Mechanisms of Drug Toxicity & Relevance to Pharmaceutical Development

21 August 2015

Evidentiary Considerations for Integration of Biomarkers in Drug Development
FDA/M-CERSI

Baltimore, MD

Prof. F. P. Guengerich
Department of Biochemistry
Vanderbilt University School of Medicine
f.guengerich@vanderbilt.edu
https://my.vanderbilt.edu/guengerichlab/
https://medschool.vanderbilt.edu/biochemistry/person/f-peter-guengerich
Total cost >$2.5 billion/new drug
—and some estimates are even higher!

(Tufts Center, November 2014)
Reasons for Termination of Drug Candidates in Development (1964 - 1985)

- Human PK (39%)
- Clinical Efficacy (29%)
- Animal Toxicity (11%)
- Human AEs (10%)
- Commercial (6%)
- Improved Candidate (2%)
- Financial (1%)
- Other (2%)

Reasons for Termination of Drug Candidates in Development (2000)

Dose-response Concepts (Paracelsus)

- **Definitions** - Effective dose = ED; Toxic dose = TD; Lethal dose = LD
- **Potency** - Range of doses over which a drug produces increasing responses
- **Efficacy** - Maximal response; plateau of the dose-response curve
Contexts of Drug Toxicity

- **On-target toxicity** (mechanisms-based): same receptor, wrong tissue (e.g., statins)
- **Hypersensitivity & immunological reactions** (e.g., penicillins)
- **Off-target pharmacology** (e.g., terfenadine & hERG channel effects)
- **Bioactivation to reactive intermediates** (e.g., acetaminophen)
- **Idiosyncratic** toxicities

Metabolic Activation of Drugs—”Reactive Metabolites”

Drug → Cellular accumulation → Excretion

Drug → Metabolism → Reactive metabolite → Stable metabolite → Excretion

DNA
- Mutations
- Carcinogenicity

Proteins

Covalent modification

Apoptosis/Necrosis
Hypersensitivity/Immune response
Idiosynchratic drug reaction

Detoxication

Direct toxicity

Excretion

Drug

Cellular accumulation
Reaction types involved in bioactivation of carcinogens (n = 799 reactions)

Fig. 6

Human Enzymes in Activation of Carcinogens, n=713

Structural Alerts for Bioactivation

Hydrazines and hydrazides
Arylacetic or aryl propionic acids
Thiophenes, furans, pyrroles
Anilines and anilides
Quinones and quinoneimines
Medium chain fatty acids
Halogenated hydrocarbons and some halogenated aromatics (Br > Cl > F)
Nitroaromatics
Moieties that form $\alpha\beta$-unsaturated enol-like structures
Thiols, thiono compounds, thiazolidinedione

So: What’s left to work with?
Also, remember that any phenyl ring is only 1-3 steps away from a reactive intermediate.

Thanks for list to Sid Nelson, U. Wash.
Comparison of Selected Adrenal Toxicants That Affect Steroidogenic Enzymes

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Adrenal Toxicity</th>
<th>Toxicity Mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMS compound</td>
<td>Adrenal vacuolar degeneration and necrosis</td>
<td>Mitochondrial bioactivation by CYP11A1</td>
<td>This study</td>
</tr>
<tr>
<td>AGT</td>
<td>Inhibition of cortisol secretion</td>
<td>Inhibition of CYP11A1</td>
<td>(34)</td>
</tr>
<tr>
<td>MTY</td>
<td>Stimulation of ACTH release</td>
<td>Inhibition of CYP11B1</td>
<td>(16)</td>
</tr>
<tr>
<td>Etomidate</td>
<td>Adrenal insufficiency</td>
<td>Inhibition of CYP11B2/1</td>
<td>(35)</td>
</tr>
<tr>
<td>Atrazine</td>
<td>Adrenal weight increase</td>
<td>Induction of CYP19</td>
<td>(36)</td>
</tr>
<tr>
<td>Letrozole</td>
<td>Mild adrenal suppression</td>
<td>Inhibition of CYP19</td>
<td>(25)</td>
</tr>
<tr>
<td>KTZ</td>
<td>Reversible adrenal insufficiency</td>
<td>Inhibition of CYP17 and 11 beta hydroxylase</td>
<td>(37)</td>
</tr>
<tr>
<td>Pfizer compound</td>
<td>Formation of vacuoles in adrenal</td>
<td>Inhibition of CYP21</td>
<td>(38)</td>
</tr>
<tr>
<td>MeSO₂-DDE</td>
<td>Adrenal disorganization loss of central cristae of mice</td>
<td>Cytotoxic to parenchymal cells, bioactivation by and inhibition of CYP11B1</td>
<td>(39, 40)</td>
</tr>
<tr>
<td>Mitotane</td>
<td>Membrane disruption an dissolution of adrenal</td>
<td>Bioactivation by CYP11B1 and other enzymes</td>
<td>(32, 41)</td>
</tr>
<tr>
<td>DMBA</td>
<td>Adrenal capillary endothelial lesion to bleeding</td>
<td>Oxidation of mitochondrial GSH, involvement of CYP11B1</td>
<td>(42, 43)</td>
</tr>
<tr>
<td>Lindane Hexachlorocyclohexane</td>
<td>Adrenal weight increase</td>
<td>Inhibition of stAR</td>
<td>(44)</td>
</tr>
</tbody>
</table>

P450s:  
11A1  
11B1  
11B2  
19A1  
17A1  
21A2

Covalent binding of chemicals to proteins: Issue or not?

