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Efficient Solute Carrier (SLC) Transporter-Mediated Drug-Drug Interaction Testing in a 96-well Plate Format

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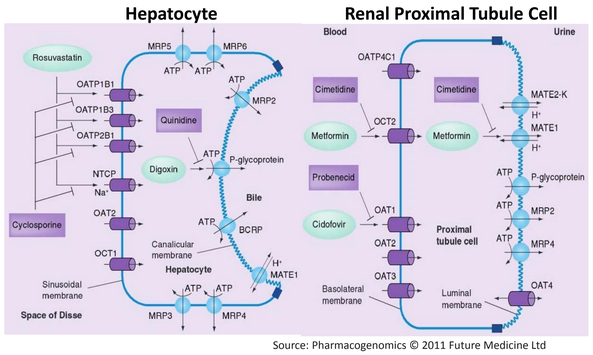
Abstract

Membrane transporters can be major determinants of pharmacokinetic properties of drugs and their metabolites, and can mediate drug-drug interactions (DDIs). We have established an *in-vitro* testing platform for systematic analysis of solute carrier (SLC) transporter interactions, for both screening and definitive DDI studies recommended by regulatory agencies. Corning® TransportoCells™ are HEK-293 cells that transiently over-express a single SLC transporter protein and provide a common cell-based model. The transporters examined were OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1 and MATE2-K, and assays were established using prototypical and/or clinically relevant substrates and inhibitors. TransportoCells were thawed, seeded in 24- and 96-well plates coated with poly-D-lysine, and cultured for 24h. Before assay, cells were washed with pre-warmed HBSS buffer (with Ca^{2+} and Mg^{2+}) and pre-incubated in HBSS buffer for 10 min at 37° C. MATE1 and MATE2-K cells were subsequently pre-treated with 40 mM NH_4Cl in HBSS for 20 min. The uptake assays were initiated by addition of radiolabeled substrate and non-radiolabeled inhibitor, if applicable, and incubated at 37° C. Reactions were stopped by removing the dosing solutions and washing cells with cold HBSS. The cells were lysed with M-Per Mammalian Protein extraction reagent, and the uptake activity was quantified using LSC normalized for protein concentration in each sample. K_m and IC_{50} values for OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1 and MATE2-K are shown in the table below. We found robust performance in a 96-well plate format with only minimum medium volumes (to conserve consumption of certain costly radiolabeled substrates). Efficiency was further improved by eliminating use of sodium butyrate pretreatment for OATP1B1 and OAPT1B3 for prototypical substrates. We have established a robust *in-vitro* testing system based on a common cell model of HEK-293 cells transiently over-expressing a single SLC transporter, for conducting screening and definitive transporter-mediated DDI studies recommended by regulatory agencies.

Figure 1. Expression of clinically important hepatic and renal transporters



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Materials and Methods

Materials: Corning® TransportoCells™ products: OATP1B1*1a (Cat. No. 354859), OATP1B3 (Cat. No. 354851), OAT1 (Cat. No. 354857), OAT3 (Cat. No. 354858), OCT1 (Cat. No. 354852), OCT2 (Cat. No. 354853), MATE1 (Cat. No. 354855), MATE2-K (Cat. No. 354856), and empty vector (control) cells (Cat. No. 354854) and cell culture reagents were obtained from Corning Life Sciences. Radiolabeled and non-radiolabeled substrates and inhibitors: estradiol-17 beta-D-glucuronide ($E_2$17-ß-Gluc), cholecystokinin octapeptide (CCK-8), estrone-3-sulfate (E3S), p-amino hippuric acid (PAH), metformin, rifampicin, probenecid, and cimetidine were obtained from Corning, PerkinElmer, American Radiolabeled Chemicals, or Moravek.

Inhibition Assay: Corning® TransportoCells™ products transiently over-expressing a single SLC transporter and control cells transfected with empty vector were thawed, seeded in 24-well or 96-well plates coated with poly-D-lysine, and cultured for 24 h. Before assay, cells were washed with pre-warmed HBSS buffer (with Ca^{2+} and Mg^{2+}) and pre-incubated in HBSS buffer for 10 min at 37˚C. MATE1 and MATE2-K were subsequently pre-treated with 40 mM NH_4Cl in HBSS for 20 min. The uptake assays were initiated by addition of radiolabeled substrate and non-radiolabeled inhibitors, if applicable, and incubated at 37˚C for 2 min (MATE1/2K), 5 min (OATP1B1*1a, OATP1B3, OAT1/3), or 10 min (OCT1/2). Reactions were stopped by removing the dosing solutions and washing the cells with cold HBSS. The cells were lysed with M-Per Mammalian Protein extraction reagent, and the uptake activity was quantified using liquid scintillation counting normalized for protein concentration in each sample.

