P434 Characterization of a Novel Transporter Model for Studying Drug Interactions with Organic Anion-Transporting Polypeptide (OATP)

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Introduction

SLC Transporters are multi-transmembrane proteins, present in all the human tissues that facilitate the uptake of endogenous compounds and xenobiotics. During the past decade, the significant role of transporters in drug disposition and safety has been strengthened due to the increasing evidence of transporter mediated drug-drug interaction (DDI). Regulatory authorities recommend evaluating specific SLC transporters of emerging importance in drug development, among which include two Organic Anion-transporting Polypeptides, OATP1B1/SLCO1B1 and ATP1B3/SLCO1B3 (1,2).

OATP1B1 and 1B3, localized on the sinusoidal membrane of hepatocytes, are major hepatic uptake transporters that facilitate the efficient cross membrane transport of drugs or xenobiotics. OATP1B1 can transport a wide variety of drugs, including many statins (e.g., atorvastatin, rosuvastatin, pravastatin and pitavastatin) and some oral antibiotics (repaglinide, troglitazone) (3,4,5). The substrate specificity of OATP1B3 commonly overlaps that of OATP1B1, but there are some differences as far as substrate recognition and infinity. Clinical evidence has demonstrated that inhibition of OATP mediated uptake can lead to significant alteration of the pharmacokinetic profiles of the "victim" drug, resulting in toxicity; e.g., the immunosuppressive drug, cyclosporin A, can cause a DDI with statins with up to 20-fold increases in systemic exposure (6). In addition, a series of genetic polymorphisms of OATP1B1 has been characterized, i.e., the individuals with OATP1B1*5 and OATP1B1*15 haplotypes have increased exposure to statin drugs (7).

Appropriate *in vitro* characterization of drug interactions with these hepatic uptake transporters facilitates decision-making and development of appropriate clinical plans in drug development. Corning Life Sciences have recently developed a novel SLC transporter model to help the pharmaceutical industry address regulatory authorities recommendation in a fast and easy manner (Refer to **Poster 465**). In this poster, the studies focus on validating the novel model for evaluating drug interaction with OATP1B1 and OATP1B3. Time-dependent, concentration dependent uptake of endogenous substrates and drugs, and the inhibition profile of known transporter modulators were evaluated. The kinetic and inhibition profiles are compared with current existing SLC transporter models. To address substrate-dependent inhibition, OATP1B1 inhibition profiles using three different substrates, estradiol-17β-glucuronide, rosuvastatin and pitavastatin, were tested and compared. Together, this study demonstrates the newly developed SLC transporter model is a robust and compliant *in vitro* tool to study OATP1B1 and OATP1B3 drug interaction.

Materials and Methods

Materials. Corning® TransportoCells™ Cryopreserved SLC transporter cells, human OATP1B1*1a (Corning Cat. No. 354859) and human OATP1B3 (Corning Cat. No. 354851), were prepared using HEK293 cells which transiently over-express OATP1B1*1a (NM_006446) or OATP1B3 (NM_019844) cDNA, respectively. The control cells (Corning Cat. No. 354854) are the parent HEK293 cells transiently expressing the empty vector. [^{3}H]Estradiol-17β-glucuronide, [^{3}H]estrone-3-sulfate, [^{3}H]rosuvastatin, [^{3}H]CCK-8 were purchased from PerkinElmer (Waltham, MA). All the non-radiolabeled compounds were purchased from Sigma-Aldrich (St. Louis, MO), except glibenclamide and simvastatin which were obtained from Toronto Research Laboratory (Toronto, Canada). Corning BioCoat™ Poly-D-Lysine coated plate, Corning cellgro® DMEM high glucose, MEM non-essential amino acid solution (100X), Fetal Bovine Serum (FBS) and Hank's Balanced Salt Solution with Ca^{2+} and Mg^{2+} (1X) (HBSS buffer) were obtained from Corning Life Sciences. Mammalian Protein Extraction Reagent (M-PER) was purchased from Thermo Scientific. Sodium butyrate (500 mM) was purchased from EMD-Millipore (Billerica, MA).

Thawing and Plating SLC Transporter Cells. The cryopreserved SLC transporter cells were thawed in a 37°C water bath. The cells were resuspended in DMEM supplemented with non-essential amino acid, L-glutamine, and 5 mM sodium butyrate, then seeded in 24 well or 96 well Poly-D-Lysine coated plates at a density of 400,000 cells per well or 100,000 cells per well, respectively. After 3-4 hours incubation, the cells were re-fed with plating media supplemented with 5 mM sodium butyrate. The cells were kept in a 37°C incubator with 8% CO_2 overnight.

