Drug Interactions with Human Renal Transporters
Development of Cell-based Assays for Assessing

Purpose

Human organic cation and anion transporters (OCTs and OATs, respectively) are two major drug transporter families involved in active renal tubular secretion of drugs and other xenobiotics. Members of these families (specifically OAT1, OAT3, and OCT2) are expressed on the basolateral (blood-facing) membrane of proximal tubule cells (Figure 1). These transporters function in concert with apical efflux carriers to excrete drugs and their metabolites from blood into urine. Inhibition of OCTs and OATs may cause drug-drug interactions in cases where a drug that is cleared by a particular renal transporter is co-administered with another drug that inhibits the same transporter. The result could be reduced renal clearance and elevated systemic exposure [1]. The objective of this study was to establish in vitro cell-based assays for identification of potential drug interactions involving human OCT2, OAT1, and OAT3.

Methods

HEK293 cells were transfected in a stable manner with plasmids coding for OCT2, OAT1, and OAT3. Control cells and stably transfected cells were cultured in multi-well plates. Probe substrates were 1-methyl-4-phenylpyridinium (MPP\(^+\)) for OCT2, para-aminohippurate (PAH) for OAT1, and furosemide for OAT3. LC-MS/MS was used to determine cellular uptake of probe substrates.

Results

After demonstrating that stably transfected HEK cells expressed the appropriate transporter (mRNA and western blot data not shown), transporter function was confirmed using known substrates. An assay matrix consisting of 3 concentrations x 4 time points (12 different conditions; data not shown) was used for each probe substrate. Based on the criterion of the ratio of influx rate in transfected cells vs. control cells > 2, each substrate was positive for the appropriate transporter(s) (data not shown). One was chosen for use as the probe substrate for each transporter: MPP\(^+\) for OCT2, PAH for OAT1, and furosemide for OAT3. A concentration and time for each transporter/probe pair was selected from the matrix to give the optimal combination of signal-to-noise ratio (transfected vs. control cells) and linearity (with time). Optimal conditions for OCT2 (5 \(\mu\)M MPP\(^+\), 5 min), OAT1 (40 \(\mu\)M PAH, 20 min), and OAT3 (5 \(\mu\)M Furosemide, 5 min) were determined in the same manner (data not shown) and these conditions were used in the subsequent inhibition experiments. In the presence of a known inhibitor of each transporter, the uptake of the probe substrate was significantly reduced (Figure 2 for OCT2, PAH for OAT1, and furosemide for OAT3). LC-MS/MS was used to determine cellular clearance and elevated systemic exposure [1]. The objective of this study was to establish in vitro cell-based assays for identification of potential drug interactions involving human OCT2, OAT1, and OAT3.

Conclusion

We have demonstrated, through accurate classification of known substrates and inhibitors as well as the determination of literature-comparable \(V_{\text{max}}\) and \(K_m\) values for specific probe substrates, that our cell-based systems can be utilized as in vitro models for drug transport. These results demonstrate their usefulness for predicting potential drug-drug interactions involving human OCT2, OAT1, and OAT3 transporters.

References


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Figure 1. Human drug transporters expressed in kidney proximal tubules

Figure 2. Furosemide Uptake +/- Probenecid

Figure 3. \(K_m\) and \(V_{\text{max}}\) for Selected Probe Substrates

Table 1A: Furosemide Uptake Inhibition in HEK-OAT3

Table 1B: PAH Uptake Inhibition in HEK-OAT1

Table 1C: MPP\(^+\) Uptake Inhibition in HEK-OCT2

Table 1D: Determination of IC_{50}