Data Analysis: Kinetic parameters were determined by non-linear regression using XLfit (IDBS). For each substrate concentration, the initial uptake rate was calculated by subtracting the initial rate determined in HEK-293 cells expressing empty vector from those obtained in HEK-293 cells over-expressing a SLC transporter. For the inhibition assays, IC_{50} values were determined by using Sigmoidal Hill four- parameter equation.

Results and Discussion

Initially, we established the Corning TransportoCells assay for contract research services using the 24-well format, obtaining K_m, V_{max}, and IC_{50} results with prototypical substrates, which are consistent with reported values in the literature. Assay conditions were then modified to incorporate operational efficiencies while retaining robust performance:

- The assay was established in the 96-well plate format, allowing volume reduction of TransportoCells product, medium, and certain costly radiolabeled substrates.
- The savings in reagent volumes using 96-well format vs. 24-well, were further increased by reducing the well volumes 33% from 120 µL to 80 µL.
- Sodium butyrate can be added to culture medium to enhance transporter activity. We found that omission of sodium butyrate from the medium (OATP1B1 and OATP1B3 assays with prototypical substrates) resulted with adequate activity of the substrates. Moreover, we observed an enhancement of cell attachment when sodium butyrate was not added to the medium. Therefore, supplementing the medium with sodium butyrate is recommended when additional activity is required, however, the step can be omitted when signal is adequate, thereby reducing the steps in the assay.

The K_m, V_{max}, and IC_{50} values for OATP1B1*1a, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1, and MATE2-K generated in the 96-well format were in good agreement with our 24-well results. Representative results of our evaluation are summarized in **Table 1**, **Table 2**, and **Table 3**.

Results from a representative Corning Gentest℠ Contract Research study performed in the 96-well Corning TransportoCells assay are shown in **Table 5**.

Table 1. Comparison of uptake (K_m) results for prototypical substrates of SLC transporters in 96-well vs. 24-well Corning TransportoCell assays

Transporter	Substrate	Plate Format	K_m (µM)	V_{max} (pmol/mg/min)	Literature K_m Value (µM)	Literature Reference
OATP1B1*1a	$E_2$17-ß-Gluc	24	9.8	337	6.3	4
		96	8.5	424		
OATP1B3	CCK-8	24	39	4998	17	5
		96	52	4649		
OAT3	E3S	24	3.7	276	6.3	6
		96	8.1	618		

Table 2. Uptake (K_m) data for prototypical substrates of SLC transporters in 96-well Corning TransportoCell assays

Transporter	Substrate	K_m (µM)		V_{max} (pmol/mg/min)		Literature K_m	Literature Reference
		Assay 1	Assay 2	Assay 1	Assay 2		
OATP1B1*1a	$E_2$17-ß-Gluc	6.0	11	337	511	6.3	4
OATP1B3	CCK-8	37	67	3277	6021	17	5
OAT1	PAH	99	118	3941	6305	28	6
OAT3	E3S	9.1	7.1	863	373	6.3	6
OCT1	Metformin	8165	5679	22899	17287	5450	7
OCT2	Metformin	3766	6618	52545	31703	3356	8
MATE1	Metformin	541	333	34281	22384	227	9
MATE2-K	Metformin	2408	2476	48932	29134	1980	10

Table 3. Inhibition of uptake (IC_{50}) data for prototypical substrates of SLC transporters in 96-well Corning TransportoCell Assays

Transporter	Substrate	Inhibitor	IC_{50} (µM)		Literature IC_{50}	Literature Reference
			Assay 1	Assay 2		
OATP1B1*1a	$E_2$17-ß-Gluc	Rifampicin	1.4	1.9	1.5	11
OATP1B3	CCK-8	Rifampicin	5.0	4.6	no data	no data
OAT1	PAH	Probenecid	6.5	5.7	6.5	12
OAT3	E3S	Probenecid	7.8	7.7	9.0 (K_i)	13
OCT1	Metformin	Cimetidine	109	251	166 (K_i)	14
OCT2	Metformin	Cimetidine	446	642	373	15
MATE1	Metformin	Cimetidine	2.2	2.0	3.8 (K_i)	16
MATE2-K	Metformin	Cimetidine	6.7	7.4	6.9	16

In addition, we conducted further characterization of OATP1B1*1a- and OATP1B3-expressing Corning TransportoCells products with clinically relevant substrates, statin drugs pravastatin and rosuvastatin. The results are shown in **Table 4** and representative K_m/IC_{50} curves are provided in **Figure 2**.