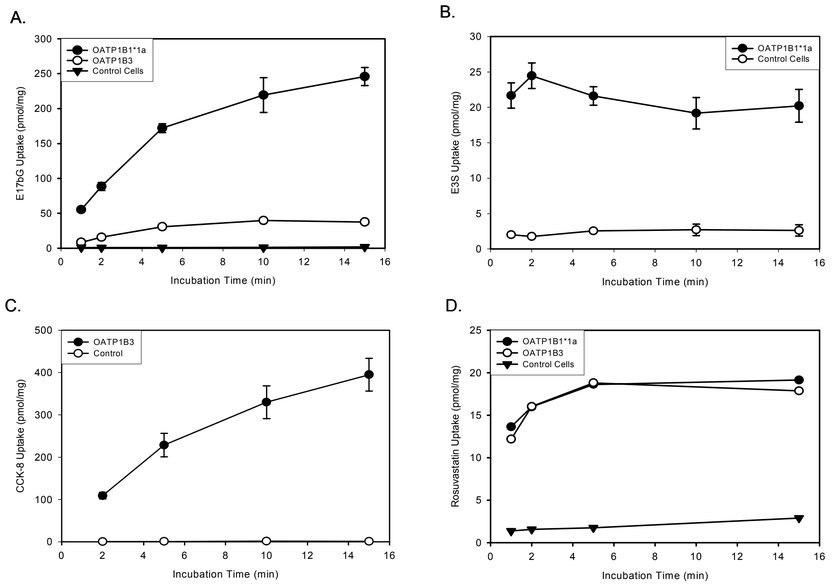
Uptake Study using SLC Transporter Cells. At 24 hours post plating, cells were washed twice and pre-incubated with HBSS buffer at 37°C for 10 min. Uptake was initiated by adding HBSS buffer containing radiolabeled and unlabeled substrates. The uptake was terminated at a designated time by adding ice-cold HBSS buffer after removal of the incubation buffer. Cells were washed twice with ice-cold HBSS buffer and lysed in M-PER for 5 min at room temperature. Aliquots of 300 µL (24 well) or 50 µL (96 well) cell lysates were transferred into scintillation vials containing 5 mL scintillation liquid. The radioactivity was measured in a liquid scintillation counter (LS6000SE, Beckman Coulter, Inc., Fullerton, CA). 25 µL of cell lysate was used to determine the protein concentration by the BCA protein assay kit (Thermo Scientific).

Data Analysis. Kinetic parameters were determined by non-linear regression using SigmaPlot™. For each substrate concentration, the initial uptake rate was calculated by subtracting the initial rate determined in HEK cells expressing empty vector from those obtained in HEKs overexpressing SLC transporter. For inhibition assay, IC_{50} values were determined by using a Sigmoidal Hill three parameter equation.

Reference

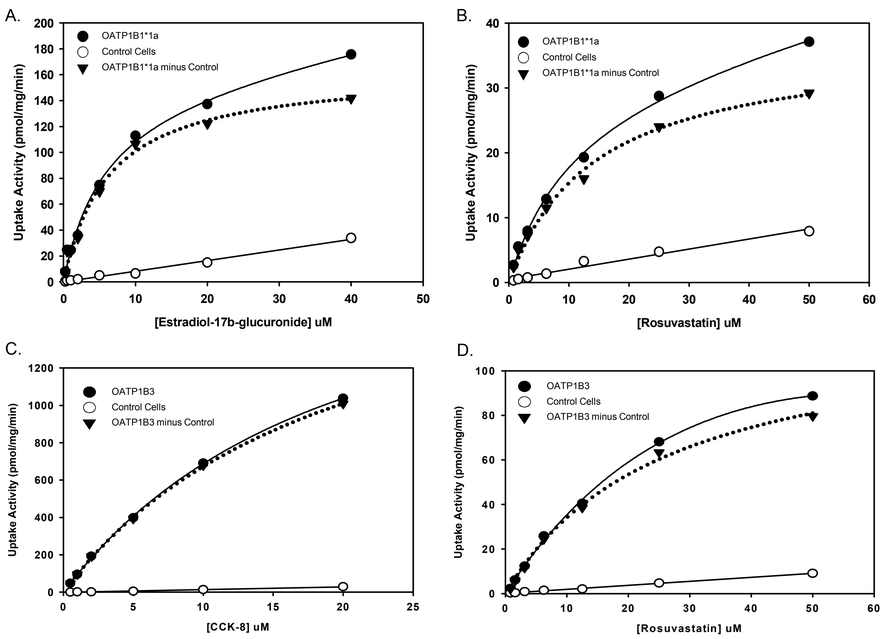
1. USFDA Draft Guidance for Industry: Drug Interaction Studies (2012)
2. EMA Guidance on Investigation of Drug Interaction. (2013)
3. Nozawa T, et al., *Drug Metab Dispos* 32:291-294 (2004)
4. Niemi M, et al., *Clin Pharmacol Ther* 77:468-478 (2005)
5. Fahrmayr C, et al., *Drug Metab Rev* 42:380 (2010)
6. Kalliokoski and Niemi, M, *Br J Pharmacol* 158:693-705 (2009)
7. Giacomini KM, et al., *Nature Reviews-Drug Discovery* (9):215-236 (2010)

