Poster#: ITC-008
ITC Workshop 3
Mar 13th-14th, 2017
Washington, DC

Rapid and Low-Cost Assessment of Compound DDI Potentials using MDCK-II Cells Co-Expressing Regulatory Transporters OATP1B1, OATP1B3, OAT1, OAT3, OCT2, P-gp, BCRP and MATE1

Xuexiang Zhang, Yewei Du, Jianlu Chen, Mark S. Warren, Yong Huang

OPTIVIA BIOTECHNOLOGY

ABSTRACT

BACKGROUND: Transporter substrate test has become a common practice in drug development to inform potential drug-drug interactions (DDI). Applying such test to early stage compounds can minimize expensive clinical DDI liabilities, which however is hindered by high costs of assessing many DDI transporters. This work presents a novel assay method for accurate and low-cost substrate assessment for eight regulatory transporters OATP1B1, OATP1B3, OAT1, OAT3, OCT2, P-gp, BCRP and MATE1.

METHODS: Polarized MDCK-II cells were transfected to express GFP (control), uptake transporters OATP1B1, OATP1B3, OAT1, OAT3 and OCT2 (uptake model), the uptake transporters plus three efflux transporters P-gp, BCRP and MATE1 (uptake+efflux model). Apparent B>A permeability ($P_{app,B>A}$), cellular concentration (C_{cell}) of 20+ compounds were measured with these models. A new parameter, apparent apical efflux permeability ($P_{eff,ap}$) defined as B>A transport rate divided by C_{cell}, was introduced to assess apical efflux activity. Differences in $P_{app,B>A}$, C_{cell} or $P_{eff,\,ap}$ among the three models were used to determine whether a compound is transported by one or more uptake and/or efflux transporters.

RESULTS: Compared to GFP control, the uptake+efflux model exhibited >2x increases in $P_{app,B>A}$, C_{cell} or $P_{eff,ap}$ for all reported substrates of the eight transporters. Uptake and efflux substrate activities were further delineated by comparing the parameters among control, uptake, and uptake+efflux models. Compared with $P_{app,B>A}$, $P_{eff,ap}$ is more accurate in assessing apical efflux activities, especially for highly permeable compounds.

CONCLUSION: The presented assay method using multi-transporter models can be used for accurate and low-cost substrate assessment for the eight common DDI transporters.

INTRODUCTION

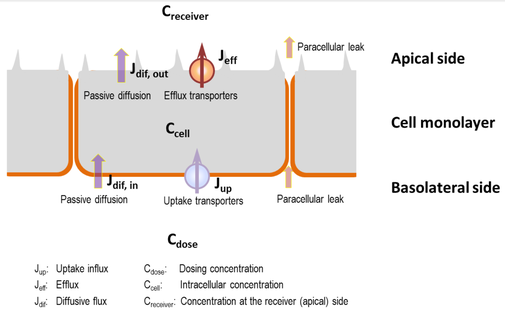

Fig 1. Schematic showing the expression of five uptake transporters (OATP1B1, OATP1B3, OCT2, OAT1, OAT3) on the basolateral side of polarized MDCK cells, and three efflux transporters (BCRP, P-gp and MATE1) on the apical side. The test article, dosed on the basolateral side, may flux into the intracellular space via the uptake transporter(s) or through passive diffusion, and then be pumped out to the apical space by the efflux transporters.

The US Food and Drug Administration (FDA) currently requests that seven (7) transporters be assessed to inform potential clinical drug-drug interactions (DDI), namely OATP1B1, OTP1B3, OCT2, OAT1, OAT3, BCRP and P-gp. With the increasing knowledge of MATE transporters, MATE1 and MATE2-K are considered to be assessed for regulatory submission as well. Based on the PK profile and clearance route, a test article is probed as a potential substrate of transporters selected from the above. Conventionally, the test article is evaluated against each transporter individually. However, in an early drug development stage with multiple leads, applying transporter substrate test to each individual DDI transporter could be time consuming and costly. We have been trying to develop cell based models by putting five uptake transporters together in one cell, with or without co-expression of 3 efflux transporters (BCRP, P-gp and MATE1) (Fig 1). We expect by analyzing a number of parameters, we can readily determine if a test article interacts with any of the eight transporters. Furthermore, we can identify whether the interaction is applied to the uptake transporters and/or efflux transporters. To this end, 20 compounds with some known transporter profiles were used to evaluate these models. The results are consistent with the transporter profile of the compounds, suggesting the proposed cell models are suitable to be used as a rapid screening to evaluate if a test article is a substrate of the five uptake and three efflux common DDI transporters.

MATERIALS AND METHODS

CELL CULTURE AND TRANSFECTION: MDCK-II cells were seeded in Millipore Millicell 96-well insert plate (PCF-0.4 µm). Approximately 24 hr later, cells were transfected using a proprietary *in situ* transfection technology, Opti-Expression™, which allows consistent and effective transfection of polarized cell monolayers. Cells were transfected with a mixture of plasmids encoding FDA DDI panel transporters including the uptake transporters OATP1B1, OATP1B3, OCT2, OAT1 and OAT3 at 15 ng/µL DNA plasmid each, with or without co-transfection of the efflux transporters P-gp, BCRP and MATE1 at 30, 20 and 10 ng/µL, respectively. Hence, three cells models were constructed, ie:

1. **GFP mock** (no expression of the transporters of interest);
2. **Uptake only** (co-expression of 5 uptake transporters);
3. **Uptake + Efflux** (co-expression of 5 uptake and 3 efflux transporters).

