*) and PEPT2 (*SLC15A2*) localized on the brush-border membrane of renal proximal tubule could contribute to reabsorption of substrate compounds (Terada and Inui, 2004; Hediger et al., 2005). These renal transporters have been shown to directly transport or interact with a number of clinically used drugs, and the evidence for pharmacokinetic drug-drug interaction at the level of tubular secretion has been reported (Shitara et al., 2005; Li et al., 2006). However, the extent of interactions is generally modest. This is probably due to the relatively low plasma concentrations of inhibitors compared with their K_i values and the contribution of glomerular filtration to total renal clearance (Shitara et al., 2005; Li et al., 2006). Drug interactions at the level of renal secretion can be important, especially for drugs with a narrow therapeutic index, as for example methotrexate or digoxin (Shitara et al., 2005).

The goal of this study was to identify the transporters potentially involved in the active renal secretion of sitagliptin in humans. Our *in vitro* studies indicated that sitagliptin was transported by hOAT3, OATP4C1, and MDR1 Pgp, but not by hOAT1, hOCT2, hPEPT1, OATP2B1, MRP2, and MRP4. Based on these findings, we evaluated the propensity

of sitagliptin to cause drug-drug interactions at the level of hOAT3 and Pgp *in vitro*.

Materials and Methods

Materials. [14 C]Sitagliptin (56.3 mCi/mmol) and [3 H]sitagliptin (3.7 Ci/mmol and 9.4 Ci/mmol) were synthesized by the Labeled Compound Synthesis Group, Department of Drug Metabolism (Merck Research Laboratories, Rahway, NJ). Unlabeled sitagliptin was obtained from the Department of Process Research (Merck Research Laboratories). The purity of radiolabeled and unlabeled sitagliptin was verified by high-performance liquid chromatography (>99.9%). [14 C]Tetraethylammonium (TEA; 55 mCi/mmol) and [3 H]quinidine (20 Ci/mmol) were obtained from American Radiolabeled Chemicals (St. Louis, MO). [3 H]Glycylsarcosine (Gly-Sar; 2 Ci/mmol), [3 H]cidofovir [(S)-1-[3-hydroxy-2-(phosphonyl-methoxy)propyl]-cytosine; 15 Ci/mmol], [3 H]ritonavir (1 Ci/mmol), and [3 H]vinblastine (5 Ci/mmol) were purchased from Moravék Biochemicals (Brea, CA). [3 H]Aminohippuric acid, *p*-[glycyl-2- 3 H] (PAH; 4.3 Ci/mmol), [3 H]estrone sulfate (57.3 Ci/mmol), [3 H]verapamil (85 Ci/mmol), and [3 H]digoxin (17.0 and 23.5 Ci/mmol) were purchased from PerkinElmer Life and Analytical Sciences (Boston, MA). [3 H]Cimetidine (25 Ci/mmol) was from GE Healthcare (Little Chalfont, Buckinghamshire, UK). Digoxin and cyclosporine A (CsA) were obtained from Sigma-Aldrich (St. Louis, MO). All other reagents were commercially obtained with the highest analytical purity grade.

Transfection and Cell Culture. hOAT1 cDNA was amplified from total human kidney cDNA (Clontech, Mountain View, CA) by PCR. In brief, oligonucleotide primers specific to the 5' and 3' coding regions of the hOAT1 sequence (5'-AGT GCC TTT AAT GAC CTC CTG CAG CAG GTG G-3' (bases 258–288) and 5'-TCA GAGTCC ATTCTT CTC TTG TGC TGA GGC C-3' (bases 1880–1910)) were synthesized (QIAGEN, Germantown, MD) and used in a standard PCR reaction with *Pfu* Ultra polymerase (Stratagene, La Jolla, CA) according to manufacturer's instructions. The resulting PCR product was cloned into the pcDNA3.1/V5-His-TOPO expression vector (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. OATP4C1 cDNA was amplified by PCR using human kidney QUICK-clone cDNA (Clontech) and cloned into pcDNA5/FRT vector. The resulting cDNAs were sequenced to confirm fidelity to the published sequence (ACGT, Inc., Wheeling, IL). hOAT3 and hOCT2 cDNAs were provided by Dr. Richard Kim (Vanderbilt University School of Medicine, Nashville, TN), and they were cloned into the pcDNA5/FRT or pCEP4 vector, respectively (Invitrogen). hPEPT1 cDNA was generated by gene synthesis by Genscript Corporation (Piscataway, NJ), and it was cloned into the expression vector pcDNA3.1 (Invitrogen, Carlsbad, CA). hOAT1/pcDNA3.1 construct was transiently transfected into CHO-K1 cells (American Type Culture Collection, Manassas, VA) using the Lipofectamine 2000 transfection reagent (Invitrogen) according to the manufacturer's protocol. hOCT2/pCEP4 and hPEPT1/pcDNA3.1 constructs were transiently transfected into HEK-293 cells (American Type Culture Collection) using Lipofectamine 2000 as described above. CHO-K1 cells stably transfected with hOAT3 or OATP4C1 cDNA were generated by using the Flp-In system (Invitrogen). Positive clones were identified by Western blotting. The transport activity by each cell line was confirmed by examining the uptake time course of 1 μ M [3 H]PAH, 1 μ M [3 H]estrone sulfate, 0.1 μ M [3 H]digoxin, 5 μ M [14 C]TEA, and 1 mM [3 H]Gly-Sar in hOAT1-, hOAT3-, OATP4C1-, hOCT2-, and hPEPT1-transfected cells, respectively.

CHO-K1 cells were cultured in F-12K medium supplemented with 10% fetal bovine serum, 2 mM GlutaMAX, 100 U/ml penicillin, and 100 μ g/ml streptomycin. HEK-293 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μ g/ml streptomycin. LLC-PK1 cells and LLC-PK1 cells expressing cDNAs encoding human MDR1 Pgp (LLC-MDR1) and mouse Mdr1a (LLC-Mdr1a) were obtained from The Netherlands Cancer Institute (Amsterdam, The Nether-

lands), and they were used under a license agreement. The cells were maintained in medium 199 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 50 U/ml penicillin and 50 μ g/ml streptomycin. All cells were maintained at 37°C in an atmosphere of 95% air, 5% CO₂ and 90% relative humidity.

Western Blot Analysis. Lysates obtained from cells transfected with hOAT1, hOAT3, hOCT2, OATP4C1, or hPEPT1 and their control cells were solubilized in Laemmli sample buffer (Bio-Rad, Hercules, CA) containing 2.5% of β -mercaptoethanol and separated in a 7.5% denaturing polyacrylamide gel (Bio-Rad). Gels were immunoblotted onto nitrocellulose membranes. Blotting efficiency was assessed by staining blots with Ponceau S. hOAT1, hOCT2, OATP4C1, and hPEPT1 were detected with custom-made polyclonal antiserum recognizing hOAT1, hOCT2, OATP4C1, and hPEPT1, respectively (BioSource International, Hopkinton, MA). The antibodies were raised against synthetic peptides corresponding to 12 to 18 amino acids of each transporter described previously (Motohashi et al., 2002). The peptide used for OATP4C1 and hPEPT1 was [NH₂]-LKACHTECKGAKAEFGC-[COOH] and [NH₂]-LEKSNPYFMSGANSQKQM-[COOH], respectively. hOAT3 was detected with polyclonal antiserum recognizing hOAT3 (Cosmo Bio Co., Ltd., Tokyo, Japan). Anti-rabbit horseradish peroxidase-labeled secondary antibody was obtained from GE Healthcare. Blots were developed with the ECL kit (GE Healthcare). Enhanced chemiluminescence signals were detected with a GeneGnome System (Syngene, Frederick, MD).

Uptake Studies in Cells Transiently or Stably Transfected with hOAT1, hOAT3, hOCT2, OATP4C1, and hPEPT1. For uptake studies with hOCT2 or hPEPT1 transiently transfected HEK-293 cells, or OATP4C1 stably transfected CHO-K1 cells, cells were dislodged with cell dissociation buffer (Invitrogen) or trypsin EDTA (Invitrogen). Then, they were washed and suspended in Hanks' balanced salt solution (HBSS; Invitrogen) containing 10 mM HEPES (pH 7.4 for hOCT2- or OATP4C1-transfected cells and pH 6.0 for hPEPT1-transfected cells) at a density of 3×10^6 cells/mL. Aliquots (200 μ l) of cell suspension were transferred to 96-deep-well plates (Fisher Scientific Co., Pittsburgh, PA), uptake was initiated by the addition of radiolabeled test compound, and cells were incubated for the indicated time at room temperature. Uptake was stopped by the addition of ice-cold phosphate-buffered saline (PBS; Invitrogen), immediate centrifugation (1800g) at 4°C (model 5180R; Eppendorf, Hamburg, Germany), followed by washing of the cell pellets with ice-cold PBS three times. Cell pellets were lysed in 50% acetonitrile in water (v/v), scintillation fluid was added (Scintisafe Econo 2; Fisher Chemicals, Fairlawn, NJ), and radioactivity was determined by liquid scintillation counting in a LS 6500 multipurpose scintillation counter (Beckman Coulter, Fullerton, CA).

