Integrating DILI Hazards to Predict Toxicity of Drug Candidates

ASSESSMENT AND MANAGEMENT OF HUMAN RISK IN THE PHARMACEUTICAL INDUSTRY

Gerry Kenna
Safer Medicines Trust  gerry@safermedicines.org; www.safermedicines.org
Drug Safety Consultant  jgerrykenna@gmail.com

Presentation outline

- Drug toxicity
- Predictive toxicity challenges
- Mechanism-based in vitro assays
- In vivo exposure-based data interpretation
- Data integration: in vitro Hazard Matrix
- PBPK based simulations
- Conclusions
Consequences of drug toxicity

Inefficient development

<table>
<thead>
<tr>
<th>Year</th>
<th>Preclinical</th>
<th>Clinical</th>
</tr>
</thead>
<tbody>
<tr>
<td>1970s</td>
<td>1,300</td>
<td>1,000</td>
</tr>
<tr>
<td>1980s</td>
<td>1,100</td>
<td>900</td>
</tr>
<tr>
<td>1990-2000</td>
<td>1,000</td>
<td>900</td>
</tr>
<tr>
<td>2000-2010</td>
<td>1,200</td>
<td>1,100</td>
</tr>
</tbody>
</table>

Cautionary labelling, e.g. bosentan

WARNING: RISKS OF LIVER INJURY and TERATOGENICITY
See full prescribing information for complete boxed warning.
Tracleer can be prescribed and dispensed only through a restricted
distribution program (Tracleer Access Program) because of these risks:
Elevations of liver aminotransferases (ALT, AST) and liver failure have
been reported with Tracleer (5.1).
- Measures liver aminotransferases prior to initiation of treatment
  and then monthly (5.1).
- Discontinues Tracleer if aminotransferase elevations are
  accompanied by signs or symptoms of liver dysfunction or injury
  or increases in bilirubin 2 x ULN (2.2, 5.1).
Based on animal data, Tracleer is likely to cause major birth defects if
used during pregnancy (4.1, 8.1).
- Must exclude pregnancy before and during treatment (4.1, 8.1).
- To prevent pregnancy, females of childbearing potential must
  use two reliable forms of contraception during treatment and for
  one month after stopping Tracleer (2.4, 8.1).

Drug withdrawal
(1971 - 2010 data)

- Many drugs cause serious human toxicities
  - e.g. halothane, troglitazone, sitaxentan, bromfenac etc.
- But many “similar” drugs do not
  - e.g. desflurane, pioglitazone, ambrisentan, ibuprofen etc.

Tools are needed which enable
design and selection of safe
compounds in drug discovery
Predictive toxicity challenges

- Which methods?
  - Mechanistic relevance?
  - Robustness, throughput, turnaround time, cost?
- How to interpret the data the assays provide?
- How to evaluate and validate them?

How drugs cause toxicity

Drug

Step 1: Drug ADME

Step 2: Chemical insult to target cells

Step 3: Biological response in cell

Step 4: Biological response in tissue

No toxicity: tolerance & adaptation

Outcome

Preclinical species vs. man

Compounds related effects

Can be explored using simplified “in vitro” model systems

Patient related effects

Can be explored only in vivo

Protection

e.g. stress response

Propagation and amplification

e.g. innate and adaptive immunity

Toxicity
BSEP inhibition

- Inverted plasma membrane vesicles from BSEP-transfected Sf21 cells
- Inhibition of ATP-dependent probe substrate ([3H]-taurocholate) uptake

Concern cut-off value = 300 μM

- Increased frequency and potency of BSEP inhibition amongst drugs which cause human cholestatic DILI
- But numerous drugs which inhibited BSEP did not cause DILI
DILI risk is due to potency of BSEP inhibition plus *in vivo* drug exposure

- All tested drugs with BSEP IC_{50} < 300 \mu M and C_{max} > 2 \mu M caused DILI

Dawson *et al.* 2012, DMD 40:130–138
See also: Morgan *et al.* 2013, Tox Sci 136:216–41

---

**Hazard and Risk**

- **Hazard** = any source of potential adverse health effect, harm or damage
- **Risk** = the likelihood that a person exposed to a hazard will be harmed
- **Exposure** = the extent to which someone is subjected to a hazard

HAZARD + EXPOSURE = RISK
Covalent binding to human hepatocyte proteins *in vitro* “flagged” many high ADR concern drugs, when considered alongside oral drug dose.