TRANSPORT ASSAY: Assays were conducted 48 hr after transfection to allow the cells to become polarized and transporters being appropriately localized. Most of the assays were done in the so-called transcellular mode. Briefly, cells were pre-incubated with HBSS for 15 min at 37°C. Then the basolateral side was supplied with 5 µM (except for quinidine at 1 µM, and PAH at 2 µM) cold or radiolabelled test articles in HBSS-HEPES, pH7.4, containing radiolabelled mannitol as a paracellular leakage marker. The apical buffer was replaced by HBSS-MES (pH 6.7) to establish the pH gradient necessary for MATE1 to operate as an efflux transporter. At the end of ninety minutes dosing, the test article and mannitol contents in the apical, intracellular and basolateral compartments were quantified by radiometry or LC/MS/MS. In some studies, another assay mode with short dosing time was also employed. Cells were pre-incubated for 15 min in HBSS, and then dosed for 5 min from the basolateral side in HBSS with the apical side was left dry. The dry apical side minimized the exit of the compound from the intracellular space to the apical side. Only the intracellular content was measured.

DATA ANALYSIS:

1. **Cellular concentration (C_{cell})**: Total cellular concentration at the end of 90-min dosing was calculated by dividing the mass of the test article inside the cells by the volume of cell monolayer previously measured by Optivia.
2. **Apparent transcellular permeability ($P_{app,\,B>A}$)**: The total B>A transmonolayer transport of the test article consisted of transcellular and paracellular components (Fig 1). However, the flux of mannitol through cytoplasmic lipid bilayer was minimal. In other words, the transmonolayer B>A flux of mannitol was almost exclusively via the paracellular route. Based on the Stokes-Einstein equation and assuming molecules were spherical, the relative diffusivity of the test article to mannitol was a factor of their molecular weight and vapor pressure. After it was estimated by that of mannitol, the paracellular flux of the test article was then subtracted from the total B>A transmonolayer transport to yield a corrected transcellular transport, which comprised J_{eff} and $J_{dif,\,out}$. The apparent transcellular permeability:
$$P_{app,\,B>A} = (J_{eff} + J_{dif,\,out})/C_{dose}$$
3. **Apparent apical efflux permeability ($P_{eff,ap}$)**: For the apical efflux transporters, the driving concentration was the intracellular C_{cell} instead of C_{dose}. Thus the apparent apical efflux permeability:
$$P_{eff,ap} = (J_{eff} + J_{dif,\,out})/C_{cell}$$
Note that the apparent $P_{eff,\,ap}$ in most cases was not the "true" or intrinsic apical efflux permeability because total, instead of unbound, intracellular concentration was used in the equation.

RESULTS & DISCUSSION

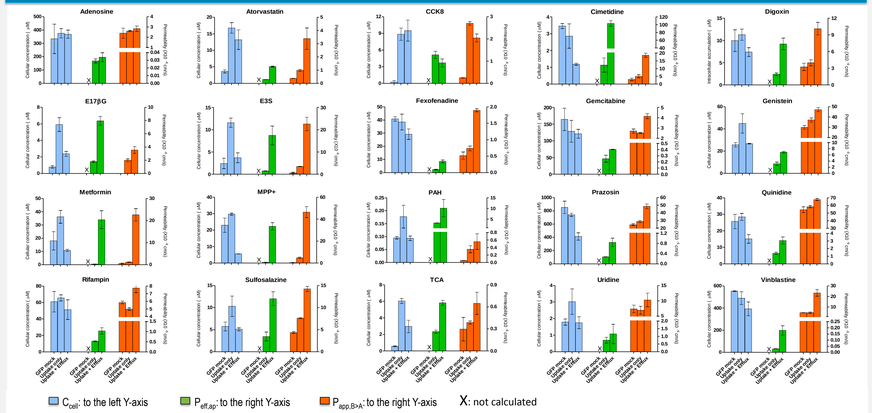

Fig 2. Cellular concentration, apparent B>A transcellular permeability and apparent apical efflux permeability of 20 representative compounds evaluated in cells expressing GFP control, uptake transporters only, and both uptake and efflux transporters.

RESULTS & DISCUSSION (CONT'D)

Tab 1. Comparison of the reported transporter profile of 20 compounds to the calculated C_{cell}, $P_{app,B>A}$, and $P_{eff,\,ap}$.