Table 4. Kinetic data for statins in 96-well Corning TransportoCell assays

Transporter	Substrate	Km (µM)	Vmax (pmol/mg/min)	Inhibitor	IC_{50} value (µM)
OATP1B1*1a	Pravastatin	58	238	Rifampicin	5.2
OATP1B1*1a	Rosuvastatin	11	76	Rifampicin	2.0
OATP1B3	Pravastatin	70	107	Rifampicin	0.74
OATP1B3	Rosuvastatin	9.7	140	Rifampicin	14

Figure 2. K_m and IC_{50} curves for statins in 96-well Corning TransportoCells assays

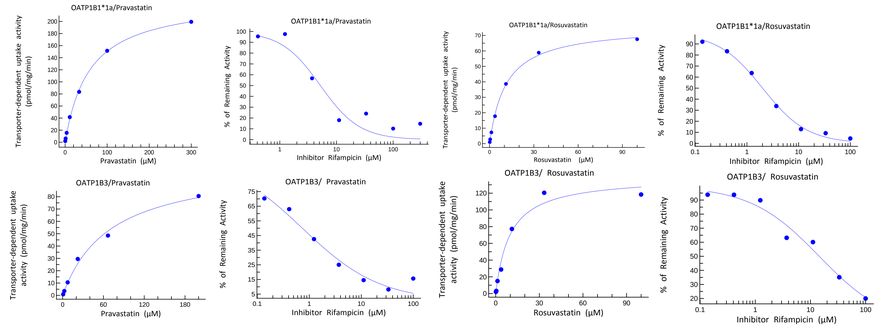

Table 5. Results from a representative Corning® Gentest℠ Contract Research Services study (Transporter DDI) conducted in Corning TransportoCells

A

Transporter	Probe Substrate	Inhibitor	Inhibitor Conc. (µM)	% of Remaining Activity	Uptake Ratio
OATP1B1	1 µM E_2-17-ß-Gluc	Test Article X	0	100%	10
			300	64%	7.6
		Rifampicin	100	0.26%	1.0
OATP1B3	2 µM cholecystokinin octapeptide (CCK-8)	Test Article X	0	100%	12
			300	76%	13
		Rifampicin	100	7.8%	2.5
OAT1	15 µM PAH	Test Article X	0	100%	22
			300	109%	20
		Probenecid	100	16%	3.8
OAT3	4 µM E3S	Test Article X	0	100%	9.5
			300	23%	3.7
		Probenecid	100	7.0%	1.6
OCT1	10 µM Metformin	Test Article X	0	100%	11
			300	33%	5.8
		Cimetidine	1000	23%	3.5
OCT2	10 µM Metformin	Test Article X	0	100%	20
			300	45%	11
		Cimetidine	1000	16%	4.8

B

Transporter	Probe Substrate	Inhibitor	Inhibitor Conc. (µM)	% of Remaining Activity			Mean % of Remaining Activity	IC_{50} (µM)
				Replicate 1	Replicate 2	Replicate 3		
OAT3	4 µM E3S	Test Article X	0	100	100	100	100	**42**
			0.41	102	96	114	103	
			1.2	93	91	109	97	
			3.7	84	85	101	89	
			11	73	69	83	75	
			33	60	53	64	58	
			100	29	27	33	29	
			300	15	13	11	13	
		Probenecid	100	6.3	5.7	8.0	6.6	N/A
OCT1	10 µM Metformin	Test Article X	0	100	100	100	100	**178**
			0.41	113	68	101	94	
			1.2	105	77	91	91	
			3.7	112	82	95	96	
			11	77	69	97	81	
			33	93	82	97	90	
			100	60	74	68	67	
			300	32	38	30	34	
		Cimetidine	100	22	21	23	22	N/A
OCT2	10 µM Metformin	Test Article X	0	100	100	100	100	**>300**
			0.41	91	92	87	90	
			1.2	96	83	74	85	
			3.7	88	57	84	77	
			11	98	94	95	96	
			33	98	94	101	98	
			100	87	98	102	95	
			300	46	56	62	54	
		Cimetidine	100	14	16	18	16	N/A

Data generated in an SLC Transporter DDI study for a pharmaceutical Sponsor.

The inhibition screening assay measuring transporter-mediated uptake activity of prototypical substrates with six SLC transporters , identified inhibitory potential of Test Article X with three of the transporters **(A).**

The concentration-dependent inhibitory effect was further characterized in the subsequent IC_{50} assay **(B).**

Conclusions

We have established a robust *in-vitro*, 96-well testing system based on a common cell model (Corning TransportoCells assay – HEK-293 cells transiently over-expressing a single SLC transporter) for conducting screening and definitive DDI studies required by regulatory agencies.

Operational efficiencies and cost savings were gained by adapting the methods to the 96-well format, which included omission of a medium supplement and reduction of expensive reagent volumes.

The method has been successfully used in the Corning Gentest℠ Contract Research Services organization for both screening and DDI studies contracted by pharmaceutical Sponsors.

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