Evaluation of Organic Anion Transporting Polypeptide 1B1 and 1B3 Humanized Mice as a Translational Model to Study the Pharmacokinetics of Statins


ABSTRACT

Organic anion transporting polypeptide (Oatp) 1a/1b knockout and OATP1B1 and -1B3 humanized mouse models are promising tools for studying the roles of these transporters in drug disposition. Detailed characterization of these models will help to better understand their utility for predicting clinical outcomes. To advance this approach, we carried out a comprehensive analysis of these mouse lines by evaluating the compensatory changes in mRNA expression, quantifying the amounts of OATP1B1 and -1B3 protein by liquid chromatography–tandem mass spectrometry, and studying the active uptake in isolated hepatocytes and the pharmacokinetics of some prototypical substrates including statins. Major outcomes from these studies were 1) mostly moderate compensatory changes in only a few genes involved in drug metabolism and disposition, 2) a robust hepatic expression of OATP1B1 and -1B3 proteins in the respective humanized mouse models, and 3) functional activities of the human transporters in hepatocytes isolated from the humanized models with several substrates tested in vitro and with pravastatin in vivo. However, the expression of OATP1B1 and -1B3 in the humanized models did not significantly alter liver or plasma concentrations of rosuvastatin and pitavastatin compared with Oatp1a/1b knockout controls under the conditions used in our studies. Hence, although the humanized OATP1B1 and -1B3 mice showed in vitro and/or in vivo functional activity with some statins, further characterization of these models is required to define their potential use and limitations in the prediction of drug disposition and drug-drug interactions in humans.

Introduction

The human organic anion transporting polypeptides (OATPs) 1B1 and 1B3 are members of the OATP class of proteins, which are encoded by the SLCO (Solute Carrier Organic Anion) gene family.

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ABBREVIATIONS: AUC, area under the curve; AUCpo, AUC determined from oral administration; BSP, bromosulfophthalein; CCK-8, [3H] cholecystokinin octapeptide; Ces, carboxylesterase; CsA, cyclosporin A; DDI, drug-drug interaction; E2/17βG, [3H] estradiol-17β-D-glucuronide; FVB, Friend Virus B; hOATP1B1, OATP1B1 humanized mice; hOATP1B3, OATP1B3 humanized mice; hOATP1B1/1B3, OATP1B1 and -1B3 double humanized mice; NTCP, Na+-taurocholate cotransporting; OATP, organic anion transporting polypeptide; Oatp1a/1b KO, Slco1a/1b gene cluster knockout mice; OAT, Organic Cation Transporter; PBS, phosphate-buffered saline; PO, orally; qRT-PCR, quantitative real-time polymerase chain reaction; Rif SV, rifamycin SV; SLCO, Solute Carrier Organic Anion; TCA, taurocholic acid; WT, wild type.
with an increased risk of statin-induced myopathy in a genome-wide association study (Link et al., 2008). Accordingly, assessment of the potential for DDIs of new chemical entities with OATP1B1 and -1B3 has been recommended during drug development (Giacomini et al., 2010; http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129606.pdf).

The extrapolation of in vitro results to in vivo models is challenging, and the predictability of traditional preclinical animal models is limited by the specific species differences in the level of sequence identity, expression pattern, and substrate specificity of OATP1B proteins (Hagenbuch and Meier, 2003; Chu et al., 2013a; Grime and Paine, 2013). In addition, human livers express OATP1B1, -1B3, and -2B1, whereas mouse livers contain Oatp1a1, -1a4, -1b2, and -2b1. In an attempt to overcome these limitations, humanized mouse models for OATP1B1 or -1B3 have been generated (van de Steeg et al., 2012) by crossing OATP1B1 and -1B3 transgenic mice to a mouse model lacking all mouse Scl/aa and -ib genes (Oatp1a1/ib KO) (van de Steeg et al., 2012). In these models, the human transporters are expressed under the control of the liver-specific apoE (apolipoprotein E) promoter, and it was estimated by Western blot analysis that the hepatic expression levels of the transgenic proteins were similar to those seen in pooled human liver samples. However, the protein levels were not determined quantitatively. Furthermore, the expression of both human transporters reversed the significant increase in plasma and urine levels of bilirubin monoglucuronide and bilirubin diglucuronide observed in Oatp1a1/ib KO mice (van de Steeg et al., 2012). These results demonstrated 1) the in vivo role of human OATP1B1 and -1B3 in bilirubin glucuronide uptake and 2) the functional activity of the transgenic transporters in the humanized models. Similarly, subsequent studies in the humanized models showed partial reversal of the changes in liver and/or plasma levels observed in Oatp1a1/ib KO animals versus wild-type (WT) controls with the anticancer drug methotrexate (van de Steeg et al., 2013) and the glucuronic acid metabolite of sorafenib (Zimmerman et al., 2013). Transgenic OATP1B3, but not OATP1B1, also was found to correlate with transport of paclitaxel in vivo (van de Steeg et al., 2013). Furthermore, three different statins (pravastatin, atorvastatin, and simvastatin) and carbamazepine were very recently tested in Oatp1a1/ib KO, hOATP1B1, and hOATP1B3 mice, and it was concluded that systemic exposure and liver distribution changes in the humanized versus knockout mice may be used to predict the impact of the human transporters on clinical pharmacokinetics if the differences in protein expression of OATP1B1 and -1B3 in the humanized mice versus human liver are taken into account (Higgins et al., 2014).

Despite these reports, a more comprehensive characterization of the humanized models and understanding of the pharmacokinetics of different OATP1B substrates in these mice are needed before they can be used for mechanistic understanding and translational studies in drug development. To this end, we investigated potential compensatory gene expression changes in the humanized OATP1B1 and -1B3 mice by mRNA analysis, quantified the protein amount of OATP1B1 and -1B3 in the liver of the humanized mice and compared those with human livers, tested various OATP1B substrates in isolated hepatocytes, and conducted pharmacokinetic studies of three hydrophilic statins: pravastatin, rosuvastatin, and pitavastatin, which are recommended OATP1B probe substrates for clinical DDI studies (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm292362.pdf). Furthermore, we generated an OATP1B1/1B3 double humanized mouse model (hOATP1B1/1B3) to allow the assessment of the combined role of both transporters in the disposition of substrates in vivo, and we included these mice in some of our studies.

