How non-animal approaches can aid the design and selection of safe drugs

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Drugs = prescription medicines

Not drugs of abuse
Outline

• Human Adverse Drug Reactions (ADRs)
• Predictive screening need and opportunity
• A proposed predictive screening cascade
• Value and limitations of where we are now
• Future opportunities
• About Safer Medicines Trust
Human Adverse Drug Reactions (ADRs)

Type A (reproducible) cause:
- Toxicity observed in animal safety studies, or early clinical trials
- Candidate drug attrition or dose-capped clinical exposure

Type B (idiosyncratic) cause:
- Serious human ill health, fatality
- Drug attrition in late clinical trials
- Failed registration
- Adverse labelling (boxed warnings etc.)
- Withdrawal of licensed drugs
Many drugs can cause severe human ADRs
   Halothane, troglitazone, sitaxentan, bromfenac etc.

But many “similar” drugs can be used safely
   Desflurane, pioglitazone, ambrisentan, ibuprofen etc.

An important reason why drug development is so inefficient
Consequences of ADRs

Inefficient development

<table>
<thead>
<tr>
<th>Year</th>
<th>Preclinical</th>
<th>Clinical</th>
</tr>
</thead>
<tbody>
<tr>
<td>1970s</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>1980s</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td>1990-2000</td>
<td>300</td>
<td>200</td>
</tr>
<tr>
<td>2000-2010</td>
<td>1,400</td>
<td>700</td>
</tr>
</tbody>
</table>

Cost, $ millions

Drug withdrawal (1971 - 2010 data)

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:

- Elevation of liver aminotransferases (ALT, AST) and liver failure have
  been reported with Tracleer (5.1).
- Measure liver aminotransferases prior to initiation of treatment
  and then monthly (5.1).
- Discontinue Tracleer if aminotransferase elevations are
  accompanied by 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).

Liver = 49 (35%)
CV = 23 (17%)
Renal = 8 (6%)
Haematology = 23 (17%)
Immune = 16 (12%)
Rodent carc = 9 (7%)
Dev tox = 3 (2%)
Other = 45 (32%)
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
The best way to manage human ADR risk is to design and select safe drugs.

This avoids the need to deal with the consequences of ADRs.
The predictive toxicity challenge

• Is predicting human ADRs a realistic goal?

• Even if it is:
  – Which methods to use?
  – Which endpoints?
  – How to evaluate and validate them?
  – How best to interpret and integrate data?
Predictive screening opportunity

Toxicity is a multi-step process

Drug

Step 1
Drug absorption and disposition

Step 2
Chemical insult
  e.g. reactive metabolite mediated

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

Step 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

Preclinical species vs. man

Tolerance & adaptation

Toxicity

Compound related effects
Can be explored in simplified model systems, in vitro and in vivo
Multiple types of chemical insults can cause human ADRs

Reactive metabolites
- necrosis
- immunoallergic toxicity

Cell death pathways

Mitochondrial impairment

BSEP inhibition
Drug dose and human ADR risk

- Very low dose/exposure drugs are likely to be safe
- But few drugs are effective at low doses....
- And many safe and unsafe drugs are given at similar doses and achieve similar plasma exposures

Drug Metab Dispos 2012; 40:130
A way forwards

- Focus on chemical insults likely to be relevant to human drug toxicity.
- Select robust, high volume, reasonable cost assays that quantify the insults.
- Take account of *in vivo* drug exposure when interpreting assay data.
- Generate validation data using toxic and non-toxic drugs.
Many possible assays

Simple
- Cultured liver cell lines

Intermediate
- Supportive Stromal Fibroblasts
- Micropatterned Hepatocytes

Complex
- Bioreactors
- Spheroids

Cost
- Low
- High

Complexity
- Low
- High

Volume
- Low
- High

Turnaround time
- High
- Low
Some useful assays

<table>
<thead>
<tr>
<th>Chemical insult</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></td>
<td>Covalent binding to human hepatocyte proteins</td>
</tr>
<tr>
<td>Mitochondrial injury</td>
<td>HepG2 cell toxicity in glucose vs. galactose media</td>
</tr>
<tr>
<td></td>
<td>Seahorse® analyzer</td>
</tr>
<tr>
<td>Membrane transporter inhibition</td>
<td>Bile Salt Export Pump (BSEP) inhibition</td>
</tr>
</tbody>
</table>

Drug Metab Dispos 2012; 40:130
Toxicol Sci 2014;137:189
In vitro cell cytotoxicity

