DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
DMD Fast Forward. Published on April 12, 2006 as doi:10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

Title page

Predominant contribution of OATP1B3 to the hepatic uptake of telmisartan, an angiotensin II receptor antagonist, in humans

Naoki Ishiguro, Kazuya Maeda, Wataru Kishimoto, Asami Saito, Akiko Harada, Thomas Ebner, Willy Roth, Takashi Igarashi, Yuichi Sugiyama

Department of Pharmacokinetics and Non-Clinical Safety, Kawanishi Pharma Research Institute, Nippon Boehringer Ingelheim Co., Ltd. 3-10-1, Yato, Kawanishi, Hyogo 666-0193, Japan (N.I., W.K., A.S., A.H., T.I.)

Department of Molecular Pharmacokinetics, Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan (K.M., Y.S.)

Department of Pharmacokinetics and Drug Metabolism, Boehringer Ingelheim Pharma KG, Birkendorfer Strasse D-88397 Biberach on der Riss, Germany (T.E., W.R.)

Copyright 2006 by the American Society for Pharmacology and Experimental Therapeutics.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

Running title page

a) Hepatic uptake of telmisartan by OATP1B3

b) Yuichi Sugiyama, Ph.D.
Department of Molecular Pharmacokinetics, Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
Tel: +81-3-5841-4770
Fax: +81-3-5841-4766
E-mail: sugiyama@mol.f.u-tokyo.ac.jp

c) Numbers of text pages: 37
Numbers of table: 1
Numbers of figures: 7
Numbers of references: 30
Numbers of words in abstract: 246
Numbers of words in introduction: 740
Numbers of words in discussion: 1490

d) AUC: area under the plasma concentration-time curve
CCK-8: cholecystokinin octapeptide
E-sul: estrone-3-sulfate
$E_2$17βG: estradiol 17β-D-glucuronide
HSA: human serum albumin
OATP: organic anion transporting polypeptide
TEA: tetraethylammonium

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

Abstract

Telmisartan, a nonpeptide angiotensin II receptor antagonist, is selectively distributed to liver. In the present study, we have characterized the contribution of organic anion transporting polypeptide (OATP) isoforms to the hepatic uptake of telmisartan by isolated rat hepatocytes, human cryopreserved hepatocytes and human transporter-expressing cells. Because it is difficult to evaluate the transport activity of telmisartan due to its extensive adsorption to cells and culture materials, we performed the uptake study in the presence of human serum albumin. The saturable uptake of telmisartan into isolated rat hepatocytes took place in a Na^+-independent manner and was inhibited by pravastatin, taurocholate and digoxin, which are Oatp substrates and inhibitors, but not by organic cation, tetraethylammonium, indicating the involvement of Oatp isoforms in its uptake into rat hepatocytes. To identify which human OATP transporters are important for the hepatic uptake of telmisartan, the uptake assay was carried out using OATP1B1- and OATP1B3-expressing HEK293 cells and cryopreserved human hepatocytes. The uptake of telmisartan by OATP1B3-expressing cells was saturable (K_m= 0.81 μM) and significantly higher than that by vector-transfected cells. In contrast, no significant uptake was observed in OATP1B1-expressing cells. We also observed the saturable uptake of telmisartan by

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

human hepatocytes. Thirty μM estrone-3-sulfate, which can selectively inhibit OATP1B1-mediated uptake compared with OATP1B3, did not inhibit the uptake of telmisartan in human hepatocytes, while it could inhibit the uptake of estradiol 17β-D-glucuronide mediated by OATP1B1. These results suggest that OATP1B3 is predominantly involved in the hepatic uptake of telmisartan in humans.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

Introduction

The renin-angiotensin-aldosterone system plays a central role in blood pressure regulation (Hedner, 1999). This system leads to the production of the hormone angiotensin I, which is converted to the active hormone angiotensin II by angiotensin-converting enzyme (ACE). Angiotensin II receptor antagonists prevent angiotensin II from exerting its vasoconstrictive effects on blood vessels (Oliverio and Coffman, 1997). Five nonpeptide angiotensin receptor antagonists, losartan, candesartan cilexetil, valsartan, telmisartan and olmesartan medoxomil, are commercially available in Japan. Losartan, candesartan cilexetil and olmesartan medoxomil are prodrugs, whereas valsartan and telmisartan are themselves pharmacologically active. All of them are mainly excreted into feces from the liver.

Telmisartan (Fig. 1) is a lipophilic compound with a log P value of 3.2 and it exists in anionic form at neutral pH (Wienen et al., 2000). Telmisartan is metabolized to an inactive acylglucuronide conjugate by UDP-glucuronosyltransferases in the intestinal wall and liver (Stangier et al., 2000a). The acylglucuronide is rapidly excreted into the bile and accounts for 10 % of the circulating drug-related material 1 h after oral administration of telmisartan (Stangier et al., 2000a; Stangier et al., 2000b). Telmisartan is selectively distributed to the liver in rats with a liver-to-plasma

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

concentration ratio of more than 40 (Wienen et al., 2000). Following a single oral and intravenous administration in humans, more than 98% of the total radioactivity was recovered in feces as a parent drug and less than 1% of the radioactivity was recovered in urine (Stangier et al., 2000b). Telmisartan shows a large inter-individual variability in its plasma concentrations and both the maximum concentration in plasma (C_{max}) and area under the plasma concentration-time curve (AUC) increased in a slightly more than the dose-proportional manner after oral administration (Stangier et al., 2000a; Stangier et al., 2000b). Since liver is a major clearance organ of telmisartan, it is essential to assess the uptake mechanism of telmisartan by human hepatocytes to gain an insight into the mechanism for its non-linear pharmacokinetics and large inter-individual variability.

Several transporters such as Na^+-taurocholate co-transporting polypeptide and organic anion transporting polypeptide (OATP) 1B1 (previously called OATP-C/OATP2/LST-1), OATP1B3 (previously called OATP8/LST-2), OATP2B1 (previously called OATP-B), organic anion transporter 2, and organic cation transporter 1, are expressed on the sinusoidal membrane of hepatocytes and are thought to be involved in the transport of a wide variety of compounds including clinically-used drugs, such as 3-hydroxy-3-methylglutaryl CoA reductase inhibitors (statins), from blood into

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

hepatocytes (Abe et al., 1999; Hsiang et al., 1999; Kok et al., 2000; Tamai et al., 2000; Konig et al., 2000a; Konig et al., 2000b; Faber et al., 2003; Hirano et al., 2004). In particular, OATP1B1 and OATP1B3 are mainly expressed in human liver (Hagenbuch and Meier, 2003) and the substrate specificity of OATP1B3 commonly overlaps that of OATP1B1, so several compounds can be bisubstrates of both OATP1B1 and OATP1B3, such as estradiol 17β-D-glucuronide ($E_2$17βG), pitavastatin and rifampicin (Vavricka et al., 2002; Hirano et al., 2004). Hirano et al. (2004) have recently established methods for estimating the contribution of OATP1B1 and OATP1B3 to the hepatic uptake of a number of compounds. They have demonstrated that pitavastatin and $E_2$17βG are taken up in human hepatocytes mainly by OATP1B1. On the other hand, the uptake of fexofenadine, an H_1 receptor antagonist, was mainly mediated by OATP1B3 rather than OATP1B1 (Shimizu et al., 2005). This kind of information is helpful for predicting the effect of changes in expression level and function of certain transporters caused by genetic polymorphisms, pathophysiological conditions and transporter-mediated drug-drug interactions on the overall hepatic uptake clearance and subsequent pharmacokinetics of drugs. Besides the methods developed by Hirano et al., if compounds can selectively inhibit OATP1B1- or OATP1B3-mediated transport, we can also easily calculate the contribution of each transporter to the uptake of particular

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

compounds in human hepatocytes by estimating the fraction of their uptake that can be inhibited by transporter-selective inhibitors. However, our preliminary study as well as a previous report demonstrated that cholecystokinin octapeptide (CCK-8) could also inhibit OATP1B1-mediated transport (Nozawa et al., 2003), indicating that it cannot be used as an OATP1B3-selective inhibitor, though it is selectively transported by OATP1B3 (Ismair et al., 2001). On the other hand, there is little information about selective inhibitors against OATP1B1.

