Overview

• Drug Induced Liver Injury (DILI)

• BSEP inhibition by drugs and its DILI association
  – Importance of *in vivo* drug exposure scaling
  – Vesicular vs. hepatocyte assays

• BSEP inhibition hazard and risk assessment
  – PBPK-based simulations
  – Integrating BSEP inhibition with other DILI liabilities (Hazard Matrix)

• *In vivo* biomarkers of BSEP inhibition

• Chemical descriptors

• Current ITC and regulatory recommendations

• THE future? *In vitro* BSEP inhibition screening

• Summary
Drug Induced Liver Injury (DILI)

Two patterns in humans:

Type A (reproducible)
- Candidate drug attrition in animal safety studies, or early clinical trials
- Dose-capped clinical exposure

Type B (idiosyncratic)
- Serious human ill health, fatality
- Drug attrition in late clinical trials
- Failed registration
- Adverse labelling (boxed warnings etc.)
- Withdrawal of licensed drugs
Many drugs which cause DILI are similar in structure and pharmacology to safe drugs

Severe DILI risk

- Halothane
  - Black Box Warning, labelling, restricted use

Marked DILI risk

- Troglitazone
  - withdrawn
- Clozapine
  - labelling, restricted use
- Sitaxentan
  - withdrawn
- Benoxaprofen
  - withdrawn
- Bromfenac
  - withdrawn
- Enflurane, isoflurane
  - labelling
- Olanzapine
  - labelling
- Diclofenac
  - labelling + monitoring

Minimal/no DILI risk ("safe")

- Pioglitazone
  - labelling
- Sevoflurane
- Quetiapine
- Ambrisentan
- Ibuprofen
  - labelling
- Bromfenac (topical)
  - labelling
DILI is a complex process

Drug

Step 1
- Hepatic uptake

Step 2
- Chemical insult in liver

Step 3
- Biological response in target cell
  - e.g. cell toxicity, stress response, transporter up-regulation

Steps 4...
- Biological response in tissue
  - e.g. cytokine release, inflammatory cell response

Protection
- e.g. stress response

Propagation and amplification
- e.g. innate and adaptive immunity

Outcome
- Preclinical species vs. man

Tolerance & adaptation

Drug related properties

Patient related properties

Toxicity

Hepatocyte transporter defects cause severe human diseases

PFIC2 = Progressive Familial Intrahepatic Cholestasis type 2
- Point mutations result in truncated, non functional protein in homozygotes
- Progressive cholestatic liver damage
- Fatality within ~10 years unless treated by liver transplantation

Quantifying BSEP inhibition by drugs

- Inverted plasma membrane vesicles from BSEP-transfected Sf21 insect cells
- Inhibition of ATP-dependent probe substrate ([³H]-taurocholate) uptake
Many drugs which cause DILI inhibit BSEP activity.

Morgan et al. 2010, Tox Sci 118:485-500
Increased frequency and potency of BSEP inhibition amongst drugs which cause human cholestatic DILI

But numerous drugs which do not cause DILI also inhibit BSEP…..
Some drugs are markedly more potent inhibitors of human vs. rat BSEP

- Good overall correlation between potency of hBSEP and rBsep inhibition

Dawson et al. 2012, DMD 40:130–138
DILI risk is due to potency of BSEP inhibition plus _in vivo_ drug exposure

All tested drugs with BSEP IC$_{50}$ < 300 µM _and_ $C_{\text{max}}$ > 2 µM caused DILI

Dawson _et al._ 2012, DMD 40:130–138
See also: Morgan _et al._ 2013, Tox Sci 136:216-41
A simple way to take account of potency of BSEP inhibition plus plasma drug concentration ($C_{\text{max}}$, or $C_{\text{ss}}$)