For uptake studies using hOAT1 transiently transfected CHO-K1 cells, cells were seeded onto Costar 24-well cell culture plates (Corning Glassworks, Corning, NY) at a density of 1.5×10^5 cells/well and transiently transfected with cDNAs encoding hOAT1, or vector only, as described above. CHO-K1 cells stably transfected with hOAT3 cDNA were seeded onto 24-well plates at a density of 1.5×10^5 cells/well and cultured with 10 mM sodium butyrate for 24 h. To study the concentration dependence and inhibition of hOAT3-mediated sitagliptin uptake, the cells were washed three times with HBSS buffer containing 10 mM HEPES, pH 7.4, and preincubated with 5 mM glutarate for 30 min at 37°C. Uptake was initiated by the addition of radiolabeled test compounds and inhibitors at various concentrations. Cells were then incubated for the indicated times at room temperature. Uptake times for kinetic analyses and inhibition studies were within the linear range of uptake for the test compounds in hOAT1- or hOAT3-transfected cells. Uptake was stopped by the addition of ice-cold PBS. After washing the cells with PBS, the cells were solubilized in 500 μ l of 1% Triton X-100, and they were transferred to scintillation vials containing 4 ml of scintillation cocktail. The radioactivity was determined as described above.

Transepithelial Transport across LLC-MDR1, LLC-Mdr1a, and LLC-PK1 Monolayers. Transepithelial transport of sitagliptin

was evaluated in LLC-MDR1, LLC-Mdr1a, and LLC-PK1 cells. LLC-MDR1, LLC-Mdr1a, and LLC-PK1 cell lines were cultured in 12-well transwell culture plates (Costar Life Sciences, Acton, MA). Sitagliptin (10 μ M) was prepared in HBSS buffer with 10 mM HEPES, pH 7.4. Substrate solution (700 μ l) was added to either the apical (A) or the basolateral (B) compartment of the culture plate, and 700 μ l of buffer was added to the compartment opposite to that containing the substrate. After incubation for 1, 2, 3, and 4 h at 37°C, 50- μ l aliquots were removed from both sides for analysis by LC-MS/MS as described below. For inhibition studies, LLC-PK1 and LLC-MDR1 cell lines were cultured in 24-well transwell culture plates (BD Biosciences, Bedford, MA). [³H]Digoxin (2 μ M), 1 μ M [³H]verapamil, 5 μ M [³H]vinblastine, 5 μ M [³H]quinidine, 5 μ M [³H]ritonavir, and 2 μ M [³H]sitagliptin stock solutions were prepared in HBSS buffer containing 10 mM HEPES, pH 7.4. Substrate solution (500 μ l) was added to either the A or the B compartment of the culture plate, and 500 μ l of buffer was added to the compartment opposite to that containing the test compound. Inhibitors were added to both compartments. After 4-h incubation at 37°C, 50- μ l aliquots were taken from both sides, and they were transferred to a 96-well plate. After addition of 200 μ l of scintillation cocktail, the total radioactivity was measured by a 1450 MicroBeta liquid scintillation counter (PerkinElmer Wallac, Gaithersburg, MD).

In Vivo Studies. Mdr1a^{+/+} and ^{-/-} male CF-1 mice (body weights of approximately 25–35 g) were purchased from Charles River Laboratories (Hollister, CA). All animal handling was performed according to Animal Procedure Statements approved by the Merck Rahway Institutional Animal Care and Use Committee. Sitagliptin was dissolved in ethanol/polyethylene glycol/saline (10:40:50, v/v) at a concentration of 0.25 mg/ml. After intravenous administration of 1 mg/kg sitagliptin via tail vein injection, three mice from each group were sacrificed at 15, 60, and 120 min, and blood and brain were collected for analyses. Blood was collected via cardiac puncture, and plasma samples were separated by centrifugation immediately. Brain tissues were homogenized with 1 ml of distilled water. All samples were kept at -80°C until LC-MS/MS analysis.

Quantitation of Sitagliptin by LC-MS/MS. Concentrations of sitagliptin in brain and plasma were determined by LC-MS/MS following protein precipitation by acetonitrile. In brief, 60 μ l of brain homogenate or 50 μ l of plasma was treated with internal standard and an equal volume of water. Samples were then precipitated with 880 or 900 μ l of acetonitrile, respectively, followed by vortexing and centrifugation. Supernatants were transferred to 96-well collection plates and evaporated to dryness under nitrogen. Residues from brain or plasma extracts were reconstituted in the initial mobile phase composition of the corresponding high-performance liquid chromatography condition.

The samples for transepithelial transport of sitagliptin in LLC-PK1-derived cell lines were analyzed by LC-MS/MS, following solid phase extraction. In brief, 25 μ l of samples treated with internal standard was loaded onto an Oasis extraction plate (Waters, Milford, MA) preconditioned with methanol and water. The extraction plate was then washed with 3 ml of water and eluted with 2 ml of methanol. Sample extracts were dried under nitrogen and reconstituted in 100 μ l of 1:1 (v/v) acetonitrile/water.

Samples were analyzed using a PE Sciex API 365 (for the bidirectional study) or a PE Sciex API 3000 (for the in vivo study) triple quadrupole mass spectrometer interfaced via a turbo IonSpray source in positive ion mode to two PerkinElmer Series 200 pumps and a PerkinElmer Series 200 autosampler (PerkinElmerSciex Instruments, Boston, MA). The chromatography conditions for brain samples were as follows. A FluoroPhase PFP column (5 μ m, 50 \times 2.1 mm; Keystone Scientific, Waltham, MA) was used. Mobile phases consisted of A, 0.1% formic acid in water/acetonitrile (60:40) with 5 mM ammonium formate and B, 0.1% formic acid in water/acetonitrile (10:90) with 5 mM ammonium formate. The flow rate was 0.2 ml/min, and isocratic conditions at 20% A and 80% B were used. The chromatography conditions for plasma samples were as follows. A

FluoroSep-RP phenyl/HS column (5 μm , 100 \times 2 mm) was used. Mobile phases consisted of A, 0.1% formic acid in water/acetonitrile (95:5) and B, 0.1% formic acid in water/acetonitrile (5:95). The flow rate was 0.4 ml/min. The gradient increased linearly from 40 to 100% mobile phase B. The chromatography conditions for the samples from in vitro bidirectional studies were as follows. FluoroSep-RP phenyl/HS columns (5 μm , 50 \times 2 mm) were used. Mobile phases were A, 0.1% formic acid in water/acetonitrile (95:5) and B, 0.1% formic acid in acetonitrile/water (95:5). The flow rate was 0.4 ml/min. The gradient was increased linearly from 40 to 100% mobile phase B. Sitagliptin and the internal standard were monitored by selected reaction monitoring of the transitions m/z 408.1 \rightarrow 235.1 and 460.2 \rightarrow 280.6, respectively.

Data Analysis. Kinetic parameters for hOAT3-mediated sitagliptin uptake were obtained by fitting the data to eq. 1 by a nonlinear least-squares method using KaleidaGraph (Synergy Software, Reading, PA).

$$V_o = V_{\max} \times S / (K_m + S) \quad (1)$$

where V_o is the initial uptake rate of substrate (picomoles per minute per 2×10^5 cells), S is the substrate concentration (micromolar), K_m is the Michaelis constant (micromolar), and V_{\max} is the maximum uptake rate (picomoles per minute per 2×10^5 cells). The initial uptake rate for hOAT3-mediated sitagliptin uptake was obtained by subtracting the transport velocity in control cells from that in hOAT3-transfected cells.

The B-A/A-B ratio of test compounds in LLC-MDR1, Mdr1a, or LLC-PK1 cells was calculated according to eq. 2:

$$B\text{-}A/A\text{-}B = P_{\text{app}}(B \text{ to } A) / P_{\text{app}}(A \text{ to } B) \quad (2)$$

The apparent permeability (P_{app}) (B to A) and P_{app} (A to B) represent the P_{app} of B-to-A or A-to-B transport. The P_{app} was calculated using eq. 3:

$$P_{\text{app}} = \frac{1}{S \times C_o} \times \left(\frac{dQ}{dt} \right) \quad (3)$$

where S represents membrane surface area, C_o is donor concentration at time 0, and dQ/dt is amount of drug transported per time. Data are presented as the average P_{app} (centimeters per second) for A-to-B and B-to-A transport.