- Correlates with *in vivo* toxicity
- Treatment of a purified enzyme with a chemical modifier can destroy activity
- Block covalent binding (e.g. N-Ac Cys), prevent toxicity
- Knock out P450s —> prevent acetaminophen toxicity
- Idiosyncratic toxicity:
  - Majority of culprits show covalent binding
  - Only seen with higher dose drugs (>10 mg/day), consistent with binding overload
- No direct proof of involvement in toxicity
- Alternative mechanisms, e.g. ox stress, would show similar profiles re N-AcCys
- Some drugs have high covalent binding but no apparent toxicity
- Delete other genes (non-P450) & see effects on toxicity, implying downstream issues
Scatter plot of % dGSH adduct formation (a) and estimated total daily burden (b) in the DIT and non-DIT groups. The open circles and triangles represent drugs not associated and associated with DIT, respectively. For illustrational purposes, a horizontal dotted line is plotted at 0.2% adduct level in panel a, and another is plotted at the 1 mg level in panel b. Adduct levels of omeprazole, lansoprazole, and montelukast are not shown in this figure.
Significance of acetaminophen metabolism in toxicity in mice

P450 2e1⁻⁻

P450 2e1⁺⁺

See also Zaher et al. (1998) Toxicol. Sci. 152, 193-199 regarding deletion of both 2e1 and 1a2
Abacavir use associated with immune hypersensitivity syndrome
- occurs in individuals with HLA-B*57:01 allele

X-ray cocrystal of abacavir bound to HLA-B*57:01
- binds to bottom of antigen binding cleft of the F pocket

Abacavir binds to 2 amino acids unique to HLA-B*57:01

Cyclopropyl moiety projects into F pocket
- reduces pocket size; alters peptide binding preference
- smaller Leu and Ile side chains preferred over Trp and Tyr

Co-crystal structure of carbamazepine with HLA-B*15:02
- indicates similar mechanism of hypersensitivity

# Mechanistic Causes of Toxicology Attrition

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Percent of All Advanced Molecules&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotransformation-related</td>
<td>27</td>
</tr>
<tr>
<td>Target-based</td>
<td>28</td>
</tr>
<tr>
<td>Single or multiple ion channel inhibition</td>
<td>18</td>
</tr>
<tr>
<td>Immune-mediated</td>
<td>7</td>
</tr>
<tr>
<td>All other mechanisms</td>
<td>36</td>
</tr>
</tbody>
</table>

<sup>a</sup>Based on experience from DuPont-Merck and Bristol-Myers Squibb, 1993-2006. Information kindly provided by B. Car.

<sup>b</sup>n=88, note as categories are partially overlapping, the total is > 100%.
## Trends in safety assessment

### Assessing toxicity earlier

<table>
<thead>
<tr>
<th>Early Discovery</th>
<th>Late Discovery</th>
<th>Preclinical Research</th>
<th>Clinical Trials</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vitro expression profiling</td>
<td>Expression profiling</td>
<td>Animal studies</td>
<td>Clinical trials</td>
</tr>
<tr>
<td>hours-days</td>
<td>1-3 days dosing</td>
<td>Weeks of dosing</td>
<td>Days-weeks of dosing</td>
</tr>
<tr>
<td>1-5 mg drug</td>
<td>1-10 g drug</td>
<td>100s-1000s g drug</td>
<td>kgs of drug</td>
</tr>
<tr>
<td>$1K</td>
<td>$1K-$10K</td>
<td>$100K-$1M</td>
<td>$1M-$100M</td>
</tr>
</tbody>
</table>

Thanks to Eric Blomme, Abbott
Overview of assays and their interrelationship.

Published in: Richard A. Thompson; Emre M. Isin; Yan Li; Lars Weidolf; Ken Page; Ian Wilson; Steve Swallow; Brian Middleton; Simone Stahl; Alison J. Foster; Hugues Dolgos; Richard Weaver; J. Gerry Kenna; Chem. Res. Toxicol. Article ASAP
DOI: 10.1021/tx300091x
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Integrated *in vitro* Hazard Matrix. IADR categories are Severe concern (black inverted triangles), Marked concern (red triangles), and Low concern (green circles).

Published in: Richard A. Thompson; Emre M. Isin; Yan Li; Lars Weidolf; Ken Page; Ian Wilson; Steve Swallow; Brian Middleton; Simone Stahl; Alison J. Foster; Hugues Dolgos; Richard Weaver; J. Gerry Kenna; *Chem. Res. Toxicol.* Article ASAP
DOI: 10.1021/tx300091x
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No provision for bioactivation!
Introduction: *W. G. Humphreys, BMS; Y. Will, Pfizer; F. Guengerich, Vanderbilt*

Physicochemical properties of molecules: *N. Meanwell, BMS*

*In silico* stratification/computational models: *Grace Patlewicz, EPA*

Transporters: *Yurong Lai, BMS*

Reactive metabolites: *Richard Thompson, AZ*

Hepatic issues: *Gerry Kenna, FRAME*

Cardiovascular issues: *Paul Levesque, BMS*

New methods in reproductive toxicology: *Karen Augustine, MBS*

New technologies: *Donna Dambach, Genentech*

Overview—pulling it all together: *Eric Blomme, Abbott; Y. Will, Pfizer*
Summary

• General issues in the pharmaceutical industry
  - Toxicity/safety is a big issue

• Bioactivation is an important issue but not the only one

• Issues with “endogenous substrate” P450s

• Covalent binding: general, issues-bad, good

• In vitro strategies in discovery toxicology