### Materials and Methods

**Chemicals and Reagents**

**Protein Quantification.** The ProteoExtract native membrane protein extraction kit was purchased from Calbiochem (Temecula, CA). The bicinchoninic acid assay kit and the in-solution trypsin digestion kit were purchased from Pierce Biotechnology (Rockford, IL). Synthetic surrogate peptides and the corresponding stable isotope labeled internal standards for human OATP1B1 and OATP1B3 were obtained from New England Peptides (Boston, MA) and Thermo Fisher Scientific (Rockford, IL), respectively. High-performance liquid chromatography-grade acetonitrile and formic acid were purchased from Fischer Scientific (Fair Lawn, NJ) and Sigma-Aldrich (St. Louis, MO), respectively.

**In Vitro Studies.** [3H] Estradiol-17β-glucuronide (E217βG) (34.3 Ci/mmol), [3H] cholecytokinin octapeptide (CCK-8) (96 Ci/mmol), and [3H] taurocholic acid (5 Ci/mmol) were purchased from PerkinElmer Life Sciences (Boston, MA). [3H] Pitavastatin (5 Ci/mmol), [3H] rosuvastatin (10 Ci/mmol), [3H] pravastatin (5 Ci/mmol), [3H] atorvastatin (10 Ci/mmol), and [14C] tetrathyllumonium (TEA) (0.055 Ci/mmol), as well as unlabeled pitavastatin, rosuvastatin, pravastatin, and atorvastatin were purchased from American Radiolabeled Chemicals (St. Louis, MO), Rifiarycin SV (RIF SV), rifampin, and cyclosporin A (CsA) were purchased from Sigma-Aldrich. Bromosulfophthalain (BSP) was purchased from MP Biomedicals (Solon, OH).

**In Vivo Studies.** Pravastatin sodium, rosuvastatin, and pitavastatin were purchased from Toronto Research Chemicals (Toronto, Canada), Sequoia Research Products (Berkshire, UK), and Allichem LLC (Baltimore, MD), respectively. All other reagents were commercially obtained with the highest analytical purity grade.

**Mouse Models, Husbandry, and Experimentation**

Oatp1a1/ib KO, hOATP1B1, and hOATP1B3 mice were described previously (van de Steeg et al., 2010, 2012, 2013). hOATP1B1/1B3 mice on a mouse Scl/aaib gene cluster knockout background were generated from hOATP1B1 and hOATP1B3 mice by breeding. Homozygous males from each strain and age-matched WT controls, all on FVB (Friend Virus B) genetic background, were obtained from Taconic Farms, Inc. (Hudson, NY). Animals were allowed to acclimatize for at least 5 days prior to an experimental procedure at all experimental locations. Mice were kept in accord with local laws and regulations and in temperature-controlled environments with a 12-hour light cycle and were given standard diets and water ad libitum. All animal procedures were approved by the local Institutional Animal Care and Use Committees.

**mRNA Measurements**

**Quantitative Real-Time Polymerase Chain Reaction: RNA Extraction and Reverse-Transcriptase Polymerase Chain Reaction.** Total RNA was extracted from the livers of WT, Oatp1a1/ib KO, hOATP1B1, and hOATP1B3 mice (n = 3/genotype) with the RNeasy Mini Kit (Qiagen, Valencia, CA), according to the kit instructions, and stored at −80°C after isolation. Total RNA (up to 1 μg) was reverse transcribed using the SuperScript VILO cDNA Synthesis Kit (Life Technologies, Carlsbad, CA) according to the manufacturer’s instructions. The resulting cDNA samples were stored at −80°C.

Real-time polymerase chain reaction (RT-PCR) reactions were carried out in 96-well reaction plates in a volume of 10 μl using the TaqMan Fast Universal Master Mix, 5 ng of template cDNA, and TaqMan probes (Life Technologies) for mouse and human genes listed in Fig. 1. Reactions were run in duplicate for each gene per sample on an Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA) with the following profile: 95°C for 20 seconds followed by 40 cycles of 95°C for 3 seconds and 60°C for 30 seconds. The relative expression of each gene was calculated based on the ΔΔCt comparative expression method (Pfaffl, 2001). The ΔCt values for all the genes in each sample were calculated by subtracting the mean Ct for two non-targeting housekeeping genes (Gapdh and β-actin) from the Ct for each target gene. The relative quantity of each gene was then determined by calculating 2-ΔΔCt.

**Microarray Analysis.** Animals were euthanized via CO2 asphyxiation, and a midline incision was made. The median lobe of the liver was removed, and 400-mg tissue pieces were snap frozen in liquid nitrogen. Following...
weighing, the frozen tissue was homogenized in Buffer RLT from a Qiagen Miniprep kit (Qiagen, Alameda, CA). Homogenized lysate was transferred to a clean conical tube and centrifuged at 5100 rpm for 10 minutes. Seventy percent ethanol was added to the recovered supernatant and centrifuged for 5 minutes in a spin column. RNA on the column filter was washed and recovered from the filter. Samples were stored at $-80^\circ$C until reverse transcription. Fifty nanograms of total RNA was reverse transcribed and amplified using the Ovation RNA Amplification System V2 (NuGEN, San Carlos, CA). cDNA was purified using Agencourt magnetic beads (Beckman Coulter, Brea, CA) and normalized to 17 ng/µl on a Caliper SciClone ALH3000 robotic workstation (PerkinElmer, Waltham, MA). cDNA was then fragmented and labeled for hybridization using the Encore Biotin Module (NuGEN) according to the manufacturer’s protocols. Then 1.875 µg of prepared target cDNA was hybridized to HT MG-430 PM arrays (Affymetrix, Santa Clara, CA) for 16 hours at 48°C. Arrays were washed, stained, and scanned using a GeneTitan instrument with MC Scanner (Affymetrix) according to manufacturer’s protocols. Raw data for each sample were saved in standard CEL file format. The microarray raw data (CEL files) were processed and analyzed using ArrayStudio version 6.0 software (Omicsoft, Cary, NC). The expression intensity of genes in each sample was extracted from CEL files using RMA algorithms and normalized across all samples using quantile normalization. Data quality was assessed by intensity distribution of each sample and Pearson correlation between samples in each genetic variation group. The average correlation in each group was 0.99, and all samples passed data quality assessment. Differential gene expression was identified by applying a general linear model that compares different genetic variations on normalized and log-2–transformed intensity data. Adjusted $P$ value (Benjamini-Hochberg procedure) ($P < 0.05$) and expression fold change $> 2$ were applied as cutoffs for selecting significant genes.

