Drug Transporters: ‘Not as Easy as ABC’
A case study demonstrating benefits and limitations of test systems and experimental designs when elucidating complex transporter interactions

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Objective
The goal of this study was to evaluate multiple test systems and experimental approaches using rosuvastatin as a model compound to elucidate complex transporter mediated interactions.

Methods
In Vitro Permeability
Caco-2, CPT-P1 (Caco-2 P-gp knockdown), CPT-B1 (Caco-2 BCRP knockdown), CPTM1 (Caco-2 MR2 knockdown), MDR1-MDCK, and OATP1B1-MDR1-MDCK cell monolayers were grown to confluence on filter membranes in a humidified atmosphere (5% CO2, 37°C). Cell monolayer integrity was verified by measuring transepithelial electrical resistance (TER) and apparent permeability (Papp) values of selected reference compounds. Established probe substrates were assessed in parallel to confirm functional expression of transporters in the various test systems. Dosing solutions of the compounds were prepared in HBSG buffer, pH 7.4. Permeability was assessed bidirectionally (A→B and B→A), and, for certain cell systems, in the presence or absence of P-gp and OATP1B1 inhibitors.

Nominal dosing concentrations of test compound were used for determination of permeability. If assessed, cell accumulation was determined at the end of the permeability assay by lysing the cell monolayers using an organic solvent. Concentrations of test compound were combined with measured donor and receiver concentrations to establish mass balance. Extent of test compound accumulation inside the cells was also used to better understand interaction with known inhibitors used during our evaluation.

Conclusion
The goal of this study was to evaluate multiple test systems and experimental approaches using rosuvastatin as a model compound to elucidate complex transporter mediated interactions.

Results
The transport of rosuvastatin (10µM) was determined across the monolayers of Caco-2 and transporter knockdown (KD) cells. Each bar represents the mean S.D.; n=3. The permeability of E3S shows expected increase in the B→A permeability in OATP1B1-MDR1-MDCK compared to MDR1-MDCK.

The permeability of rosuvastatin in OATP1B1-MDR1-MDCK cells was evaluated in the presence of various inhibitors of OATP1B1 (Valspodar and LY335979), P-gp (E3S and FTC), and BCRP (Rifamycin SV and L335979). The presence of OATP1B1 or P-gp inhibitors, the contribution of the uptake versus efflux transporter can be clearly distinguished using both data from apparent permeability and cell accumulation.

The permeability of rosuvastatin across MDR1-MDCK and double transfected OATP1B1-MDR1-MDCK cells was measured. Each bar represents the mean S.D.; n=3. The results indicate that rosuvastatin is taken up through the basolateral membrane by OATP1B1 and extruded through the apical membrane by P-gp.

The bi-directional permeability of rosuvastatin across MDR1-MDCK and double transfected OATP1B1-MDR1-MDCK cells was measured. Each bar represents the mean S.D.; n=3. The results indicate that rosuvastatin is taken up through the basolateral membrane by OATP1B1 and extruded through the apical membrane by P-gp.

The effect of titrating verapamil concentration on rosuvastatin (10µM) transport in Caco-2 cells was evaluated. Each bar represents the mean S.D.; n=3. The 150 µM concentration of verapamil which is > 20 fold higher than its IC50 of verapamil when the change in RER is comparable to the RER obtained in the KD cell line CPT-P1.

The permeability of rosuvastatin across MDR1-MDCK and double transfected OATP1B1-MDR1-MDCK cells was measured. Each bar represents the mean S.D.; n=3. The results indicate that rosuvastatin is taken up through the basolateral membrane by OATP1B1 and extruded through the apical membrane by P-gp.

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The permeability of E3S shows expected increase in the B→A direction in the double transfected cell line OATP1B1-MDR1-MDCK compared to MDR1-MDCK.