Functional Assessment of OATP1B1 and BCRP Polymorphisms in an OATP1B1/BCRP Co-Expressing Model

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P423

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ABSTRACT

OATP1B1 and BCRP are two major hepatic transporters that mediate vectorial transport/hepatic clearance and biliary secretion of various organic anion compounds. Expressed in hepatocytes on basolateral and apical membranes respectively, genetic variants of the two transporters have been linked to altered pharmacokinetic and pharmacodynamic properties of various drugs, including statins[1-3].

To evaluate the effects of OATP1B1 or BCRP polymorphisms on biliary secretion of drugs, we have developed and characterized polarized cell monolayer models that express the wild type or a SNP variant of one or both transporters, with their relative expression levels quantitatively controlled to a certain extent[4].

Using these in vitro models, we confirmed that OATP1B1 and BCRP together mediate basal to apical (B → A) transport of their common substrates, including estradiol-17β-D-glucuronide (E17βG), estrone sulfate (E3S), atorvastatin, rosuvasatin and pravastatin. Protein quantitation analysis confirmed that under the same conditions, transfecting BCRP 421C>A SNP gene (encoding the $BCRP_{Q141K}$ variant), conferred ~56% less protein expression compared to the wild type gene in our OATP1B1/BCRP dual model (Courtesy of Millennium Pharmaceuticals). For the first time, we demonstrated that compared to $OATP1B1/BCRP_{WT}$ expressing cells, $OATP1B1/BCRP_{Q141K}$ cells had substantially lower B>A transport of atorvastatin, pravastatin and estradiol-17β-D-glucuronide. Strikingly, no statistically significant differences were observed between the two models in rosuvastatin and E3S transport.

A new method of calculating efflux transport kinetics using intracellular unbound concentration, is presented in calculating V_{max} and K_m of $BCRP_{WT}$ and $BCRP_{Q141K}$. In order to understand the differential effect of reduced BCRP activity on B>A transport of its substrates, in silico simulations were performed using OptiSim™, a multi-transporter mechanistic modeling tool employing novel mathematical descriptors for active transport processes. Using parameters measured from the in vitro studies, we were able to model why substrates like rosuvastatin and E3S were not sensitive to BCRP polymorphism in these in vitro studies. Furthermore, we hypothesized that one reason that rosuvastatin hepatic clearance is sensitive to BCRP polymorphism in vivo, but not in our in vitro models, is likely because the protein level and relative activity of BCRP to OATP1B1 is much lower in hepatocytes compared to our models[5]. This hypothesis was further confirmed using mathematical simulation.

These studies demonstrated that novel OATP1B1/BCRP co-expressing cellular models are useful tools to study vectorial transport of drugs and the effects of transporter polymorphisms. Properly designed in vitro studies using cell based assays and data analysis can generate important parameters for mechanistic modeling of drug transport in complex multi-transporter environments; while modeling and simulation can be extremely useful not only for interpreting in vitro study results, but also for potentially predicting or correlating in vivo outcomes.

BACKGROUND

Transporter gene polymorphisms can have profound effects on statin pharmacokinetics. In particular, a common genetic variant of organic anion-transporting polypeptide 1B1 (OATP1B1) reduces the hepatic uptake of many statins, increasing the risk of statin-induced myopathy[1]. Similarly, reduced efflux activity in $BCRP_{Q141K}$ compared to $BCRP_{wt}$ results in a marked increase in systemic exposure to various statins[6-7]. Importantly, the effects of these polymorphisms differ depending on the specific statin that is used. This provides a rational basis for the individualization of lipid-lowering therapy.

MATERIALS AND METHODS

Methods of quantitatively expressing transporters in MDCK monolayers and B → A transport measurements were previously described[4,8]. Briefly, MDCK-II cell monolayers 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 transporters or a plasmid encoding green fluorescent protein (GFP) as a mock control. Intracellular retention and B→A flux of were measured in cells expressing either GFP as a control, OATP1B1 only, BCRP only, $OATP1B1/BCRP_{WT}$, or $OATP1B1/BCRP_{Q141K}$.
Radiolabeled estrone-3-sulfate, estradiol-17β-D-glucuronide, atorvastatin, pravastatin and rosuvastatin were used for transport assessment.
Simulations were performed with OptiSim™, an internally developed modeler for simulating active transport in the context of multi-transporter environment.

SUMMARY

1. Functional assessment of transporter SNP can be done in multi-transporter expressing models.
2. Appropriate in vitro experiment designs (such as using OATP1B1 only cells) and data analysis (such as using intracellular concentration to calculate efflux kinetic parameters) is important in order to extrapolate meaningful data from in vitro studies.
3. Mechanistic modeling (using proper experimental data) is very useful in understanding active transport resulting from complex interactions among multiple transporters.
4. Both in vitro results and mechanistic modeling are needed to fully understand results.

