FUNCTIONAL CHARACTERIZATION OF MAJOR SOLUTE CARRIER (SLC) TRANSPORTERS INVOLVED IN DRUG-DRUG INTERACTIONS USING A POLARIZED MDCK MODEL

Mark S. Warren, Mirza Jahic, Alan Kosaka, John Zhang, Jane Huang, and Yong Huang
Optivia Biotechnology Inc., 115 Constitution Dr. Suite 7, Menlo Park, CA, USA

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Optivia Biotechnology Inc.
www.optiviabio.com

ABSTRACT

Recent regulatory interest in the role of drug transporters in drug-drug interactions (DDI) has resulted in explicit requirements for new chemical entities (NCEs) to be evaluated as substrates and inhibitors of major transporters.

The FDA Draft Guidance on Drug Interaction Studies recommends seven major transporters to be routinely tested: Organic Anion Transporters **OAT1** (SLC22A6) and **OAT3** (SLC22A8), Organic Cation Transporter **OCT2** (SLC22A2), Organic Anion Transporting Polypeptides **OATP1B1** (SLCO1B1) and **OATP1B3** (SLCO1B3), and efflux transporters Breast Cancer Resistance Protein **BCRP** (ABCG2) and P-glycoprotein **P-gp/MDR1** (ABCB1). The EMA guidance recommends two additional transporters – Organic Cation Transporter **OCT1** (SLC22A1) and the Bile Salt Export Pump (**BSEP**) – for evaluating transporter related DDI.

Among the six solute carrier (SLC) transporters, OAT1, OAT3 and OCT2 are predominantly expressed on basolateral membranes of renal proximal tubule cells; OCT1, OATP1B1 and OATP1B3 are the major basolateral transporters in hepatocytes. Despite these transporters operating in polarized cellular environments of the kidney and liver *in vivo*, most labs conduct transporter studies using non-polarized cellular models such as frog oocytes, CHO and HEK293 cells.

Here, we present characterization data for expression of these important SLC transporters in **polarized MDCK cell monolayers**, which more closely mimic the physiology of real organ boundaries. These data are published to facilitate comparison with expression models that do not closely match *in vivo* physiology.

APPROACH

MDCK cells were transfected using a novel *in situ* transfection technology, Opti-Expression, which allows consistent and effective transfection of polarized cell monolayers. Cells were either transfected with plasmids encoding the SLC transporters or a plasmid encoding green fluorescent protein (GFP) as a control. Observations confirmed the SLC transporters are properly localized on the basolateral membranes of polarized MDCK cells.

Nearly perfect controls have been achieved using cells transfected at the same time with either the transporter of interest or GFP. Using this approach, the effects of endogenous transporters and the effects of passive uptake on the compound of interest can be easily subtracted, allowing measurement of only the transporter specific uptake.

For each of these transporters, evaluation of known substrates including time course and K_m determination is presented, as well as IC_{50} determination for multiple known inhibitors.

REFERENCES

1. FDA Guidance for Industry (Draft) – Drug Interaction Studies, February 2012
2. EMA Guideline on the Investigation of Drug Interactions (Final) June 2012

OBJECTIVE

To characterize the expression and function of clinically-relevant SLC drug transporters expressed in polarized MDCK cell models, and to demonstrate the robustness of these models for use in DDI studies.

REPRESENTATIVE DATA

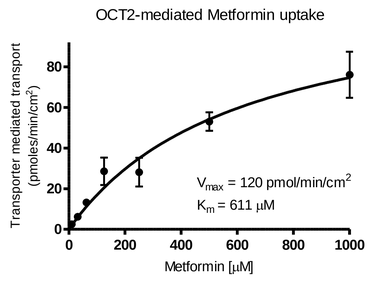

Figure 1. OCT2-mediated transport using 10 µM Metformin. For kinetic determinations, the assay time was 5 minutes.

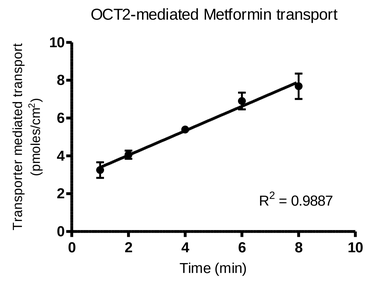

Figure 2. OCT2-mediated transport of 10 µM Metformin is shown to be linear to at least 8 minutes.

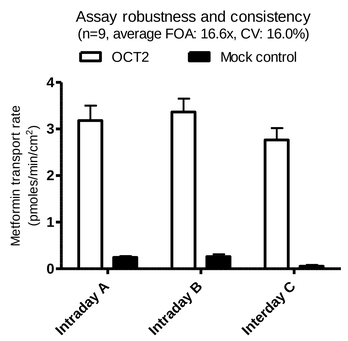

Figure 3. Robust and reproducible assay for OCT2, with an average uptake 16.6 times higher in OCT2-expressing cells compared to perfectly matched control cells.