Therefore, the aim of our study is to show the involvement of OATP family transporters in the hepatic uptake process of telmisartan and estimate the contribution of OATP1B1 and OATP1B3 to its uptake in human hepatocytes by a newly-developed estimation method using OATP1B1-selective inhibitor, E-sul.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

Methods

Chemicals

[^{3}H]-Telmisartan (762 GBq/mmol, radiochemical purity >98%), 4'-[(1,4'-dimethyl-2'-propyl[2,6'-bi-1H-benzimidazol]-1'-yl)methyl]-[1,1'-biphenyl]-2-carboxylic acid, and unlabeled telmisartan were synthesized by Boehringer Ingelheim Pharma KG (Biberach, Germany)(Ries et al., 1993). [^{3}H]-$E_2$17βG, [^{3}H]-estrone-3-sulfate (E-sul), and [^{3}H]-taurocholate were purchased from PerkinElmer Life and Analytical Sciences (Boston, MA). [^{3}H]-CCK-8 was purchased from Amersham Biosciences UK Ltd. (Little Chalfont, Buckinghamshire, UK). Unlabeled $E_2$17βG, E-sul, taurocholate, CCK-8, and digoxin were purchased from Sigma-Aldrich (St. Louis, MO). Pravastatin and tetraethylammonium (TEA) were obtained from Wako Pure Chemicals (Kyoto, Japan). All other chemicals and reagents were commercial products of reagent grade.

Cell culture

OATP1B1-, OATP1B3-, and OATP2B1-expressing or vector-transfected HEK293 cells were established previously (Hirano et al., 2004; Shimizu et al., 2005). HEK293 cells were grown in Dulbecco's modified Eagle's medium low glucose (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA),

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

100 U/ml penicillin, 100 µg/ml streptomycin, and 0.25 µg/ml amphotericin B at 37 ºC with 5 % CO_2 and 95 % humidity. Cells were then seeded in 12-well plates (coated with 50 mg/l poly(L-lysine) and 50 mg/l poly(L-ornithine); Sigma) at a density of 1.5 × 10^5 cells/well. For the transport study, the cell culture medium was replaced with culture medium supplemented with 5 mM sodium butyrate for 24 h before transport assay to induce the expression of transporters.

Transport study using transporter expression systems

The transport study was carried out as described previously (Hirano et al., 2004). Uptake was initiated by adding Krebs-Henseleit buffer (118 mM NaCl, 23.8 mM $NaHCO_3$, 4.8 mM KCl, 1.0 mM KH_2PO_4, 1.2 mM $MgSO_4$, 12.5 mM HEPES, 5.0 mM glucose, and 1.5 mM $CaCl_2$ adjusted to pH 7.4) containing radiolabeled and unlabeled substrates after cells had been washed twice and preincubated with Krebs-Henseleit buffer at 37ºC for 15 min. The uptake was terminated at designated times by adding ice-cold Krebs-Henseleit buffer after removal of the incubation buffer. Then, cells were washed twice with 1 ml ice-cold Krebs-Henseleit buffer, solubilized in 1 N NaOH, and kept for 1 h at 37ºC. Aliquots were transferred to scintillation vials after adding a half volume of 2 N HCl. The radioactivity associated with the cells and incubation buffer was measured in a liquid scintillation counter (TRI-CARB 2500TR, Perkin Elmer,

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

Boston, MA) after adding 2 ml scintillation fluid. The remaining 50 µl cell lysate was used to determine the protein concentration by the method of Lowry with bovine serum albumin as a standard.

Preparation of rat and human hepatocytes

Isolated rat hepatocytes were prepared from Sprague-Dawley rats weighing 200-300 g by the collagenase perfusion method described previously (Yamazaki et al., 1993). Isolated hepatocytes (viability > 80%) were suspended in Krebs-Henseleit buffer, adjusted to 2.0×10^6 cells/ml, and stored on ice before the uptake experiment. Cryopreserved human hepatocytes (Lot. OCF, MYO and 094) were purchased from In Vitro Technologies, Inc. (Baltimore, MD). The preparation of hepatocytes was performed as described previously (Shitara et al., 2003). The cryopreserved human hepatocytes were resuspended in Krebs-Henseleit buffer to give a final cell density of 1.0×10^6 viable cells/ml for the uptake study. The number of viable cells was determined by trypan blue staining. To measure the uptake in the absence of Na^+, sodium chloride and sodium bicarbonate in Krebs-Henseleit buffer were replaced with choline chloride and choline bicarbonate.

Transport study using hepatocytes

Prior to the uptake studies, the cell suspensions were prewarmed at 37ºC for 3 min.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

The uptake studies were initiated by adding an equal volume of buffer (120-200 µl) containing labeled and unlabeled substrates to the cell suspension. After incubation at 37ºC for 0.5, 2 or 5 min, the reaction was terminated by separating the cells from the substrate solution. For this purpose, an aliquot of 80 µl incubation mixture was collected and placed in a centrifuge tube (450 µl) containing 50 µl 2 N NaOH under a layer of 100 µl oil mixture (density, 1.015, a mixture of silicone oil and mineral oil; Sigma-Aldrich, St. Louis, MO), and subsequently the sample tube was centrifuged for 20 s using a standard centrifuge (17500 g, MX-100, TOMY). During this process, hepatocytes passed through the oil layer into the alkaline solution. After an overnight incubation in alkali to dissolve the hepatocytes and then the centrifuge tube was frozen in liquid nitrogen, the centrifuge tube was cut and each compartment was transferred to a scintillation vial. The compartment containing the dissolved cells was neutralized with 50 µl 2 N HCl. The aliquots were mixed with scintillation cocktail and the radioactivity was measured in a liquid scintillation counter.

Estimation of protein unbound concentration of telmisartan in the presence of human serum albumin

The unbound concentration of 1 µM telmisartan in the presence of human serum albumin (HSA) (0, 0.1, 0.3, 1, 3, 5%) was determined after a 2 h incubation at 37ºC by

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

equilibrium dialysis (DIANORM, Dainippon Pharmaceutical Ltd., Osaka, Japan).