The IC_{50} values for inhibition of hOAT1-mediated cidofovir uptake, hOAT3-mediated cimetidine uptake, and hOAT3- and MDR1-Pgp-mediated sitagliptin transport were obtained by fitting the data to eq. 4 by nonlinear regression analysis using KaleidaGraph:

$$\% \text{ Control} = 100 / (1 + I/IC_{50}) \quad (4)$$

where I was the inhibitor concentration (micromolar); % control was calculated according to eq. 5:

$$\% \text{ Control} = (R_1/R_0) \times 100 \quad (5)$$

where R_1 represents the hOAT1-, hOAT3-, or MDR1 Pgp-mediated transport rate measured in the presence of various concentrations of inhibitor, and R_0 represents the transport rate in the absence of inhibitor. For bidirectional transport studies, the MDR1 Pgp-mediated transport rate was obtained by subtracting the A-to-B from the B-to-A transport rate in LLC-MDR1 cells.

Results

Detection of hOAT1, hOAT3, hOCT2, OATP4C1, and hPEPT1 Protein and Activity in Stably or Transiently Transfected CHO-K1 and HEK-293 Cells. By Western blotting, hOAT1, hOAT3, hOCT2, OATP4C1, and hPEPT1 were confirmed to be produced in cells transiently or stably expressing these transporters. As shown in Fig.

2, hOAT1 (A, lane 2), hOAT3 (B, lane 2), hOCT2 (C, lane 2), OATP4C1 (D, lane 2), and hPEPT1 (E, lane 2) were detected in hOAT1, hOAT3, hOCT2, OATP4C1, and hPEPT1 transiently or stably transfected cells but not in their respective control cells (lane 1 in A–E). Due to the different antibodies used, it was not possible to compare the relative expression levels of the transporter proteins in the various cell lines. Uptake of 1 μM [^3H]estrone sulfate, 0.1 μM [^3H]digoxin, 1 μM [^3H]PAH, 5 μM [^{14}C]TEA, and 1 mM [^3H]Gly-Sar, prototypic substrates for hOAT3, OATP4C1, hOAT1, hOCT2, and hPEPT1, respectively (Lee and Kim, 2004; Mikkaichi et al., 2004; Terada and Inui, 2004), was time-dependent and significantly greater than in control cells (Figs. 3A, 4A, and 5, A, C, and E), demonstrating the functional activity of each transporter.

Transport of Sitagliptin by hOAT3, OATP4C1, hOAT1, hOCT2, hPEPT1, and OATP2B1. To evaluate whether sitagliptin was transported by the renal uptake transporter hOAT3, the time-dependent uptake of [^{14}C]sitagliptin into CHO-K1 cells stably transfected with hOAT3 cDNAs was measured. The uptake of 10 μM [^{14}C]sitagliptin into CHO-hOAT3 cells was time-dependent and significantly higher than in control cells at all time points (Fig. 3B). hOAT3-mediated sitagliptin uptake was completely inhibited by 1 mM probenecid, an inhibitor of organic anion transporters (data not shown). The uptake window for hOAT3-mediated transport of sitagliptin was \sim 2-fold (6.6 ± 0.1 versus 3.2 ± 0.1 pmol/ 2×10^5 cells at 5 min for hOAT3-transfected cells and control cells, respectively). Since it has been demonstrated that hOAT3 substrates are transported into the cell in exchange for intracellular α -ketoglutarate (Wright and Dantzer, 2004), we examined whether the uptake window in CHO-hOAT3 cells would increase after preloading cells with 5 mM glutarate. Under these conditions, hOAT3-mediated sitagliptin uptake was *trans*-stimulated 2-fold (data not shown). We therefore determined the concen-

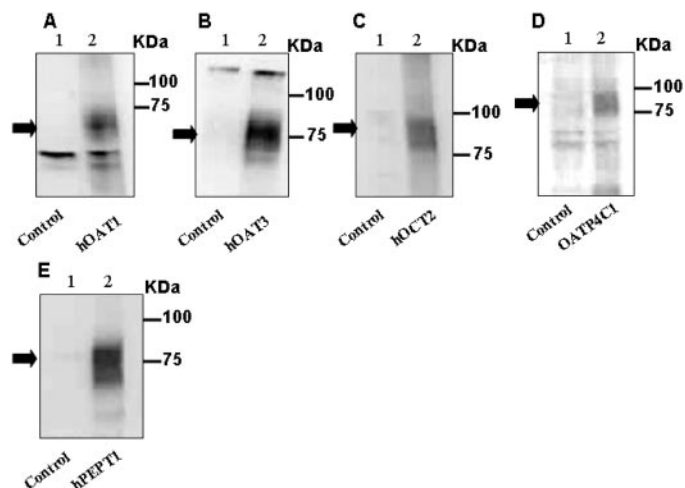


Fig. 2. Western blot detection of hOAT1, hOAT3, hOCT2, OATP4C1, and hPEPT1 in transiently or stably transfected CHO-K1 and HEK-293 cells. Cell lysates obtained from hOAT1 (10 μg) (A), hOAT3 (10 μg) (B), hOCT2 (10 μg) (C), OATP4C1 (10 μg) (D), and hPEPT1 (15 μg) (E) transiently or stably transfected and control cells were separated in a 7.5% denaturing polyacrylamide gel and immunoblotted as described under *Materials and Methods*. Lanes 1 and 2 contained samples from control and transfected cells, respectively. Relevant bands are indicated by arrows. A, hOAT1. B, hOAT3. C, hOCT2. D, OATP4C1. E, hPEPT1. Detection was achieved using polyclonal antibodies specific for each transporter.

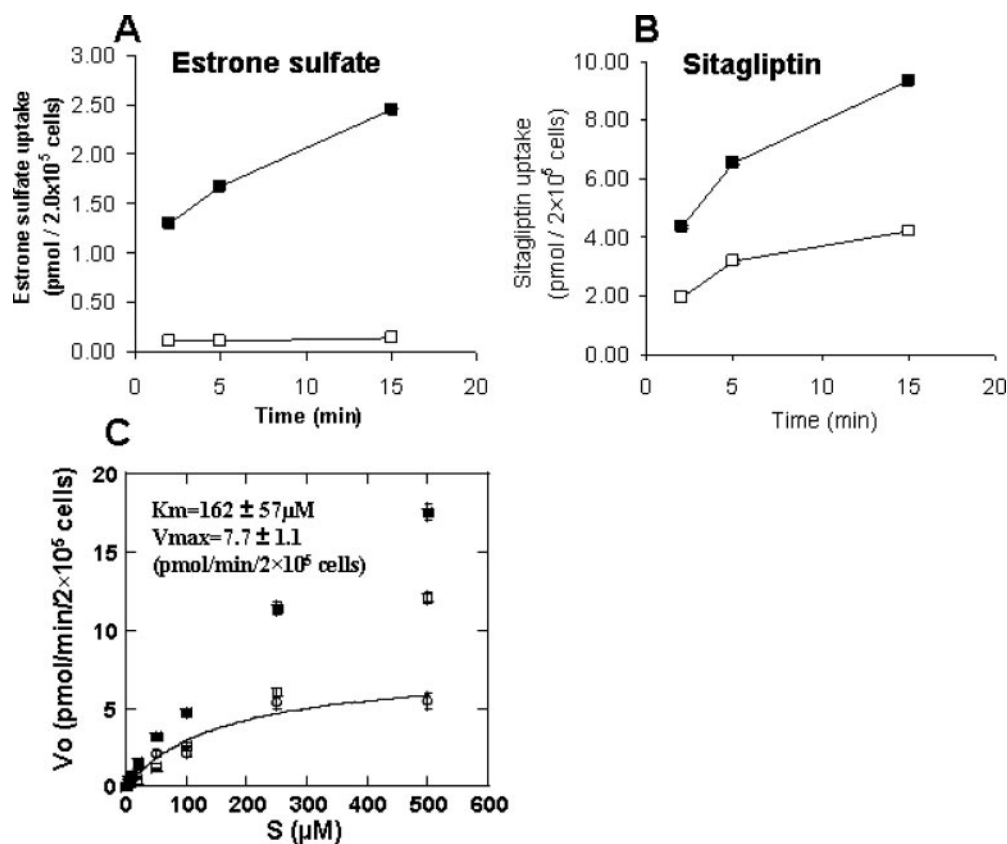


Fig. 3. Uptake of estrone sulfate and sitagliptin by CHO-hOAT3 cells. Time-dependent uptake of $1 \mu\text{M}$ [^3H]estrone sulfate (A) and $10 \mu\text{M}$ [^{14}C]sitagliptin (B) in CHO-K1 cells stably transfected with hOAT3 cDNA (closed squares) or vector only (open squares). C, concentration dependence of the uptake of sitagliptin in CHO-hOAT3 (closed squares) or control cells (open squares). The hOAT3-mediated transport (open circles) was obtained by subtracting the transport velocity in vector control cells (open squares) from that in hOAT3-transfected cells (closed squares). Kinetic parameters were obtained by fitting the uptake data to the Michaelis-Menten equation (eq. 1, using Kaleidagraph). Values shown are mean \pm S.E. of experiments performed in triplicate.