**OATP1B1 and -1B3 Humanized Mice**

Mice were euthanized at Taconic Farms, Inc. by exposure to a rising concentration of CO$_2$, and livers from 8- to 12-week-old male Oatp1a/1b KO...
Liver-to-blood ratio determinations, six mice from each strain were given a PO processed as described for the i.v. administration. For liver concentration and analysis. After a 7-day washout period, the same mice received a PO (5 mg/kg) was added to 60 mL of radiolabeled substrate compounds. The reaction mixtures were incubated at 37°C for the time indicated, and uptake was stopped by the addition of ice-cold PBS, followed by immediate centrifugation for 1 minute at 3000g at 4°C (model 5180R; Eppendorf, Hamburg, Germany) and washing of the cell pellets with ice-cold PBS three times. Cell pellets were resuspended in 50 mM acetonitrile, scintillation fluid (ScintiSafe Econo 2; Fisher Chemicals, Pittsburgh, 4°C (model 5180R; Eppendorf, Hamburg, Germany) and washing of the cell lines and with numbers per dose group as described in

In Vitro Studies
Isolation of Primary Mouse Hepatocytes. Fresh primary mouse hepatocytes were isolated from male WT (FVB), Oatplai/b KO, hOATP1B1, and hOATP1B3 mouse livers (abbreviated as WT, Oatplai/b KO, hOATP1B1, and hOATP1B3 hepatocytes, respectively) using a two-step collagenase perfusion method (Li et al., 2010). In brief, mice were anesthetized with 50 mg/ml nembutal. A catheter was placed into the inferior vena cava with flow (5 ml/min) through the portal vein. At 37°C, the first solution containing 0.5 mM EGTA in Earle’s basic salt solution without Ca2+/Mg2+ was perfused to weaken the intercellular junctions of liver cells by removing extracellular calcium ions. The second solution containing 300 µM collagenase IV with Ca2+/Mg2+ in Hanks’ basic salt solution was used to break down the extracellular compartment to easily release both nonparenchymal and parenchymal cell fractions. The isolated hepatocyte suspension was obtained after mechanical dissociation, filtration, and low-speed centrifugation. Following isolation of cells and 36% percoll purification, cell viability was measured (~70%), and hepatocytes were then prepared for uptake studies.

Uptake Studies in Hepatocytes. Uptake studies were conducted as previously described (Chu et al., 2013b) in fresh mouse hepatocyte suspensions. In brief, cells were resuspended in Krebs-Henseleit modified buffer (pH 7.4; Sigma-Aldrich) in 96-deep-well plates (Analytical Sales, Pompton Plains, NJ) at a final concentration of 0.2 x 10^6 cells/well. The cells and dosing solution were preincubated at 37°C for 5 minutes. Uptake studies were initiated by the addition of 50 µM of radioabeled substrate compounds. The reaction mixtures were incubated at 37°C for the time indicated, and uptake was stopped by the addition of ice-cold PBS, followed by immediate centrifugation for 1 minute at 3000g at 4°C (model 5180R; Eppendorf, Hamburg, Germany) and washing of the cell pellets with ice-cold PBS three times. Cell pellets were resuspended in 50 mM acetonitrile, scintillation fluid (ScintiSafe Econo 2; Fisher Chemicals, Pittsburgh, PA) was added, and radioactivity was determined by liquid scintillation counting in a LS6500 Multipurpose Scintillation Counter (Beckman Coulter). Inhibitory effect of several prototypical inhibitors of hepatic uptake transporters, including rifampycin SV, rifampin, cyclosporin A, BSP, and quinidine (Niem et al., 2011), on uptake of test compounds was also measured at 3 minutes at 37°C.

In Vivo Studies
Pravastatin. Nine- to 14-week-old mice from each of the different mouse lines and with numbers per dose group as described in Results were used in the studies. Pravastatin was administered in saline either i.v. or orally (PO) at 5 mg/kg. A crossover design was used in the pharmacokinetic studies; four mice from each strain were given an i.v. dose of 5 mg/kg pravastatin, and 15 µl of blood was taken via tail nick at 0.033, 0.83, 0.25, 0.5, 1, and 2 hours postdose. Blood was added to 60 µl of EDTA water then vortexed and stored at ~80°C until analysis. After a 7-day washout period, the same mice received a PO (5 mg/kg) dose of pravastatin. Following oral administration, blood was collected and processed as described for the i.v. administration. For liver concentration and liver-to-blood ratio determinations, six mice from each strain were given a PO dose (5 mg/kg). Animals were euthanized 5 or 30 minutes postdose by terminal bleeding through cardiac puncture under isoflurane anesthesia, and tissues were isolated. Liver was rinsed in phosphate-buffered saline, weighed, and stored at ~80°C until analysis. Approximately 200 µl of blood was collected into a microtainer containing EDTA and centrifuged at 11,000 rpm for 5 minutes, and plasma was collected and stored at ~80°C until analysis. Seventy-five microliters of blood was added to 300 µl of EDTA water, vortexed, and stored at ~80°C until analysis.

Rosuvastatin. Four mice per strain (WT, Oatplai/b KO, hOATP1B1, and hOATP1B3) were used for i.v. or PO administration of rosuvastatin. For i.v. bolus administration, the rosuvastatin dose was 5 mg/kg, in 2% N-methyl-2-pyrrolidone:10% Solutol via tail vein. Following a 2-week washout period, rosuvastatin was administered orally at 15 mg/kg in 0.5% hydroxypropyl methylcellulose to the same groups of mice through a gavage needle. Serial blood sampling procedures were followed as described by Peng et al. (2009). Plasma samples were collected at 2, 5, 15, and 30 minutes and 1, 2, 6, and 24 hours following i.v. administration, and at 5, 15, and 30 minutes and 1, 2, 6, and 24 hours after PO dosing (Peng et al., 2009).

Pitavastatin, hOATP1B1, hOATP1B3, Oatplai/b KO, and FVB WT mice (9, 9, 9, and 12 animals) were dosed at 5 mg/kg PO. Blood samples were collected via composite sampling with three animals per group through microsampling from the saphenous vein at 6, 15, and 30 minutes and 1, 2, 3, 5, and 8 hours. At 15 minutes, 2, 5, 15, and 30 minutes, the groups of animal were deeply anesthetized with isoflurane and euthanized; terminal bleeds taken from cardiac puncture and liver was isolated and stored at ~20°C until use. At each time point in both studies, blood samples were centrifuged at 4000 rpm for 10 minutes, and plasma was transferred to Micronic PP tubes (Micronic, Asten, PA) and stored at ~20°C until use.

Liquid Chromatography–Tandem Mass Spectrometry Analysis
Details on the measurement of pravastatin, rosuvastatin, and pitavastatin blood, plasma, liver, and/or urine concentration using liquid chromatography–tandem mass spectrometry are provided in the Supplemental Materials and Methods.