Kinetic analyses

Ligand uptake was expressed as the uptake volume (microliters per milligram protein), given as the radioactivity associated with the cells (disintegrations per milligram protein) divided by its concentration in the incubation media (disintegrations per microliter). Specific uptake was obtained by subtracting the uptake into vector-transfected cells from the uptake into cDNA-transfected cells. Kinetic parameters were obtained using the following equation (Eq. 1):

$$v = \frac{V_{\max} \times S}{K_m + S} \qquad \text{(Eq. 1)}$$

where v is the uptake velocity of the substrate (picomoles per minute per milligram protein), S is the substrate concentration in the medium (micromolar), K_m is the Michaelis constant (micromolar) and V_{max} is the maximum uptake rate (picomoles per minute per milligram protein). Fitting was performed by the nonlinear least-squares method using a MULTI program (Yamaoka et al., 1981). The half-inhibitory concentration (IC_{50}) of inhibitors was obtained by examining their inhibitory effects on the uptake of CCK-8, $E_2$17βG and telmisartan based on (Eq. 2).

$$CL_{+I} = CL / \left(1 + \frac{I}{IC_{50}}\right) \qquad \text{(Eq. 2)}$$

where CL and CL_{+I} represent the uptake clearance in the absence and presence of

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

inhibitor, respectively, and I is the concentration of inhibitor. IC_{50} values were estimated by the nonlinear least-squares method using a MULTI program (Yamaoka et al., 1981). To determine the saturable hepatic uptake clearance in rat and human hepatocytes, we first determined the hepatic uptake clearance [$CL_{(2\ \text{min-}0.5\ \text{min})}$] (microliters per minute per 10^6 cells) by calculating the slope of the uptake volume (V_d) (microliters per 10^6 cells) between 0.5 and 2 min (Eq. 3). The hepatic uptake clearance was fitted to Eq. 1 by means of nonlinear least-squares regression analysis using a MULTI program (Yamaoka et al., 1981). The saturable hepatic uptake clearance (CL_{hep}) was determined by subtracting $CL_{(2\ \text{min-}0.5\ \text{min})}$ in the presence of an excess of unlabeled substrate (excess) from that in the absence of unlabeled substrate (tracer) (Eq. 4).

$$CL_{(2\,\text{min}-0.5\,\text{min})} = \frac{V_{d,2\,\text{min}} - V_{d,0.5\,\text{min}}}{2 - 0.5} \qquad \text{(Eq. 3)}$$

$$CL_{hep} = CL_{(2\,\text{min}-0.5\,\text{min}),tracer} - CL_{(2\,\text{min}-0.5\,\text{min}),excess} \qquad \text{(Eq. 4)}$$

Estimation of the maximum unbound concentration of inhibitors at the liver inlet

The maximum unbound concentration at the liver inlet ($I_{in,\ max,\ u}$) was calculated from the following equation (Eq. 5) as described previously (Ito et al., 1998).

$$I_{in,\max,u} = \left(C_{\max,ss,blood} + \frac{k_a \cdot D \cdot F_a}{Q_h} \right) \times f_{u,blood} \qquad \text{(Eq. 5)}$$

where $C_{\text{max, ss, blood}}$ and $f_{\text{u, blood}}$ are estimated by the reported values of the maximum

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

blood concentration of drug after oral administration of the clinical dose (D =160 mg) and the protein unbound fraction (0.005) and f_{blood}/f_{plasma} ratio (about 1.0) in humans (Stangier et al., 2000b; Stangier et al., 2000c). Q_h is the hepatic blood flow rate (96.6 l/h). To avoid the false negative prediction, k_a is set to a theoretically maximum absorption rate constant (6 h^{-1}) and F_a is set to one.

Statistical analysis

The two-tailed Dunnett test was used to assess the significance of differences between three sets of data. Differences were considered to be statistically significant when P was < 0.05.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

Results

Uptake of telmisartan into isolated rat hepatocytes Telmisartan was taken up into isolated rat hepatocytes in a time-dependent manner. While saturation of the uptake of telmisartan by an excess of unlabeled telmisartan (40 μM) was not clearly observed in the incubation media with 0 and 0.1 % HSA, it could be observed in the presence of 0.3-5 % HSA (Figs. 2A-F). Thus, we decided to evaluate the telmisartan uptake with more than 0.3 % of HSA in the incubation media to prevent its extensive adsorption to the cells and culture materials. The protein unbound fraction of telmisartan in the incubation media with 0.3, 1, 3, and 5 % HSA was 0.056, 0.018, 0.006 and 0.004, respectively. Both the uptake and the unbound fraction of telmisartan were reduced in parallel as the concentration of HSA was increased (Fig. 2G). In the presence of 1% HSA, telmisartan was taken up into isolated rat hepatocytes linearly up to 5 min (Fig. 3A). The concentration-dependence of the uptake of telmisartan was studied over concentration range of 0.1 μM to 40 μM in the presence of 1% HSA. Eadie-Hofstee plot showed one-saturable component (Fig. 3B) and the apparent K_m and V_{max} values for telmisartan uptake in the presence of 1% HSA were 21.7 ± 4.4 μM and 371 ± 58 pmol/min/10^6 cells, respectively. Depletion of Na^+ in the incubation media did not affect the uptake of telmisartan (Fig. 4A) and the uptake was inhibited by pravastatin,

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

digoxin and taurocholate, which are substrates and inhibitors of Oatp isoforms, with IC_{50} values of 58.6 ± 15.5, 45.3 ± 11.7, and 300 ± 99 μM, respectively. However, 1 mM TEA, a typical substrate of OCTs, did not affect the uptake of telmisartan (Fig. 4B).

Uptake of telmisartan by transporter-expressing HEK293 cells In the transport study using transporter expression systems, we reduced the HSA concentration from 1% to 0.3% in the incubation media, because only a minimal transport activity of $E_2$17βG, which is used as the probe substrate for OATP1B1, was detected in OATP1B1-expressing cells in the presence of 1% HSA due to the significant decrease in its unbound concentration by binding to HSA (vector-transfected control cells: 2.32 ± 0.21 μl/2 min/mg protein; OATP1B1-expressing cells: 3.95 ± 0.21 μl/2 min/mg protein). To identify which transporters are important for the hepatic uptake of telmisartan in humans, the uptake assay was carried out using OATP1B1- and OATP1B3-expressing HEK293 cells in the presence of 0.3% HSA in the incubation media. Under these conditions, significant uptake of $E_2$17βG by OATP1B1- and OATP1B3-expressing HEK293 cells was observed (Fig. 5B). On the other hand, telmisartan was taken up by OATP1B3, but not by OATP1B1 (Fig. 5A). Because the difference in the degree of uptake between vector- and OATP1B3-transfected cells was too small to assess its saturation kinetics in the presence of 0.3% HSA, the concentration-dependence of

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

telmisartan uptake was evaluated over the concentration range of 0.05 μM to 10 μM in the absence of HSA at 5 min. The K_m and V_{max} values of telmisartan transport by OATP1B3 were calculated to be 0.81 ± 0.18 μM and 6.7 ± 0.91 pmol/min/mg protein, respectively (Fig. 6).

Inhibitory effect of E-sul on OATP1B1- and OATP1B3-mediated uptake of telmisartan in transporter-expression systems The inhibitory effect of E-sul on OATP1B1- and OATP1B3-mediated uptake of $E_2$17βG, CCK-8 and telmisartan was evaluated using OATP1B1- and OATP1B3-expressing HEK293 cells in the presence of 0.3% HSA. E-sul strongly inhibited OATP1B1-mediated $E_2$17βG uptake with an IC_{50} value of 0.79 ± 0.51 μM, while E-sul did not inhibit the OATP1B3-mediated CCK-8 uptake up to 30 μM (IC_{50} = 97.1 ± 37 μM) (Fig. 7A). In addition, the OATP1B3-mediated uptake of telmisartan was not inhibited by 30 μM E-sul (Fig. 7B).