tration dependence of uptake of sitagliptin by hOAT3 and inhibition of hOAT3-mediated sitagliptin uptake after preloading with glutarate. Kinetic analysis showed that hOAT3-mediated sitagliptin uptake was saturable with a K_m of $162 \pm 57 \mu\text{M}$ and a V_{max} of $7.7 \pm 1.1 \text{ pmol/min/} 2 \times 10^5 \text{ cells}$ (Fig. 3C).

To examine whether sitagliptin was transported by the recently identified renal uptake transporter OATP4C1 (Mikkaichi et al., 2004), the uptake of $5 \mu\text{M}$ [^{14}C]sitagliptin into CHO-K1 cells stably transfected with OATP4C1 cDNA (CHO-OATP4C1) was evaluated. As shown in Fig. 4B, uptake of $5 \mu\text{M}$ [^{14}C] sitagliptin in transfected cells was time-dependent and significantly higher than that in control cells at all time points tested, suggesting that sitagliptin was a substrate of OATP4C1. However, transport activity of sitagliptin by OATP4C1 was low (1.85 ± 0.19 versus $1.22 \pm 0.07 \text{ pmol/} 2 \times 10^5 \text{ cells/} 5 \text{ min}$ at $5 \mu\text{M}$ sitagliptin). Therefore, it

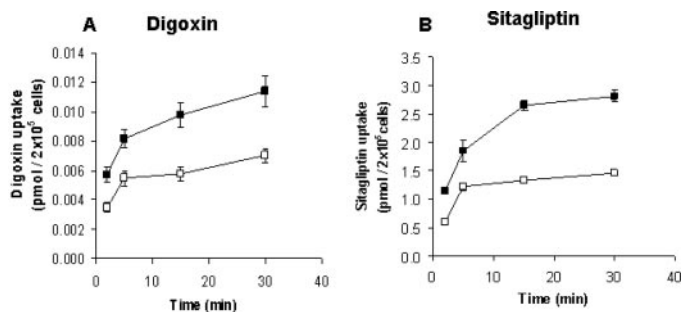


Fig. 4. Uptake of digoxin and sitagliptin by CHO-OATP4C1 cells. Time-dependent uptake of $0.1 \mu\text{M}$ [^3H]digoxin (A) and $5 \mu\text{M}$ [^{14}C]sitagliptin (B) in CHO-K1 cells stably transfected with OATP4C1 cDNA (closed squares) or vector only (open squares). Values shown are mean \pm S.E. of experiments performed in triplicate.

was not possible to obtain reliable kinetic parameters (K_m and V_{max}). Likewise, the uptake of digoxin, a reported substrate of OATP4C1 (Mikkaichi et al., 2004), was only approximately 1.5-fold higher in CHO-OATP4C1 cells than that in controls (0.0082 ± 0.0007 versus $0.0055 \pm 0.0005 \text{ pmol/} 2 \times 10^5 \text{ cells/} 5 \text{ min}$ at $0.1 \mu\text{M}$ digoxin) (Fig. 4A).

To evaluate whether sitagliptin was transported by the renal uptake transporters hOAT1, and hOCT2, the time-dependent uptake of [^{14}C]sitagliptin into CHO-K1 or HEK-293 cells transiently transfected with hOAT1 or hOCT2 cDNAs was measured. The uptake of $5 \mu\text{M}$ [^{14}C]sitagliptin in hOAT1 and hOCT2 transfected cells was similar to that in control cells (Fig. 5, B and D).

Because sitagliptin has a peptide-like structure, we tested whether it was a substrate of the oligopeptide transporter hPEPT1, a transporter that could play a role in reabsorption by renal proximal tubule cells or intestinal absorption (Terada and Inui, 2004). Uptake of $100 \mu\text{M}$ [^{14}C]sitagliptin was evaluated in HEK-293 cells transiently transfected with an hPEPT1 cDNA. The results showed that transport of sitagliptin was similar between hPEPT1- and vector-transfected cells (Fig. 5F).

We also examined whether sitagliptin was transported by the organic anion transporting polypeptide OATP2B1 (OATP-B; SLCO2B1), which is located in the basolateral membrane of hepatocytes; apical membrane of the small intestine; and also in pancreas, lung, ovary, testes, and spleen (Tamai et al., 2000). Transport of sitagliptin was evaluated in MDCKII cells stably transfected with OATP2B1 cDNA. The results indicated that sitagliptin was not a substrate of this transporter under the conditions tested (data not shown).

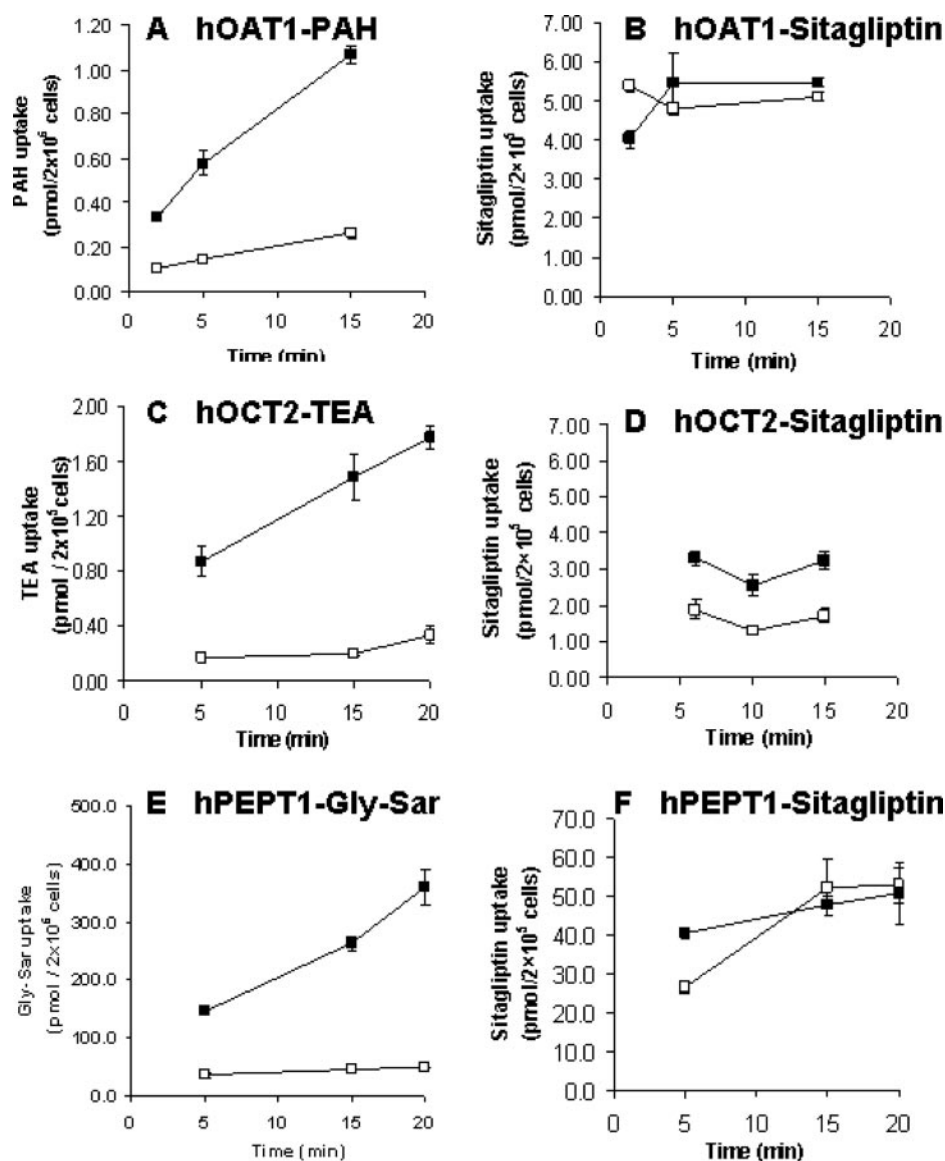


Fig. 5. Uptake of control substrates and sitagliptin by hOAT1, hOCT2, and hPEPT1 transiently transfected cells. Time-dependent uptake of 5 μM [^{14}C]sitagliptin was evaluated in hOAT1 transiently transfected CHO-K1 cells (B) and hOCT2 transiently transfected HEK-293 cells (D). F, uptake of 100 μM [^{14}C]sitagliptin in hPEPT1 transiently transfected HEK-293 cells at pH 6.0. A, C, and E show time-dependent uptake of the prototypical substrates 1 μM [^3H]PAH, 5 μM [^{14}C]TEA, and 1 mM [^3H]Gly-Sar for hOAT1, hOCT2, and hPEPT1, respectively. Closed squares represent the uptake in transfected cells, and open squares represent the uptake by control cells. Values shown are means \pm S.E. of experiments performed in triplicate.