Panel 1: Time-dependent Uptake of Prototypical Substrates in OATP1B1 and OATP1B3 Corning® TransportoCells™ SLC Transporter Cells



Uptake of 1 µM Estradiol-17β-glucuronide (**A**) and 1 µM Estrone-3-sulfate (**B**) in OATP1B1*1a cells or OATP1B3 cells were determined at 1, 2, 5, 10 and 15 min respectively at 37°C. Uptake of 1 µM CCK-8 (**C**) in OATP1B3 were determined at 2, 5, 10 and 15 min at 37°C. Uptake of 1 µM Rosuvastatin (**D**) in OATP1B1*1a and OATP1B3 cells were determine at 1, 2, 5, and 15 min, respectively. Control Cells were used in all time-course experiment to demonstrate the time-dependent uptake of selected probe substrates via OATP. The results represent the mean ± S.D. of three replicates.

Panel 2: Kinetic Analysis of Prototypical Substrate Uptake Activity in OATP1B1 TransportoCells

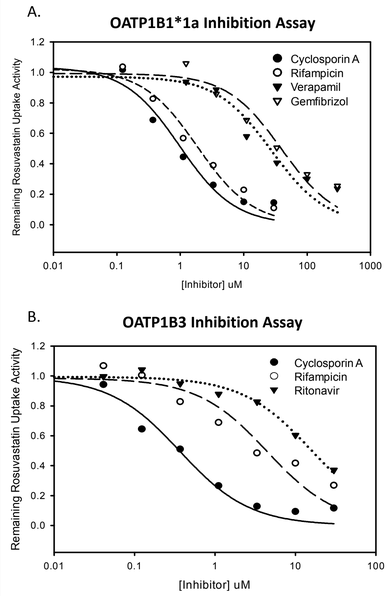


Determination of kinetics of the prototypical substrates of OATP1B1 and OATP1B3. (**A**) and (**B**) concentration-dependent uptake of [^{3}H]-E17βG and [^{3}H]-Rosuvastatin in OATP1B1*1a cells after 1 min or 2 min incubation, respectively. (**C**) and (**D**) concentration-dependent uptake of [^{3}H] CCK-8 and [^{3}H] Rosuvastatin in OATP1B3 cells after 5 min or 2 min incubation, respectively. Six to eight substrate concentrations were used in each kinetic determination. The dotted line represents the nonlinear fit of the uptake into the Corning® TransportoCells™ corrected for the uptake in the control cells.

Table 1. Comparison of Kinetic Parameters with Existing SLC Transporter Models

Corning TransportoCells SLC Transporter Cells			Reported Kinetic Parameters in Literature		
Transporter	**Substrate**	**K_m (µM)**	**K_m (µM)**	**Test System**	**Literature**
OATP1B1*1a	Estradiol-17β-glucuronide	6.2	6.3	HEK293 stable	P. Sharma, et al., Xenobiotica (2010)
			7	HEK293 stable	E. van de Steeg, et al., DMD (2013)
			8.2	HEK293 stable	Hirano M, et al., JPET (2004)
	Estrone-3-sulfate	0.4	0.46	HEK293 stable	Hirano M, et al., JPET (2004)
			0.23	CHO stable	Noé J, et al., DMD (2007)
	Rosuvastatin	14.2	13.1	HEK293 stable	E. van de Steeg, et al., DMD (2013)
			7.4	HeLa stable	Ho RH, et al., Gastroenterology (2006)
			8.5	Oocytes	Simonson SG, et al., Clin Pharmacol Ther (2004)
OATP1B3	CCK-8	20.2	16.5	CHO stable	Poirier A, et al., J Pharmacokinet Pharmacodyn (2009)
			3.8	HEK293 stable	Hirano M, et al., JPET (2004)
	Rosuvastatin	26.2	14.2	HEK293 stable	Kitamura S, et al., DMD (2008)
			9.8	HeLa stable	Ho RH, et al., Gastroenterology (2006)