Pharmacokinetic Analysis
Pharmacokinetic parameters were calculated by noncompartmental methods as described in Gibaldi and Perrier (1982) using Phoenix WinNonlin version 6.3 or WinNonlin 5.2 (Pharsight Corporation, Mountain View, CA).

Statistical Analysis
Student’s t test (P < 0.01) or one-way analysis of variance was applied to determine statistical significance in in vitro hepatocyte uptake studies (compared with Oatplai/b KO mice in uptake time course studies and corresponding no inhibitor control in inhibition studies). Pharmacokinetic parameters obtained with pravastatin and rosuvastatin were compared between the Oatplai/b KO mice and each of the other mouse strains by t test and overall through one-way analysis of variance. Values were considered statistically different when P < 0.05. Analyses were performed using Microsoft Excel (Microsoft, Redmond, WA) or GraphPad Prism 5 (GraphPad Software, La Jolla, CA).

Results
Gene Expression Changes in Oatplai/b KO, hOATP1B1, and hOATP1B3 Mice
To better understand potential compensatory changes in the expression of genes encoding proteins involved in drug metabolism and disposition, we compared hepatic mRNA levels of selected genes by quantitative real-time PCR (qRT-PCR) as well as the whole genome by microarray analysis between WT, Oatplai/b KO, hOATP1B1, and hOATP1B3 mice.

qRT-PCR. Liver expression of genes was determined by qRT-PCR for 17 transporters and 9 drug-metabolizing enzymes selected for their involvement in drug transport or reported expression change in KO and humanized mice (van de Steeg et al., 2013). As expected, Slco1a1,
Slco1a4, and Slc10a2 were only detected in WT mice, whereas Slco1b1 and Slco1b3 were only detected in hOATP1B1 and hOATP1B3 mice, respectively (Fig. 1). This confirmed the successful deletion of mouse Slco1b genes in the liver of Oatp1a1/b KO, hOATP1B1, and hOATP1B3 mice. Overall, few genes showed altered expression in the absence and/or presence of different Slco/SLCO transporters. Of the remaining 13 transporter genes, which were analyzed, only Abcb1b showed an altered expression pattern, with mRNA expression increased by 4- to 6-fold in Oatp1a1/b KO and both strains of humanized mice relative to WT mice. Five metabolizing enzyme genes had altered expression patterns (2- to 2-fold change) across the different strains of mice (Fig. 1). Aox1 (Aldehyde Oxidase 1 gene; 2- to 3-fold reduction), carboxylesterase (Ces) 1c (2- to 200-fold reduction), Ces1d (3- to 8-fold reduction), Cesle (3- to 19-fold reduction), and Ces2a (2-fold reduction) all had reduced mRNA expression in Oatp1a1/b KO and both strains of humanized mice relative to WT mice. The strongest reduction in Ces gene expression was observed in hOATP1B1 followed by hOATP1B3 mice. Interestingly, Oatp1a1/b KO mice showed increased levels of hepatic Ces1 expression in the study by Iusuf et al. (2014), which were normalized in hOATP1B1 and hOATP1B3 mice, whereas we found unchanged or slightly decreased Ces1 levels in Oatp1a1/b KO mice in our analysis and a more pronounced repression in the humanized mice (Fig. 1; Supplemental Table 1). This discrepancy might be explained by the different origins of the mouse lines (Netherlands Cancer Institute versus Taconic Farms, Inc.) and the possible impact of genetic drift or housing and/or diet.

Microarray Analysis. Microarray analysis on mouse livers from WT, hOATP1B1, hOATP1B3, and Oatp1a1/b KO mice revealed a total of 294 unique genes (356 total genes, including genes that are duplicated within the array) altered by greater than 2-fold (P < 0.05). The full list of genes and the fold changes observed can be found in Supplemental Table 1. As expected, in Oatp1a1/b KO, hOATP1B1, and hOATP1B3 mice, the hepatic expression of the mouse Slc10a1, Slc10a4, and Slc10b2 genes was significantly suppressed compared with WT controls (43- to 239-fold decrease). No other significant changes in known “drug” transporter genes were noted with the exception of Abcc9, which compared with the WT mice was decreased by 2.3-, 2.3-, and 2.2-fold in Oatp1a1/b KO, hOATP1B1, and hOATP1B3 mice, respectively. Genes coding for members of the solute carrier superfamily of transporters that showed significant downregulation included Slc6a4 (serotonin transporter), Slc13a2 (sodium/dicarboxylate cotransporter), Slc26a3 (chloride anion exchanger), and Slc30a10 (zinc transporter). Slc transporter genes that were significantly induced included Slc5a3 (sodium/myo-inositol cotransporter), Slc10a2 (sodium/bile acid cotransporter), Slc25a32 (mitochondrial folate transporter), and Slc4a1 (large neutral amino acid cotransporter).

In terms of genes encoding for drug-metabolizing enzymes, in agreement with the qRT-PCR results, Ces1d and Je were significantly repressed, particularly when comparing hOATP1B1 to WT or Oatp1a1/b KO mice (23- to 364-fold repression, respectively). In general, Ces1 gene expression in Oatp1a1/b KO mice was comparable to WT controls, whereas they were markedly repressed in the humanized models. The arylhydrocarbon receptor responsive cytochrome P450 gene Cyp2b9 showed greater than 4-fold repression in the knockout and humanized mice, as compared with WT controls. The peroxisome proliferator–activated receptor α-responsive Cyp4a10 and Cyp4a14 genes were also repressed in the knockout and humanized mouse, when compared with WT mice. In contrast, two members of the flavin monooxygenase family (Fmo2 and 3) were upregulated >2-fold in the humanized versus WT mice. Finally, nicotinamide N-methyltransferase (Nmnt) was also induced in both the knockout and humanized mice, compared with WT controls (>2.6-fold).