Interaction of Sitagliptin with Known Substrates or Inhibitors of hOAT1/3 and Several Clinically Used Drugs.

To evaluate the potential inhibitory effect of sitagliptin on hOAT1- and hOAT3-mediated transport, the effect on uptake of the known hOAT1 substrate cidofovir (Cihlar et al., 1999) and the hOAT3 substrate cimetidine (Tahara et al., 2005) was investigated. As shown in Fig. 6A, uptake of 1 μM [^3H]cidofovir into CHO-hOAT1 cells was time-dependent and significantly higher than in control cells. Uptake was saturable with a K_m of $30 \pm 6 \mu\text{M}$ (data not shown), which is comparable with what has been reported by others (Cihlar et al., 1999). Sitagliptin (0.1–500 μM) did not inhibit hOAT1-mediated [^3H]cidofovir (10 μM) uptake (Fig. 6B). In contrast, probenecid potently inhibited hOAT1-mediated [^3H]cidofovir uptake with an IC_{50} of $3.9 \pm 0.9 \mu\text{M}$ (Fig. 6B). Uptake of 5 μM [^3H]cimetidine into CHO-hOAT3 cells was time-dependent and significantly higher than in control cells (Fig. 6C). Kinetic analysis showed that transport was saturable with a K_m of $174 \pm 68 \mu\text{M}$ (data not shown), which is comparable with previous reports (Tahara et al., 2005). Sitagliptin showed weak inhibition of hOAT3-mediated [^3H]cimetidine

uptake with an IC_{50} of $160 \pm 17 \mu\text{M}$ (Fig. 6D), whereas probenecid inhibited uptake of [^3H]cimetidine strongly with an IC_{50} of $3.1 \pm 1.2 \mu\text{M}$ (Fig. 6D).

The effect of several known hOAT3 substrates or inhibitors, including probenecid, ibuprofen, furosemide, and cimetidine, on hOAT3-mediated [^{14}C]sitagliptin uptake was evaluated. In addition, effects of several commonly prescribed drugs that are eliminated into the urine as parent drugs or their active metabolites (>50% of dose), including gabapentin, indapamide, quinapril, enalapril, and its active metabolite, enalaprilat, fenofibrate and its active metabolite, fenofibric acid were also evaluated. The results showed that probenecid, ibuprofen, furosemide, fenofibric acid, quinapril, indapamide, and cimetidine inhibited hOAT3-mediated sitagliptin uptake with IC_{50} values of 5.6 ± 1.4 , 3.7 ± 0.3 , 1.7 ± 0.4 , 2.2 ± 0.1 , 6.2 ± 1.7 , 11 ± 1.7 , and $79 \pm 20 \mu\text{M}$, respectively (Table 1). Enalaprilat, fenofibrate, and gabapentin up to 100 μM had no effect on uptake of sitagliptin (Table 1). Enalapril at 100 μM inhibited hOAT3-mediated sitagliptin uptake by $43 \pm 0.1\%$ of control (Table 1). To evaluate the potential for drug-drug interactions at the level of hOAT3,

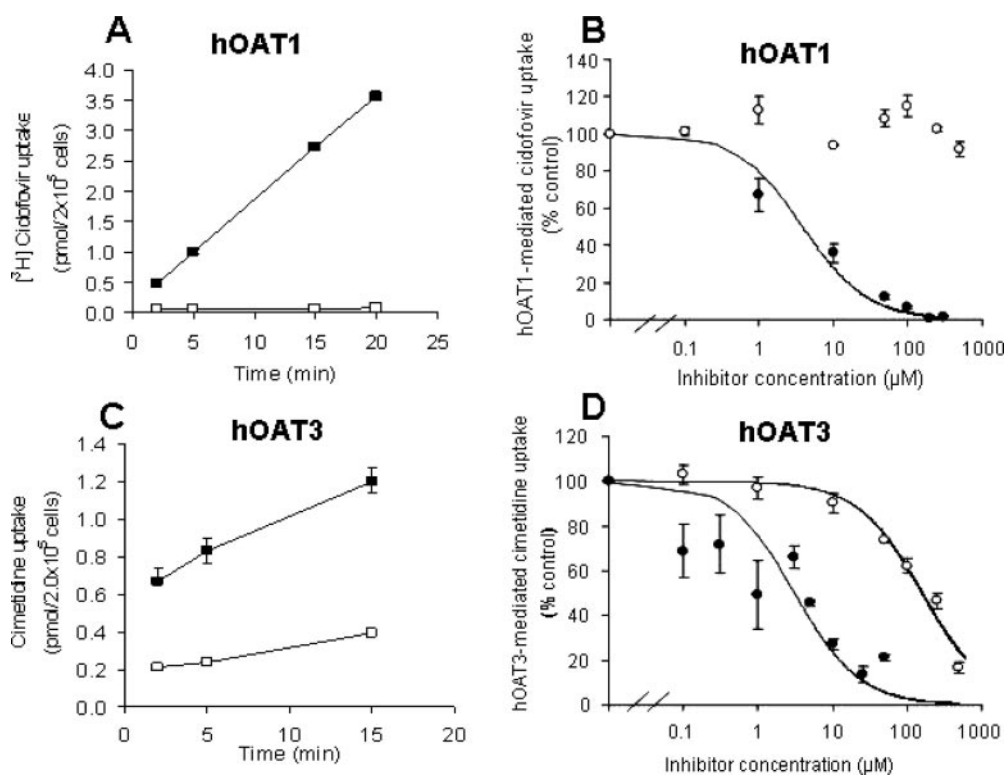


Fig. 6. Effect of sitagliptin and probenecid on hOAT1-mediated cidofovir and hOAT3-mediated cimetidine uptake by hOAT1 and hOAT3 transiently or stably transfected CHO-K1 cells. **A**, time-dependent uptake of 1 μM [^3H]cidofovir in CHO-hOAT1 (closed squares) and control cells (open squares). **B**, effects of sitagliptin (open circles) and probenecid (closed circles) on hOAT1-mediated 10 μM [^3H]cidofovir uptake. The data are expressed as a percentage of hOAT1-mediated cidofovir uptake in the presence of inhibitors versus that in the absence of inhibitors. hOAT1-mediated cidofovir uptake was examined by subtracting cidofovir uptake in control cells from that in hOAT1-transfected cells. The IC_{50} for inhibition by probenecid was $3.9 \pm 0.9 \mu\text{M}$. **C**, time-dependent uptake of 5 μM [^3H]cimetidine in CHO-hOAT3 (closed squares) and control cells (open squares). **D**, effect of sitagliptin (open circles) and probenecid (closed circles) on hOAT3-mediated 5 μM [^3H]cimetidine uptake. The data were expressed as a percentage of hOAT3-mediated cimetidine uptake in the presence of inhibitors versus that in the absence of inhibitors. IC_{50} values were $3.1 \pm 1.2 \mu\text{M}$ for probenecid and $160 \pm 17 \mu\text{M}$ for sitagliptin inhibition, respectively. Values shown are mean \pm S.E. for experiments performed in triplicate.