THLE cells = SV40 - T antigen immortalised Human Liver Epithelial Cells

- Immortal and stable cell background, excellent growth properties
- No P450 expression/activity
- Retains phase II activities (GST, ST, EH), not UGT
  Pfeifer et al. PNAS USA 1996;90: 5123
- Limited transporter expression
  Soltanpour et al. Drug Metab Dispos. 2012;40:2054
THLE-P450 cells

• Sub-lines prepared by transfection with constructs encoding individual human P450s
  
  Mace et al. (1997) Carcinogenesis 18: 1291

• No CYP construct = “THLE-Null”

• Individual cell lines stably expressing human P450s (CYP1A2, 2E1, 2C9, 2C19, 2D6, 3A4) at levels similar to hepatocytes = “THLE-CYP”
Numerous drugs which cause human DILI exhibited CYP-independent THLE-Null cell toxicity

One “false positive”: rimonabant
Potentiated THLE-3A4 cell toxicity caused by drugs which cause ADRS

\[ \text{Ratio} = \frac{\text{THLE-Null EC}_{50}}{\text{THLE-3A4 EC}_{50}} \]
Potentiated cell toxicity to THLE-3A4 cells (Δ----Δ)
Rimonabant toxicity to THLE-3A4 cells is due to bioactivation

![Graph showing the levels of [14C]-rimonabant (pmoles eq./mg protein) for different conditions.](image)

![Diagram illustrating the bioactivation of rimonabant and its effects on THLE-3A4 cells.](image)
Mitochondrial Impairment

Toxicity in glucose vs. galactose

Seahorse® analyzer

**Oligomycin** inhibits ATP synthase

**FCCP** uncouples mitochondrial respiration

**Rotenone/antimycin** blocks mitochondrial electron transport
Bile Salt Export Pump (BSEP)

- BSEP transports bile acids from hepatocytes into bile
- Genetic defects cause human cholestatic liver injury (PFIC2 etc.)
  - Toxic bile salts accumulate in hepatocytes
- *In vitro* assays quantify BSEP inhibition by drugs
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.....
Integrating human plasma exposure and BSEP inhibition potency

- Considering *in vivo* human plasma exposure markedly improves the correlation between in vitro BSEP inhibition and DILI risk

*Drug Metab Dispos 2012; 40:130*
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
<table>
<thead>
<tr>
<th><strong>In vitro cell toxicity</strong></th>
<th>THLE-Null cells (CYP independent)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>THLE-P450 cells (P450 potentiated)</td>
</tr>
<tr>
<td><strong>HepG2 MitoTox</strong></td>
<td>HepG2 toxicity potentiated in glucose vs galactose media</td>
</tr>
<tr>
<td></td>
<td>Seahorse assay</td>
</tr>
<tr>
<td><strong>BSEP inhibition</strong></td>
<td>Inhibition of human BSEP</td>
</tr>
<tr>
<td><strong>Mrp2 inhibition</strong></td>
<td>Inhibition of Mrp2</td>
</tr>
</tbody>
</table>
# Aggregating in vitro Safety Assays

*Chem. Res. Toxicol. 2012: 25;1616*

<table>
<thead>
<tr>
<th>Assay</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSEP inhibition</td>
<td>Inhibition of human BSEP transport activity</td>
</tr>
<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>THLE-3A4 toxicity</td>
<td>THLE-3A4 (CYP3A4 potentiated) toxicity</td>
</tr>
</tbody>
</table>

Each Y scores 1, each N scores 0

Binary scores
Aggregated *in vitro* Panel scores

**Selectivity and Specificity for the *in vitro* Panel**

<table>
<thead>
<tr>
<th></th>
<th>Severe &amp; Marked concern</th>
<th>Low concern</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 or more Signals</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>1 or less Signals</td>
<td>14</td>
<td>8</td>
</tr>
</tbody>
</table>

PPV (13/14) = 93%

NPV (8/22) = 58%

Sensitivity (13/27) = 48%

Specificity (8/9) = 89%

Correct = 58%

PPV, positive predictive value; NPV, negative predictive value
Human hepatocyte covalent binding (CVB)


- Can be split into 3 zones
- All **Severe** or **Marked ADR** drugs are in the top two zones
- Separation insufficient for use in isolation as a compound selection tool

$f_{cvb} = \text{fraction of metabolism leading to CVB}$

Severe ADR = withdrawn or black box warning
Marked ADR = warning on label
Low ADR = Widely used, no special concern
Integrated in vitro Hazard Matrix


<table>
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<tr>
<td>THLE toxicity</td>
<td>Toxicity to THLE-Null (CYP independent) and THLE-3A4 (CYP3A4 potentiated) toxicity</td>
</tr>
</tbody>
</table>

Covalent binding (CVB)

- CVB of radiolabelled drug to human hepatocyte proteins
- $F_{cvb} = \text{Fraction of metabolism leading to CVB}$
- CVB Burden = $f_{cvb} \times \text{Daily dose (mg/day)}$
In Vitro Approach to Assess the Potential for Risk of Idiosyncratic Adverse Reactions Caused by Candidate Drugs


See also: J Pharmacol Exp Ther. 2015; 352:281.