Some useful *in vitro* assays

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell cytotoxicity</td>
<td>THLE-Null cell toxicity</td>
</tr>
<tr>
<td>Reactive metabolite toxicity</td>
<td>THLE-3A4 cell toxicity</td>
</tr>
<tr>
<td>Mitochondrial injury</td>
<td>Covalent binding to human hepatocyte proteins</td>
</tr>
<tr>
<td></td>
<td>HepG2 cell toxicity in glucose vs. galactose media</td>
</tr>
<tr>
<td>Membrane transporter inhibition</td>
<td>Bile Salt Export Pump (BSEP) inhibition</td>
</tr>
<tr>
<td></td>
<td>Seahorse® analyzer</td>
</tr>
</tbody>
</table>

Drug Metab Dispos 2012; 40:130
Toxicol Sci 2014;137:189
THLE cell toxicity

- THLE = SV40 - T antigen immortalised Human Liver Epithelial Cells
- Immortal and stable cell background, excellent growth properties
  - No CYP expression/activity
  - Retains phase II activities (GST, ST, EH), not UGT. (Pfeifer et al. PNAS USA 1996;90: 5123)
- Sub-lines prepared by transfection with constructs, encoding individual human P450s (Macé et al.1997, Carcinogenesis 18:1291)
  - No CYP construct = “THLE-Null”
  - Individual cell lines expressing CYP 1A2, 2E1, 2C9, 2C19, 2D6, 3A4 = “THLE-CYP”

THLE-null cell toxicity

Gustafsson et al. 2014, Toxicol. Sci. 137:189-211.

- CYP-independent THLE-Null cytotoxicity caused by numerous drugs which caused DILI
- One “false positive”: rimonabant, a very low dose/exposure drug
Potentiated THLE-3A4 cell toxicity

$$\text{Ratio} = \frac{\text{THLE-Null IC}_{50}}{\text{THLE-3A4 IC}_{50}}$$

Gustafsson et al. 2014, Toxicol. Sci. 137:189-211.

Integrating \textit{in vitro} assay data

Integrating dose adjusted $f_{CVB}$ ("CVB burden") with other in vitro liabilities


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

Covalent binding (CVB)

$F_{CVB} =$ Fraction of metabolism leading to CVB

CVB Burden = $f_{CVB} \times$ Daily dose (mg/day)

Binary scores

Each V scores 1, each N scores 0

Integrated in vitro Hazard Matrix

CVB in human hepatocytes


- Good discrimination between 27 toxic drugs and 9 non-toxic drugs (100% sensitivity, 78% specificity)
- Many High Concern drugs exhibit multiple in vitro liabilities
Value of *in vitro* Hazard Matrix

- Provides objective evidence of whether a candidate drug exhibits properties that raise human safety concern
  - If Low/No Concern, can progress into the clinic with confidence
  - Individual assays can be used to address liabilities of High Concern compounds
- But cannot be used to predict toxicity in individual patients.

Case study: Endothelin Receptor Antagonists (ETRAs)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose, mg/day</th>
<th>Number of patients treated</th>
<th>Human DILI observed</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitaxentan -Thelin*</td>
<td>100</td>
<td>2,000</td>
<td>• 4 deaths</td>
<td>Withdrawn 2010</td>
</tr>
<tr>
<td>Bosentan -Tracleer*</td>
<td>250</td>
<td>80,000</td>
<td>• Elevated LFT common</td>
<td>Black box warning</td>
</tr>
<tr>
<td>Ambrisentan -Letairis™ (US), -Volibris™ (EU)</td>
<td>10</td>
<td>10,000</td>
<td>None, but precautionary label when licensed</td>
<td>Safe drug, no DILI label</td>
</tr>
</tbody>
</table>


- Can the *in vitro* Hazard Matrix correctly rank the ETRAs?
### ETRA Integrated *in vitro* Hazard Matrix

**Graph:**
- **X-axis:** CVB Burden (mg/day)
- **Y-axis:** Aggregated *in vitro* Panel score
- **Points:**
  - Sitaxentan
  - Bosentan
  - Ambrisentan

**Table:**

<table>
<thead>
<tr>
<th></th>
<th>hBSEP IC&lt;sub&gt;50&lt;/sub&gt; (μM)</th>
<th>hMRP2 IC&lt;sub&gt;50&lt;/sub&gt; (μM)</th>
<th>Binary score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitaxentan</td>
<td>14</td>
<td>67</td>
<td>2</td>
</tr>
<tr>
<td>Bosentan</td>
<td>28</td>
<td>157</td>
<td>2</td>
</tr>
<tr>
<td>Ambrisentan</td>
<td>285</td>
<td>Activator*</td>
<td>1</td>
</tr>
</tbody>
</table>

