

CeeTox Kidney Specific Toxicity (KST) Screen

Background Rationale

Drug-induced adverse effects in the kidney are a major reason for late stage attrition of promising new candidates. The ability to identify renal specific risk for toxicity early in the drug discovery process would greatly improve the probability of success in animal and human safety studies. *In vitro* models that can provide reliable information on expected adverse effects must consist of organ specific cells that maintain *in vivo* physiological functions. The biochemical or molecular endpoints measured must be unique to the organ of interest, and the origin of the test cells should be species-specific.

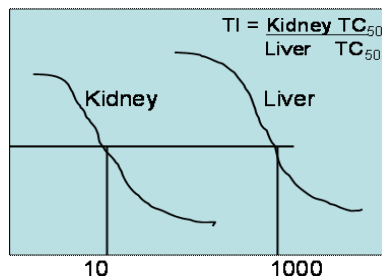
Determining Kidney Specific Toxicity

CeeTox developed the use of human proximal tubule epithelial cells (hRPTECs) as its primary model for assessing a compound's potential to cause renal toxicity. Developing a species-specific cell-based model is important because the renal proximal tubule cells possess many different transporter proteins that play key roles in the elimination and toxicity of drugs. The cells are grown in transwell plates (figure below) to establish polarity and to set up a transport environment similar to that found *in vivo*. Test compounds may be added to the system in a manner that mimics apical or basolateral exposure scenarios.

Predicting kidney specific toxicity from *in vitro* data sets cannot be accomplished by simply using kidney cells and then measuring general cell health following the administration of test compounds. In order for *in vitro* data to provide information specific to the kidney, the *in vitro* model must have three components: 1) an organ specific cell type, 2) biomarkers that monitor signaling pathways specific to the kidney, and 3) a means of comparing the relative sensitivity (acute toxicity) of the target cell (hRPTEC) type to another cell type, such as liver. The combination of all three components allows the *in vitro* data to predict kidney toxicity.

Renal specificity is achieved by 1) determining whether the cytotoxicity of the test compound is due to transporter uptake, and 2) determining toxicity in hRPTECs relative to hepatocytes.

The probability that a test compound will produce renal toxicity over hepatotoxicity is determined by comparing general toxicity in human proximal tubule epithelial cells (hRPTECs) to toxicity in hepatocytes (Hep). The same exposure concentrations and times are used in both cell models. A mean TC50 value is determined across the endpoints measured in each cell type. The mean TC50 in hRPTECs is compared to the mean TC50 in Hep to determine the toxicity index (TI). Activation of signaling pathways known to initiate adverse renal events that may not result in cell death can also be monitored (See optional assays below).



If TI = 1 then equal toxicity
 If TI < 1 then kidney toxicity
 If TI > 1 then liver toxicity

Kidney Toxicity Screen Assays

Primary human kidney cells (hRPTECs) and human hepatocytes (Hep)

- Membrane integrity
 - hRPTEC - (NAG) leakage
 - Hep - LDH, ALT, or GST
- Mitochondrial toxicity (ATP)
- Steatosis (Nile red)
- KIM1 (Expression) (hRPTEC only)
- Solubility

Optional kidney assays (hRPTECs)

- | | |
|---------------|-----------|
| • NGAL | • MRP 2,4 |
| • Clusterin | • MDR1 |
| • OAT 1,3,4,5 | • OATP4 |
| • OCT 1,2 | • PKC |

Summary

The kidney toxicity model is a cost-effective way to quickly identify kidney-specific toxicity using multiple biochemical endpoints and gene expression markers. It is of considerable value in early drug development because multiple biochemical markers can be used to rank-order compounds from greatest to least toxicity.

CeeTox is a GLP compliant laboratory, therefore assays may be performed and reported in compliance with GLP if desired by the client.

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