

CeeTox Liver Specific Toxicity (LST) Screen

Background Rationale

Liver toxicity is a major reason for drug discovery failure and is the number one adverse event leading to FDA regulatory action on drugs, including non-approval, postmarketing warnings added to the label, and withdrawal from the market. Pharmaceutical companies, not surprisingly, rank liver toxicity first in research priorities in adverse drug events.

The study of potential drug-induced liver toxicity aims to answer the following questions:

- early in discovery, does the new molecule have a risk of liver toxicity?
- once marketed, will there be a risk of idiosyncratic toxicity?

It is important to note that the tissue from which a cell is derived does not dictate the target organ for toxicity. General markers of cell health such as MTT and ATP for mitochondrial toxicity or GST for membrane integrity, are not linked to an organ specific effect. Thus, a toxic chemical placed on a liver cell, a renal cell or a heart cell may produce the same effect. The key to identifying target organ toxicity with *in vitro* models is the incorporation of organ specific markers of toxicity with tissue specific cell types.

Determining Liver Specific Toxicity

The model is designed to identify liver specific toxicity of the non-metabolized (parent) test article.

Hepatocytes (liver epithelial cells) make up about 80% of the mass of the liver. The primary hepatocyte culture represents a well studied and validated cell model consisting of normal liver parenchymal cells. The culture system that offers the most *in vivo*-like morphology is when cells are grown between two layers of gelled collagen or a bottom layer of collagen and a top layer of Matrigel, known as "sandwich" culture. The collagen interaction stimulates cells to repolarize and form extensive bile canalicular networks.

Unlike standard monolayer cultures of hepatocytes, the cells in sandwich culture are able to form a functional hepatobiliary system. This enables one to evaluate markers for liver toxicity specific to the bile canalicular membrane. It also allows examination of metabolic stability, activation, inhibition of CYP enzymes, induction of CYP enzymes, and inhibition/down-regulation of BSEP (Bile Salt Export Pump). Drugs that inhibit BSEP/UGT1A1 can lead to cholestatic liver toxicity. General cell health is monitored by evaluating compound effects on the canalicular membrane, BSEP, UGT1A1, steatosis, mitochondrial function and membrane integrity.

Liver Toxicity Screen Assays

Primary hepatocytes in sandwich culture:

- Rat
- Dog
- Monkey
- Human

Liver Toxicity Screen Assays (Acute Liver Toxicity)

- Membrane integrity (LDH or ALT)
- Hepatobiliary toxicity (GGT)
- BSEP (Bile Salt Export Pump)
- Mitochondrial toxicity (ATP)
- Solubility

Optional Assays (Chronic Liver Toxicity)

- Steatosis (Nile red)
- UGT1A1 inhibition (cell free assay)
- PPAR α and PPAR γ (mRNA and ELISA)
- Metabolic activation (GSH, CYP induction (1A1/2, 3A1/2, 4A mRNA))
- PXR mRNA/binding
- LXR mRNA/binding
- CAR mRNA/binding
- Oxidative stress (GSH, 8ISP, DCFDA)
- Normal metabolism (PEPCK, G-6pase, etc.)

Summary

The liver toxicity screen is a cost-effective way to quickly identify liver-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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