TABLE 1

Effect of various compounds on the hOAT3-mediated uptake of 2 μM sitagliptin and comparison with C_{max}

Compound	IC_{50}^a	Plasma C_{max}^b	References
		μM	
Cimetidine	79 ± 20	7.0 (400 mg)	Van Crugten et al. (1986)
Enalapril	$43 \pm 0.1\%^c$	0.83 (10 mg)	Arafat et al. (2005)
Enalaprilat	>100	0.16 (10 mg)	Arafat et al. (2005)
Fenofibrate	>100	40 (145 mg)	Gustavson et al. (2006)
Fenofibric acid	2.2 ± 0.1	$0.26\text{--}0.81^d$	Yamazaki et al. (2005)
Furosemide	1.7 ± 0.4	5.1 (40 mg)	Hardman and Limbird (2001)
		0.07 (40 mg) d	Hardman and Limbird (2001)
Gabapentin	>100	28 (400 mg)	Blum et al. (1994)
Ibuprofen	3.7 ± 0.3	135 (400 mg)	Albert and Gernaat (1984)
		0.7 (400 mg) d	Albert and Gernaat (1984)
Indapamide	11 ± 1.7	0.91 (5 mg) e	Grebow et al. (1982)
Probenecid	5.6 ± 1.4	12 to 44 (0.5 to 2 g) d	Selen et al. (1982)
Quinapril	6.2 ± 1.7	1.31 (20 mg) f	Breslin et al. (1996)

^a hOAT3-mediated sitagliptin uptake was examined by subtracting sitagliptin uptake in control cells from that in hOAT3-transfected cells. The IC_{50} value for inhibition by the compounds tested was obtained by fitting the data to eq. 4 by nonlinear regression analysis using KaleidaGraph.

^b Maximal total concentration in plasma, unless otherwise indicated; the number in parentheses represents the clinical dose.

^c Percentage of control at 100 μM enalapril.

^d Maximal concentration of unbound drug.

^e Maximal total concentration in blood.

^f Maximal total concentration of active carboxylic acid.

clinically relevant maximum plasma or blood concentrations of the compounds tested above are included in Table 1.

Transcellular Transport of Sitagliptin across LLC-MDR1, LLC-Mdr1a, and LLC-PK1 Cell Monolayers. To evaluate whether sitagliptin is a substrate of human MDR1 or mouse Mdr1a Pgp, transcellular transport of 10 μM sitagliptin across monolayers of LLC-MDR1, LLC-Mdr1a, and LLC-PK1 cells was measured. In parental LLC-PK1 cell monolayers, the ratio of transport of sitagliptin from B-A and A-B was close to unity (1.3; Fig. 7A), and the compound showed a poor diffusion rate (P_{app} of 1.7×10^{-6} cm/s). In both LLC-MDR1 (Fig. 7B) and LLC-Mdr1a (Fig. 7C) monolayers, sitagliptin exhibited a significantly greater vectorial transport from B-A than from A-B in transfected versus control

cell monolayers, resulting in a B-A/A-B ratio of 9.6 for LLC-MDR1 and 14.4 for LLC-Mdr1a, respectively. These data indicated that sitagliptin was a substrate of both human MDR1 and mouse Mdr1a.

In Vivo Disposition of Sitagliptin in Wild-Type and Mutant Mdr1a^{-/-} CF-1 Mice. To study the in vivo significance of the in vitro results with Pgp-expressing cell lines, the in vivo disposition of sitagliptin was investigated in Mdr1a-deficient and control CF-1 mice. After intravenous administration of 1 mg/kg sitagliptin, the mice were sacrificed at the designated time points, and the concentration of sitagliptin in brain and plasma was measured. Brain penetration of sitagliptin in CF-1 control mice was relatively low (<10 ng/g brain), whereas penetration of sitagliptin into

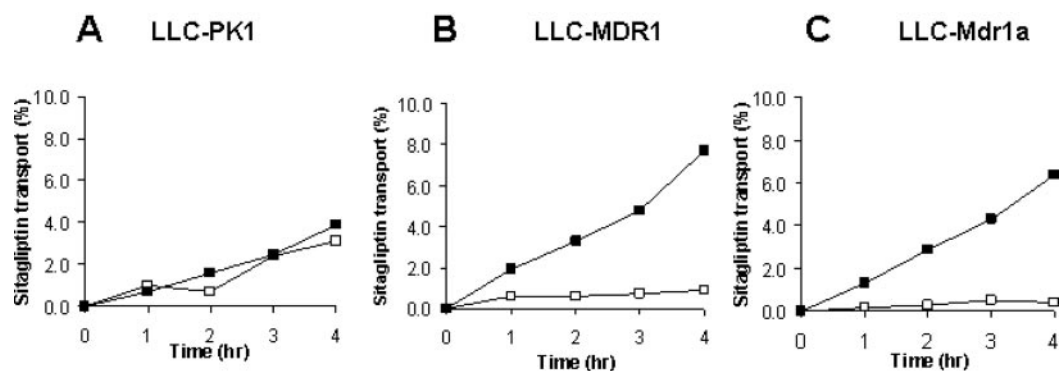


Fig. 7. Transcellular transport of sitagliptin across LLC-MDR1, LLC-Mdr1a, and LLC-PK1 cell monolayers. Transcellular transport of 10 μM sitagliptin was evaluated in LLC-PK1 (A), LLC-MDR1 (B), and LLC-Mdr1a monolayers (C) from the B-A compartment (closed squares) and A-B compartment (open squares). The percentage of sitagliptin occurring in the opposite compartment at the time points indicated was measured. The B-A/A-B ratio was 1.3 in LLC-PK1, 9.6 in LLC-MDR1, and 14.4 in LLC-Mdr1a cells, respectively. Data shown are average values for experiments performed in duplicate.

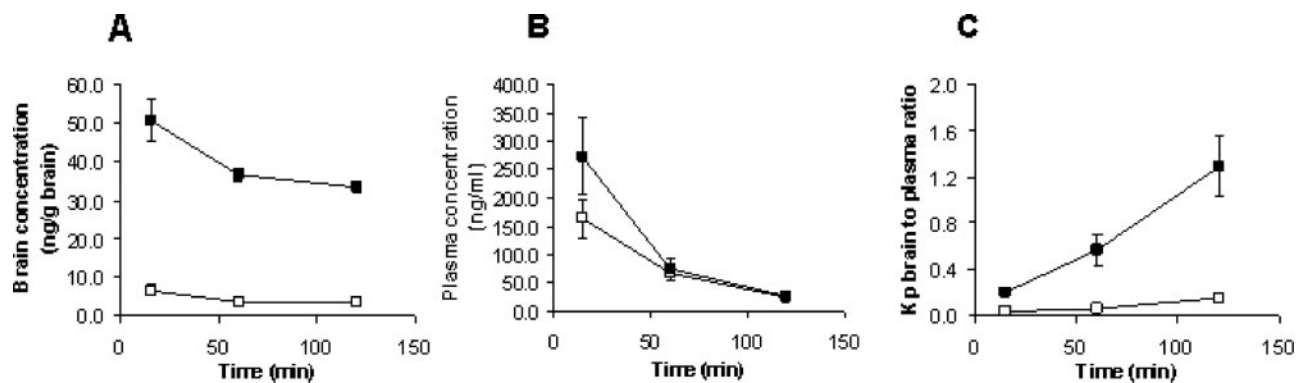


Fig. 8. Brain and plasma concentrations of sitagliptin in Mdr1a-deficient and wild-type CF-1 mice after intravenous injection of sitagliptin. After intravenous injection of 1 mg/kg sitagliptin, mutant (closed squares) and wild-type CF-1 mice (open squares) were sacrificed at 15, 60, and 120 min. The brain (A) and plasma (B) concentrations of sitagliptin were measured by LC-MS/MS. Three mice were analyzed in each group. $K_{p, \text{brain}}$ represents the brain-to-plasma concentration ratio (C). Values shown are mean \pm S.E.

brain of mutant mice was approximately 8 to 10 times higher (Fig. 8A). There was no significant difference in the plasma concentration time profile of sitagliptin between mutant and control mice (Fig. 8B). The brain-to-plasma concentration ratio in wild-type mice was time-dependent and increased from 0.04 to 0.15 after dosing, whereas the ratio in mutant mice increased from 0.20 to 1.30 (Fig. 8C). The higher brain to plasma ratios in the mutant mouse strain confirmed that sitagliptin was a substrate for mouse Mdr1a Pgp. Consistent with the low P_{app} measured in vitro, the ratios at the early time points were low in both mutant and control mice, indicating a low intrinsic brain penetrability of this compound.