Quantification of Hepatic OATP1B1 and -B3 Protein Expression in hOATP1B1 and hOATP1B3 Mice

The OATP1B1 and OATP1B3 protein amount in hOATP1B1 and hOATP1B3 mice was 1.63 ± 0.08 and 3.83 ± 1.31 fmol/μg of total membrane protein, respectively. The level of OATP1B3 in the liver of hOATP1B3 mice was on average 2.4-fold higher than the level of OATP1B1 in hOATP1B1 animals. The relative differences in hepatic OATP1B1 and -B3 amounts between the hOATP1B1 and hOATP1B3 mice were similar as measured by three independent groups who contributed to this paper (data not shown). Whereas the amount of hepatic OATP1B1 in hOATP1B1 mice (1.63 ± 0.08 fmol/μg protein) was marginally lower than the previously determined average amount in human liver (2.0 ± 0.9 fmol/μg protein) (Prasad et al., 2013), OATP1B3 was ~3.5-fold higher in hOATP1B3 mice than the average human levels (1.1 ± 0.5 fmol/μg protein) (Fig. 2). There was no signal for OATP1B1 peptide in hOATP1B3 mice or for OATP1B3 in hOATP1B1 mice, nor for any of the two human peptides in Oatp1a1/b KO animals. In the present study, to avoid variability in transporter quantification in human versus animal tissues due to differences in laboratories or methods, the transporters were quantified by the same laboratory (and method) that quantified the transporters in the human liver tissues (Prasad et al., 2013).
Fig. 3. Uptake time profile of E217βG, CCK-8, TEA, and TCA in hepatocytes isolated from Oatp1a/1b KO, hOATP1B1, hOATP1B3, and WT mouse livers, and the inhibition of uptake by several inhibitors. Time-dependent uptake of E217βG (0.1 μM) (A), CCK-8 (0.01 μM) (C), TEA (0.1 μM) (E), and TCA (0.1 μM) (G) was determined in hepatocytes isolated from Oatp1a/1b KO (red circles), hOATP1B1 (blue circles), hOATP1B3 (green circles), and WT (black circles) mouse livers at 37°C. *p < 0.01, statistically significant compared with Oatp1a/1b KO mice.

Inhibitory effect of RIF SV (100 μM), rifampin (200 μM), CsA (10 μM), quinidine (100 μM), and BSP (100 μM) on uptake (measured at 3 minutes at 37°C) of E217βG (0.1 μM) (B), CCK-8 (0.01 μM) (D), TEA (0.1 μM) (F), and TCA (0.1 μM) (H) was also measured. *p < 0.01, statistically significant compared with corresponding no inhibitor controls. Values shown are the mean ± S.D. for experiments performed in triplicate.
confirming that mouse Oatp1a/1b is responsible for hepatic uptake of this compound. In the WT mouse, hepatocyte uptake of E217B was time-dependent and was abolished nearly completely in the presence of the OATP inhibitors Rif SV (100 μM), rifampin (200 μM), and CsA (10 μM). Uptake of E217B in hOATP1B1 and hOATP1B3 hepatocytes was 3- to 10-fold higher than in Oatp1a/1b KO mice at all time points tested, but 134- and 40-fold lower than in hepatocytes from WT mice (Fig. 3). As a selective probe substrate for OATP1B3, uptake of CCK-8 in hOATP1B3 mouse hepatocytes was time-dependent and substantially higher than the uptake measured in hepatocytes from all other mouse strains (Fig. 3). The uptake of CCK-8 in both hOATP1B3 and WT hepatocytes was completely inhibited by Rif SV (100 μM), rifampin (200 μM), and CsA (10 μM), indicating that OATP1B3 was functional in hOATP1B3 hepatocytes. Uptake of TEA, a prototypical substrate of OCT1/Oct1, was time-dependent and comparable in hepatocytes from all mouse strains tested, and significantly inhibited by quinidine, an inhibitor of OCT1. Similarly, TCA, a substrate for hepatic NTCP/Ntcp, showed comparable hepatic uptake in all mouse strains, although its uptake in WT mouse hepatocytes was slightly higher than in other strains at 3 and 5 minutes (P < 0.01) (Fig. 3G). Uptake of TCA in all strains was completely inhibited by BSP (100 μM), an inhibitor of Ntcp. These data suggested that hepatocytes isolated from the various mouse strains retained comparable functional activity of Oct and Ntcp.

Uptake of Several Clinically Used OATP1B Substrates into Mouse Hepatocytes. To further assess the functional activity of OATP1B1 and -1B3 in hOATP1B1 and hOATP1B3 mice, uptake of pitavastatin, rosuvastatin, pravastatin, and atorvastatin was evaluated in hepatocytes isolated from all mouse strains. Compared with WT mice, uptake of all compounds tested was reduced substantially in Oatp1a/1b KO hepatocytes (Fig. 4), confirming that Oatp1a/1b is the predominant contributor to the hepatic uptake of these compounds in WT mouse hepatocytes. In hOATP1B1 hepatocytes, uptake of pitavastatin, rosuvastatin, and atorvastatin was higher than in Oatp1a/1b KO hepatocytes, but significantly lower than in WT hepatocytes. In the case of pravastatin, uptake in hOATP1B1 hepatocytes was only slightly higher than in Oatp1a/1b KO hepatocytes at 3 and 5 minutes (P < 0.01) (Fig. 4E). Uptake measured in hOATP1B1 hepatocytes likely could be attributed to OATP1B1 as it was inhibited by Rif SV, rifampin, and CsA. Notably, the residual hepatic uptake of the statins (relatively high for pitavastatin, atorvastatin, and rosuvastatin, but low for pravastatin) observed in Oatp1a/1b KO hepatocytes was significantly inhibited by the aforementioned inhibitors. By contrast, uptake of statins in hOATP1B3 hepatocytes was compound-dependent. Uptake of pravastatin and atorvastatin in hOATP1B3 hepatocytes was significantly higher than in hOATP1B1 hepatocytes, whereas uptake of other statins was within the same range as in hOATP1B1 hepatocytes. Hepatic uptake of all statins tested in hOATP1B3 hepatocytes was sensitive to Rif SV, rifampin, and CsA.

Pharmacokinetics of Pravastatin, Rosuvastatin, and Pitavastatin in WT, Oatp1a/1b KO, hOATP1B1, and hOATP1B3 Mice

In vivo activities of OATP1B1 and -1B3 toward the OATP1B substrates pravastatin, pitavastatin, and rosuvastatin were assessed in pharmacokinetic studies in hOATP1B1 and -1B3 mice. In these studies, the effect of the transgenic transporters on systemic (plasma or blood) and liver exposure of the aforementioned statins was investigated.