Uptake of telmisartan into cryopreserved human hepatocytes The uptake of 1 μM $E_2$17βG and 0.1 μM telmisartan by three different batches of cryopreserved human hepatocytes (Lot. OCF, 094 and MYO) in the presence of 0.3% HSA was increased from 0.5 to 2 min (uptake of $E_2$17βG and telmisartan by cryopreserved human hepatocytes (OCF): 6.3 ± 1.0 and 56.7 ± 3.6 μl/min/10^6 cells, respectively) and their uptake was reduced in the presence of an excess of unlabeled $E_2$17βG (200 μM) and

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

telmisartan (40 μM) to 0.6 ± 1.1 and 8.2 ± 4.7 μl/min/10^6 cells, respectively. The uptake of $E_2$17βG into human hepatocytes was inhibited by more than half at 30 μM E-sul, whereas that of telmisartan was not significantly inhibited by 30 μM E-sul (Table. 1).

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

Discussion

In the present study, we have shown that telmisartan is likely to be taken up into rat and human hepatocytes by OATP family transporters since the uptake was Na^+-independent and inhibited by some OATP substrates/inhibitors. And we have also suggested that OATP1B3 predominantly contributes the hepatic uptake of telmisartan in human hepatocytes.

Since it is difficult to evaluate the transport of telmisartan in the absence of HSA due to the extensive adsorption of lipophilic telmisartan to cells and/or culture materials, we examined the effect of different concentrations of HSA (0.1-5 %) in the incubation media on the uptake of telmisartan by isolated rat hepatocytes (Figs. 2A-F). In 0.3-5 % HSA, saturable time-dependent uptake of telmisartan was clearly observed. The uptake of telmisartan into isolated rat hepatocytes was almost proportional to the protein unbound concentration of telmisartan in the incubation media, suggesting that the uptake of telmisartan followed the ‘‘free’’ hypothesis in which only unbound ligand can be recognized by transporters (Fig. 2G).

Taking the balance between the absolute uptake amount and the avoidance of extensive adsorption of telmisartan to cells and/or culture materials by HSA into consideration, we decided to use 1 and 0.3 % HSA in the incubation media for the

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

further evaluation of telmisartan uptake by rat and human hepatocytes, respectively.

Initially, we characterized the transport property of telmisartan using isolated rat hepatocytes. Telmisartan was transported into isolated rat hepatocytes in a time- and concentration-dependent manner (Fig. 3). The K_m and V_{max} values of telmisartan uptake into isolated rat hepatocytes and the protein unbound fraction of telmisartan in the presence of 1 % HSA were 21.7 μM, 371 pmol/min/10^6 cells, and 0.018, respectively. Then, the K_m value normalized by the unbound concentration in the incubation media was estimated to be 0.4 μM. To evaluate the non-saturable uptake of telmisartan, we defined 40 μM telmisartan as an excess concentration due to the limited solubility of telmisartan in the incubation media. The uptake of telmisartan into isolated rat hepatocytes was Na^+-independent, indicating that telmisartan is not transported by Na^+-taurocholate co-transporting polypeptide (NTCP), the uptake by which is Na^+-dependent (Fig. 4A). Furthermore, the uptake of telmisartan was inhibited by digoxin, pravastatin and taurocholate with the IC_{50} value of 45.3, 58.6 and 300 μM, respectively. In contrast, a high concentration of TEA (1 mM) did not inhibit telmisartan uptake (Fig. 4B). Taurocholate, pravastatin and digoxin are the substrates and inhibitors of Oatp1a1, 1a4 and 1b2 in rats (Noe et al., 1997; Kouzuki et al., 1999; Tokui et al., 1999; Cattori et al., 2000; Sasaki et al., 2004). It is reported that 100 μM

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

digoxin completely inhibited the Oatp1a4 activity, but at most inhibited Oatp1a1-mediated uptake of digoxin by 70% (Shitara et al., 2002). Based on these results, it appears that telmisartan is taken up into rat hepatocytes by Oatp isoforms, and at least Oatp1a4 is involved in the uptake of telmisartan.

Next, to examine which human OATP transporters are involved in the uptake of telmisartan into human hepatocytes, we evaluated the transport properties of telmisartan using HEK293 cells expressing individual OATPs and cryopreserved human hepatocytes. In this transport study, we reduced the HSA concentration from 1 % to 0.3 % in the incubation media, because no significant uptake of $E_2$17βG, which is used as a probe substrate for OATP1B1, was detected in OATP1B1-expressing cells in the presence of 1 % HSA. Telmisartan could be taken up by OATP1B3, but not OATP1B1 (Fig. 5A). Because the transporter-mediated uptake of telmisartan in the presence of HSA was not high enough to evaluate the saturation kinetics, we investigated the saturable uptake of telmisartan in the absence of HSA. The K_m and V_{max} values of telmisartan uptake by OATP1B3 in the absence of HSA were 0.81 μM and 6.7 pmol/min/mg protein, respectively (Fig. 6). This K_m value was almost comparable with that in isolated rat hepatocytes normalized by the unbound concentration of telmisartan in the incubation media. Telmisartan was taken up into cryopreserved

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

human hepatocytes in a saturable manner. Considering that telmisartan was taken up by OATP1B3, but not OATP1B1, the uptake of telmisartan seems to be mediated by OATP1B3. To confirm the minor contribution of OATP1B1 to the hepatic uptake of telmisartan, we planned to perform an inhibition study using OATP1B1- and OATP1B3-selective inhibitor. From our analyses, E-sul inhibited OATP1B1-mediated $E_2$17βG uptake with an IC_{50} value of 0.8 μM, whereas E-sul did not inhibit OATP1B3-mediated CCK-8 uptake up to 30 μM (Fig. 7A). These results confirmed that 30 μM E-sul can selectively inhibit the OATP1B1-mediated uptake. On the other hand, CCK-8 inhibited both OATP1B1- and OATP1B3-mediated $E_2$17βG uptake with IC_{50} value of 6.79 ± 0.59 and 14.6 ± 2.2 μM, respectively, indicating that CCK-8 cannot be used as a selective inhibitor for OATP1B3 as reported previously (Nozawa et al., 2003). The uptake of telmisartan into OATP1B3-expressing cells and cryopreserved human hepatocytes was not inhibited by 30 μM E-sul (Fig. 7B and Table 1). On the contrary, 30 μM E-sul inhibited more than half of $E_2$17βG uptake in all the batches of cryopreserved human hepatocytes (Table 1). Hirano et al. (2004) have reported that the uptake of $E_2$17βG in human hepatocytes is mediated mainly by OATP1B1. These results suggest that telmisartan is transported into cryopreserved human hepatocytes by OATP1B3 rather than OATP1B1.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

In a previous study, the ratio of the relative expression level of OATP1B1 and OATP1B3 in cryopreserved human hepatocytes to that in expression systems, determined by Western blot analysis, was 1.79 and 0.96, respectively (Hirano et al., 2004). In human liver, OATP2B1 is also expressed on the basolateral membrane (Kullak-Ublick et al., 2001). We checked that telmisartan was significantly taken up into OATP1B3- and OATP2B1-expressing HEK293 cells (2.6 ± 0.4 and 1.7 ± 0.9 μl/5 min/mg protein, respectively). The ratio of the protein expression level of OATP2B1 in human hepatocytes to that in our expression systems was less than 0.2 (Hirano et al., submitted). Therefore, it is suggested that the contribution of OATP2B1 to the hepatic uptake of telmisartan into human hepatocytes is at most one-fifth that of OATP1B3.