Interaction of Sitagliptin with Several Known Substrates and Inhibitors of MDR1 Pgp. To evaluate the potential for drug-drug interactions between sitagliptin and coadministered Pgp substrates or inhibitors, the effect of sitagliptin on the bidirectional transport of 2 μM [^3H]digoxin, 1 μM [^3H]verapamil, 5 μM [^3H]vinblastine, 5 μM [^3H]ritonavir, and 5 μM [^3H]quinidine was investigated in LLC-MDR1 and LLC-PK1 cells. Digoxin, verapamil, vinblastine, ritonavir, and quinidine exhibited MDR1 Pgp-mediated transport with B-A/A-B ratios of 6.3, 4.3, 11.0, 7.7, and 8.2 in LLC-MDR1 cells and of 2.5, 1.0, 2.2, 2.2, and 0.8 in LLC-PK1 cells, respectively (data not shown). At the concentrations tested (0.3–500 μM), sitagliptin did not inhibit MDR1 Pgp-mediated transport of any of the drugs tested (Fig. 9, A–E). In addition, sitagliptin did not affect their transport in the parental LLC-PK1 cells (data not shown). In con-

trast, 10 μM CsA, a known Pgp inhibitor (Tanigawara, 2000), completely blocked the Pgp-mediated transport of all Pgp substrates tested (data not shown).

The effect of CsA (0.05–15 μM) on the bidirectional transport of 2 μM [^3H]sitagliptin was evaluated in LLC-MDR1 and LLC-PK1 cells. The B-A/A-B ratio in the absence of CsA was 1.3 in LLC-PK1 and 5.6 in LLC-MDR1 cells (data not shown). As shown in Fig. 9F, CsA inhibited MDR1 Pgp-mediated transport of sitagliptin in a concentration-dependent manner, with a calculated IC_{50} of $1.1 \pm 0.3 \mu\text{M}$.

Transport of Sitagliptin by MRP2 and MRP4. Like MDR1 Pgp, MRP2 and MRP4 have been detected in the luminal membrane of renal proximal tubule cells and therefore they may also contribute to the efflux of substrates into the urine (Launay-Vacher et al., 2006). Transport of sitagliptin by MRP2 and MRP4 was evaluated in uptake studies using membrane vesicles isolated from baculovirus-infected Sf9 cells containing MRP2 or MRP4. In addition, the bidirectional transport of sitagliptin was studied using MDCKII cells stably transfected with MRP2 cDNA. The results indicated that sitagliptin was not a substrate of MRP2 or MRP4 (data not shown).

Discussion

In this report, the potential contribution of a range of transporters to the active renal secretion of sitagliptin was

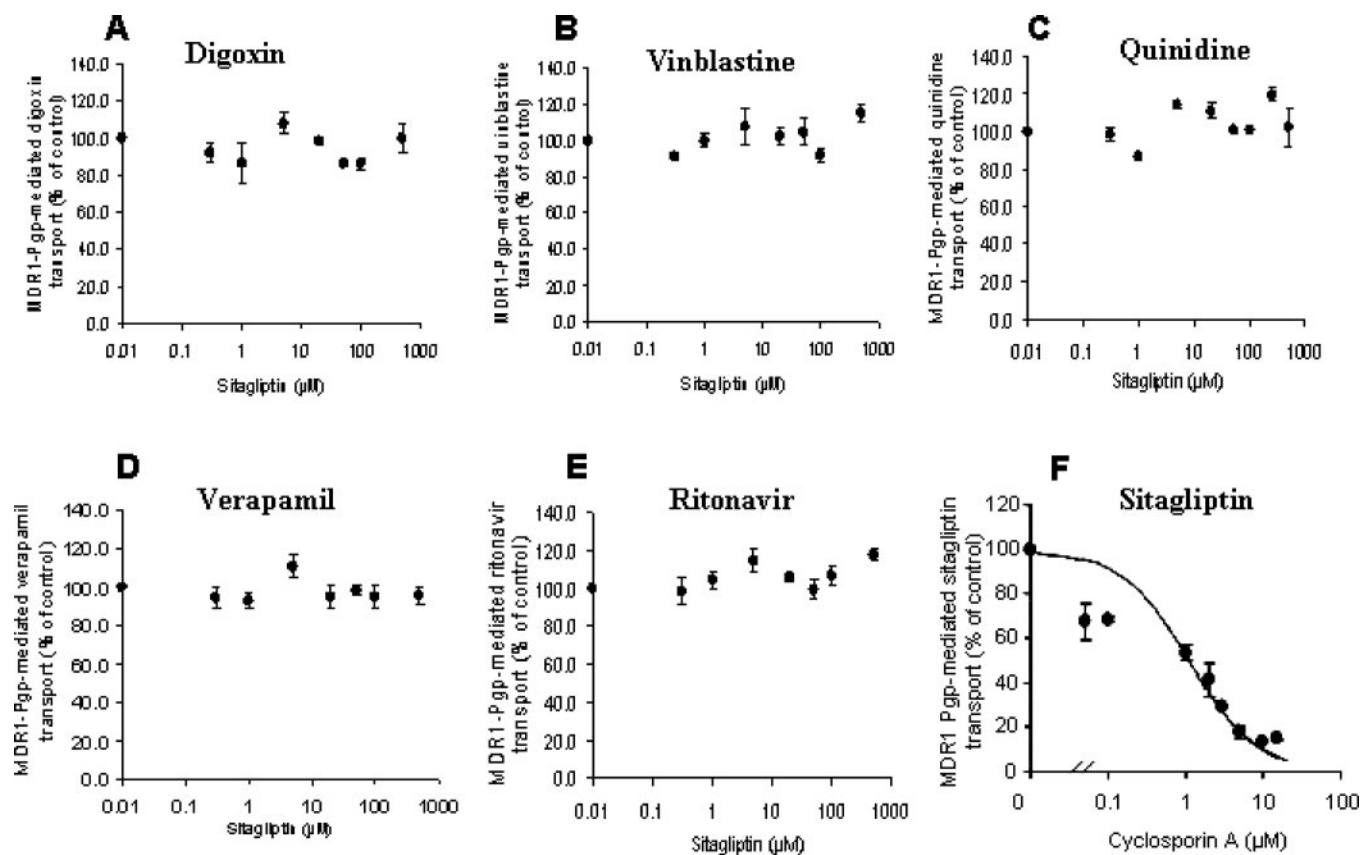


Fig. 9. Effect of sitagliptin on MDR1-Pgp-mediated transport of prototypical substrates and the effect of CsA on MDR1-Pgp-mediated sitagliptin transport across LLC-MDR1 and LLC-PK1 cell monolayers. Effect of 0.3 to 500 μM sitagliptin on MDR1-Pgp-mediated transport of 2 μM [^3H]digoxin (A), 5 μM [^3H]vinblastine (B), 5 μM [^3H]quinidine (C), 1 μM [^3H]verapamil (D), and 5 μM [^3H]ritonavir (E) in LLC-MDR1 and LLC-PK1 cell monolayers. MDR1-Pgp-mediated transport of test compounds expressed as percentage of control was calculated according to eq. 5. (F) Effect of CsA on MDR1-Pgp-mediated 2 μM [^3H]sitagliptin transport in LLC-MDR1 and LLC-PK1 cell monolayers. [^3H]Sitagliptin at 2 μM was applied to the basal or apical compartment of the cell monolayer, and the percentage of radioactivity occurring in the opposite compartment was measured at $t = 4$ h. Experiments were conducted with sitagliptin alone or in the presence of 0.05, 0.1, 1, 2, 3, 5, 10, or 15 μM CsA. The B-A/A-B ratio in the absence of CsA was 1.3 in LLC-PK1 cells and 5.6 in LLC-MDR1 cells. The IC_{50} value for CsA inhibition was 1.1 ± 0.3 μM . MDR1-Pgp-mediated sitagliptin transport expressed as percentage of control was calculated according to eq. 5, and the IC_{50} value was obtained by fitting the data to eq. 4 by nonlinear regression analysis using KaleidaGraph. Values shown are mean \pm S.E. for experiments performed in triplicate.

investigated. Our studies indicated that sitagliptin was a substrate for the renal uptake transporters hOAT3, OATP4C1, and the efflux transporter MDR1 Pgp but not for hOAT1, hOCT2, hPEPT1, OATP2B1, MRP2, and MRP4. We therefore postulate that hOAT3 and MDR1 Pgp could contribute to the renal uptake and efflux of sitagliptin, respectively. We also present evidence that sitagliptin is a substrate for OATP4C1, the only OATP expressed in the kidney at relatively high levels (Mikkaichi et al., 2004). Because the *in vivo* role of OATP4C1 in drug transport has not been well defined, and the expression level of this transporter is low compared with hOAT3 (unpublished data), the physiological relevance of this observation requires further study.