Pravastatin. In a first study, pravastatin was tested in Oatp1a/1b KO, hOATP1B1, hOATP1B3, and WT mice. Following i.v. (5 mg/kg) administration of pravastatin, systemic exposure measured by blood area under the curve (AUC) was 4-fold higher in Oatp1a/1b KO mice when compared with WT animals (Fig. 5A; Table 1), with blood concentrations between these two strains significantly different at all time points tested. Pravastatin blood concentrations in the hOATP1B1 and -1B3 mice were only slightly lower than those measured in Oatp1a/1b KO animals (Fig. 5A), with AUC remaining 3.4- and 2.7-fold higher, respectively, than in the WT mice. In contrast, a more pronounced impact was observed following PO administration (Fig. 5B). Pravastatin AUC 0-2hr in Oatp1a/1b KO mice was 18-fold higher than in the WT animals, and exposure in the humanized mice pointed to a recovery of the liver uptake activity with systemic exposure in the hOATP1B1 and -1B3 mice 8.6- and 3.7-fold higher than in the WT, respectively, and significantly lower than that measured in the KO mice (Fig. 5B; study 1 in Table 1). Pravastatin liver concentrations measured 5 minutes following PO administration were 2- to 5-fold lower in Oatp1a/1b KO than in WT mice (data not shown). In hOATP1B1 animals, liver concentrations were 35% higher than in Oatp1a/1b KO mice, whereas in hOATP1B3 mice, they were similar to Oatp1a/1b KO animals (data not shown). Liver concentrations were not significantly different between the four strains 30 minutes postdose (data not shown). Changes in hepatic uptake were reflected more profoundly in the liver-to-blood ratio, which was significantly reduced in Oatp1a/1b KO mice (1.3 vs. 166 in WT mice). Although the ratio was only moderately restored in the humanized mice, it was significantly different from that measured in Oatp1a/1b KO animals 5 and 30 minutes postdose (Fig. 5C; 2.1-4.4 in humanized mice versus 1.3 in KO mice).

In a follow-up study, pravastatin was tested in Oatp1a/1b KO, hOATP1B1/1B3, and WT mice. The significant increase in systemic exposure in Oatp1a/1b KO mice compared with WT controls following i.v. administration was confirmed in this study. Interestingly, the pravastatin blood concentrations in hOATP1B1/1B3 mice were comparable to WT levels (Fig. 5D), in contrast to the minor reversion observed in the corresponding single humanized models (Fig. 5A). The pravastatin AUC 0-2hr in hOATP1B1/1B3 mice was also significantly (3.7-fold) lower than in Oatp1a/1b KO mice after PO administration (Fig. 5E; study 2 in Table 1), whereas sufficient data points above the limit of detection could not be collected in WT controls to establish a pharmacokinetic profile in this study. Nevertheless, measured blood concentrations at 0.25, 0.5, and 1 hour were substantially lower than in both Oatp1a/1b KO, and hOATP1B1/1B3 mice.

Pitavastatin. A 5-mg/kg PO dose of pitavastatin was administered to Oatp1a/1b KO, hOATP1B1, hOATP1B3, and WT mice. Whereas pitavastatin plasma exposure in Oatp1a/1b KO mice was increased 11-fold compared with WT controls, the plasma concentration-time profile in hOATP1B1 and hOATP1B3 mice was not different from Oatp1a/1b KO animals (Fig. 6A; Table 2). Furthermore, the liver-to-plasma ratio was markedly decreased in Oatp1a/1b KO relative to WT animals (Fig. 6B), although the liver concentrations were not significantly different between the two mouse lines (data not shown). In contrast, pitavastatin liver concentrations in hOATP1B1 mice were numerically slightly (up to 2-fold) increased compared with Oatp1a/1b KO animals, contributing to an up to 2-fold higher liver-to-plasma ratio in hOATP1B1 compared with Oatp1a/1b KO mice at all three time points (Fig. 6B). Although these results might suggest a role of OATP1B1 in liver uptake of this compound, they were not statistically significant (P > 0.05; Student’s t test) at any of the three time points (Fig. 6B). The liver concentrations and liver-to-plasma ratio in hOATP1B3 mice were indistinguishable from that measured in Oatp1a/1b KO animals (Fig. 6B).

Rosuvastatin. Oatp1a/1b KO mice showed higher systemic exposure than WT mice after both i.v. (5 mg/kg) and PO (15 mg/kg) administration of rosuvastatin, with 2.3- (i.v.) and 45-fold (PO) increased mean AUC0-last (AUC from time 0 to last timepoint with measurable concentration) and 2.1- (i.v.) and 33-fold (PO) increased Cmax, respectively (Table 3). Surprisingly, neither in the hOATP1B1
Fig. 4. Uptake time profile of pitavastatin, rosuvastatin, pravastatin, and atorvastatin in hepatocytes isolated from Oatp1a/1b KO, hOATP1B1, hOATP1B3, and WT mouse livers, and the inhibition of uptake rate by several inhibitors. Time-dependent uptake of pitavastatin (0.1 μM) (A), rosuvastatin (0.01 μM) (C), pravastatin (0.1 μM) (E), and atorvastatin (0.1 μM) (G) was determined in hepatocytes isolated from Oatp1a/1b KO (red circles), hOATP1B1 (blue circles), hOATP1B3 (green circles), and WT (black circles) mouse livers at 37°C. *P, 0.01, statistically significant compared with Oatp1a/1b KO mice. Inhibitory effect of RIF SV (100 μM), rifampin (200 μM), and CsA (10 μM) on uptake rate (measured at 3 minutes at 37°C) of pitavastatin (0.1 μM) (B), rosuvastatin (0.01 μM) (D), pravastatin (0.1 μM) (F), and atorvastatin (0.1 μM) (H) was also measured. *P < 0.01, statistically significant compared with corresponding no inhibitor controls. Values shown are the mean ± S.D. for experiments performed in triplicate.
nor the hOATP1B3 mice was there a reversion toward WT levels, with both humanized mouse lines showing similar systemic exposures as the Oatp1a/1b KO animals (Fig. 7; Table 3).

Discussion

In this paper, Oatp1a/1b KO, hOATP1B1, and hOATP1B3 mice have been characterized further to assess their potential use in translational research. For this purpose, compensatory gene expression changes were assessed, the amount of OATP1B1 and -1B3 with those in human livers were compared, and the functional activity of various OATP1B probe substrates was tested both in vitro and in vivo.

In general, no remarkable compensatory changes in the expression of drug transporter genes were observed in the transgenic mouse models compared with WT controls except for a moderate increase in the expression of Abcb1b (4- to 6-fold, qRT-PCR) (Fig. 1; Supplemental Fig. 5).

