Inhibition of Hepatobiliary Transporters in Drug-Induced Liver Injury

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Hepatobiliary transport systems

• Formation of bile is a vital physiological function of the liver
  • Bile is a complex fluid which contains water, electrolytes, bile acids, cholesterol, phospholipids, bilirubin

• Fundamental functions of bile
  • Bile acids are important for digestion & absorption of fats & fat-soluble vitamins
  • Elimination of endogenous & exogenous waste products, e.g. bilirubin, cholesterol

• Transporters have a key role in generating bile
  • Bile salt export pump BSEP
  • Multidrug-resistance associated protein 2 MRP2

Trauner et al., J Clin Gastroenterol 2005
BSEP & MRP2

Transports
- bile salts
- conjugated bile salts, bilirubin, xenobiotics & their conjugates

Genetic defects in man
- severe liver injury in man (PFIC2)
- Dubin-Johnson Syndrome

Mutations & polymorphisms
- associated with cholestatic DILI
- associated with cholestasis & various forms of hepatitis

Knockout animals
- liver dysfunction in ko mice
- Hyperbilirubinaemia in in korats (TR-, EHBK)

Numerous drugs reported to cause cholestatic injury in man inhibit BSEP & MRP2 in vitro
BSEP deficiency causes severe liver disease

- **PFIC2** (progressive familial intrahepatic cholestasis type 2)
  - Progressive cholestatic liver damage
  - Causes fatality within approx 10 years
  - Point mutations result in truncated, non functional protein in homozygotes
  - Intrahepatic accumulation of cytotoxic bile salts is considered pivotal

Stieger et al., Pflügers Arch - Eur J Physiol 2007; Strautnieks et al., Gastroenterology 2008
Bilirubin transport & metabolism

• Genetic defects in human
  • Mutations in uptake & efflux transporters as well as metabolising enzymes lead to a range of syndromes resulting in conjugated or unconjugated hyperbilirubinaemia
  • The phenotype is generally mild, requiring no treatment; except Crigler-Najjar type I

Erlinger et al., Gastroenterology 2014; Strassburg, Best Pract Res Clin Gastroenterol 2010
BSEP inhibition in vitro

• BSEP inhibition in vesicles
  • Inverted plasma membrane vesicles derived from Sf21 insect cells expressing BSEP
  • Measures ATP-dependent transport of a probe substrate into the vesicle
  • Fairly high throughput method allowing direct measure of inhibition of one target
  • Transporters expressed in isolation, lack of metabolism & compensatory mechanisms

Experimental system

Transport of substrate S under control conditions

Reduced substrate uptake in presence of inhibiting drug D

Concentration response curves

Human BSEP Activity (%)

0 25 50 75 100

S S S S
ADP

S S S S
ATP

S S
ADP

S

taurocholate

Bosentan
Bezafibrate
Labetalol
Acetaminophen

Dawson et al., DMD 2012; Warner et al., DMD 2014
BSEP inhibition *in vitro*

- Evaluation of >80 pharmaceuticals for inhibition of BSEP in vesicles
  - Higher frequency & potency of BSEP inhibition amongst drugs associated with cholestatic & cholestatic/mixed DILI
  - Numerous DILI drugs do not inhibit BSEP
  - Some drugs are “false positives”

DILI classification taken from Gustafsson et al., Tox Sci 2014.
IN SILICO/SAR
**Transporter in silico models**

- **Limited number of inhibitor models published**
  - Pharmacophore models exist for e.g. NTCP, ASBT, OCT2, OCTN2
  - Regression models are less frequent
  - First QSAR model for BSEP published by Hirano et al. in 2006
  - BSEP pharmacophore model published by Ritschel et al. in 2014

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**Table 1**

<table>
<thead>
<tr>
<th>Descriptor (CFC)</th>
<th>C(i)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M132 (M132)</td>
<td>35.07</td>
</tr>
<tr>
<td>ESTR (J211)</td>
<td>31.47</td>
</tr>
<tr>
<td>R-CC (M531)</td>
<td>14.41</td>
</tr>
<tr>
<td>MN- HC (M521)</td>
<td>9.66</td>
</tr>
<tr>
<td>Constant</td>
<td>-9.50</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>81.11</strong></td>
</tr>
</tbody>
</table>

