ESTABLISHMENT OF A COMPLETE IN VITRO DRUG TRANSPORTER PANEL TO MEET GLOBAL REGULATORY GUIDELINES

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Introduction

- Drug transporters play a key role in the absorption, distribution, metabolism, elimination, and toxicity (ADME-Tox) of many drugs. Due to the importance of transporters in these processes and possible contributions to drug-drug interactions, in vitro transporter studies are required by the FDA, EMA, and PDMA regulatory agencies to evaluate the potential interactions between investigational drugs acting as substrates and/or inhibitors of the standard panel of drug transporters.
- The current drug transporter panel includes P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1, and MATE2-K, although regulatory agencies are routinely requesting data on transporters in addition to the current panel.
- Alliance Pharma has established a complete set of cell-based or membrane- vesicle–based assays to satisfy the studies required by regulatory agencies. The data presented here describes our work to show that transiently transfected HEK293 cells are capable of characterizing transporter-specific substrates and inhibitors.

Materials and Methods

- ***Materials***

  SLC TransportoCells™ (Transporter-transiently transfected HEK293 cells), poly-D-lysine–coated 96-well plates, high-glucose DMEM, and FBS were from Corning Inc. Sodium-butyrate (500 mM) was from EMD-Millipore. Rosuvastatin, estrone-3-sulfate, Na-aminohippuric acid, metformin, rifampicin, probenecid, and cimetidine were from Sigma-Aldrich, Inc.

- ***Cell Culture***

  SLC TransportoCells™ were thawed and seeded at an optimized density in a poly-D-lysine–coated 96-well plate. The medium was changed after 4-6 hr post-seeding, and cells were cultured for a total of 24 hr before the assay. Medium was supplemented with Na-butyrate (2 mM) for OATP1B1, OATP1B3, MATE1, and MATE2-K.

Transporter-transiently transfected cells stored in liquid N_2 → **Cell seeding in poly-D-lysine coated plate** → **Culture for 24 hours** → **Uptake assay**

- ***Uptake Assay***

  Cells were washed with HBSS (10 mM HEPES) and preincubated for 10 min (simple uptake) or 30 min (uptake inhibition). Preincubation buffer was replaced with assay buffer containing substrate to initiate uptake. At the end of incubation, the uptake was stopped by removing the assay buffer and washing with ice-cold HBSS, two times.

  Cells were crashed with acetonitrile containing internal standard for LC/MS/MS analysis of probe substrates or test compounds, or lysed with Thermo Scientific RIPA lysis buffer for BCA assay to determine protein concentration.

- ***Calculations***

  Uptake (pmol/mg protein) = Drug cellular accumulation (pmol) / Protein amount (mg)

