

Development of a Rat Sandwich-Cultured Hepatocytes Model Expressing Functional Human Organic Anion Transporting Polypeptide (OATP) 1B3: A Potential Screening Tool for Liver-Targeting Compounds

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ABSTRACT – Purpose: Organic anion transporting polypeptide (OATP) 1B3 transports many clinically important drugs, including statins, from blood into the liver. It exclusively expresses in human liver under normal physiological conditions. There is no rodent ortholog of human OATP1B3. Tissue targeting of therapeutic molecules mediated by transporters, including liver-targeting via liver-specific OATPs, is an emerging area in drug development. Sandwich-cultured primary hepatocytes (SCH) are a well characterized *in vitro* model for assessment of hepatic drug uptake and biliary excretion. The current study was designed to develop a novel rat SCH model expressing human OATP1B3 to study the hepatic disposition of OATP1B3 substrates. **Methods:** Primary rat hepatocytes transduced with adenoviral vectors expressing FLAG-tagged OATP1B3 (Ad-OATP1B3), a control vector Ad-LacZ, or that were non-transduced were cultured in a sandwich configuration. FLAG immunoblot and immunofluorescence-staining determined expression and localization of OATP1B3. Uptake of [³H]-cholecystinin octapeptide (CCK-8), a specific OATP1B3 substrate, was determined. Taurocholate (TC) is a substrate routinely used in SCH to assess biliary excretion via bile canaliculi (BC) and is also a substrate of OATP1B3. [³H]-TC accumulation in cells+BC, cells, biliary excretion index (BEI) and *in vitro* Cl_{biliary} were determined using B-CLEAR® technology. **Results:** OATP1B3 protein was extensively expressed and primarily localized on the plasma membrane in day 4 Ad-OATP1B3-transduced rat SCH. [³H]-CCK-8 accumulation in cells+BC was significantly greater (~5-13 folds, *p*<0.001) in day 4 SCH with vs. without Ad-OATP1B3-transduction. Expressing OATP1B3 in rat SCH significantly increased [³H]-TC accumulation in cells+BC and cells, without affecting BEI and *in vitro* Cl_{biliary}. **Conclusions:** Rat SCH expressing human OATP1B3 is a novel *in vitro* model allowing simultaneous assessment of hepatic uptake, hepatocellular accumulation and biliary excretion process of a human OATP1B3 substrate. This model could be a potential tool for screening for liver-targeting compounds mediated by OATP1B3.

INTRODUCTION

Organic Anion Transporting Peptide (OATP) 1B3 is a member of the solute carrier organic anion (SLCO) transporter superfamily. OATP1B3 is localized to the basolateral membrane of human hepatocytes and has ~80% amino acid homology with another basolateral transport protein, OATP1B1 (1). Under normal physiological conditions, both OATP1B1 and OATP1B3 specifically express in the human liver (2). They can transport many clinically important drugs, including widely prescribed lipid-lowering statins, anti-diabetic, antibiotics, and anti-cancer agents, from blood into the liver (3). Tissue targeting of therapeutic molecules mediated by

transporters, including liver targeting via liver-specific OATP1B1 and OATP1B3, is an emerging area in drug development (4-6). There is a need to develop a screening tool that can simultaneously assess OATP1B3-mediated hepatic uptake and hepatocellular accumulation of drug candidates *in vitro*.

Hepatocytes cultured in a sandwich configuration between two layers of extracellular matrix exhibit proper localization of basolateral and canalicular transport proteins and functional metabolic activity (7). The SCH system is a physiologically relevant and well characterized *in vitro* model with which to evaluate hepatic uptake, hepatocellular accumulation, and biliary excretion of

drugs and derived metabolites (7). It is a valuable tool used in drug discovery and development to characterize *in vitro* hepatobiliary disposition of many drugs. SCH has been established successfully using primary hepatocytes for humans (8-11) and preclinical species, including rats (12, 13). Due to the lack of rodent ortholog of OATP1B1 and OATP1B3, currently, there is no rodent SCH model available to study hepatobiliary disposition of human OATP1B1 and OATP1B3 substrates *in vitro*. The current study was designed to develop a novel rat SCH model that expresses human OATP1B3 and to utilize this model to assess the hepatic disposition of human OATP1B3 substrates. Such a model would allow simultaneous detection of OATP1B3-mediated hepatic uptake and hepatocellular accumulation of OATP1B3 substrate.

