Developing an *In Vitro* Model to Determine Kidney Specific Toxicity (KST)

**JF Pregenzer**, K Leach, B Feng, BL Wallace, D Keller, III, JA Willoughby, Sr, and JM McKim, Jr

*CeTox, Inc*., Kalamazoo, MI 49008; 1Compound Safety Prediction; 2Pharmacokinetics and Drug Metabolism, Pfizer Global Research and Development, Groton CT.

### ABSTRACT

Kidney toxicity is induced by a wide spectrum of marketed drugs, and renal toxicity is one of the major reasons for drug failure in early preclinical safety studies. Cellular models that can be used to predict compounds that may cause renal toxicity would improve our ability to manage drug development risks. Hence, we developed a renal proximal tubule cell (RPTC) model and a renal specific transporter assay to predict renal cell toxicity. The model includes the cryopreserved human primary proximal tubule cell (hRPTC) and the LLC-PK1 renal proximal tubule cell line. hRPTCs were seeded into Corning transwell-COL 24-well tissue culture plates at a density of 7.4 x 10^4 cells/well and incubated with modified Dulbecco’s Modified Eagle Medium (DMEM)/High Glucose (1:1) with 5% FBS and 1% Penicillin-Streptomycin. hRPTC viability at 24 hours was greater than 80%. The RPTCs were used at 24-48 hours post-seeding for experiments. hRPTCs were transfected with the CDH1 gene, which encodes the tight junction protein cadherin-1, to increase cell-cell adhesion and barrier function. The RPTCs were used in this study at 60 days post-seeding. At this stage, the cells had achieved tight cell-cell junctions and proper polarity. In this study, we developed a novel cell-based assay for high-throughput screening of renal specific transporters. We used this assay to screen the hRPTCs for the presence of renal specific transporters and compared the results with those obtained with the LLC-PK1 cell line. The results show that the hRPTCs have a higher proportion of renal specific transporters than the LLC-PK1 cell line. The results also show that the hRPTCs have a higher expression of the renal specific transporter OAT3, which is involved in the uptake and secretion of organic anions. The results also show that the hRPTCs have a higher expression of the renal specific transporter OCT2, which is involved in the uptake and secretion of organic cations. The results also show that the hRPTCs have a higher expression of the renal specific transporter MATE1, which is involved in the uptake and secretion of organic cations. 

The results show that the hRPTCs have a higher expression of the renal specific transporter OAT3, which is involved in the uptake and secretion of organic anions. The results also show that the hRPTCs have a higher expression of the renal specific transporter OCT2, which is involved in the uptake and secretion of organic cations. The results also show that the hRPTCs have a higher expression of the renal specific transporter MATE1, which is involved in the uptake and secretion of organic cations.

### RESULTS

**Figure 1** Kidney Injury Can Occur at Multiple Locations in the Nephron

**Figure 2** Transporter Location in Human Renal Proximal Tubule Cells

**Figure 3** Transwell Culture Plates Provide a Two Compartment Model

**Figure 4** A Comparison of OCT2 Transport Activity in Porcine and Human Proximal Tubule Cells

**Figure 5** Cell Dependent Sensitivity to Cisplatin Toxicity

### REFERENCES

M. Taddei et al. (2005) J Pharmacol Exp Therap. 309, 185-191