**TABLE 1**

Pharmacokinetic parameters after i.v. and PO administrations of 5 mg/kg pravastatin to FVB WT, Oatp1a/1b KO, hOATP1B1, hOATP1B3, and hOATP1B1/1B3 mice

<table>
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<th>PO Administration</th>
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<tr>
<td></td>
<td>CL</td>
<td>AUC&lt;sub&gt;0-2hr&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>(ml/min/kg)</td>
<td>(μM·h)</td>
</tr>
<tr>
<td>Study 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FVB WT</td>
<td>150 ± 22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.32 ± 0.20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oatp1a/1b KO</td>
<td>36 ± 2.8</td>
<td>5.46 ± 0.40</td>
</tr>
<tr>
<td>hOATP1B1</td>
<td>45 ± 9.1</td>
<td>4.50 ± 0.81</td>
</tr>
<tr>
<td>hOATP1B3</td>
<td>56 ± 7.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.55 ± 0.48</td>
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<tr>
<td>Study 2</td>
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<td></td>
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<tr>
<td>FVB WT</td>
<td>132 ± 8.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.49 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oatp1a/1b KO</td>
<td>39 ± 3.6</td>
<td>5.07 ± 0.48</td>
</tr>
<tr>
<td>hOATP1B1/1B3</td>
<td>83 ± 13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.39 ± 0.36&lt;sup&gt;a&lt;/sup&gt;</td>
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</table>

AUC<sub>0-2hr</sub>, Area under the blood concentrations-time curve from time 0 to 2 hours; CL, Blood clearance; NC, not calculated.

<sup>a</sup>Significantly different from Oatp1a/1b KO mice (P < 0.05).
When translating the data from humanized OATP1B mice to the human situation, it is important to compare the amount of hepatic OATP1B protein in the humanized mice relative to human liver. Using liquid chromatography–tandem mass spectrometry–based protein quantification, we found that the amount of OATP1B3 in hOATP1B3 mice was 3.5-fold higher than the average level in human liver, whereas the expression of OATP1B1 in hOATP1B1 mice was comparable to that in human liver (Prasad et al., 2013). These results are in reasonable agreement with those recently reported by Higgins et al. (2014). Overall, these findings suggested that the effect of hOATP1B3 could be overestimated in this mouse model. It should be noted that the amount of OATP1B1 and -1B3 in this study was determined using crude membrane preparations from liver samples, without considering the specific spatial expression of the functional OATP1B in the plasma membrane within the liver.

Given that the in vivo disposition of many OATP1B substrates, such as statins, is complex, involving multiple elimination pathways via various drug transporters and metabolizing enzymes (Elsby et al., 2012; Yoshida et al., 2012), we evaluated the functional activity of hOATP1B mice using several OATP1B substrates both in isolated hepatocytes and in vivo. Using this comprehensive approach, we could directly measure the functional activity of OATP1B as active uptake transporters in vitro and assess their roles in in vivo disposition. For most of the compounds tested, the expected increase in uptake in hepatocytes from the hOATP1B1 and hOATP1B3 mice relative to the Oatp1a/1b KO hepatocytes was observed, albeit not with a full recovery to WT levels in most cases (with the exception of CCK-8 in hOATP1B3 hepatocytes) (Figs. 3 and 4). The lack of full recovery was not unexpected, because five functional mouse Oatp1a/1b proteins were replaced with only one functional OATP1B transporter, OATP1B1 or -1B3, in the humanized mice. At the concentrations tested (0.1 μM, typically at least 10-fold lower than reported Km values of these OATP substrates) (Hirano et al., 2006; Kalliokoski and Niemi, 2009), a very obvious trend was the weak recovery of pravastatin uptake in hOATP1B1 relative to Oatp1a/1b KO hepatocytes, except for pitavastatin and rosuvastatin, for which it was consistently lower uptake in hOATP1B1 compared with hOATP1B3 hepatocytes (see below).

To assess in vivo functional roles of OATP1B in hOATP1B mice, pharmacokinetic studies were conducted with pravastatin, pitavastatin, and rosuvastatin. In contrast to minimal in vitro functional activity of OATP1B1, a 2-fold decrease of pravastatin blood AUC in hOATP1B1 relative to Oatp1a/1b KO mice following PO dosing was observed. Despite the minimal changes measured after i.v. dosing of pravastatin, results obtained following PO administration (AUC and liver-to-blood ratios) suggested that OATP1B1 was functionally active in vivo in the hOATP1B1 mice. Nevertheless, our study design did not allow us to

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**Fig. 6.** Pitavastatin plasma concentration-time profiles and liver-to-plasma concentration ratio in WT, Oatp1a/1b KO, hOATP1B1, and hOATP1B3 mice. (A) Pharmacokinetic profiles after 5 mg/kg PO administration of pitavastatin to male WT (black circles), Oatp1a/1b KO (red circles), hOATP1B1 (blue circles), and hOATP1B3 (green circles) mice. Values shown are the mean ± S.D. of at least three mice per strain. *P < 0.05, statistically significant compared with Oatp1a/1b KO mice.

**TABLE 2**

<table>
<thead>
<tr>
<th>Mouse Line</th>
<th>AUC&lt;sub&gt;0-8h&lt;/sub&gt;</th>
<th>C&lt;sub&gt;max&lt;/sub&gt;</th>
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<tbody>
<tr>
<td></td>
<td>μM*h</td>
<td>μM</td>
</tr>
<tr>
<td>FVB WT</td>
<td>0.33 ± 0.1</td>
<td>0.39 ± 0.17</td>
</tr>
<tr>
<td>Oatp1a/1b KO</td>
<td>3.87 ± 0.55</td>
<td>5.27 ± 2.64</td>
</tr>
<tr>
<td>hOATP1B1</td>
<td>3.70 ± 0.92</td>
<td>4.59 ± 1.12</td>
</tr>
<tr>
<td>hOATP1B3</td>
<td>4.01 ± 0.47</td>
<td>6.91 ± 0.63</td>
</tr>
</tbody>
</table>
One of the unexpected findings was the lack of differences in systemic exposure for pitavastatin and rosuvastatin in the hOATP1B1 and -1B3 mice compared with Oatp1a/1b KO animals. This was surprising because of the established role of specifically OATP1B1 in the transport of these compounds in humans (Ieiri et al., 2009; Niemi et al., 2011; Williamson et al., 2013; Prueksaritanont et al., 2014) and the fact that the uptake of both compounds in hepatocytes derived from hOATP1B1 and hOATP1B3 mice was increased relative to Oatp1a/1b KO hepatocytes (Fig. 4). Lack of OATP1B1 functional activity with pitavastatin and rosuvastatin in vivo might be related to 1) potentially low transport activity in the liver of the hOATP1B1 mice. This is supported by a less than 2-fold difference in uptake rate for both pitavastatin and rosuvastatin in hOATP1B1 relative to Oatp1a/1b KO hepatocytes (Fig. 3). Consistently, lower in vitro transport activity of OATP1B1 relative to OATP1B3 was also observed with several other OATP1B prototypical substrates tested (Figs. 3 and 4). 2) Potential predominant effects of some other mouse transporters on hepatic uptake at in vivo relevant exposure. The residual hepatic uptake of pitavastatin and rosuvastatin observed in Oatp1a/1b KO hepatocytes was significantly inhibited by RIF, rifampicin, and CsA (Fig. 4, B and D), suggesting the differential involvement of other transporters in the hepatic uptake of these compounds (Ho et al., 2006; Bi et al., 2013). Relatively low impact and selectivity of OATP1B1 on overall in vivo disposition of rosuvastatin in humans may also explain minimal restoration of its systemic exposure in hOATP1B1 mice (Elsby et al., 2012; Prueksaritanont et al., 2014). 3) Potential change of alternative elimination pathways in hOATP1B mice. According to Iusuf et al. (2013), renal clearance of rosuvastatin is only 9.4% of total clearance in WT mice. However, renal clearance increased to 29% of total clearance in Oatp1a/1b KO mice, which is similar to humans. In fact, we observed increased renal excretion of rosuvastatin in humanized OATP1B1 and -1B3 and Oatp1a/1b KO mice relative to WT animals (preliminary observations, data not shown). This change in renal clearance may still not be the major explanation for the lack of in vivo functional activity of OATPs with rosuvastatin in the humanized mice. 4) Differences in the metabolism of these statins in mice and humans, which might obscure the in vivo impact of the OATP1B transporters on drug disposition in the mouse models. The metabolism of rosuvastatin in mice was very limited (Hirano et al., 2005; Kitamura et al., 2008; Iusuf et al., 2013), so that