- Requires accurate determination of *in vivo* plasma drug concentrations

**Data from:** Dawson *et al.* 2012, DMD 40:130–138
Why *in vitro*/*in vivo* “BSEP exposure scaling” is problematic

- Actual *in vitro* drug concentrations are unknown
  - Apparent IC$_{50}$ values assume all added drug is available in solution
  - True values likely to be much lower, due to binding to proteins and lipids
- Drug concentrations within human hepatocytes *in vivo* are unknown
  - Likely to be much higher than plasma concentrations
- BSEP inhibition by drug metabolites not evaluated
  - May be markedly more potent than parent e.g. troglitazone sulfate
- Short *in vitro* assay time interval, focussed on competitive inhibition
  - Time dependent / irreversible inhibition not addressed
- 1% DMSO vehicle, required to solubilize many compounds
- Unclear what extent of BSEP inhibition is functionally *significant in vivo*

- Unsurprising that apparent *in vitro* hBSEP IC$_{50}$ values are much lower than unbound plasma C$_{max}$ values
Estradiol glucuronide trans-inhibits BSEP

- Bsep inhibition by estradiol glucuronide requires co-expression of Bsep and Mrp2
  - Detectable in hepatocytes and in vesicles which co-express both transporters
  - Not evident in membrane vesicles which express only Bsep

Stieger et al. 2000, Gastroenterology 118:422–430
BSEP inhibition *in vitro* in hepatocytes

- Biliary efflux activity inhibited by nefazodone (withdrawn due to DILI), but not buspirone or trazodone (safer licensed drugs)

Kostrubsky *et al.* 2006, Tox Sci 90:451-459
Inhibition of vectorial bile flow in vitro: Hepatocyte high content cell imaging

Barber et al., Toxicol Sci 2015, 148:48-59

Cholyllysylfluorescein (CLF) = fluorescent bile salt analogue

- Uptake and canalicular efflux in SC hepatocytes
- Quantified via High Content Imaging
- Transported by Mrp2, but not Bsep
- However, CLF inhibition correlates well with inhibit Bsep inhibition

Barber et al., Toxicol Sci 2015, 148:48-59
Translating from BSEP inhibition to DILI risk

Integrating BSEP inhibition with other DILI liabilities

Thompson et al., 2012. Chem. Res. Toxicol. 25:1616

<table>
<thead>
<tr>
<th><strong>BSEP inhibition</strong></th>
<th>Inhibition of human BSEP transport activity</th>
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<td><strong>Mrp2 inhibition</strong></td>
<td>Inhibition of rat Mrp2 transport activity</td>
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<td><strong>HepG2 MitoTox</strong></td>
<td>HepG2 toxicity in glucose vs galactose media (mito-independent) (mito-dependent)</td>
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<td><strong>THLE toxicity</strong></td>
<td>Toxicity to THLE-Null (CYP independent)</td>
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<td>THLE-3A4 (CYP3A4 potentiated) toxicity</td>
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**Covalent binding (CVB)**

- CVB of radiolabelled drug to human hepatocyte proteins
- $F_{cvb} = \text{Fraction of metabolism leading to CVB}$
- CVB Burden = $f_{cvb} \times \text{Daily dose (mg/day)}$

Binary scores

In vitro Panel

- BSEP
- Mrp2
- HepG2 Glu/Gal ratio
- THLE-Null
- THLE-Null/3A4 ratio

Integrated in vitro Hazard Matrix

Each Y scores 1, each N scores 0
Zone 1 = CVB + multiple safety assay signal concerns
Zone 2 = Multiple assay signal concerns
Zone 3 = CVB concerns
Zone 4 = No CVB or safety signal concerns
Multiple compound related adverse properties contribute to liver injury caused by endothelin receptor antagonists

J. Gerry Kenna, Simone H. Stahl, Julie A. Eakins, Alison J. Foster, Linda C. Andersson, Jonas Bergare, Martin Billger, Marie Elebring, Charles S. Elmore, Richard A. Thompson