In general, it is accepted that OATP1B1 is responsible for the hepatic uptake of several compounds. Hirano et al. (2004) have demonstrated that pitavastatin and $E_2$17βG are taken up mainly via OATP1B1. However, a recent report suggested that fexofenadine, an H_1-receptor antagonist, is transported by OATP1B3 rather than OATP1B1 (Shimizu et al., 2005). Moreover, in the case of valsartan, which is in the same therapeutic class as telmisartan, the contribution of OATP1B1 and OATP1B3 to its hepatic uptake is estimated to be almost similar (Yamashiro et al., submitted). So, the relative contribution of OATP1B1 and OATP1B3 to the hepatic uptake of organic

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

anions depends on the substrate properties and chemical structures, and we cannot a priori decide which transporters are responsible for hepatic uptake without using dedicated experiments for estimating the contribution of each proposed transporter.

The C_{max} value of telmisartan increases disproportionately with the dose (10-160 mg). In clinical situations, 160/25 mg/day telmisartan/hydrochlorothiazide combination therapy is approved for the treatment of hypertension in the USA. The C_{max} values of telmisartan after single and multiple 160 mg doses were 3.0 and 5.6 μM, respectively (Stangier et al., 2000c). Considering that 99.5% of the telmisartan in blood is bound to plasma proteins (Stangier et al., 2000c), the unbound concentration of telmisartan is estimated to be 0.015 and 0.028 μM. These values are more than 20-times lower than the K_m value of telmisartan uptake by OATP1B3 obtained in this study. In addition, to avoid the false negative prediction of the contribution of OATP1B3 on its non-linear pharmacokinetics, we calculated the maximum unbound concentration of telmisartan at the inlet to the liver ($I_{in, max, u}$) to be 0.12 μM after multiple 160 mg dose using a method established (Ito et al., 1998). However, the K_m value of telmisartan uptake by OATP1B3 is still more than 5-times higher than the $I_{in, max, u}$ of telmisartan. If the conventional assumption applies, in which only the unbound drug can interact with OATP1B3, the saturation of OATP1B3-mediated telmisartan uptake seems to have a

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

minor effect on the non-linear increase of C_{max} and AUC over the clinical dose range. Furthermore, a large inter-individual variability in the plasma profile of telmisartan has been observed in clinical situations (Stangier et al., 2000a; Stangier et al., 2000b). Letschert et al. (2004) have reported two naturally-occurring mutations in the *SLCO1B3* gene which cause a substrate-dependent functional change in OATP1B3. These genetic polymorphisms in OATP1B3 may be one of the reasons for the inter-individual variability of the pharmacokinetics of telmisartan. In addition, the glucuronidation process of telmisartan and hepatobiliary transport of telmisartan glucuronide may also affect its inter-individual variability and the further quantitative analyses in each process will be needed.

In conclusion, we have demonstrated that telmisartan is taken up into human hepatocytes by OATP1B3 rather than OATP1B1. In addition, these findings support and further extend the important role of OATP1B3 in overall hepatic elimination of some drugs.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

References

Abe T, Kakyo M, Tokui T, Nakagomi R, Nishio T, Nakai D, Nomura H, Unno M, Suzuki M, Naitoh T, Matsuno S and Yawo H (1999) Identification of a novel gene family encoding human liver-specific organic anion transporter LST-1. *J Biol Chem* **274:**17159-17163.

Cattori V, Hagenbuch B, Hagenbuch N, Stieger B, Ha R, Winterhalter KE and Meier PJ (2000) Identification of organic anion transporting polypeptide 4 (Oatp4) as a major full-length isoform of the liver-specific transporter-1 (rlst-1) in rat liver. *FEBS Lett* **474:**242-245.

Faber KN, Muller M and Jansen PL (2003) Drug transport proteins in the liver. *Adv Drug Deliv Rev* **55:**107-124.

Hagenbuch B and Meier PJ (2003) The superfamily of organic anion transporting polypeptides. *Biochim Biophys Acta* **1609:**1-18.

Hedner T (1999) Management of hypertension: the advent of a new angiotensin II receptor antagonist. *J Hypertens Suppl* **17:**S21-25.

Hirano M, Maeda K, Shitara Y and Sugiyama Y (2004) Contribution of OATP2 (OATP1B1) and OATP8 (OATP1B3) to the hepatic uptake of pitavastatin in humans. *J Pharmacol Exp Ther* **311:**139-146.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

Hsiang B, Zhu Y, Wang Z, Wu Y, Sasseville V, Yang WP and Kirchgessner TG (1999) A novel human hepatic organic anion transporting polypeptide (OATP2). Identification of a liver-specific human organic anion transporting polypeptide and identification of rat and human hydroxymethylglutaryl-CoA reductase inhibitor transporters. *J Biol Chem* **274:**37161-37168.

Ismair MG, Stieger B, Cattori V, Hagenbuch B, Fried M, Meier PJ and Kullak-Ublick GA (2001) Hepatic uptake of cholecystokinin octapeptide by organic anion-transporting polypeptides OATP4 and OATP8 of rat and human liver. *Gastroenterology* **121:**1185-1190.

Ito K, Iwatsubo T, Kanamitsu S, Ueda K, Suzuki H and Sugiyama Y (1998) Prediction of pharmacokinetic alterations caused by drug-drug interactions: metabolic interaction in the liver. *Pharmacol Rev* **50:**387-412.

Kok LD, Siu SS, Fung KP, Tsui SK, Lee CY and Waye MM (2000) Assignment of liver-specific organic anion transporter (SLC22A7) to human chromosome 6 bands p21.2-->p21.1 using radiation hybrids. *Cytogenet Cell Genet* **88:**76-77.

Konig J, Cui Y, Nies AT and Keppler D (2000a) A novel human organic anion transporting polypeptide localized to the basolateral hepatocyte membrane. *Am J Physiol Gastrointest Liver Physiol* **278:**G156-164.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

Konig J, Cui Y, Nies AT and Keppler D (2000b) Localization and genomic organization of a new hepatocellular organic anion transporting polypeptide. *J Biol Chem* **275:**23161-23168.

Kouzuki H, Suzuki H, Ito K, Ohashi R and Sugiyama Y (1999) Contribution of organic anion transporting polypeptide to uptake of its possible substrates into rat hepatocytes. *J Pharmacol Exp Ther* **288:**627-634.

Noe B, Hagenbuch B, Stieger B and Meier PJ (1997) Isolation of a multispecific organic anion and cardiac glycoside transporter from rat brain. *Proc Natl Acad Sci U S A* **94:**10346-10350.

Nozawa T, Tamai I, Sai Y, Nezu J and Tsuji A (2003) Contribution of organic anion transporting polypeptide OATP-C to hepatic elimination of the opioid pentapeptide analogue [D-Ala2, D-Leu5]-enkephalin. *J Pharm Pharmacol* **55:**1013-1020.

Oliverio MI and Coffman TM (1997) Angiotensin-II-receptors: new targets for antihypertensive therapy. *Clin Cardiol* **20:**3-6.

Ries UJ, Mihm G, Narr B, Hasselbach KM, Wittneben H, Entzeroth M, van Meel JC, Wienen W and Hauel NH (1993) 6-Substituted benzimidazoles as new nonpeptide angiotensin II receptor antagonists: synthesis, biological activity, and

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

structure-activity relationships. *J Med Chem* **36:**4040-4051.