**Figure 1**

*Hirano et al., Mol Pharm 2006; Ritschel et al., Chem Res Tox 2014*
**Categorical model**

- **BSEP inhibition potencies for 624 chemically diverse compounds**
  - Experimental system: membrane vesicles
  - BSEP inhibition observed over a continuous range of potencies - 0.2 to several hundred µM
  - $K_m$ value for taurocholate 8-10 µM

**Potency range**

**Ion class distribution**

**IC$_{50}$ (µM)**

- BSEP IC$_{50}$ >300 µM
  - Non-inhibitors: 3%
  - Inhibitors: 20%
- BSEP IC$_{50}$ <300 µM
  - Acid: 38%
  - Base: 39%
  - Cationic: 3%
  - Neutral: 32%
  - Zwitterion: 35%

*Warner et al., DMD 2012*
Categorical model

Recursive partitioning scheme based on MW & cLogP

Probability contour plot of a compound being BSEP positive

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
<th>Matthews Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.84</td>
<td>0.83</td>
<td>0.83</td>
<td>0.67</td>
</tr>
</tbody>
</table>

- Improvement in false-negative classification through use of the SVM_AZdesc model versus simple property-based recursive partitioning
- Matched pair analysis of anecdotal value

Warner et al., DMD 2012
BEYOND A SIMPLE IC$_{50}$ THRESHOLD APPROACH
Incorporation of exposure
Use of the IC$_{50}$ value on its own has limitations

- **Total C$_{\text{max}}$ used together with BSEP IC$_{50}$ values – C$_{\text{max}}$/IC$_{50}$ ratio**
  - Akin to evaluation of drug-drug interaction potential; proposed by Morgan et al., Tox Sci 2013
  - Dataset for 60 DILI drugs & 16 No DILI drugs

<table>
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<tr>
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<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC$_{50}$ threshold</td>
<td>25/60 = 42%</td>
<td>11/16 = 69%</td>
</tr>
<tr>
<td>C$<em>{\text{max}}$/IC$</em>{50}$ ≥ 0.1</td>
<td>21/60 = 35%</td>
<td>16/16 = 100%</td>
</tr>
</tbody>
</table>

⇒ Small reduction in sensitivity but considerable improvement in specificity

Data taken from Dawson et al., DMD 2012; DILI classification as published in Gustafsson et al., Tox Sci 2014.
Incorporation of exposure
Use of the IC$_{50}$ value on its own has limitations

- Inhibition of MRP2 is an additional DILI risk factor
  - Dataset for 96 DILI drugs & 18 No DILI drugs

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<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC$_{50}$ threshold</td>
<td>25/96 = 26%</td>
<td>17/18 = 94%</td>
</tr>
<tr>
<td>C$<em>{max}$/IC$</em>{50}$ ≥ 0.1</td>
<td>15/96 = 16%</td>
<td>18/18 = 100%</td>
</tr>
</tbody>
</table>

57 DILI drugs IC$_{50}$>1000 µM

DILI classification as published in Gustafsson et al., Tox Sci 2014.

Thompson et al., CRT 2012
IN VIVO TRANSLATION
# Example - antidepressants

<table>
<thead>
<tr>
<th>Drug</th>
<th>DILI</th>
<th>Status</th>
<th>Dose (mg)</th>
<th>$C_{\text{max}}$ (µM)</th>
<th>BSEP IC$_{50}$ (µM)</th>
<th>$C_{\text{max}}$/IC$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nefazodone</strong>&lt;br&gt;<strong>Serzone®</strong></td>
<td>Acute severe liver injury &amp; cases of liver failure&lt;br&gt;With withdrawn in EU&lt;br&gt;Black Box warning US</td>
<td>600 qd</td>
<td>4.3</td>
<td>4.2</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td><strong>Buspirone</strong></td>
<td>Infrequent LFT elevations but no liver injury&lt;br&gt;Marketed</td>
<td>60 qd</td>
<td>0.01</td>
<td>104.5</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

**Nefazodone**<br>Phenylpiperazine antidepressant<br>MW 470<br>cLogP 5.7

**Buspirone**<br>Antianxiety agent<br>MW 386<br>cLogP 2.2<br>logD 2.2
Example - antidepressants

• Administration of nefazodone results in a transient increase in plasma bile acids after a single dose in rats

75 mg Nefazodone, p.o. 75 mg Buspirone, p.o.