RESULTS

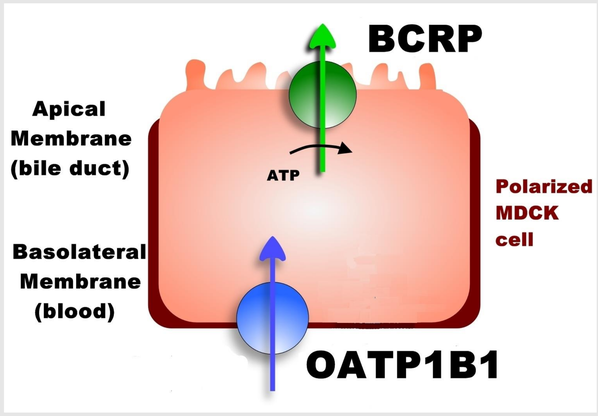

Figure 1. MDCK model co-expressing OATP1B1 and BCRP. OATP1B1 will transport substrates into the polarized MDCK monolayer cells, while BCRP will efflux those substrates across the apical membrane.

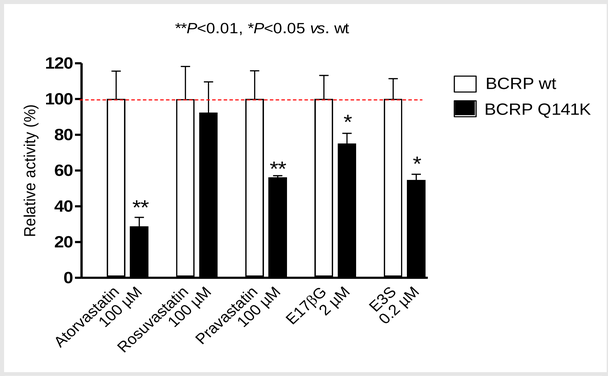

Figure 3. Differential effect of $BCRP_{Q141K}$ variant on the transport of common substrates of OATP1B1 and BCRP, across OATP1B1/BCRP co-expressing MDCK monolayers.

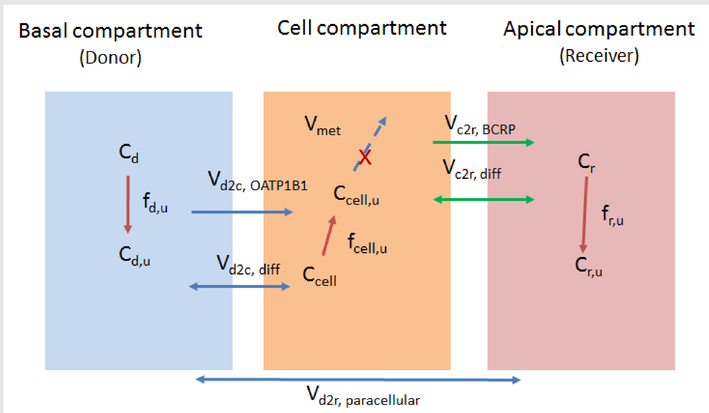

Figure 5. Schematic illustration of a three-compartment model for simulating transport in OATP1B1 and BCRP co-expressing polarized MDCK cells. (C: concentration, V: transport or metabolic rate, f: unbound fraction)

DISCUSSION

1. OATP1B1 and BCRP jointly dictate the intracellular concentration. The intracellular concentration and BCRP activity determine apical efflux.
2. In our system, the BCRP SNP effect is substrate dependent, caused by the complex interaction between OATP1B1 and BCRP (Figure 3).
3. The uptake and efflux transporters have different transport efficiency (V_{max}/K_m).
4. The Transport Asymmetry Ratio (Efflux efficiency/Uptake efficiency) determines the nonlinear dependence of vectorial transport and intracellular retention.
5. Determining the unbound intracellular concentration is crucial for determining BCRP kinetics (V_{max} and K_m) from experimental measurements (Figure 4).
6. Mathematic modeling of atorvastatin and rosuvastatin confirmed our experimental findings. We hypothesize that the cause of insensitivity of rosuvastatin to the BCRP SNP is likely due to the relative transport efficiency of BCRP being higher than that of OATP1B1 in this system (Figure 6).
7. Simulation results showing that decreasing the relative BCRP efficiency compared to OATP1B1 efficiency will lead to more sensitivity in measuring the actual activity of the BCRP polymorphism.

