Chemically Reactive Drug Metabolites in Drug Discovery and Development
Detection, Evaluation, and Risk Assessment

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Figure 1: **Routes of elimination of the top 200 most prescribed drugs in 2002.** Metabolism represents the listed clearance mechanism for ~73% of the top 200 drugs. Of the drugs cleared via metabolism, about three-quarters are metabolized by members of the cytochrome P450 (CYP) superfamily. For the CYP-mediated clearance mechanisms, the majority of drug oxidations (46%) were carried out by members of the CYP3A family; followed by 16% by CYP2C9; 12% for both CYP2C19 and CYP2D6; 9% for members of the CYP1A family; and 2% for both CYP2B6 and CYP2E1 (REF. 9). UGT, uridine diphosphate glucuronyl transferase.
Chemically Reactive Drug Metabolites
Role in Liver Toxicity

The Presence and Significance of Bound Aminoazo Dyes in the Livers of Rats Fed p-Dimethylaminoazobenzene*

Elizabeth C. Miller,** Ph. D., and James A. Miller, Ph. D.
(From the McArdle Memorial Laboratory, University of Wisconsin Medical School, Madison 6, Wisconsin)
(Received for publication February 3, 1947)

*Cancer Res., 7, 468-480 (1947)

ACETAMINOPHEN-INDUCED HEPATIC NECROSIS.
I. ROLE OF DRUG METABOLISM

J. R. MITCHELL, D. J. JOLLOW, W. Z. POTTER,** J. R. GILLETTE AND B. B. BRODIE

Laboratory of Chemical Pharmacology, National Heart and Lung Institute, National Institutes of Health, Bethesda, Maryland
Accepted for publication May 30, 1973

Bioactivation and Liver Toxicity

Acetaminophen (Paracetamol)

Acetaminophen (APAP)

\[ \text{APAP Sulfate} \]
\[ \text{APAP Glucuronide} \]

CYP1A2
CYP2E1
CYP3A4

NAPQI

\[ \text{Depletion of GSH Pools} \]
\[ \text{Oxidative Stress} \]
\[ \text{Covalent Binding to Proteins} \]

APAP-GSH

\[ \text{Liver Toxicity} \]

I. M. Copple et al., Hepatology, 48, 1292-1301 (2008)
“Current recommendations say that the maximum single dose is 1,000 milligrams -- the amount in two Extra Strength Tylenol tablets; the advisory panel recommended lowering that amount to 625 milligrams. The current maximum total daily dose is 4 grams; the panel recommended reducing that as well, to 3.25 grams or less.”

“People vary in their responses, so it's hard to say what an overdose is for any particular individual. Poison control experts generally consider 10 to 12 grams at one time an overdose, but even 8 grams can be dangerous in someone who weighs 120 pounds, and 3 grams can be risky for a 40-pound child. In addition, people who regularly consume three or more alcoholic drinks per day tend to be more sensitive to the toxic effects of acetaminophen, which means they should be more careful in limiting dose.”

Acetaminophen "is the most common cause of acute liver failure in the US"
P450-Mediated Quinoid Formation
Toxicological Implications

Note: Metabolism can often introduce / expose –OH / –NH functionalities

Oxidative Damage (DNA, Proteins)

Target Organ Toxicity

Nuc = GSH, protein
“Structural Alerts” for Metabolic Activation

- Evolved from consideration of genotoxic carcinogens ("hard" electrophiles)
- Do not translate as readily to "soft" electrophilic drug metabolites which usually demonstrate a "threshold" for toxicity

Structural alerts must be supplemented by experimental data!

Structural Alerts and Idiosyncratic Drug Toxicity

Structural Alert/Reactive Metabolite Concept as Applied in Medicinal Chemistry to Mitigate the Risk of Idiosyncratic Drug Toxicity: A Perspective Based on the Critical Examination of Trends in the Top 200 Drugs Marketed in the United States

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Supporting Information

## Role of Dose in Drug-Induced Liver Injury

### Table 7. DILI Requiring Liver Transplantation in the United States From 1990 to 2002 According to the UNOS Database

<table>
<thead>
<tr>
<th>Dosage Groups</th>
<th>≤10 mg/day</th>
<th>11-49 mg/day</th>
<th>≥50 mg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Individual agents (number of cases)</td>
<td>Cerivastatin (2)</td>
<td>Fialuridine (3)</td>
<td>Isoniazid (24)</td>
</tr>
<tr>
<td></td>
<td>Lisinopril (1)</td>
<td>Propylthiouracil (13)</td>
<td></td>
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<tr>
<td></td>
<td>Simvastatin (1)</td>
<td>Phenytion (10)</td>
<td></td>
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<tr>
<td></td>
<td>Pemoline (1)</td>
<td>Valproate (10)</td>
<td></td>
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<tr>
<td></td>
<td>Zafirkulast (1)</td>
<td>Nitrofurantoin (7)</td>
<td></td>
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<tr>
<td></td>
<td>Paroxetine (1)</td>
<td>Ketoconazole (6)</td>
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<td></td>
<td></td>
<td>Disulfiram (6)</td>
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<td></td>
<td></td>
<td>Troglitazone (4)</td>
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<tr>
<td></td>
<td></td>
<td>Sulfasalazine (3)</td>
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<td></td>
<td></td>
<td>Methyldopa (3)</td>
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<tr>
<td></td>
<td></td>
<td>Nefazadone (2)</td>
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<td></td>
<td></td>
<td>Labetalol (2)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Amoxicillin/Clavulan (1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bromfenac (1)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Ibuprofen (1)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Hydrocodone (1)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>6-Mercaptopurine</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Itraconazole (1)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Carbamazepine (1)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Trimethoprim/Sulfametho</td>
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<td></td>
<td></td>
<td>Bupropion (1)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Iron (1)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Naproxen (1)</td>
<td></td>
</tr>
</tbody>
</table>

Total number: 2, 8, 101

NOTE. Excluded are inhalation agents (4), intravenous agents (3), herbal agents (7), *Amanita* mushrooms (9), and combination of agents (3).