Kostrubsky et al., Tox Sci 2006

• Additional mechanisms implicated in nefazodone DILI
  • Reactive metabolism
  • Effects on protein synthesis
Example: Endothelin receptor antagonists (ERA)

- Pulmonary arterial hypertension (PAH) is a progressive disease leading to
  - increased pulmonary artery pressure and vascular resistance
  - right-ventricular failure and death
- ERAs target ETₐ or ET₅ receptors on smooth muscle cells facilitating
  vasoconstriction
- Some ERAs are associated with liver function test abnormalities
# Example - ERA

<table>
<thead>
<tr>
<th>Drug</th>
<th>DILI</th>
<th>Status</th>
<th>Dose (mg)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitaxentan</td>
<td>Cases of liver failure requiring transplantation &amp; deaths</td>
<td>Withdrawn 2010</td>
<td>100 qd</td>
<td>28.6</td>
</tr>
<tr>
<td>Thelin®</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bosentan</td>
<td>Elevated LFT (10%); cases of severe liver injury but not liver failure</td>
<td>Black Box warning</td>
<td>125 bid</td>
<td>4.2</td>
</tr>
<tr>
<td>Tracleer®</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ambrisentan</td>
<td>Low risk, liver injury warning removed from label 2011</td>
<td>Marketed</td>
<td>10 qd</td>
<td>3.2</td>
</tr>
<tr>
<td>Letairis™ (US)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volibris® (EU)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Sitaxentan**
sulfonamide  
1,3-benzodioxazole  
MW 455  
cLogP 3.44

**Bosentan**
sulfonamide  
MW 552  
cLogP 4.2

**Ambrisentan**
propanoic acid  
MW 378  
cLogP 3.8
Example - ERA

- ERAs interact with hepatic transporters

**Table 1: IC₅₀ and Cmax/IC₅₀ flags for BSEP and MRP2**

<table>
<thead>
<tr>
<th></th>
<th>BSEP</th>
<th>MRP2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitaxentan</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Bosentan</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Ambrisentan</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

**Graphs: [H] TCA uptake (%) vs Concentration (µM)**

- **IC₅₀**
  - Sitaxentan: [Graph]
  - Bosentan: [Graph]
  - Ambrisentan: [Graph]

- **BSEP/MRP2**
  - [Graph]

**Kenna et al., JPET 2015**
Integration with other hazards
Multiple mechanisms are implicated in DILI

In Vitro Approach to Assess the Potential for Risk of Idiosyncratic Adverse Reactions Caused by Candidate Drugs
Richard A. Thompson,1,2,3 Enme M. Isin,4 Yan Li,5 Lars Weidel,6 Ken Page,7 Ian Wilson,8 Steve Swallow,9 Brian Middleton,9 Simone Stahl,9 Alison J. Foster,9 Hugues Dolgos,7,10 Richard Weaver,6 and J. Gerry Kenna6

CVB Burden

In vitro Panel

In vitro Panel Score
Min 0, Max 5

Integrated in vitro Hazard Matrix

CVB Burden

Maximum daily dose

CVB in human hepatocytes

Thompson et al., CRT 2012
Integration with other hazards

Multiple mechanisms are implicated in DILI
Summary

- **Transporter proteins have vital physiological functions**
  - Important for generation of bile flow & excretion of waste products
  - Genetic defects help to elucidate role of transporters in physiology

- **A number of tools are available to assess transporter inhibition**
  - Vesicles & hepatocytes can be employed in drug discovery
  - Biomarkers such as bile acids & bilirubin can provide follow-up in preclinical species

- **BSEP inhibition is considered to be an important mechanism in DILI**
  - Understanding of role & contribution of effects on uptake & alternative efflux transporters is still evolving

- **Context of exposure is important for interpretation of in vitro data**
  - Leads to an improvement in specificity, i.e. reduction of false positive compounds across datasets

- **For many compounds additional mechanisms will be important to explain the clinical DILI**
  - Additional drug related properties together with dose/exposure
  - Patient susceptibility factors
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Alison Foster             Jamie Scott
Jane Barber               Steve Swallow
Clare Walker
Julie Eakins

And many more colleagues....
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