Sasaki M, Suzuki H, Aoki J, Ito K, Meier PJ and Sugiyama Y (2004) Prediction of in vivo biliary clearance from the in vitro transcellular transport of organic anions across a double-transfected Madin-Darby canine kidney II monolayer expressing both rat organic anion transporting polypeptide 4 and multidrug resistance associated protein 2. *Mol Pharmacol* **66:**450-459.

Shimizu M, Fuse K, Okudaira K, Nishigaki R, Maeda K, Kusuhara H and Sugiyama Y (2005) Contribution of OATP (organic anion-transporting polypeptide) family transporters to the hepatic uptake of fexofenadine in humans. *Drug Metab Dispos* **33:**1477-1481.

Shitara Y, Li AP, Kato Y, Lu C, Ito K, Itoh T and Sugiyama Y (2003) Function of uptake transporters for taurocholate and estradiol 17beta-D-glucuronide in cryopreserved human hepatocytes. *Drug Metab Pharmacokinet* **18:**33-41.

Shitara Y, Sugiyama D, Kusuhara H, Kato Y, Abe T, Meier PJ, Itoh T and Sugiyama Y (2002) Comparative inhibitory effects of different compounds on rat oatpl (slc21a1)- and Oatp2 (Slc21a5)-mediated transport. *Pharm Res* **19:**147-153.

Stangier J, Su CA, Schondorfer G and Roth W (2000a) Pharmacokinetics and safety of intravenous and oral telmisartan 20 mg and 120 mg in subjects with hepatic

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

impairment compared with healthy volunteers. *J Clin Pharmacol* **40:**1355-1364.

Stangier J, Schmid J, Turck D, Switek H, Verhagen A, Peeters PA, van Marle SP, Tamminga WJ, Sollie FA and Jonkman JH (2000b) Absorption, metabolism, and excretion of intravenously and orally administered [14C]telmisartan in healthy volunteers. *J Clin Pharmacol* **40:**1312-1322.

Stangier J, Su CA and Roth W (2000c) Pharmacokinetics of orally and intravenously administered telmisartan in healthy young and elderly volunteers and in hypertensive patients. *J Int Med Res* **28:**149-167.

Tamai I, Nezu J, Uchino H, Sai Y, Oku A, Shimane M and Tsuji A (2000) Molecular identification and characterization of novel members of the human organic anion transporter (OATP) family. *Biochem Biophys Res Commun* **273:**251-260.

Tokui T, Nakai D, Nakagomi R, Yawo H, Abe T and Sugiyama Y (1999) Pravastatin, an HMG-CoA reductase inhibitor, is transported by rat organic anion transporting polypeptide, oatp2. *Pharm Res* **16:**904-908.

Vavricka SR, Van Montfoort J, Ha HR, Meier PJ and Fattinger K (2002) Interactions of rifamycin SV and rifampicin with organic anion uptake systems of human liver. *Hepatology* **36:**164-172.

Wienen W, Entzeroth M, van Meel JCA, Stangier J, Busch U, Ebner T, Schmid J,

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

Lehmann H, Matzek K, Kempthorne-Rawson J, Gladigau V and Huel NH (2000) A review on telmisartan: a novel. long-acting angiotensin II-receptor antagonist. *Cardiovascular drug reviews* **18:**127-156.

Yamaoka K, Tanigawara Y, Nakagawa T and Uno T (1981) A pharmacokinetic analysis program (multi) for microcomputer. *J Pharmacobiodyn* **4:**879-885.

Yamazaki M, Suzuki H, Hanano M, Tokui T, Komai T and Sugiyama Y (1993) Na(+)-independent multispecific anion transporter mediates active transport of pravastatin into rat liver. *Am J Physiol* **264:**G36-44.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

Footnotes

This work was partly supported by a Health and Labour Sciences Research Grants from Ministry of Health, Labour and Welfare for the Research on Advanced Medical Technology and Grant-in Aid for Young Scientists (B) (17790113) from the Ministry of Education, Culture, Sports, Science and Technology.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

Legends for Figures

Figure 1: Chemical structure of [^{3}H]telmisartan. The asterisk denotes the position of the [^{3}H]-label.

Figure 2: Effect of various concentrations of human serum albumin on the uptake of telmisartan in isolated rat hepatocytes and the unbound fraction of telmisartan. Uptake of telmisartan was measured by incubating cells with 0.1 μM (closed circle) and 40 μM (open circle) telmisartan, and saturable uptake of telmisartan by isolated rat hepatocytes was determined using Eqs 3 and 4. HSA concentrations used were 0 (A), 0.1 (B), 0.3 (C), 1 (D), 3 (E), and 5 % (F). (G) Triangles and circles represent the uptake of telmisartan into isolated rat hepatocytes (μl/min/106 cells) and unbound fraction of telmisartan, respectively. Each point represents the mean ± S.E. of 3 separate determinations.

Figure 3: Time profile (A) and Eadie-Hofstee plot (B) of the uptake of telmisartan by isolated rat hepactocytes in the presence of 1% HSA. (A) Substrate concentrations used were 0.1 (closed circles) and 40 μM (open circles). (B)The uptake of telmisartan in isolated rat hepatocytes was measured at a concentration between 0.1 and 40 μM telmisartan. The initial uptake rate of telmisartan in isolated rat hepatocytes was determined using (Eq. 3). The solid line represents the fitted curve.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

Each point represents the mean ± S.E. of 3 separate determinations.

Figure 4: Effect of Na^+ ion (A) and various compounds (B) on the uptake of telmisartan in isolated rat hepatocytes in the presence of 1% HSA. The substrate concentration used was 0.1 μM. Saturable uptake of telmisartan by isolated rat hepatocytes was determined using (Eqs 3 and 4). (B) Data are shown as the percentage of the saturable uptake of telmisartan in the absence of inhibitors. Squares, triangles, circles, and diamonds represent the uptake of telmisartan in the presence of pravastatin, digoxin, taurocholate, and tetraethylammonium, respectively. Solid lines represent the fitted curves obtained by nonlinear regression analysis. Each bar and point represents the mean ± S.E. of 3 separate determinations.

Figure 5: Time profiles of the uptake of telmisartan (A) and $E_2$17βG (B) into transporter-expressing cells. The substrate concentrations of telmisartan and $E_2$17βG used were 0.1 μM. Squares, triangles and circles represent the uptake by OATP1B3-, OATP1B1- and vector-transfected cells. The uptake of telmisartan and $E_2$17βG was evaluated in the presence and absence of 0.3% HSA, respectively. *: a significant difference ($P < 0.05$) from the uptake by vector-transfected cells. Each point represents the mean ± S.E. of 3 separate determinations.

Figure 6: Time profile and Eadie-Hofstee plot of the uptake of telmisartan into

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

transporter-expressing cells in the absence of HSA. (Inset) The substrate concentration used was 0.1 μM. Squares, triangles and circles represent the uptake by OATP1B3-, OATP1B1- and vector-transfected cells. The uptake of telmisartan by transporter-expressing cells was measured at a concentration between 0.05 and 10 μM telmisartan in the absence of HSA. The OATP1B3-mediated telmisartan transport was obtained by subtracting the uptake in vector-transfected cells from that in OATP1B3-expressing cells for 5 min. *: a significant difference ($P < 0.05$) from the uptake by vector-transfected cells. Each point represents the mean ± S.E. of 3 separate determinations.

