Risk Assessment for Cholestatic Hepatotoxicity: Integrating Transporter Inhibition and FXR Mediated Regulation into a Predictive InVitro Assay

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Drug-Induced Liver Injury (DILI)

- DILI is the leading cause of acute liver failure in the US, and a major reason for liver transplantation. ¹
  - Approximately 55,000 cases/year in the US ²
- DILI is the #1 cause of regulatory actions
  - drug failure in clinical trials
  - drug withdrawal
- Herbals and dietary supplements are the second leading cause for liver injury ³
- DILI from many drugs involves cholestasis and accumulation of bile acids within hepatocytes. Adaptation to the harmful effects of such accumulation can mean the difference between hepatocyte death and survival. ⁴
- The adaptive response by the liver is an important component in predicting the potential for cholestatic hepatotoxicity.

¹ Reuben et al. Hepatology 2010:52: 2065-2076
² Fontana. Gastroenterology 2013;314: 1818
⁴ Roth Applied In Vitro Toxicology 2016;2:4, 1-2
Importance of Transporter Function

• Plated hepatocytes lack efflux transporters and generate high intracellular concentrations, leading to hepatotoxicity

• B-CLEAR® Technology utilizes a fully polarized system with functioning uptake and efflux transporters which generate *in vivo* relevant intracellular concentrations

Each plate configuration used the same lot of Transporter Certified™ cryopreserved human hepatocytes

**Use of Transporter Certified™ hepatocytes in a polarized system ensures more predictive data**
Cholestasis: Impairment of Bile Acid Flow

“Cholestasis is defined as a decrease in bile flow due to impaired secretion by hepatocytes or to obstruction of bile flow through intra-or extrahepatic bile ducts.”


Increased concentrations of bile acids can lead to toxicity in the:

• Liver
  – Activating death receptors and inducing oxidative damage

• Bile ducts
  – Portal inflammation, direct injury

• Systemic circulation
  – Endothelial injury in the kidney and lungs
  – Cancer-promoters indirectly through DNA damage

However, the liver routinely handles high concentrations of bile acids

World J Gastroenterol. 2009 Apr 14; 15(14): 1677–1689
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2668773/
Bile Acids in Humans: Composition and Cytotoxicity

- **Human models** are critical since rodents differ in synthesis, metabolism and regulation of bile acids.
- **Primary bile acids**: cholic acid (CA) and chenodeoxycholic acid (CDCA).
- **Secondary bile acids**: deoxycholic acid (DCA) and lithocholic acid (LCA).
  - formed by bacterial modification in the intestines.
- **Humans**: conjugated to glycine (~75%) or taurine (~25%).
- **Bile acids differ in cytotoxicity and therefore a pool of bile acids better represents the in vivo situation**.
Regulation of Bile Acid Disposition

- Bile acid disposition is tightly regulated by the Farnesoid X Receptor (FXR)

**FXR activation leads to:**
- Increased FGF19
- Suppression of CYP7A1
- Induction of BSEP, MDR3, OSTα/β

- Potential for a drug to impact multiple pathways

<table>
<thead>
<tr>
<th>Bile Acid Pathway</th>
<th>Regulation</th>
<th>Significance (normal conditions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uptake – NTCP/OATPs</td>
<td>No FXR Regulation</td>
<td>Extensive hepatic uptake</td>
</tr>
<tr>
<td>Canalicular Efflux - BSEP</td>
<td>FXR Induced</td>
<td>75 % of total clearance</td>
</tr>
<tr>
<td>Basolateral Efflux – MRP3/4</td>
<td>No FXR Regulation</td>
<td>25 % of total clearance</td>
</tr>
<tr>
<td>Basolateral Efflux - OSTα/β</td>
<td>FXR Induced</td>
<td>Not significant</td>
</tr>
<tr>
<td>Synthesis – CYP7A1</td>
<td>FXR Suppressed</td>
<td>&lt; 5% of bile acid pool daily</td>
</tr>
</tbody>
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Predicting Cholestatic DILI

• **Historical Hypothesis:** BSEP inhibition results in build up of bile acids (detergents) which can “dissolve” membranes at high intracellular concentrations, leading to hepatotoxicity