* >1000 in rat Mrp2

---

### BSEP and MRP2 inhibition, plus THLE cell cytotoxicity

**Table:**

<table>
<thead>
<tr>
<th></th>
<th>THLE-Null EC&lt;sub&gt;50&lt;/sub&gt; (μM)</th>
<th>THLE-Null/3A4 EC&lt;sub&gt;50&lt;/sub&gt; ratio (μM)</th>
<th>Binary score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitaxentan</td>
<td>160</td>
<td>1.8</td>
<td>2</td>
</tr>
<tr>
<td>Bosentan</td>
<td>&gt;300</td>
<td>1.5</td>
<td>1</td>
</tr>
<tr>
<td>Ambrisentan</td>
<td>&gt;300</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

**Total *in vitro* panel score:**

<table>
<thead>
<tr>
<th></th>
<th>Total in vitro panel score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitaxentan</td>
<td>4</td>
</tr>
<tr>
<td>Bosentan</td>
<td>3</td>
</tr>
<tr>
<td>Ambrisentan</td>
<td>1</td>
</tr>
</tbody>
</table>

---

Kenna et al. 2015, J. Pharmacol. Exp. Ther. 352(2):281-90
A simple way to take account of potency of BSEP inhibition plus total (bound plus unbound) plasma drug concentration ($C_{\text{max}}$ or $C_{ss}$)

Requires accurate determination of in vivo plasma drug concentrations

Data from: Dawson et al. 2012, DMD 40:130–138

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

This article has not been copyedited and formatted. The final version may differ from this version.

JPET Fast Forward. Published on December 2, 2014 as DOI: 10.1124/jpet.114.220491

Improved ranking of human DILI propensity

- Sitaxentan (withdrawn)
  - High CVB
  - Cytotoxic metabolites
  - Mitochondrial impairment
  - Intrinsic cell cytotoxicity
  - BSEP, MRP2 inhibition

- Bosentan (BBW)
  - CVB
  - BSEP inhibition

- Ambisentan (safe)
  - No signals
PBPK exposure-based modelling

DILI-sim consortium: http://www.dilisym.com/

Drug exposure based simulation of population variability

PBPK based exposure scaling


<table>
<thead>
<tr>
<th>Simulations</th>
<th>Clinical Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGZ 200 mg</td>
<td>TGZ 400 mg</td>
</tr>
<tr>
<td></td>
<td>(n=331)</td>
</tr>
<tr>
<td>ALT &gt; 3× ULN (%)</td>
<td>0.3</td>
</tr>
<tr>
<td>ALT &gt; 5× ULN (%)</td>
<td>0.3</td>
</tr>
<tr>
<td>ALT &gt; 8× ULN (%)</td>
<td>0.3</td>
</tr>
<tr>
<td>ALT &gt; 10× ULN (%)</td>
<td>0.6</td>
</tr>
<tr>
<td>Time to peak ALT (Days)</td>
<td>180</td>
</tr>
<tr>
<td>Total Bilirubin &gt; 2× (%)</td>
<td>0.3</td>
</tr>
<tr>
<td>Hy’s Law cases (%)</td>
<td>0.3</td>
</tr>
<tr>
<td>Jaundice (%)</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Conclusions

- Mechanistically relevant in vitro assays can discriminate between drugs that cause idiosyncratic human toxicities and “safe” drugs.
- Data interpretation needs to take account of assay potency and drug exposure in vivo.
- Integration of data from multiple assays can be achieved using a Hazard Matrix. The Matrix aids selection of compounds for clinical development which have low propensity to cause severe toxicities in humans.
- In vitro assays do not address patient-specific susceptibility factors, so cannot predict whether or not toxicity may arise in individual humans.
- However, PBPK-based data simulations suggest that variability in drug exposure could be an important factor influencing susceptibility to toxicity.

Some References

Gustafsson F, Foster AJ, Sarda S, Bridgland-Taylor MH, Kenna JG. A correlation between the in vitro drug toxicity of drugs to cell lines that express human P450s and their propensity to cause liver injury in humans. Toxicol Sci. 2014 Jan;137(1):189-211.
Safer Medicines Trust

An independent charity.

Our goal is to replace poorly performing animal studies with more predictive human biology-based methods, for human efficacy and safety testing of pharmaceuticals and other chemicals.

See: www.SaferMedicines.org