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**TABLE 3**

Pharmacokinetic parameters after i.v. (5 mg/kg) and PO (15 mg/kg) administrations of rosuvastatin to FVB WT, Oatp1a/1b KO, hOATP1B1, and hOATP1B3 mice

<table>
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<th>Mouse Line</th>
<th>i.v. Administration</th>
<th>PO Administration</th>
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<tr>
<td></td>
<td>CL</td>
<td>AUC&lt;sub&gt;0-24h&lt;/sub&gt;</td>
</tr>
<tr>
<td>FVB WT</td>
<td>88.8 ± 28.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.45 ± 0.96&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Oatp1a/1b KO</td>
<td>31.2 ± 7.20</td>
<td>5.73 ± 1.58</td>
</tr>
<tr>
<td>hOATP1B1</td>
<td>29.5 ± 12.2</td>
<td>7.02 ± 2.33</td>
</tr>
<tr>
<td>hOATP1B3</td>
<td>28.5 ± 9.9</td>
<td>7.35 ± 3.41</td>
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<sup>a</sup>Significantly different from Oatp1a/1b KO mice (P < 0.05).

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**Fig. 7.** Rosuvastatin blood concentration-time profiles in WT, Oatp1a/1b KO, hOATP1B1, and hOATP1B3 mice. Pharmacokinetic profiles after 5 mg/kg i.v. (A) or 15 mg/kg PO (B) administration of rosuvastatin to male WT (black circles), Oatp1a/1b KO (red circles), hOATP1B1 (blue circles), and hOATP1B3 (green circles) mice. Values shown are the mean ± S.D. of n = 4 mice per strain.
this appears to be an unlikely explanation for rosuvastatin. However, pitavastatin showed more profound hepatic metabolism in mice compared with rats and humans (Fujino et al., 2002). Despite more extensive metabolism, we observed 12-fold increase of pitavastatin plasma AUC in Oatp1a/1b KO versus WT mice, suggesting that Oatp1a/1b proteins play important roles in hepatic uptake of pitavastatin in mice. However, we cannot exclude that the metabolism of pitavastatin in mice could be another contributing factor to the lack of difference in plasma exposure of pitavastatin between hOATP1B1 and Oatp1a/1b KO mice. Given that we did not observe significant compensatory changes of major drug-metabolizing enzymes in these mouse strains, the change in metabolic activity of pitavastatin in these mouse strains is less likely. Nevertheless, the detailed comparison of metabolic clearance of pitavastatin between these animals and humans could be helpful to understand the mechanism underlying the lack of in vivo functional activity of OATP1B1 with pitavastatin in the humanized mice.

Although the in vivo functionality of OATP1B1 with several substrates has been demonstrated in the humanized OATP1 mouse models (van de Steeg et al., 2013; Zimmerman et al., 2013; Higgins et al., 2014), quantitative translation of these findings to humans is still premature. In this study, only pravastatin showed in vivo functional activity of OATP1B1 and -IB3. Using the approach proposed by Higgins et al. (2014), the fractional contribution of OATP1B1 to oral systemic clearance was estimated to be 0.57 (OATP1B1 relative expression factor is 1.23, Oatp1a/1b KO AUCpo/OATP1B1 AUCpo is 2.08), resulting in a maximal fold increase in systemic exposure of 2.3-fold. This is in agreement with the result by Higgins et al. (2014). However, when taking into account OATP1B3, the fractional contribution of OATP1B3 to oral systemic clearance was 0.52 (OATP1B3 relative expression factor is 0.29, Oatp1a/1b KO AUCpo/OATP1B3 AUCpo is 4.76), resulting in a maximal fold increase in systemic exposure of 2.1-fold. As such, the impact of OATP1B3 was overestimated compared with clinical observations (Ieiri et al., 2009; Elsby et al., 2012). The lack of in vivo function with pitavastatin and rosuvastatin in hOATP1B1 mice might suggest that the activity is compound-dependent and precludes the translation of the findings for these two statins from humanized mice to humans. Furthermore, appropriate in vivo study design (e.g., i.v. and PO, and dose level) is critical to ensure relevant in vivo exposure is achieved compared with clinical studies. Finally, in vitro uptake studies in hepatocytes isolated from these mouse strains could be a useful tool to directly assess active hepatic uptake of OATP1B in these models. Detailed kinetic studies in the hepatocytes will be helpful to translate the in vitro observations to the in vivo situation when taking into account relevant in vivo exposure, the ADME (absorption, distribution, metabolism, excretion) properties of drugs, and involvement of other transporters in liver uptake. Further studies will be undertaken by our group to refine the mechanistic understanding of the observations reported here and to provide additional insights into the potential use of these models in translational research.

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Conducted experiments: Salphati, Chu, Chen, Prasad, Mamari-Fishman, Geier, Kehler, Pang, Rode, van Waterschoot, Yabut.

Performed data analysis: Salphati, Chu, Chen, Prasad, Dallas, Evers, Geier, Kunta, Pang, Mezler, Soars, Unadkat, van Waterschoot, Schinkel, Scheer.

Wrote or contributed to the writing of the manuscript: Salphati, Chu, Chen, Prasad, Dallas, Evers, Geier, Kunta, Mezler, Soars, Unadkat, van Waterschoot, Schinkel, Scheer.

References


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