Adenoviral vector has been successfully used in gene expression in primary rat (7, 14) and human hepatocytes (15). In the current studies, human OATP1B3 was expressed in rat SCH by adenoviral vector-mediated gene transfer. OATP1B3-mediated hepatic uptake in rat SCH was assessed using cholecystokinin 8 (CCK-8), a specific substrate of OATP1B3 (16), as the probe substrate. Taurocholate (TC) is subject to extensive hepatic biliary excretion in rat SCH and has been routinely used as a model substrate to assess the function of bile canaliculi in SCH (7). In rat hepatocytes, uptake of TC into hepatocytes is primarily mediated by basolateral transport protein Na⁺-taurocholate co-transporting polypeptide (Ntcp) (17), while biliary excretion of TC is mediated by the bile salt export pump (BSEP) (18). TC is also a substrate of OATP1B3 (19). TC was used as a model substrate in the current study to assess the hepatobiliary disposition of substances in rat SCH when OATP1B3 is overexpressed.

MATERIALS AND METHODS

Chemicals

[³H]- CCK-8 (specific activity 88.0 Ci/mmol) and [³H]-TC (specific activity 5.0 Ci/mmol) was purchased from Perkin Elmer Life Science (Waltham, MA). Unlabeled CCK-8, Triton X-100, dimethyl sulfoxide (DMSO), and Hank's Balanced Salt Solution (HBSS) were purchased from Sigma-Aldrich (St. Louis, MO). Fetal bovine serum (FBS) was purchased from Hyclone Laboratories (Logan, Utah). Matrigel™, BioCoat™ plates, and insulin/transferrin/selenium (ITS⁺) were purchased from BD Biosciences (Bedford, MA). Bio-Safe II liquid scintillation mixture was purchased from Research Products International (Mt. Prospect, IL).

All other materials were purchased from VWR or Thermo Fisher Scientific (Waltham, MA).

Adenoviral Vectors

The adenoviral vector Ad-OATP1B3, which expresses FLAG-tagged OATP1B3, and control adenoviral vector (Ad-LacZ), which expresses β-galactosidase (lacZ), were published previously (15). The titer of adenoviral vectors was determined using the Adeno-X Rapid Titer Kit (Clontech Laboratories, Mountain View, CA).

Sandwich-culture hepatocytes (SCH) and transduction of adenoviral vectors

Male Wistar rat hepatocytes in suspension, which were isolated by the collagenase perfusion method (20), were purchased from Triangle Research Laboratories (Research Triangle Park, NC). After centrifugation of the cell suspension at 4 °C at 76 g for 6 min and decanting the shipping medium, cells were resuspended in fresh seeding medium and had viability of greater than 81% (average 85%, ranging 81-93%). Hepatocytes were then cultured in sandwich configuration as published previously (7, 14, 21). Briefly, cells were seeded onto 24-well BioCoat™ culture plates at 2.5 × 10⁵ cells per well (BD Bioscience) with seeding medium (phenol red-free Dulbecco's modified Eagle's medium [DMEM] supplemented with nonessential amino acids, 5% fetal bovine serum, L-glutamine, penicillin/streptomycin, 4 μg/mL insulin, and 1 μM dexamethasone) and incubated at 37 °C in a humidified incubator with 95% air/5% CO₂. One hour after seeding, seeding medium was replaced with fresh seeding medium without adenoviral vectors (non-transduced) or with Ad-LacZ or Ad-OATP1B3 at a multiplicity of infection (MOI) of 5. Approximately 24 h after seeding, cells were overlaid with Matrigel™ at 0.25 mg/mL in feeding medium containing 0.1 μM dexamethasone and ITS⁺ premix (feeding medium); the feeding medium was then changed every 24 h until the day of experiment.