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- *In vitro* assay panel correctly ranked human DILI propensity of ETRAs
- Sitaxentan exhibited:
  - High CVB
  - Cytotoxic metabolites
  - Mitochondrial impairment
  - Intrinsic cell cytotoxicity
  - BSEP, MRP2 inhibition
- Bosentan exhibited:
  - CVB
  - BSEP inhibition

J Pharmacol Exp Ther. 2015 Feb;352(2):281-90
In vivo biomarkers of Bsep inhibition: serum bile acids

Funk et al., 2001 Mol Pharmacol 59:627-635

- Rats treated with troglitazone and other Bsep inhibitors exhibit elevated serum bile acids
Administration of cyclosporin A to rats caused:

- Dose dependent plasma bile acid elevations
- Plus hepatic Bsep (and Mrp2) expression
Biomarkers of Bsep inhibition in vivo: MRI imaging

Ulloa et al. 2013, NMR Biomed 26:1258-1270

**Time course**

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Gadoxetate (Primovist®) radiocontrast agent: hepatic uptake via OATP, biliary excretion via MRP2
- iv administration to rats
- disposition quantified by DCE-MRI imaging
- novel compartmental PK model developed

- A failed AZ drug candidate (CKA) reduced rates of gadoxetate hepatic uptake and biliary clearance
- CKA caused liver injury in rats in vivo and inhibited multiple hepatic transporters in vitro
Chemical descriptors and BSEP inhibition

- > 600 test compounds
- Recursive partitioning scheme based on molecular weight and $C_{\log P}$ enabled good prediction of “BSEP non-inhibitors” ($IC_{50} > 300 \mu M$)

Warner et al. 2012, DMD 40:2332-2341
The emerging data linking BSEP inhibition with cholestatic DILI was recognised by the International Transporter Consortium (Hillgren et al. 2013, Clin Pharmacol Ther 94:52-63)

Pro-active BSEP inhibition testing during drug development was not recommended because at that time:

- It was impossible to define a value for BSEP inhibition that could be considered predictive of BSEP-mediated DILI
- A strategy which enabled accurate assessment of the clinical relevance of BSEP inhibition had not been devised

If clinical studies or pre-clinical safety studies provided evidence of cholestatic DILI, the potential contribution of BSEP inhibition should be considered:

- For compounds that inhibited BSEP, serum bile acid levels should be evaluated in vivo in preclinical species and potentially in humans

Incorporated in EMEA Guidance in 2012:
The future? *In vitro* BSEP inhibition screening

A possible screening cascade

- Consider phys chem props
  - MW < 250Da and ClogP < 1.5 = BSEP inhibition unlikely
  - MW > 250Da and/or ClogP > 1.5 = BSEP inhibition possible

- Quantify *in vitro* BSEP prior to *in vivo* preclinical safety testing
  - BSEP IC$_{50}$ > 300 μM
  - Elevation vs. control values not detected at estimated safety margin >10-fold
  - No elevation detected vs. control values at estimated safety margin >10-fold
  - No BSEP hazard

- Quantify and screen away from *in vitro* BSEP inhibition
  - BSEP IC$_{50}$ ≤ 300 μM
  - Elevation detected vs. control values, estimated safety margin <10-fold
  - Quantify total plasma bile acids *in vivo* in preclinical species
  - Quantify total plasma bile acids *in vivo* in humans
  - Elevation detected vs. control values, estimated safety margin <10-fold
  - BSEP hazard

- Test alternative compounds
Summary

- BSEP inhibition is a key drug-related adverse property which may cause DILI
- The safety risk posed by BSEP inhibition is a product of potency of BSEP inhibition and \textit{in vivo} drug exposure DILI risk posed by BSEP
- \textit{In vitro} assays enable pro-active detection and minimisation of BSEP inhibition during drug discovery
- Biomarkers enable \textit{in vivo} risk assessment by evaluating functional bile flow impairment
- Since BSEP inhibition is one of several DILI initiating mechanisms, it should be evaluated alongside other contributory mechanisms and not separately from them
- Proactive BSEP inhibition screening may enable selection of safer drugs
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