Kinetic analysis showed that sitagliptin was a low-affinity substrate of hOAT3 ($K_m = 162$ μM ; $V_{\text{max}} = 7.7$ pmol/min/ 2×10^5 cells). The V_{max}/K_m , which represents the intrinsic clearance of hOAT3-mediated sitagliptin transport in the CHO-hOAT3 cells, was 0.05 $\mu\text{l}/\text{min}/2 \times 10^5$ cells, which was comparable with that for hOAT3-mediated cimetidine uptake ($V_{\text{max}}/K_m = 0.03$ $\mu\text{l}/\text{min}/2 \times 10^5$ cells; $K_m = 174$ μM ; $V_{\text{max}} = 5.7$ pmol/min/ 2×10^5 cells; data not shown), indicating that sitagliptin demonstrates a similar transport efficiency by hOAT3 as cimetidine. Therefore, it is likely that hOAT3 is

involved in the uptake of sitagliptin into renal proximal tubule cells.

We evaluated *in vitro* whether sitagliptin had the potential to act as a victim or perpetrator if coadministered with other OAT1 or OAT3 substrates or inhibitors. In evaluating the potential of compounds to cause drug-drug interactions, we considered the free drug concentration in plasma as the relevant concentration available for interaction with transporters (Shitara et al., 2005). Sitagliptin up to 500 μM did not inhibit hOAT1-mediated cidofovir uptake (Fig. 6B), in agreement with our finding that sitagliptin was not a substrate of hOAT1. In contrast, probenecid could cause almost complete inhibition of hOAT1-mediated cidofovir uptake in humans ($\text{IC}_{50} = 3.9$ μM ; Fig. 6B), because the unbound plasma concentration of probenecid at clinical dosages (0.5–2.0 g), ranges from 12 to 52 μM (Selen et al., 1982). This is consistent with a clinical report that showed a nearly complete inhibition of the active tubular secretion of cidofovir by probenecid (Cundy et al., 1995). Probenecid caused strong inhibition of hOAT3-mediated cimetidine uptake ($\text{IC}_{50} = 3.1$ μM ; Fig. 6D), whereas sitagliptin only showed a weak inhibition with an IC_{50} of 160 μM (Fig. 6D), which was approximately 160-fold higher than the observed steady-state plasma con-

centration of sitagliptin in humans (0.9 μM) after multiple oral doses (100 mg) at day 10 (Bergman et al., 2006). Taken together, our studies suggest that sitagliptin is unlikely to be a perpetrator of clinical drug interactions with hOAT1 or hOAT3 substrates.

The effect of several clinically used drugs on hOAT3-mediated sitagliptin uptake was investigated. The results showed that probenecid, ibuprofen, furosemide, and cimetidine, known hOAT3 substrates or inhibitors, inhibited sitagliptin uptake with IC_{50} values of 5.6, 3.7, 1.7, and 79 μM , respectively (Table 1). Because the IC_{50} value for probenecid was below its unbound concentration in plasma (Table 1), probenecid could potentially inhibit active secretion of sitagliptin. The IC_{50} of ibuprofen and fenofibric acid is higher than but within the range of the clinically relevant unbound plasma concentration, indicating that there is potential for interaction when coadministered with sitagliptin (Table 1). The IC_{50} of furosemide and cimetidine is much higher than their therapeutically relevant unbound plasma concentrations, making these drugs unlikely to affect renal clearance of sitagliptin. Quinapril and indapamide demonstrated a similar inhibitory potency of sitagliptin transport as probenecid. Interestingly, these compounds have not been reported as hOAT3 substrates or inhibitors. The potential of these compounds to cause clinically relevant interactions with sitagliptin at the level of hOAT3 is low, because the therapeutic plasma concentrations of these drugs are lower than their IC_{50} values (Table 1). Because active renal secretion clearance accounts for approximately 50% of total clearance of sitagliptin in healthy human subjects following a 100-mg intravenous dose (Bergman et al., 2005), even if the active renal secretion would be completely blocked by inhibitors of renal transport, the increase in systemic exposure (i.e., the plasma concentration of sitagliptin) would not be more than 2-fold. Such a change would not be considered clinically relevant, given the large therapeutic window of sitagliptin, with phase I studies showing no dose-related adverse experiences and generally good tolerance to single doses of 800 mg and multiple doses of 600 mg. In addition, in large clinical trials, a dose of 200 mg per day was well tolerated over 24 weeks (Charbonnel et al., 2006).

Our *in vitro* data demonstrate that sitagliptin is a substrate of human MDR1 and mouse *Mdr1a* Pgp (Fig. 7). Therefore, Pgp expressed in the brush-border membrane of renal proximal tubular cells might play a role in the efflux of sitagliptin into the urine. In agreement with the high expression of *Mdr1a* in the blood-brain barrier, the brain concentration of sitagliptin in CF-1 control mice following intravenous administration of sitagliptin was very low (<10 ng/g brain), whereas the concentration in brain of mutant *Mdr1a*-negative mice was approximately 8 to 10 times higher (Fig. 8A).

To evaluate the potential for drug-drug interactions due to inhibition of MDR1 Pgp, the effect of sitagliptin on Pgp-mediated transport of several known Pgp substrates and inhibitors was investigated *in vitro*. Sitagliptin did not inhibit Pgp-mediated transport of digoxin, vinblastine, quinine, verapamil, and ritonavir (Fig. 9, A–E), suggesting a low potential for sitagliptin to cause drug interactions at the Pgp level. This is in line with a clinical drug-drug interaction study, in which the effect of sitagliptin on the plasma concentrations of digoxin was evaluated in healthy subjects

(Miller et al., 2006). Concomitant administration of 100 mg of sitagliptin and 0.25 mg of digoxin once daily for 10 days only slightly increased the exposure of digoxin (plasma $\text{AUC}_{0-\infty}$) on day 10 by ~11% compared with digoxin alone, whereas renal clearance of digoxin was not changed.

Pgp-mediated transport of sitagliptin was inhibited *in vitro* by the potent Pgp inhibitor CsA ($\text{IC}_{50} = 1.1 \mu\text{M}$; Fig. 9E). A clinical drug-drug interaction study was conducted to investigate whether an interaction between CsA and sitagliptin would be observed in humans (Krishna et al., 2007). After coadministration of a single oral dose of 600 mg of CsA with 100 mg of sitagliptin (single oral dose) in healthy male subjects, the sitagliptin plasma area under the curve was increased by approximately 29%, whereas there was no change in its renal clearance. It does not seem likely that the increase in plasma sitagliptin exposure can be accounted for solely by inhibition of Pgp in the intestine, given its absolute bioavailability of 87% when given without CsA (Bergman et al., 2005; Herman et al., 2005). Other mechanisms potentially involved remain unknown.

In addition to MDR1 Pgp, other efflux transporters expressed in the luminal membrane of renal proximal tubules might also contribute to the excretion of sitagliptin into the urine. Our studies have indicated that sitagliptin is not transported by MRP2 and MRP4. Recently, the human multidrug and toxin extrusion proteins 1/2, localized in the luminal membrane of renal proximal tubules have been identified (Omote et al., 2006). The contribution of these transporters to the renal excretion of sitagliptin remains to be identified.

Diffusion of sitagliptin through LLC-PK1 cell monolayers was slow ($P_{\text{app}} = 1.7 \times 10^{-6} \text{ cm/s}$). Interestingly, sitagliptin was well absorbed in humans and preclinical species, bioavailability in humans was high, and the time to reach the maximal plasma concentration ranged from 1 to 4 h (Bergman et al., 2006). This suggests that luminal uptake transporters might be involved in the intestinal absorption of sitagliptin. Since sitagliptin has a peptide-like structure, one potential candidate is the human oligopeptide transporter hPEPT1 (Terada and Inui, 2004). However, no uptake of sitagliptin was detected in hPEPT1-transfected cells (Fig. 5F). Sitagliptin was also not transported by OATP2B1, an uptake transporter localized in the apical membrane of the intestine.

In conclusion, we demonstrated that sitagliptin is a substrate for hOAT3, MDR1 Pgp, and OATP4C1. These transporters potentially could contribute to the active renal secretion of sitagliptin in humans. Our *in vitro* drug-drug interaction studies suggested that sitagliptin has a low propensity to act as a perpetrator in drug-drug interactions at clinically relevant drug concentrations. Sitagliptin could be a victim of drug-drug interactions if coadministered with potent hOAT3 inhibitors such as probenecid, although the effects likely will not be of clinical significance due to the contribution of glomerular filtration to renal clearance, the contribution of CYP3A4 to hepatic clearance and the relatively high safety margin of the drug.

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