• However, bile acids can also cause toxicity through signaling events e.g. death receptor activation

• Drugs cause cholestatic DILI by increasing bile acid levels through:
  – Direct inhibition of transporters
    • Canalicular (bile)
    • Basolateral (blood)
  and/or
  – Changes to FXR activation (e.g. antagonism)

• The liver has a high ability to compensate (Adaptive Response) - BSEP inhibition alone is not enough

Dawson et al., *Drug Metab Dispos* 40:130, 2012
Increased Intracellular Bile Acid Concentrations - Adaptive Response

- In response to high intracellular concentrations of bile acids:
  - Decreased expression of CYP7A1
  - Increased expression of BSEP
  - Increased expression of OSTα and OSTβ
- Increase in mRNA expression of transporters linked to function
- The Net Effect of the Adaptive Response is a decrease in the intracellular concentration of bile acids

In the induced state, the canalicular and basolateral efflux clearances are equal
The Adaptive Response: Time Frame and Functional Consequences

Exposure to Cyclosporine A (10 µM), a potent BSEP inhibitor leads to a **rapid, time dependent decrease** in biliary excretion of endogenous bile acids.

Inhibition of biliary excretion leads to an increase in the **intracellular concentration** of endogenous bile acids.

Increased intracellular concentrations of bile acids **activate FXR** (increased FGF19)
- This leads to **suppression of CYP7A1** (bile acid synthesis), and **induction of OST α/β** (basolateral efflux transporter)

* P ≤ 0.05
Impact of FXR Antagonism on the Adaptive Response

- Increased OSTβ expression through activation of FXR in the presence of CDCA and CDCA + CsA
- Troglitazone (weak FXR antagonist) response decreased to 46.8 % of control
- DY268 (strong FXR antagonist) response decreased to 5.6 % of control
- FXR antagonism can prevent the hepatocyte from responding to high intracellular concentrations of bile acids and increasing the potential for cholestatic hepatotoxicity

**Experimental:** 24 hours exposure, Transporter Certified™ hepatocytes in sandwich configuration (24-well) using QualGro™ media
Cholestatic DILI: Hepatocellular Injury
- Need to integrate multiple mechanisms

Initiating Insult
• BSEP Inhibition

Secondary Insult
• FXR Antagonism and/or
• Basolateral Efflux Inhibition

Compounds can Increase the Intracellular Concentration of Bile Acids through:
• BSEP Inhibition plus
• Basolateral Efflux Inhibition (MRP3/4 and/or OSTα/β) and/or
• FXR Antagonism
The C-DILI™ Assay: Cholestatic Hepatotoxicity

• System
  – Transporter Certified™ human hepatocytes in sandwich culture
  – 96 well format
  – 24 hour exposure
  – LDH readout

C-DILI™ Assay Integrates:

• Acute Effects
  – Metabolism (endogenous and exogenous)
  – Uptake and/or Efflux (basolateral and canalicular) Transporter Inhibition

• Chronic Effects (adaptive response)
  – Regulation (induction – transporters and metabolism)
  – Synthesis of endogenous bile acids

It is the NET effect of all these processes on bile acid disposition (adaptive response) that determine the cholestatic drug induced liver injury potential of a compound.
C-DILI™ Assay: Utility in Drug Discovery

Discovery
- Identify SAR for cholestasis
- Screening to identify hits (threshold effect) for cholestatic hepatotoxicity

Lead Optimization
- Differentiate between Cholestatic and Direct Toxicity
- Understand the mechanism of the interaction

PreClinical
- Predict clinical hepatotoxicity
- Evaluate drug interaction potential when multiple pathways are impacted

• Integrates multiple mechanisms that lead to hepatocellular cholestatic hepatotoxicity
  – In vivo linked intracellular concentrations of test compounds

• Use the same model system at different points in drug discovery
  – Data is directly translatable

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<th>Contrast</th>
<th>Mechanistic</th>
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<tbody>
<tr>
<td>Cholestatic Toxicity</td>
<td>✓</td>
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<td>✓</td>
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<td>Direct Toxicity</td>
<td></td>
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