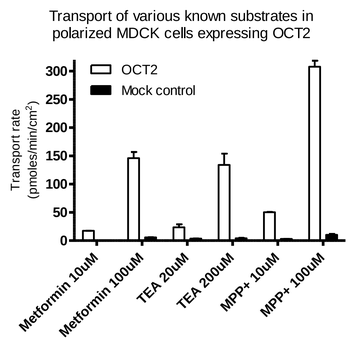

Figure 4. Uptake of a variety of cationic substrates in polarized MDCK cells expressing OCT2 compared to control cells.

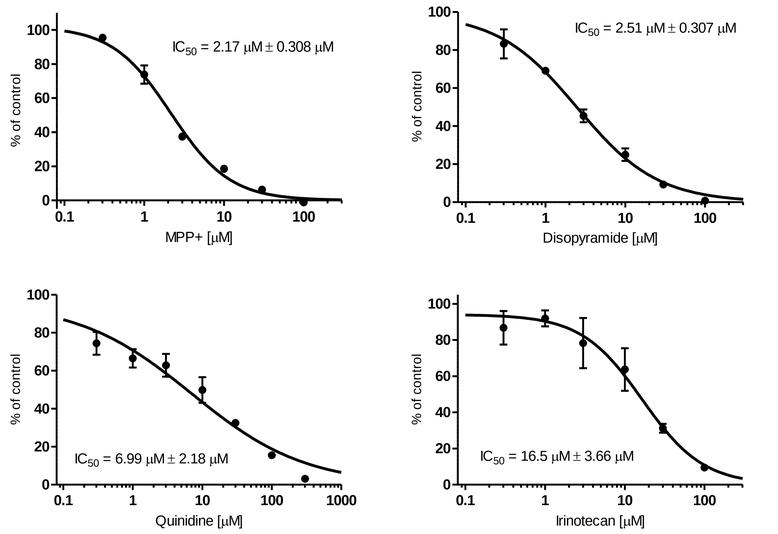

Figure 5. IC_{50} determinations for a variety of OCT2 inhibitors. 10 µM Metformin was used as the substrate; assay incubation time was 5 minutes.

RESULTS

Transporter	Reference Substrate	Test Concentration (µM)	Linearity (min)	Linear Coefficient (r^2)
hOAT1	p-aminohippurate	2	1 - 8	0.9632
hOAT3	p-aminohippurate	10	1 - 10	0.9814
hOATP1B1	estradiol-17β-D-glucuronide	2	1 - 8	0.9458
hOATP1B3	CCK8	2	1 - 10	0.9665
hOCT1	MPP+	10	1 - 8	0.9739
hOCT2	metformin	10	1 - 8	0.9887

Table 1. Linearity of assays used to investigate important SLC transporters

Transporter	Reference Substrate	K_m (µM)	V_{max} (pmol/min/cm²)	FOA	% CV
hOAT1	p-aminohippurate	15.6 ± 3.71	6.86 ± 0.678	24.1	15.6
hOAT3	p-aminohippurate	116 ± 79.6	2.16 ± 0.614	7.08	15.4
hOATP1B1	estradiol-17β-D-glucuronide	17.4 ± 3.38	8.58 ± 0.555	24.6	12.3
hOATP1B3	CCK8	48.2 ± 5.74	14.6 ± 0.827	30.4	17.4
hOCT1	MPP+	37.8 ± 11.0	66.1 ± 8.48	32.2	15.8
hOCT2	metformin	611 ± 236	120 ± 23.9	16.6	16.0

Table 2. Assay properties: Substrate kinetics, fold of activity, and interday/intraday (n=9) % CV.

Transporter	Reference Substrate	Test Concentration (µM)	Inhibitor	IC_{50} (µM)
hOAT1	p-aminohippurate	2	Mefanamic Acid	0.355 ± 0.0724
			Ibuprofen	3.43 ± 0.854
			Probenecid	3.73 ± 0.459
hOAT3	p-aminohippurate	10	Indomethacin	0.164 ± 0.0543
			Probenecid	2.28 ± 0.378
			Ibuprofen	14.4 ± 3.63
hOATP1B1	estradiol-17β-D-glucuronide	2	Cyclosporin A	1.77 ± 0.222
			Rifampicin	1.88 ± 0.328
			Gemfibrozil	226 ± 37.6
hOATP1B3	CCK8	2	Bromosulfophthalein	1.51 ± 0.416
			Cyclosporin A	2.04 ± 0.276
			Rifampicin	2.71 ± 0.273
			Rosuvastatin	6.25 ± 2.78
hOCT1	MPP+	10	Verapamil	18.9 ± 1.46
			Quinidine	88.8 ± 11.1
			Cimetidine	569 ± 210
hOCT2	metformin	10	MPP+	2.17 ± 0.308
			Disopyridamole	2.51 ± 0.307
			Quinidine	6.99 ± 2.18
			Irinotecan	16.5 ± 3.66

Table 3. Assessment of inhibition for known inhibitors of each transporter.

DISCUSSION

- The characterization data demonstrate robust and consistent substrate and inhibition studies can be achieved using transiently transfected MDCK cells.
- Nearly perfect control cells are used to subtract the effects of passive uptake and endogenous transporters, isolating the results to only the transporter of interest.
- MDCK cell-based assays represent an excellent tool for studying transporter activity in polarized cells, a more physiologically relevant polarized cellular environment.