Transport Studies in rat SCH

[³H]-CCK-8 accumulation in rat SCH was determined in protein-free standard HBSS buffer containing Ca²⁺, similar to that published previously (22). The substrate concentration of CCK-8 (1 μM) was below the K_m values of CCK-8 transport by OATP1B3 (3.82 μM) (23) and that of CCK-8 uptake in primary rat hepatocyte (6.7 μM) (16). Initial uptake of CCK-8 in non-transduced rat hepatocytes was linear over at least 1 min (16). Incubation of

[³H]-CCK-8 (1 μM) was determined at 20 sec, which was in the linear range of both non-transduced (16) and Ad-OATP1B3-transduced rat SCH determined in the current study. At the end of substrate incubation, SCH was washed three times with ice-cold standard HBSS buffer. Cells were then lysed on ice with 0.2 mL of ice-cold 0.5% Triton-X 100 in phosphate buffered saline (PBS). [³H]-CCK-8 accumulation was determined by liquid scintillation spectrometry (LS6500 scintillation counter, Beckman Coulter, Brea, CA). Uptake studies were also conducted in blank BioCoat™ plates overlaid with Matrigel without hepatocytes to correct for nonspecific binding. BCA assay (Pierce Chemical, Rockford, IL) was performed to normalize substrate accumulation to the protein concentrations.

TC accumulation in rat SCH was determined similar to the method previously described (14). Rat SCH were rinsed twice with standard HBSS and then incubated in Ca²⁺-free HBSS (to disrupt the canalicular networks by opening the tight junctions) or standard HBSS for 10 min at 37 °C. Cells were then incubated for 10 min at 37 °C in standard HBSS containing [³H]-TC (1 μM, 100 nCi). At the end of the incubation, cells were rinsed three times with ice-cold standard HBSS buffer and solubilized in ice-cold phosphate buffered saline (PBS) containing 0.5% Triton-X-100. TC biliary excretion was determined by subtracting TC accumulation in cells (rat SCH preincubated in Ca²⁺-free HBSS) from that in cells + bile canaliculi (BC) (rat SCH preincubated in standard HBSS). Substrate accumulation was determined similarly to that described above by liquid scintillation counting, normalized by protein concentration. TC uptake in a blank BioCoat™ plates overlaid with Matrigel without cells was conducted to correct for nonspecific binding.

The biliary excretion index (BEI; %) and *in vitro* biliary clearance (Cl_{biliary}) (ml/min/kg) of TC were determined in rat SCH using B-CLEAR® technology (U.S. Pat. No. 6,780,580, Pat. No. 7,604,934) (24) (Qualyst Transporter Solutions, BioIVT, Durham, NC) according to the following equations (12):

Equation 1:

$$BEI = \frac{Accumulation_{cell+BC} - Accumulation_{cells}}{Accumulation_{cells+BC}} \times 100\%$$

Equation 2:

$$In\ vitro\ Cl_{biliary} = \frac{Accumulation_{cells+BC} - Accumulation_{cells}}{AUC_{medium}}$$

AUC_{medium} was the product of the medium concentration and the incubation time (10 min). The medium concentration of TC was defined as the initial TC concentration (1 μM) as the medium concentrations at the end of the incubation did not differ by more than 10% compared with that at the beginning. The *in vitro* Cl_{biliary} (ml/min/mg protein) was scaled to kilogram of body weight, assuming 40 g of rat liver tissue/kg of body weight and 200 mg protein/g of rat liver tissue (25).

Immunoblotting

After washing once with standard HBSS buffer, SCH were lysed in lysis buffer containing 1 mM EDTA, 1% SDS, and Complete cocktail (Roche Diagnostics, Mannheim, Germany). BCA assay (Pierce, Rockford, IL) was conducted to determine protein concentrations. Proteins from whole-cell lysates (50 μg) separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis using 10% gel (Biorad, Hercules, CA). After transferring proteins to nitrocellulose membranes, blocking in 5% (w/v) milk in Tris-buffered saline containing Tween 20 (TBST), the blots were probed with mouse monoclonal antibodies against FLAG and β-Actin (Sigma-Aldrich, St. Louis, MO), followed by incubation with an HRP-conjugated anti-mouse secondary antibody after washing. Immunoblotting signals were detected by a Bio-Rad ChemiDoc XRS imaging using chemiluminescent substrate Supersignal West Duro (Pierce, Rockford, IL).