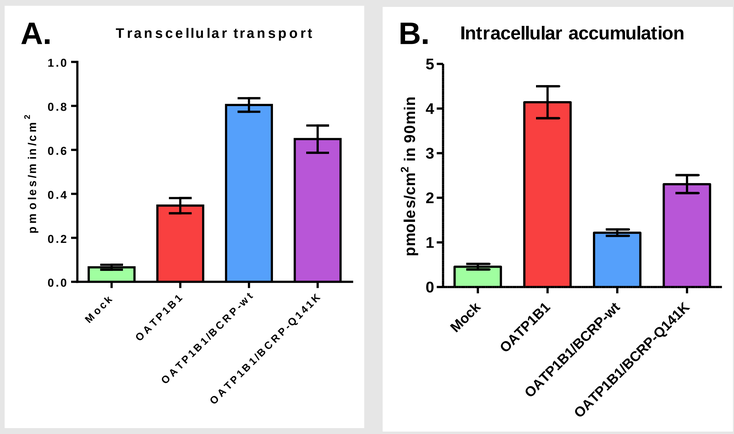

Figure 2. Function validation of OATP1B1/BCRP double transfected cells using estradiol-17β-D-glucuronide (E17βG) as a probe substrate. E17βG concentration was 1 µM. Assay time was 90 min. Panel A: transcellular transport; Panel B: intracellular retention.

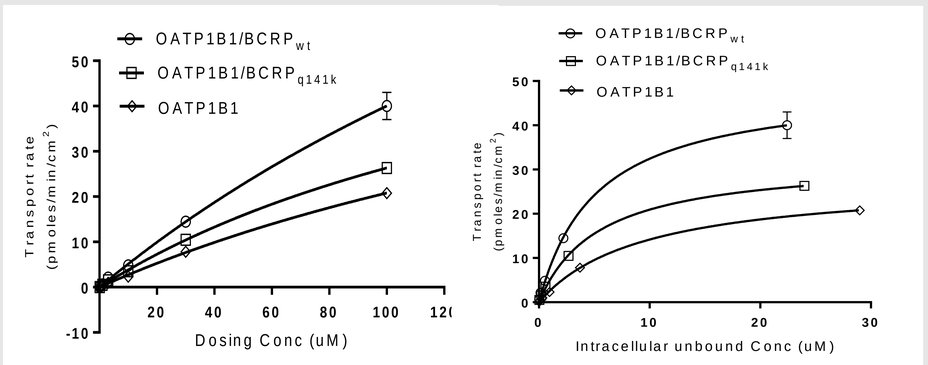

Figure 4. The importance of using matched OATP1B1 only cells and unbound intracellular concentration in determining BCRP mediated atorvastatin efflux kinetics. **Left:** 'apparent' B>A transport kinetics (transport rate vs. basal dosing concentration) **Right:** apical efflux kinetics (transport rate vs. intracellular unbound concentration)

	BCRP wild type		BCRP Q141K variant		Q141K /WT		
	V_{max} (pmol/min/cm2)	K_m (uM)	V_{max} (pmol/min/cm2)	K_m (uM)	By Vmax	By Vmax/Km	By protein expression
Apical efflux	23.9±1.5	5.4±1.1	6.2±0.4	3.2±0.7	0.26	0.44	0.44*
Apparent	89.5±51.2	365.4±260	9.7±1.6	75.2±23.7	0.11	0.53	

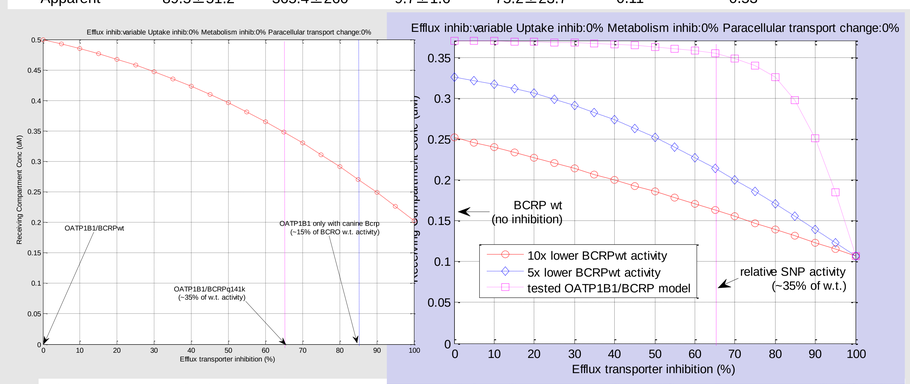

Figure 6. Simulation of effects of BCRP activity change on the efflux of atorvastatin (left) and rosuvastatin (right). For rosuvastatin, the effect of $BCRP_{Q141K}$ is predicted to become more pronounced when BCRP expression/activity is reduced.

$$\text{Transport Asymmetry Ratio} = \frac{Efflux\ Efficiency}{Uptake\ Efficiency} = \frac{Efflux\ Vmax/Km}{Uptake\ Vmax/Km}$$

Equation 1. Transport Asymmetry Ratio, determines the nonlinear dependence of vectorial transporter activities

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