  Net transport = Uptake $_{transfected}$ − Uptake $_{control}$

  Uptake Activity (pmol/mg protein/ min) = Net transport / incubation time

  %Control = Uptake with inhibitor / Uptake without inhibitor × 100

Results—Method Validation

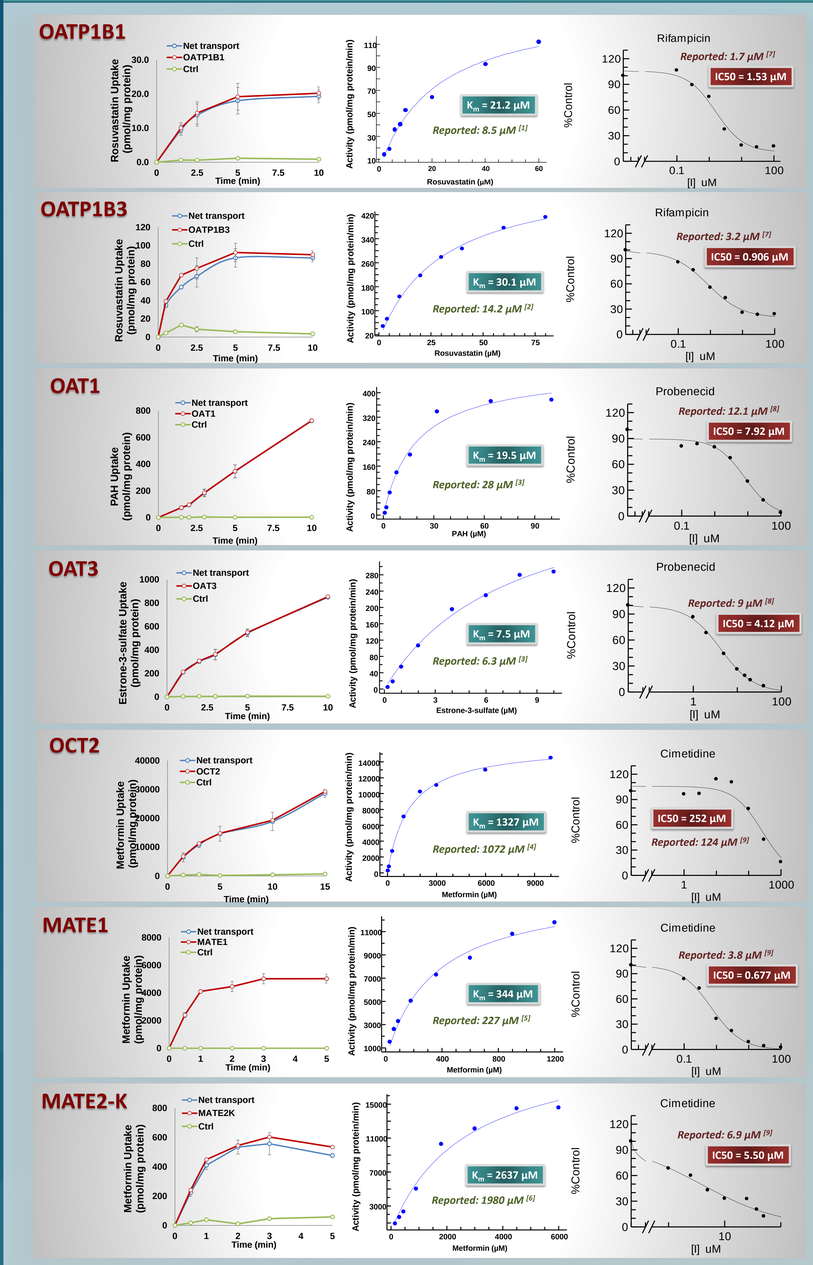

Figure 1. Results of system validation using known transporter substrates and inhibitors. Linear uptake time was first determined with a time-dependent assay (left), followed by a kinetics assay for K_m determination (middle) and an inhibition assay with a known inhibitor (right).

References

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Results—Representative Project

❑ Case 1: Substrate Assessment for Compound A

Based on uptake ratios of transfected and mock cells, Compound A is likely to be transported by OAT3 and possibly by OATP1B3 and MATE1.

Transporter	OATP1B1			OATP1B3			OAT1			OAT3			OCT2			MATE1			MATE2-K		
Conditions	Compound A 2 μM	Compound A 10 μM	Control (RSVS)	Compound A 2 μM	Compound A 10 μM	Control (RSVS)	Compound A 2 μM	Compound A 10 μM	Control (PAH)	Compound A 2 μM	Compound A 10 μM	Control (E3S)	Compound A 2 μM	Compound A 10 μM	Control (MTF)	Compound A 2 μM	Compound A 10 μM	Control (MTF)	Compound A 2 μM	Compound A 10 μM	Control (MTF)
Transfected Cell (*n*=3)	0.0220	0.0806	*0.448*	0.0624	0.215	*0.952*	0.0350	0.156	*14.6*	0.117	0.484	*7.27*	0.0242	0.0954	*1.37*	0.0588	0.234	*1.42*	0.0761	0.175	*1.25*
Mock Cell (*n*=3)	0.0162	0.0574	*0.0181*	0.0251	0.0833	*0.0808*	0.0180	0.0756	*0.267*	0.0190	0.0850	*0.0972*	0.0327	0.145	*0.0807*	0.0209	0.0847	*0.0833*	0.0577	0.123	*0.147*
Transfected/Mock Ratio	**1.36**	**1.40**	***24.7***	**2.49**	**2.58**	***11.8***	**1.94**	**2.06**	***54.6***	**6.15**	**5.69**	***74.8***	**0.739**	**0.660**	***17.0***	**2.82**	**2.76**	***17.0***	**1.32**	**1.43**	***8.51***

Abbreviations: **RSVS** = rosuvastatin; **PAH** = para-aminohippurate; **E3S** = estrone-3-sulfate; **MTF** = Metformin
Note.—Test compound is regarded to be a substrate when Transfected/Mock Ratio > 2.