Immunofluorescent Staining

Immunofluorescent staining was conducted similarly to that published previously (26, 27). In brief, rat hepatocytes were grown on rat collagen-coated coverslip. Subsequent transduction with adenoviral vectors and sandwich-culture was the same as described above. After rinsing once with PBS buffer, cells were subjected to fixation with ice-cold 100% methanol for 15 min, permeabilization with 0.25% Triton X-100 in PBS for 10 min at room temperature and blocking with 5% (w/v) BSA in PBS for 1 h at room temperature. For immunofluorescence staining of FLAG-tagged OATP1B3, cells were then co-incubated with the mouse monoclonal anti-FLAG antibody (Sigma-Aldrich, St. Louis, MO, 1:100 dilution in PBS containing 5% (w/v) BSA) at 37 °C for 2 h. Following three washes with PBS, cells were then co-incubated with an Alexa Fluor 488-conjugated goat anti-mouse IgG (Life Technologies, Grand Island, NY, 1:200 dilution in PBS containing 5% (w/v) BSA) at 37 °C for 1 h. Cells were also

incubated for 5 min in 300 nM 4',6-diamidino-2-phenylindole (DAPI) to counterstain nuclei. After washing off DAPI, the coverslips were mounted on glass slides with ProLong gold antifade reagent (Life Technologies, Grand Island, NY). Images were captured using a Olympus XL73 inverted fluorescence microscope system with 10X and 20X objectives (Olympus Scientific Solutions Americas Corp., Waltham, MA).

Lactate Dehydrogenase (LDH) Cytotoxicity Assay

Assays were conducted similarly to those published previously (21, 22) using a lactate dehydrogenase (LDH) cytotoxicity detection kit (Roche Diagnostics, Mannheim, Germany). Untreated and Triton-X (2%)-treated cells served as the negative control and 100% cytotoxicity positive control, respectively.

Statistical Analysis

As indicated in the figure legend, substrate accumulation and associated standard errors (SEs) were estimated by linear mixed models; fold changes in substrate accumulation and associated SEs were estimated by generalized linear mixed models (GLMM). Models incorporate a fixed effect (treatment group), and random effects (culture day), adjusting for group-specific overdispersion (in GLMM). In the case of multiple comparisons, we reported *p*-values after Bonferroni correction. A two-sided *p*-value of <0.05 defines statistical significance. Paired *t*-test was utilized to determine the statistical difference in BEI and *in vitro* Cl_{biliary} . SAS software (version 9.4, Cary, NC) was utilized for statistical analysis.

RESULTS

Expression of OATP1B3 in Ad-OATP1B3-transduced SCH

As shown in Figure 1A, adenoviral vector-mediated expression of OATP1B3 increased over days in culture with strong immunoblot signal detected on day 4 of culture. Immunoblot with FLAG antibody did not detect OATP1B3 expression in non-transduced cells (day 0). FLAG immunofluorescent staining showed primarily plasma membrane localization of FLAG-tagged human OATP1B3 in Ad-OATP1B3-transduced rat SCH (Figure 1B). No staining of FLAG-OATP1B3 was detected in non-transduced control cells (Figure S1)

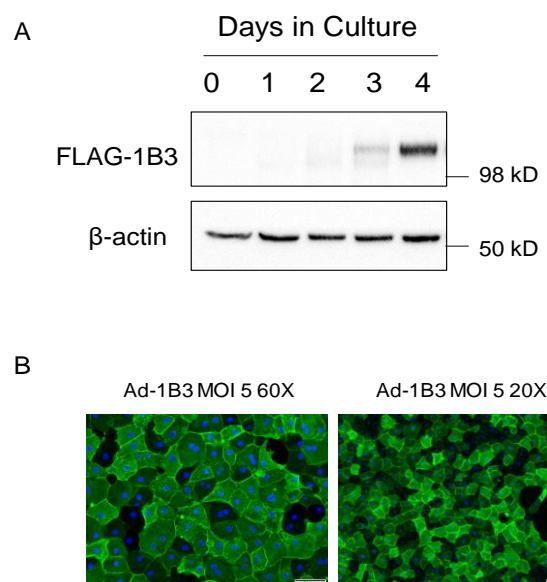


Figure 1. Adenoviral vector-mediated expression of human OATP1B3 in rat SCH. Primary rat hepatocytes were transduced with Ad-OATP1B3 at MOI of 5 and cultured in sandwich configuration. (A) Immunoblot of FLAG and β -actin in non-transduced (day 0) and Ad-OATP1B3-transduced rat SCH cultured for the indicated days. Representative images from one experiment of a total of four independent experiments ($N=4$ rat livers) are shown. (B) Immunofluorescence staining with FLAG antibody in Ad-OATP1B3-transduced rat SCH at day 4 of culture. Representative images in hepatocytes from $N=3$ rat livers are shown. Images were captured using Olympus XL73 inverted fluorescence microscope system using 10X and 20X objectives. Scale bars showed 50 μM and 100 μM in the left and right panel, respectively.

