Comparison between MDR1-MDCK and MDR1-LLC-PK1 Cells for Pgp Substrate and Digoxin Inhibition Interaction Studies
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Purpose
A recent FDA guidance recommends a clinical DDI study when plasma or gut concentrations of a drug exceed specific ratios of the drug’s Pgp IC50. There is, however, considerable variability in measured Pgp IC50 values in various laboratories (Bentz et al, 2013) and that variability could result in inconsistent decisions to conduct clinical DDI studies. The objective of this study was to calibrate our in house MDR1-MDCK and MDR1-LLC-PK1 cells for substrate and digoxin inhibition interaction studies using selected Pgp substrates and inhibitors.

Methods
Permeability and efflux of 12 marketed compounds were determined in parental LLC-PK1, MDR1 transfected LLC-PK1 cells (MDR1-LLC-PK1), vector-control MDCK, and MDR1 transfected MDCK cells (MDR1-MDCK) using 96-Transwell plates. For digoxin inhibition studies, cells were pre-incubated with inhibitor and then transport of [3H]-digoxin in both directions was measured with the inhibitor in both receiver and donor chambers. The IC50 values were calculated from efflux ratio or PappB-A flux data using the Hill Equation with SigmaPlot V.11.0. MDR1 mRNA levels were measured by qPCR. P-gp protein expression was determined by Western blot and flow cytometry.

Results
Permeability measurements and efflux ratios (ER) for the 12 compounds were comparable (within 2-fold) between MDR1-MDCK and MDR1-LLC-PK1 cells. ER IC50 values were significantly lower (2- to 34-fold) than PappB-A IC50 values for both transfected cell lines; however, MDR1-LLC-PK1 PappB-A IC50 values tended to be higher than those from MDR1-MDCK cells. Using the FDA [I2]/IC50 cut-off values, the ER IC50 predicted 2 false positives in both cell lines; however, using the PappB-A IC50 both compounds predicted to be true negatives. Using the PappB-A IC50, 1 false negative was predicted in MDR1-LLC-PK1. Though MDR1 mRNA levels was similar between the two cell lines, Pgp protein expression was 3 to 4-fold higher in MDR1-LLC-PK1 cells than MDR1-MDCK cells.

Conclusion
MDR1-MDCK and MDR1-LLC-PK1 cells provided similar efflux ratios for Pgp substrates. However, Pgp digoxin IC50 values were affected by the calculation methods and differences in Pgp expression. Our data suggest that PappB-A IC50 is more predictive than ER IC50 for a digoxin clinical DDI.