❑ Case 2: Inhibition Screening for Compound A

Compared to control activity with 0 μM test compound, Compound A showed potent inhibition of OAT1 and OAT3 with remaining activity of 17.1% and 11.1% at 50 μM concentration, respectively.

Transporter	OATP1B1			OATP1B3			OAT1			OAT3			OCT2			MATE1			MATE2-K		
Conditions	Compound A 0	Compound A 50 μM	Control (RIF)	Compound A 0	Compound A 50 μM	Control (RIF)	Compound A 0	Compound A 50 μM	Control (PBC)	Compound A 0	Compound A 50 μM	Control (PBC)	Compound A 0	Compound A 50 μM	Control (CMT)	Compound A 0	Compound A 50 μM	Control (CMT)	Compound A 0	Compound A 50 μM	Control (CMT)
Activity (Analyte/IS, *n*=3)	0.102	0.092	*0.013*	0.160	0.188	*0.048*	0.733	0.126	*0.035*	0.692	0.077	*0.070*	0.593	0.645	*0.059*	0.097	0.076	*0.020*	0.105	0.097	*0.029*
% of Remaining Activity	**100**	**90.3**	***12.3***	**100**	**117**	***30.0***	**100**	**17.1**	***4.8***	**100**	**11.1**	***10.1***	**100**	**109**	***9.9***	**100**	**78.2**	***20.8***	**100**	**92.2**	***28.0***

Abbreviations: **RIF** = rifampicin; **PBC** = probenecid; **CMT** = cimetidine; **IS** = internal standard

❑ Case 3: IC_{50} Determination for OATP Inhibitors

Inhibition potency (IC_{50} values) were determined for Compounds AA, BB, CC, and DD against OATP1B1 and OATP1B3. All four compounds showed potent inhibition of OATP1B1, while only Compound CC showed inhibition of OATP1B3 with an IC_{50} value lower than the highest tested concentration (33 μM).

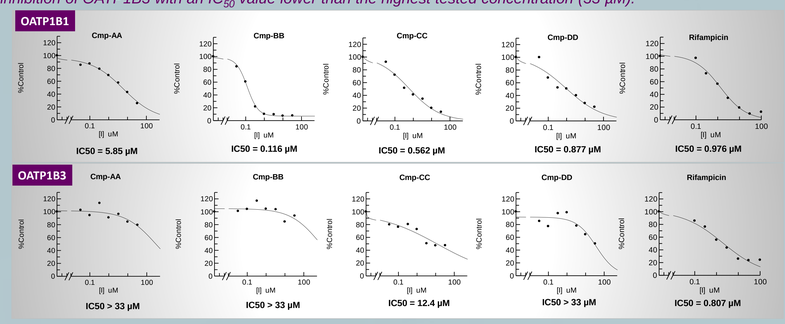

Figure 2. IC_{50} determination for the inhibition of four compounds on OATP1B1 and OATP1B3.

Conclusions

- Using SLC TransportoCells™ (Transporter-transiently transfected HEK293 cells) together with a previously established membrane vesicle assay, Caco-2 bidirectional permeability assay, and MDR1/MDCK bidirectional permeability assay, we established a full-panel platform for transporter studies required by regulatory agencies (including OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1, MATE2-K, P-gp, and BCRP).
- With the optimized and validated uptake assay platform that has been implemented for contract research services, we are able to perform both a phase I assay for substrate/inhibitor screening and a phase II definitive assay for K_m / IC_{50} determination.

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