Linear uptake range of [^3H]-CCK-8 in Ad-OATP1B3-transduced rat SCH

The time-dependent total accumulation of [^3H]-CCK-8 was characterized in rat SCH with and without Ad-OATP1B3 transduction on day 4 of culture. As shown in Figure 2, OATP1B3-mediated [^3H]-CCK-8 (1 μM) accumulation in Ad-OATP1B3-transduced cells was linear at least up to 5 min.

Total accumulation of [^3H]-CCK-8 in rat SCH with and without Ad-OATP1B3 transduction

In non-transduced rat SCH, total accumulation of [^3H]-CCK-8 in cells+BC at day 4 of culture was significantly reduced to 0.26 ± 0.06 fold of that at day 0 of culture ($p < 0.001$) (Figure 3A). These data suggest that day 4 SCH has minimal CCK-8 uptake mediated by endogenous rat SCH transporter

On day 4 of culture, total accumulation of [^3H]-CCK-8 in Ad-OATP1B3-transduced SCH was

significantly greater than that in the non-transduced or Ad-LacZ-transduced SCH; there was no statistically significant difference in total [^3H]-CCK-8 accumulation between non-transduced and Ad-LacZ-transduced cells (Figure 3B). LDH assay results showed no cytotoxicity in Ad-OATP1B3-transduced cells (supplemental Figure S2). Taken together, the findings suggest that day 4 of culture has efficient OATP1B3-mediated transport.

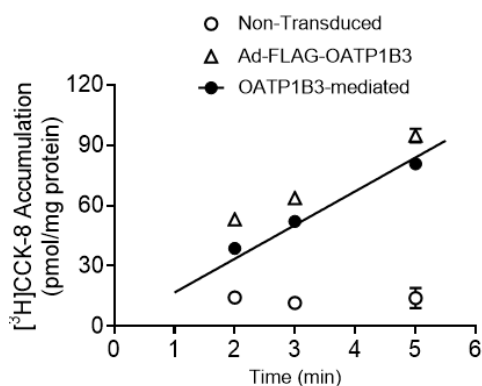


Figure 2. Time-dependent accumulation of [^3H]-CCK-8 (1 μM) cells+BC of rat SCH. Accumulation of [^3H]-CCK-8 (1 μM) was determined in standard HBSS buffer (with Ca^{2+}) on day 4 culture of non-transduced (open circles) and Ad-OATP1B3-transduced (MOI=5) (open triangles) rat SCH for 2, 3, and 5 min. For non-transduced and Ad-OATP1B3-transduced cells, data were expressed as mean \pm SD from $N=1$ experiment in triplicate. Error bars were in visible when overlapping with the symbols. OATP1B3-mediated [^3H]-CCK-8 accumulation (closed circles) was determined by subtracting the mean value in non-transduced (open circles) from that in Ad-OATP1B3-transduced (open triangles) SCH. Line represents the linear regression of the OATP1B3-mediated CCK-8 transport.

TC disposition in rat SCH with and without Ad-OATP1B3 transduction

As shown in Figure 4, compared with non-transduced SCH, expressing OATP1B3 in rat SCH significantly increased total accumulation of [^3H]-TC in cells+bile (39.3 ± 5.1 vs. 25.4 ± 5.1 pmol/mg protein, Ad-OATP1B3 vs. none, $p=0.001$), consistent with the fact that TC is a substrate of OATP1B3 (19). Over-expressing OATP1B3 in rat SCH also significantly increased accumulation of [^3H]-TC in cells (11.5 ± 4.4 vs. 4.16 ± 4.8 pmol/mg protein) (Figure 4). There was no significant difference in TC BEI or *in vitro* $\text{Cl}_{\text{biliary}}$ values between rat SCH with and without Ad-OATP1B3-transduction.