Panel 3: Inhibitory Profiles of OATP1B1 and OATP1B3 Modulators



			Corning® TransportoCells™	Reported IC_{50}s
Transporter	**Substrate**	**Inhibitor**	**IC_{50} (µM)**	**IC_{50} (µM)**
OATP1B1*1a	E17βG	Cyclosporin A	0.8	0.9[a (HEK)]
		Rifampicin	1.1	0.6[a (HEK)]
		Ritonavir	0.6	0.9[a (HEK)]
		Simvastatin	8.5	5.0[a (HEK)]
	Rosuvastatin	Cyclosporin A	0.9	0.31[b (HEK)]
		Glibenclamide	1.4	1.77[c (HEK)]
		Rifamycin SV	0.3	n.a
		Rifampicin	1.7	n.a
		Ritonavir	0.6	n.a
		Simvastatin	6.9	n.a
		Gemfibrozil	30.3	25[b (HeLa)]
		Verapamil	18	n.a
OATP1B3	CCK-8	Cyclosporin A	0.7	n.a
		Rifampicin	1.9	n.a
		Ritonavir	2.8	n.a
	Rosuvastatin	Cyclosporin A	0.3	0.06[b (HeLa)]
		Rifampicin	3.2	n.a
		Ritonavir	16.5	n.a

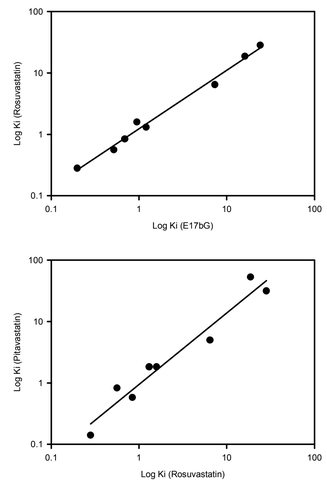
[a] Soars MG, et al. Drug Metab Dispos (2012)
[b] Ho RH, et al. Gastroenterology (2006)
[c] E. van de Steeg, et al., Drug Metab Dispos (2013)

SLC Transporter Cells Inhibition Assay. IC_{50} values for the indicated transporter modulators were determined by co-incubating the cells with the substrates (E17βG, Rosuvastatin or CCK-8) at a fixed concentration (1µM) with the selected modulator at a range of concentrations. The IC_{50} values generated using Corning TransportoCells SLC transporter cells are comparable to those published in the literature.

Panel 4: Comparison of Inhibition Profiles using Different Substrates

Inhibitor	IC_{50}		
	E17βG	**Rosuvastatin**	**Pitavastatin (Ki)**
Cyclosporin A	0.8	0.9	0.7[a]; 0.24[b]
Rifampicin	1.1	1.7	2.2[a]; 0.48[b]
Ritonavir	0.6	0.6	1.0[a]; 0.78[b]
Simvastatin	8.5	6.9	6.0[a]
Gemfibrozil	28[a]	30.3	38[a]; 25.2[b]
Verapamil	18.7	20	64[a]; 51.6[b]
Glibenclamide	1.4[a]	1.4	2.2[a]; 0.75[b]
Rifamycin SV	0.23[c]	0.3	0.17[b]

[a] Soars MG, et al., Drug Metab Dispos (2012)
[b] Hirano M, et al., Drug Metab Dispos (2006)
[c] Sharma P, et al., Xenobiotica (2010)



Comparing the IC_{50} values determined using E17βG, Rosuvastatin or Pitavastatin as substrate respectively. K_i estimates were calculated using the IC_{50} value of OATP1B1 uptake of indicated substrate at the concentration of 1µM based on equation $K_i = IC_{50}/(1+[S]/K_m)$. The linear regression analysis based on Ki estimates of eight known OATP1B1 inhibitors indicated substrates have no significant impact on OATP1B1 inhibition.

Panel 5 : Product Batch to Batch Consistency

Corning® TransportoCells™	
Batch No.	IC_{50} (µM)
1	0.5
2	0.6
3	0.7
4	0.6
5	0.8
mean	0.6
S.D.	0.1

Five batches of Corning TransportoCells OATP1B1 cells were made following the same manufacture procedure. IC_{50} of inhibition of E17βG uptake in OATP1B1 by cyclosporin A was determined using each batch of OATP1B1 cells. The average IC_{50} is 0.6 µM with S.D. of 0.1 µM.

Conclusions

- OATP1B1 and OATP1B3 transiently overexpressing HEK293 cells were validated by characterizing interaction with known substrates and inhibitors.
- Time- and concentration-dependent uptake of prototypical substrates was evaluated. Kinetic and inhibition profiles are aligned with those generated using alternative SLC transporter models described in the literature.
- No substrate dependent effect was observed on inhibition of OATP1B1 mediated uptake of E17βG, Rosuvastatin or Pitavastatin.
- The Corning TransportoCells Cryopreserved SLC transporter model is demonstrated to be an easy to use and robust assay platform with great lot to lot consistency.

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