

Background Rationale

In vitro models able to predict toxicity specific to the heart are of considerable value in early drug development. It is estimated that almost 30% of drug withdrawals in the US over the last 30 years have been due to negative cardiovascular events.

Adverse consequences of drugs on the heart are of three general types:

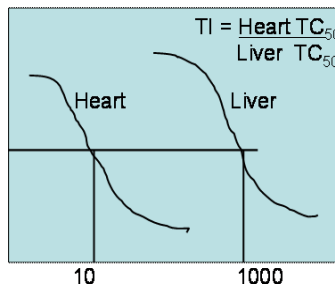
- Cytotoxicity
- QT prolongation or ion channel effects
- Hypertrophy

Predicting cardiac specific toxicity from *in vitro* data sets cannot be accomplished by simply using heart 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 heart, the *in vitro* model must have three components: 1) an organ specific cell type, 2) biomarkers that monitor signaling pathways specific to the heart, and 3) a means of comparing the relative sensitivity (acute toxicity) of the target cell (cardiomyocytes) type to another cell type, such as liver. The combination of all three components allows the *in vitro* data to predict cardiac toxicity.

The two most frequently used models in cardiac research are the isolated whole heart and cultured cardiac cells. The cultured cell model is popular because of its versatility, economy and convenience. The neonatal rat cardiomyocyte model enables researchers to study and understand the morphological, biochemical and electrophysiological characteristics of the heart. The model allows not only the study of toxicity of drugs, but also their cardioprotective effects.

Determining Cardiac Specific Toxicity

The probability that a test compound will produce cardiac toxicity over hepatotoxicity is determined by comparing general toxicity in cardiomyocytes (CM) 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 CMs is compared to the mean TC₅₀ in Hep to determine the toxicity index (TI). Activation of signaling pathways known to initiate adverse cardiac 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 heart toxicity
 If TI > 1 then liver toxicity

Heart Toxicity Screen Assays

Primary rat cardiomyocytes (CM) and rat hepatocytes (Hep)

- Membrane integrity
 - CM - Troponin 1 leakage
 - Hep - LDH, ALT, or GST
- Mitochondrial toxicity (ATP)
- Steatosis (Nile red)
- Solubility

Optional heart assays (CM only)

- Metabolic activation (GSH)
- Cell proliferation (Propidium iodide)
- Apoptosis (Caspase 3, BAX/Bcl2)
- Hypertrophy (GLUT1/GLUT4, ANP, BNP, SERCA 1/2)
- Oxidative stress (GSH, iNOS expression)
- Ion channel interaction inhibitor (hERG, Nav1.5, Kv1.3)

Summary

The heart toxicity model is a cost-effective way to quickly identify heart-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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