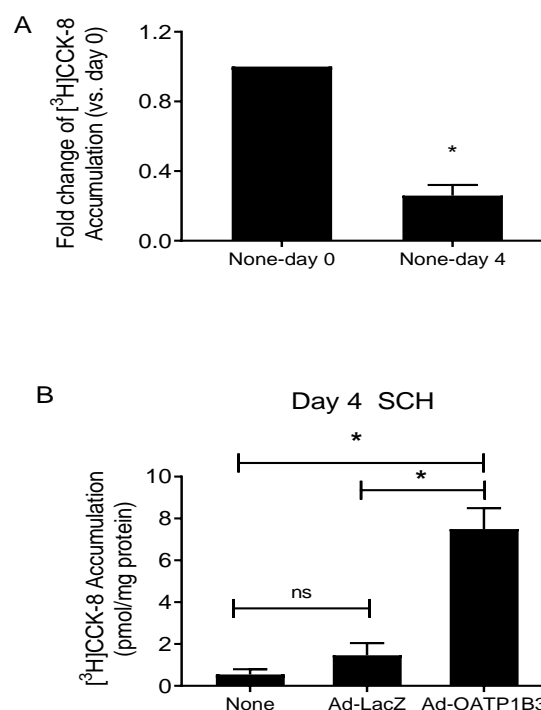


Figure 3. Comparison of [^3H]-CCK-8 accumulation in conditions of different culture day and Ad-OATP1B3-transduction. [^3H]-CCK-8 accumulation (1 μM , 20 sec) in HBSS+ Ca^{2+} buffer was determined in non-transduced (None) rat SCH on day 0 and day 4 (A), and in Ad-LacZ- or Ad-OATP1B3-transduced rat SCH on day 4 (B). (A) Model estimated fold change and associated SE of [^3H]-CCK-8 accumulation on day 4 vs. day 0 non-transduced SCH. A generalized linear mixed effect model was utilized to analyze the data. *, $p<0.05$ vs. non-transduced (none) control. (B) Model estimated [^3H]-CCK-8 accumulation and associated SE in non-transduced, Ad-LacZ- and Ad-OATP1B3-transduced day 4 rat SCH. A linear mixed effect model was utilized to analyze the data. *, $p<0.05$ vs. indicated control. ns, $p>0.05$ vs. indicated control. Experiments were conducted in triplicate using hepatocytes from $N=3$ or 4 rat livers. Data are represented as model estimated mean values and associated standard error (SE). Data from non-transduced day 4 SCH in (A) were replotted in B for comparison purposes.

DISCUSSION

The current study developed a novel rat SCH model expressing human OATP1B3. In rat hepatocytes, it has been reported that Oatp1b2 is the endogenous basolateral transport protein that mediates hepatic uptake of CCK-8 and that mRNA of Oatp1b2 in rat SCH was reduced more than 10-fold on day 4 compared with day 0 (28). The negligible [^3H]-CCK-8 uptake mediated by endogenous rat transporter in non-transduced day 4

SCH (Figure 3A) is consistent with reduced Oatp1b2 expression over days in culture in rat hepatocytes reported previously (28). In the current study, in Ad-OATP1B3-transduced SCH at day 4 of culture, there was extensive expression of OATP1B3 protein (Figure 1) and effective OATP1B3-mediated uptake of CCK-8 into hepatocytes (Figure 3). In addition, the TC BEI in Ad-OATP1B3-transduced SCH was similar to non-transduced cells without detected toxicity (Figure S2). Day 4 cultured non-transduced rat SCH has been demonstrated to be an optimal culture time to study *in vitro* drug biliary disposition, as day 4 is when extensive bile canaliculi are formed (11-13). Our data suggest that Ad-OATP1B3-transduced rat SCH can also be used to assess hepatic biliary disposition of OATP1B3 substrates at day 4 of culture.

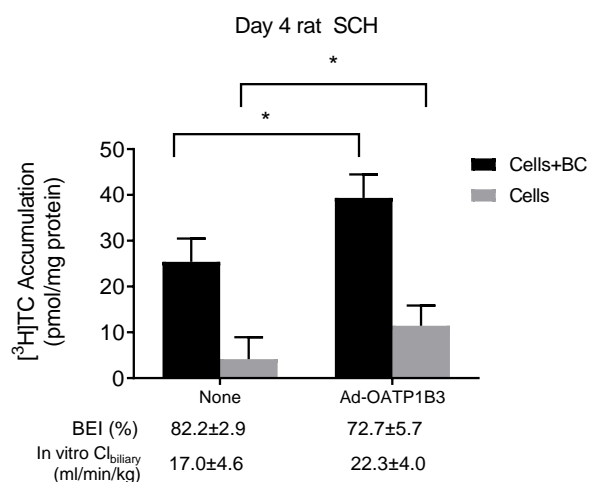


Figure 4. TC accumulation in cells+BC and cells after 10-incubation with 1 μ M TC in non-transduced (none) and Ad-OATP1B3-transduced day 4 rat SCH. Model estimated $[^3\text{H}]$ -TC accumulation (1 μ M, 10 min) and associated SE in cells+BC (closed bars) and cells (open bars). A linear mixed effect model was utilized to analyze the data. BEI and *in vitro* $Cl_{biliary}$ values are expressed as mean \pm standard error of mean (SEM). Experiments were conducted in triplicate using hepatocytes from $N=4$ rat livers. *, indicates a statistically significant difference ($p<0.05$) vs. none.