Compound	Reported transporter profile (among the 8 transporters selected)		$P_{eff,ap} \geq 2$?	$C_{cell} \geq 2$?	$P_{app,\,B>A} \geq 2$?	
	Uptake	Efflux	Uptake+Efflux *vs.* uptake only	Uptake only *vs.* GFP mock		Uptake+Efflux *vs.* GFP mock
Atorvastatin	OATP1B1, OATP1B3	BCRP, P-gp	✓	✓	✓	✓
E17bG	OATP1B1	BCRP	✓	✓	✓	✓
E3S	OATP1B1, OAT3	BCRP	✓	✓	✓	✓
Metformin	OCT2	MATE1	✓	✓	X	✓
MPP+	OCT2	MATE1	✓	X	✓	✓
PAH	OAT1, OAT3	BCRP	✓	X	✓	✓
TCA	OATP1B3	P-gp	✓	✓	X	✓
Cimetidine	X	MATE1	✓	X	X	✓
Digoxin	X	P-gp	✓	X	X	✓
Fexofenadine	X	BCRP	✓	X	X	✓
Genistein	X	BCRP	✓	X	X	X
Prazosin	X	BCRP, P-gp	✓	X	X	X
Quinidine	X	P-gp	✓	X	X	X
Rifampin	X	P-gp	✓	X	X	X
Sulfosalazine	X	BCRP	✓	X	X	✓
Vinblastine	X	P-gp	✓	X	X	✓
Adenosine	X	X	X	X	X	X
Gemcitabine	X	X	X	X	X	X
Uridine	X	X	X	X	X	X
CCK8	OATP1B3	X	X	✓	✓	✓

To identify if a compound is a substrate of the efflux transporter(s) or not, we compared the apical efflux permeability $P_{eff,ap}$ in efflux + uptake cells versus that of uptake only cells. For the compounds known as substrates of efflux transporter(s), $P_{eff,ap}$ was consistently greater (≥2) in the former cells. On the other hand, compounds that are not reported as substrates of the efflux transporter(s) demonstrated <2 fold difference in $P_{eff,ap,}$ (Tab 1, columns in orange shade). In contrast, $P_{app,B>A}$ alone failed to detect efflux transporter interactions for a number of high-permeability compounds such as quinidine and prazosin (Fig 2 and Tab 1).

To determine if a compound is a substrate of the uptake transporter(s), we compared both C_{cell} and $P_{app,B>A}$ between the uptake only and control cells. The rationale of this approach lies in that a compound crossing basolateral cell membranes can remain inside the cells or cross the apical membranes through diffusion or canine transporters expressed in MDCK cells (assuming the compound does not undergo biotransformation inside cells). Therefore, in order to accurately determine whether a compound is a substrate of the uptake transporter(s), both C_{cell} and $P_{app,B>A}$ need to be compared between the uptake only cells and control cells. As shown in Tab 1, most reported substrates of one or more of the 5 uptake transporter(s) showed ≥2 increase in C_{cell} in the former cells. However, <2 fold increase in C_{cell} was observed on MPP+ and PAH (Fig 2). On the other hand, ≥2 increases in $P_{app,\,B>A}$ of PAH and MPP+, suggested a majority of the compounds transported by the uptake transporter(s) further cross the apical membranes. To validate our hypothesis, we conducted another experiment using the same cell models by dosing the cells for 5 min (within the linear range of OAT1/3 mediated uptake) but leaving the apical side dry to demolish apical exit. Under this conditions, PAH showed significant higher C_{cell} in uptake only cells versus mock (Fig. 3). The 11.3X fold of activity was also higher than that in 90-min dosing with apical exit. These results suggest apical exit in transcellular assay model can lead to reduced changes in C_{cell} in presence of uptake transporter(s). Interestingly, metformin and TCA showed the opposite trend (Tab 1). The rest of reported substrates showed ≥2 increases in both C_{cell} and $P_{app,B>A}$. Our results demonstrated that either C_{cell} or $P_{app,B>A}$ alone is not able to give a definitive answer on whether a compound is a substrate of the uptake transporter(s). Instead, ≥2 increase in one or both C_{cell} and $P_{app,B>A}$ resulted in accurate substrate assessment for the tested compounds against these uptake transporters (Tab 1, Columns in light blue).

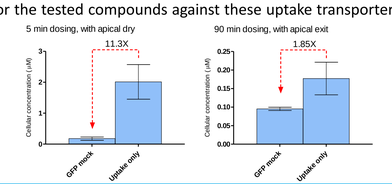

Fig 3. Cellular concentrations of 5 compounds in 5 min dosing with apical side dry, or 90 min dosing with the apical in HBSS-MES. PAH showed higher C_{cell} in the former assay conditions and also higher fold of activity.

CONCLUSIONS

Powered by novel assay and analysis method, MDCK-II cells expressing 5 uptake transporters with or without the co-expression of 3 efflux transporters can be used as a low-cost, rapid substrate screening of the 8 common transporters. As such, apical efflux permeability $P_{eff,ap}$ is a reliable parameter to determine if a test article (TA) interacts with the efflux transporters. ≥2 changes in either C_{cell} or $P_{app,\,B>A}$ indicate the TA is a substrate of one or more of the uptake transporters. If the TA is a substrate of any of the transporters, at least one of the 3 parameters, C_{cell}, $P_{app,B>A}$ and $P_{eff,ap}$ will be altered significantly. Nucleosides and analogues, which are not transported by the transporters, affect none of the parameters.