Inhibition of Human Hepatic Bile Acid Transporters as Contributing Factors to Drug-Induced Liver Injury

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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
Transporter Certified™ Hepatocytes

- **Transporter Certification™ program**
  - QTS evaluates lots of plateable cryopreserved human hepatocytes for:
    - Uptake and efflux of clinically relevant transporter probe substrates
    - Ability to inhibit uptake and efflux transporters
    - Concentrations of six (6) endogenously generated bile acids
    - Glucuronidation capacity
  - Parameters are compared to a data base from fresh primary human hepatocytes

- **Transporter Certified™ hepatocytes provide in vivo relevant transporter expression, localization, and function, when cultured under conditions QTS has defined**

- **Transporter Certified™ hepatocytes demonstrate improved prediction of in vivo function in:**
  - Transport interactions
  - Induction (transporters and metabolic enzymes)
  - Hepatotoxicity
A Polarized System is Critical for *In Vivo* Relevant Transporter Function

- **System is not polarized**
- **No bile pocket formation**
- **Canalicular transporters are not functioning**
- **Uptake transporters only**

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**B-CLEAR® Sandwich-Cultured Hepatocytes**

- **Normal cell polarity re-established**
- **Uptake and efflux transporters functioning**
- **Regulatory pathways are intact and functioning**
- **Demonstrated *in vitro* – *in vivo* correlations**
Importance of Transporter Function

• Lack of efflux transporters in plated hepatocytes generates higher intracellular concentrations, leading to hepatotoxicity
• B-CLEAR® Technology utilizes a polarized system with fully functioning uptake and efflux transporters which generate in vivo relevant intracellular concentrations – demonstrating the absence of hepatotoxicity and correlation with in vivo data.

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/
## Two Patterns of Clinical Cholestatic Toxicity

<table>
<thead>
<tr>
<th>Description</th>
<th>Hepatocellular Injury</th>
<th>Bile Duct Injury</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hepatocellular Injury</strong></td>
<td>Hepatitis-like injury</td>
<td>Bile duct flow blockage</td>
</tr>
<tr>
<td><strong>Manisfests As</strong></td>
<td>marked liver cell necrosis</td>
<td>portal inflammation, bile duct injury</td>
</tr>
<tr>
<td><strong>Clinical Symptoms</strong></td>
<td>fatigue and weakness</td>
<td>jaundice and increased itching</td>
</tr>
<tr>
<td><strong>AP and GGT</strong></td>
<td>↑↑↑</td>
<td>↑↑↑</td>
</tr>
<tr>
<td><strong>ALT and AST</strong></td>
<td>↑↑↑↑</td>
<td>↑↑↑↑</td>
</tr>
<tr>
<td><strong>Bilirubin</strong></td>
<td>↑↑↑↑</td>
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</table>

- Bile Duct Injury is typically associated with immune reactivity and cholangiocyte cell damage
- Mixed injury combines both patterns together – mechanisms may not be clear
- **Our Focus is on Hepatocellular Injury**
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>
</table>
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 sufficient

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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

**Guo, et.al. ITC Workshop, 13 March 2017**
The Adaptive Response: Synergistic Effects

- The Adaptive Response can be initiated by:
  - BSEP inhibition
  - Increased intracellular concentrations of bile acids
- Increased intracellular concentrations plus inhibition of BSEP leads to a synergistic effect on the adaptive response
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)
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: An Innovative In Vitro Assay

• Mechanism-based assay
• Integrated whole-cell model
• Transporter Certified™ primary human hepatocytes in sandwich culture
• A single toxicity readout specific for bile acid induced toxicity
• Highly predictive of cholestatic drug induced liver injury
• A test set of 50 drugs
In Vitro Potency of BSEP Inhibition and Cholestatic Drug Induced Liver Injury

Dawson et al., Drug Metab Dispos 40:130, 2012
Adverse Outcomes Pathway: Integration of the Adaptive Response to Predict Cholestasis

Vinken M. (2013) Toxicology 312 158-165
The C-DILI™ Assay: Key Features

• 96-well format
• Transporter Certified™ human hepatocytes
• **Optimized** culture conditions
  – 5 days in culture: optimizes formation of bile pockets and efflux transporter function
  – QualGro™ Sensitization Media: Creates a sensitized cellular environment using lipids and bile acids
• **Standard** Culture Media (control)
  – Non-sensitized cells to account for direct compound toxicity
• 24-hour incubation with test article
  – Integrates metabolism and FXR gene expression changes (Adaptive Response)
• A **single** readout for toxicity

Patent Pending
The Sensitization Media Enables Separation of Direct versus Cholestatic DILI

Troglitazone, is toxic only in cells cultured with Sensitization Media, is NOT toxic using standard media
C-DILI™ Assay: Troglitzone and Cyclosporine A

- CsA - only a potent BSEP inhibitor
- Troglitazone and its metabolites are BSEP inhibitors
- Troglitazone is also an FXR antagonist

- Toxicity is **only observed for compounds that impact multiple pathways**, i.e. Inhibition of BSEP and/or:
  - Inhibition of Basolateral Efflux
  - FXR antagonism

- LDH chosen for analogy to clinical readouts

**Dose selection:**
- Based on $C_{\text{max}}$
- Portal concentration (10-20X $C_{\text{max}}$) or,
- Limit of solubility

Troglitzone $C_{\text{max}} = 6 - 8 \, \mu\text{M}$
CsA $C_{\text{max}} = 0.5 - 0.8 \, \mu\text{M}$
Experimental Parameters for a Proof-of-Concept Study

• 50 drugs were selected from extensive work published by Morgan et al. (2010) and Dawson et al. (2012) based on hBSEP vesicle IC_{50} data
• Portal circulation concentration > systemic concentration for orally administered drugs
• Test concentration selected was 20X greater than systemic C_{max}
• Standard and Sensitization Media were evaluated to identify direct hepatotoxicity
C-DILI™ Assay: A Closer Look at the Data

Low Toxic Potential: <150% Solvent Control
Medium Toxic Potential: 150-200% Solvent Control
High Toxic Potential: >200% Solvent Control
Extended Proof-of Concept: 45 Drugs with hBSEP IC\textsubscript{50} Data and Verifiable Liver Effects

Contingency Table Analysis

<table>
<thead>
<tr>
<th>Hepatocellular Toxicity</th>
<th>Literature (+)</th>
<th>Literature (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>True Positive</td>
<td>7</td>
<td>False Positive 1</td>
</tr>
<tr>
<td>False Negative</td>
<td>False Positive 1</td>
<td>True Negative 36</td>
</tr>
</tbody>
</table>

Probability of Toxicity if you have a positive result

Probability of no Toxicity if you have a negative result

Sensitivity 87.5%
Specificity 97.3%

Accuracy = 96%

Summary

Prediction of Cholestatic Drug Induced Liver Injury Requires Integration of:

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

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

It is the NET effect of all these processes (adaptive response) that determine the cholestatic drug induced liver injury potential for a compound

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