The hepatobiliary elimination process of TC involves basolateral uptake and efflux, and biliary excretion (10). TC disposition in human SCH was described by a pharmacokinetic model in a previous publication (10). The model simulation showed that while both TC total concentration in cells and total amount of TC in Cells+bile was sensitive to changes in Cl_{uptake} of TC (10), BEI and total amount of TC in bile were not sensitive to changes in Cl_{uptake} (10). In

the current study, increased hepatic uptake of $[^3\text{H}]$ -TC in OATP1B3-expressing rat SCH led to a significant increase in total accumulation of $[^3\text{H}]$ -TC in cells+bile and cells without affecting *in vitro* $Cl_{biliary}$. Our data are consistent with the predicted trend of *in vitro* disposition of TC in human SCH upon changes in Cl_{uptake} , suggesting that TC disposition in this OATP1B3-expressing rat SCH may be similar to that in human SCH.

Transporter-overexpressing mammalian stable cell lines, including human embryonic kidney (HEK) 293 and Chinese Hamster Ovary (CHO) cells (21, 29-32), have been extensively utilized to study OATP1B1- and OATP1B3-mediated transport. These cell culture models are suitable to determine substrates of transporters, characterize transport kinetics, and assess inhibition potency of transporter inhibitors. Suspension hepatocytes are gold-standard to determine substance initial total accumulation. Application of the B-CLEAR[®] technology in Ad-OATP1B3-expressing rat SCH allows simultaneous determination of not only total substrate accumulation (cells+bile), but also hepatocellular accumulation (cells) and *in vitro* biliary excretion of the compound in the presence of OATP1B3.

We and others also reported that OATP1B3 mRNA and immunoreactivity is detected in various cancers (33, 34), however, the exact function of OATP1B3 in cancers remains to be elucidated. Because OATP1B1 and OATP1B3 are abundantly and primarily expressed in human liver (2, 35), they provide opportunities for liver-targeted drug design. Some therapeutic agents exert their pharmacological action via extensive uptake by the liver include 3-hydroxy-3-methyl-glutaryl coenzyme-A (HMG-CoA) reductases inhibitor statins, glucokinase activators (GKA) (36), and stearoyl-CoA desaturase-1 (SCD1) inhibitors (37). Statin can accumulate in the muscle and has potential to cause statin-induced myopathy when systemic exposure of statin increases due to drug-drug interactions (38, 39). Statins are substrates of hepatic OATPs (23, 40). The liver-targeting feature of statins is beneficial for their clinic tolerability against statin-induced myopathy. A liver-selective GKA with a liver-to-pancreas ratio greater than 50-fold reduces the risk of insulin overproduction due to excessive GK activation in pancreas (36, 41). A liver-targeting SCD1 inhibitor, which has >10-fold liver-to-plasma ratio and >30-fold liver-to-skin ratio, did not elicit adverse responses found in a systemically distributed SCD1 inhibitor, including severe hair loss and skin and eye abnormalities (37). Hence, the Ad-OATP1B3-

expressing rat SCH could be a useful model to use when screening for liver-targeting compounds during drug development. This approach can potentially be extended to other liver-specific hepatic transporters, such as OATP1B1.

CONCLUSION

In conclusion, the current study established a novel human OATP1B3-expressing rat SCH model that can be used to study hepatobiliary disposition of OATP1B3 substrates. This model has been successfully utilized to assess hepatic uptake of OATP1B3 substrate CCK-8 and hepatobiliary disposition of TC in current studies. Further studies are warranted to test the application of this model using liver-targeting drugs that are OATP substrates.

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Authorship Contributions. Participated in research design: TF, WY; Conducted experiments: TF, WY; Performed data analysis: TF, CX, WY; Wrote or contributed to writing the manuscript: TF, CX and WY

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