Figure 7: Inhibitory effect of E-sul on OATP1B1-mediated $E_2$17βG uptake (A, circle) and OATP1B3-mediated CCK-8 uptake (A, triangle) and OATP1B3-mediated telmisartan uptake (B) in the presence of 0.3% HSA. The substrate concentration used was 0.1 μM for all compounds. The OATP1B1- and OATP1B3-mediated transport was obtained by subtracting the uptake in vector-transfected cells from that in OATP1B1- or OATP1B3-expressing cells for 2 min for $E_2$17βG or 5 min for CCK-8 and telmisartan. Data are shown as the percentage of the OATP1B1- and OATP1B3-mediated substrate uptake in the absence of E-sul. The solid lines in (A) represent the fitted curves obtained by nonlinear regression analysis. Each bar and

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

point represents the mean ± S.E. (n=3).

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

Table

Table 1 Effect of E-sul on the uptake of telmisartan and $E_2$17βG by cryopreserved human hepatocytes in the presence of 0.3% HSA.

	HH-OCF		HH-094		HH-MYO	
E-sul (μM)	Telmisartan	$E_2$17βG	Telmisartan	$E_2$17βG	Telmisartan	$E_2$17βG
	μl/min/10^6 cells		μl/min/10^6 cells		μl/min/10^6 cells	
0	48.5±3.6	5.66±1.1	42.0±1.7	1.85±0.72	16.8±5.3	2.01±0.62
30	63.9±11	1.50±0.75	42.2±0.41	1.03±0.76	18.1±3.7	0.38±0.45
	(131%)	(26.6%)	(100%)	(55.8%)	(108%)	(19.0%)

The substrate concentration used was 0.1 and 1 μM for telmisartan and $E_2$17βG. Saturable uptake of telmisartan and $E_2$17βG into cryopreserved human hepatocytes was determined after the subtraction of non-saturable uptake (evaluated as the uptake clearance of the respective compounds in the presence of 40 μM telmisartan and 200 μM $E_2$17βG). In parenthesis is the percentage of the saturable uptake of telmisartan and $E_2$17βG in the absence of inhibitor.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

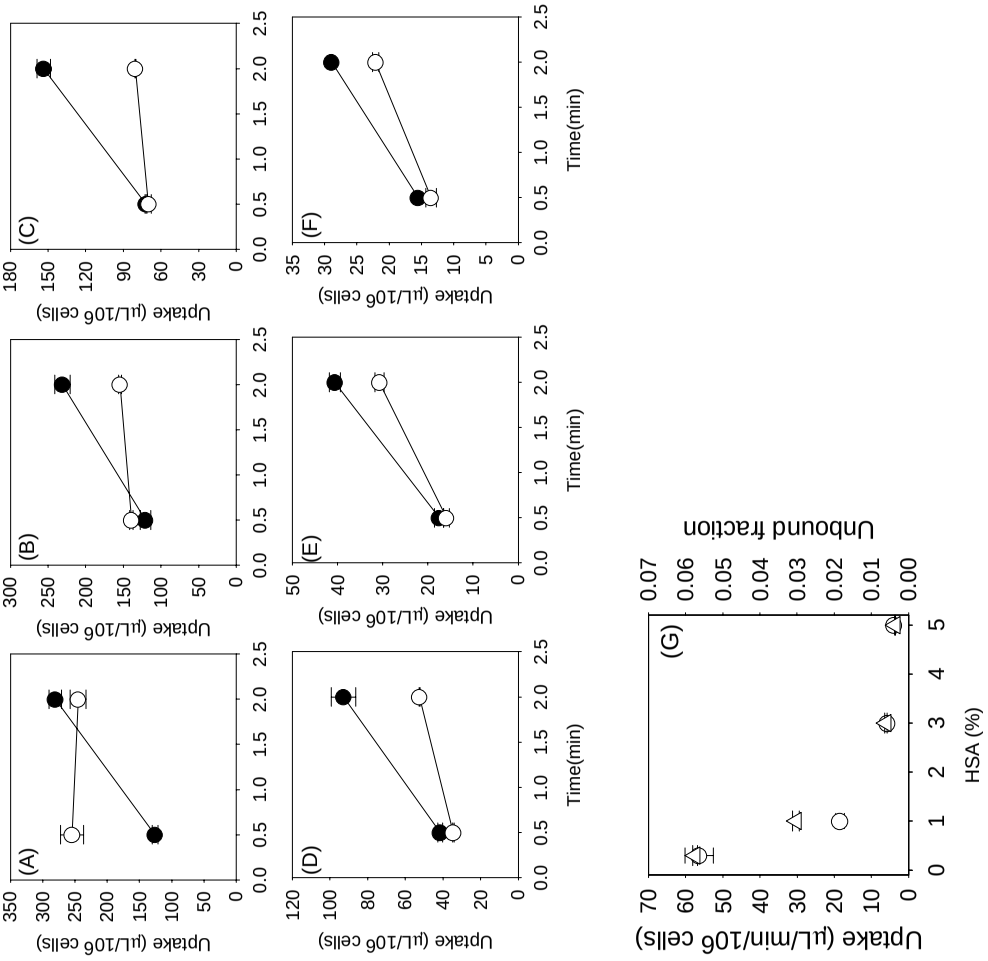
DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 1

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

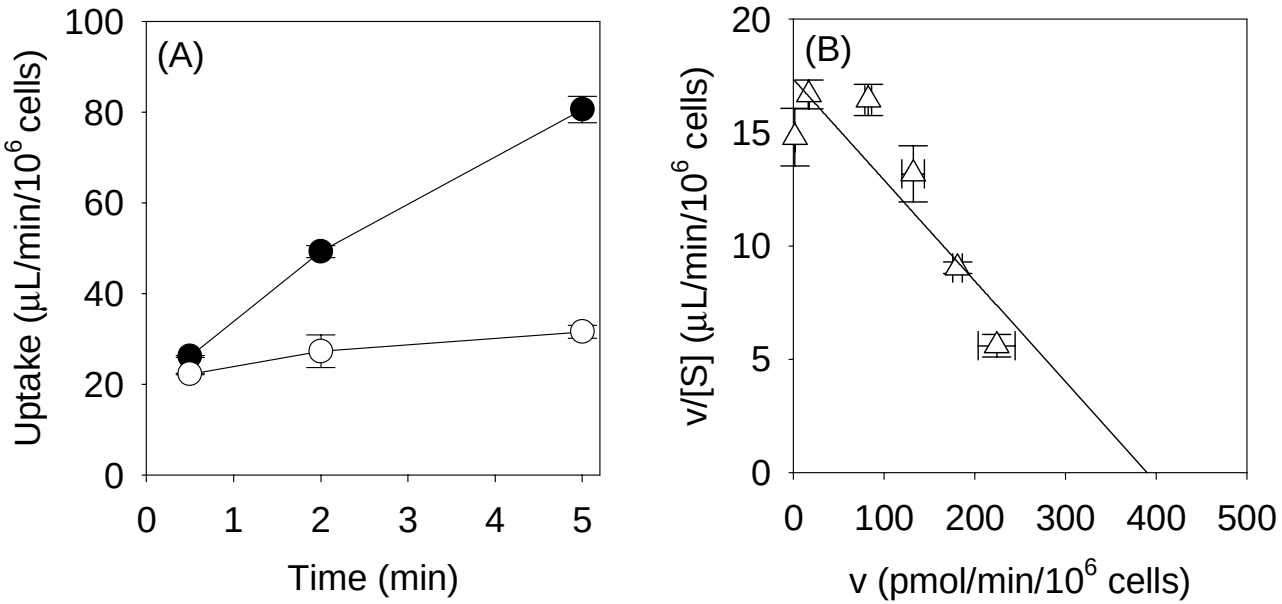
DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 2



Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

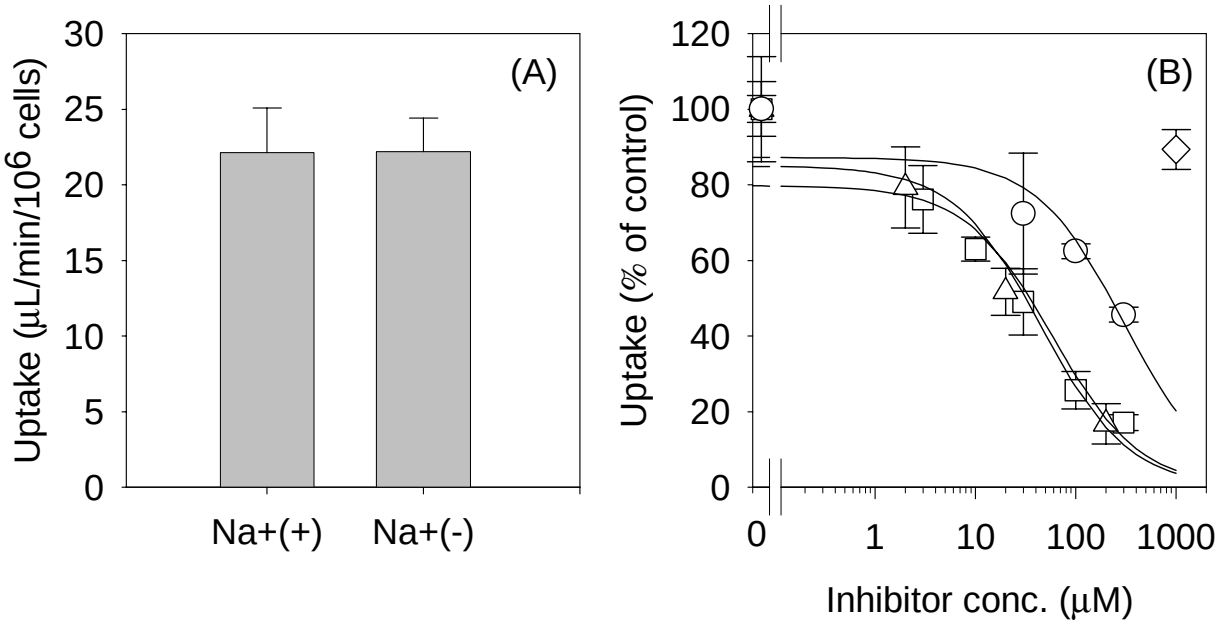
Figure 3



DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

Figure 4

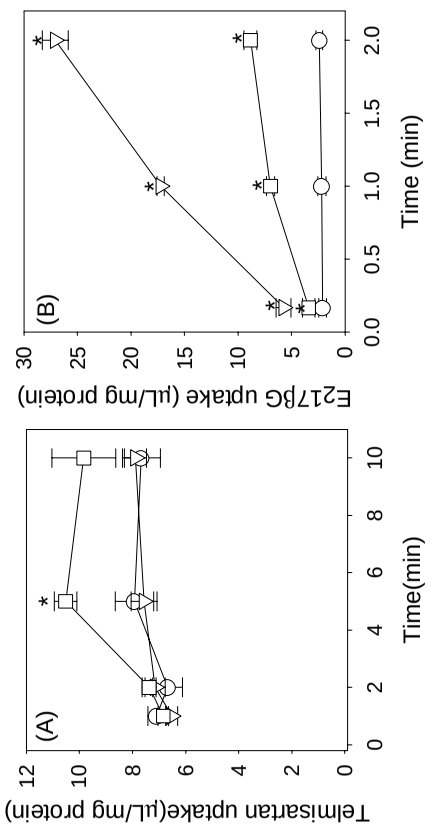


DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

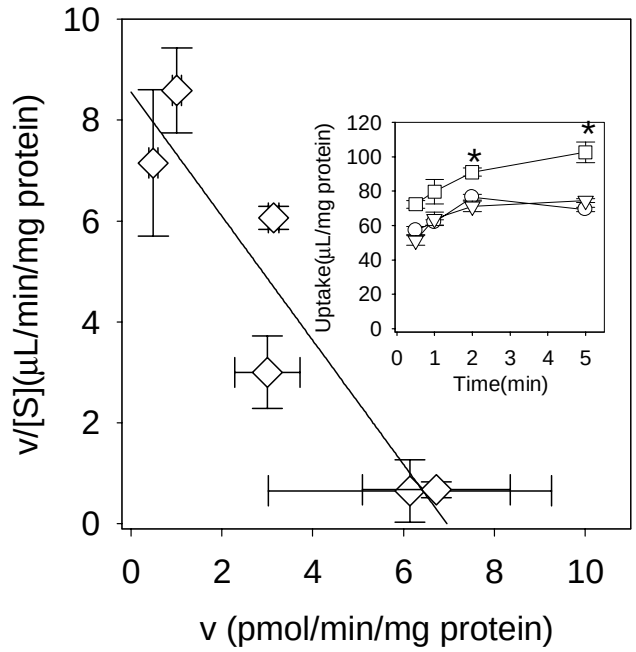
DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 5



Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

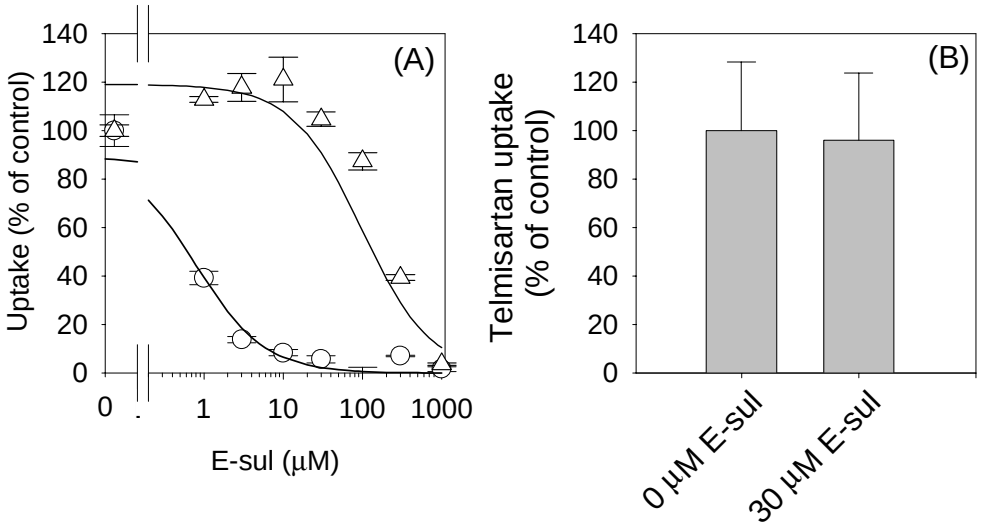
Figure 6



DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024

Figure 7



DMD Fast Forward. Published on April 12, 2006 as DOI: 10.1124/dmd.105.009175
This article has not been copyedited and formatted. The final version may differ from this version.

Downloaded from dmd.aspetjournals.org at ASPET Journals on April 16, 2024