- Excellent discrimination between 27 toxic drugs and 9 non-toxic drugs (100% sensitivity, 78% specificity)
**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

*J Pharmacol Exp Ther. 2015 Feb;352(2):281-90*

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose mg/day</th>
<th># of patients treated</th>
<th>Hepatotoxicity</th>
<th>Regulatory Status</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sitaxentan</strong></td>
<td>100</td>
<td>2,000</td>
<td>• 4 liver failure fatalities</td>
<td>Withdrawn 2010</td>
</tr>
<tr>
<td>-Thelin®</td>
<td></td>
<td></td>
<td>• 1 liver transplantation</td>
<td></td>
</tr>
<tr>
<td><strong>Bosentan</strong></td>
<td>250</td>
<td>80,000</td>
<td>• Frequent ALT elevations</td>
<td>DILI Black Box Warning</td>
</tr>
<tr>
<td>-Tracleer®</td>
<td></td>
<td></td>
<td>• Rare DILI cases, no liver failure</td>
<td></td>
</tr>
<tr>
<td><strong>Ambrisentan</strong></td>
<td>10</td>
<td>10,000</td>
<td>• Occasional ALT elevations</td>
<td>No ADR concern</td>
</tr>
<tr>
<td>-Letairis™ (US),</td>
<td></td>
<td></td>
<td>• DILI not reported</td>
<td></td>
</tr>
<tr>
<td>-Volibris® (EU)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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Multiple compound related adverse properties contribute to liver injury caused by endothelin receptor antagonists

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In vitro assay panel correctly predicts that sitaxentan causes human DILI but ambrisentan does not

DILI liability of sitaxentan is due to:
- BSEP inhibition
- cytotoxic reactive metabolites
- mitochondrial impairment
- intrinsic cell cytotoxicity

Could take account of human exposure because the drugs have been used in humans. This calculation is not feasible in drug discovery.
In Vitro Approach to Assess the Potential for Risk of Idiosyncratic Adverse Reactions Caused by Candidate Drugs

Richard A. Thompson,*† Emre M. Isin,* Yan Li,* Lars Weidolf,* Ken Page,* Ian Wilson,* Steve Swallow,* Brian Middleton,* Simone Stahl,* Alison J. Foster,* Richard Weaver,* and J. Gerry Kenna†

†DMPK Innovative Medicine, AstraZeneca, Möln达尔, 431 83, Sweden

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
Conclusions

• Mechanistically relevant *in vitro* assays can discriminate between non-toxic drugs and drugs that cause human toxicities.

• When *in vivo* human exposure is used to aid data interpretation, these assays can aid selection of safe new drugs.

• In principle, the same approaches could be used to support human risk assessment of other chemicals.
But....

• The *in vitro* assay panel addresses only drug related adverse properties and “average” drug exposure

• It takes no account of innate immunity or adaptive immunity

• Opportunities for improvement!
• Human ADRs occur infrequently, probably due to:

\[ \text{Adverse props} \times \text{Exposure} \times \text{Individual susceptibility} = \text{Individual risk} \]

• How can we realistically take account of and simulate population variability?
The future: Exposure Modelling

e.g. Hamner DILI-sim consortium: [http://www.dilisym.com/](http://www.dilisym.com/)

- Drug exposure based simulation of population variability
Bottlenecks to progress

- Underpinning science is still emerging.
- No consensus on which assays to select, or how.
- Limited scientific funding.
- Innovation largely driven from industry, not academia.
- Toxicologists are conservative.
- Lack of regulatory pressure to change.
- It’s much easier to criticise than to be constructive.
THAT WHEEL THING...WE TRIED IT ONCE BEFORE AND IT DIDN'T WORK!

NEANDERTHAL MAN'S LACK OF CURiosity DOOMED HIM TO EXTINCTION
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
Thanks to many colleagues

AstraZeneca

- Molecular Toxicology
  - Simone Stahl, Clare Walker, Sarah Dawson, Mhairi Greer, Alison Foster, Frida Gustafsson, Irene Edebert, Julie Eakins
- DMPK
  - Richard Thompson, Ian Wilson and the ARMS team

Other collaborators

- Hans Westerhoff, Suzanne Geenen, University of Manchester
- Kevin Park and MRC CDSS, University of Liverpool
- Paul Watkins and the Hamner DILI-sim team