Reactive Drug Metabolites and Idiosyncratic Drug Toxicity
**Idiosyncratic Drug Toxicity**

Susceptibility to adverse drug reactions (ADRs) is a function of:

(a) **Chemistry of drug and its interaction with biological systems**
- On- and off-target pharmacology
- Metabolic activation of accessible toxicophore (e.g., acetaminophen)
- Normally dose-dependent, predictable, reproducible in animals

(b) **Phenotype and genotype of patient**
- Not related to pharmacology of drug
- No clear dose-response relationship, unpredictable, often not reproduced in animals (“idiosyncratic”)

“Idiosyncratic” drug reactions can result from the sequence:
- Metabolic activation of parent
- Covalent modification of proteins (“chemical stress”)
- Presentation (in susceptible individuals) of adducted proteins to T cells via specific HLA proteins
- Immune-mediated ADRs (often involving liver or skin) – “Hapten Hypothesis”

Metabolism-Dependent Abacavir Hypersensitivity

### ADRs: Reaction Frequency vs. Allele Frequency

<table>
<thead>
<tr>
<th>Drug</th>
<th>Reaction</th>
<th>Allele</th>
<th>Allele Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abacavir</td>
<td>Hypersensitivity</td>
<td>HLA-B*5701</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>0.05-0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flucloxacillin</td>
<td>Hepatotoxicity</td>
<td>HLA-B*5701</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>0.000085</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>SJS</td>
<td>HLA-B*1502</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>0.0001</td>
<td>(Chinese)</td>
<td></td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Hypersensitivity</td>
<td>HLA-A*3101</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>0.029</td>
<td>(Japanese)</td>
<td></td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Hypersensitivity</td>
<td>HLA-A*3010</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>(Caucasians)</td>
<td></td>
</tr>
<tr>
<td>Lumiracoxib</td>
<td>Hepatotoxicity</td>
<td>HLA-DQA1</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>0.025</td>
<td>*0102</td>
<td></td>
</tr>
<tr>
<td>Ximelagatran</td>
<td>Hepatotoxicity</td>
<td>HLA-DRB1</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>0.06-0.13</td>
<td>*0701</td>
<td></td>
</tr>
</tbody>
</table>

Mallal, 2008; Kindmark et al., 2008; Daly et al., 2009; Chung et al., 2004; Williams et al., 2004; Levine et al., 2004; Kamali et al., 2009; McCormack et al., 2011.
Experimental Approaches for the Study of Reactive Drug Metabolites
Assessing Formation of / Exposure to Reactive Drug Metabolites

(A) Observation of time-dependent P450 inhibition \textit{in vitro}
   - Implications for clinical drug-drug interactions

(B) Formation of adducts with nucleophiles and detection by LC-MS/MS
   \textit{In vitro} “trapping” experiments with GSH or CN\textsuperscript{-} (or radiolabeled counterparts)
   \textit{In vivo} metabolic profiling studies (eg GSH conjugates in bile)
   - Invaluable in enabling rational structural re-design

(C) Covalent binding studies with radiolabeled drug
   - Measures “total” burden of protein-bound drug residue
   - Helpful complement to trapping studies

\textit{These approaches employ different end-points and serve different purposes!}
Covalent Binding as an Index of Metabolic Activation
The Discovery of Merck’s CB-1 Inverse Agonist, Taranabant

ADDRESSING METABOLIC ACTIVATION IN DRUG DISCOVERY

1 (3900)

Figure 1 Metabolic activation of an aryloxy-substituted drug candidate (1).

(1690) (911) (303) (88)

Figure 2 Improved analogs of the lead compound 1 showing reduced levels of covalent binding (pmol-equiv/mg protein, shown in parentheses).

Evolution of Taranabant (CB-1 Inverse Agonist)

Lead compound
(CB1 IC$_{50}$ = 2.03 nM; covalent binding = 3900 pmol-equiv./mg; rat F = 9%)

Taranabant
(CB1 IC$_{50}$ = 0.29 nM nM; covalent binding = 27 pmol-equiv./mg; rat F = 74%)

Body Burden of Reactive Metabolites and Risk of Hepatotoxicity
Combining Reactive Metabolite Body Burden and In Vitro Hepatic Screening Panel in Risk Assessment AstraZeneca

**Hepatic Screening Panel**
- Cytotoxicity in immortalized human hepatocyte-derived cell lines (THLE) with or without CYP3A activity
- Mitochondrial impairment – HepG2 cytotoxicity in galactose vs. glucose medium
- Inhibition of hepatic biliary transport – BSEP and Mrp2

**Estimated Reactive Metabolite Body Burden**
- Product of fraction of metabolism leading to covalent binding in human hepatocytes and estimated therapeutic dose

R. A. Thompson *et al.*, *Drug Metab. Rev.*, **43** (S2): 144-145 (2011)
From the test group of 36 drugs, Zone 1 contained only “safe” compounds, while Zones 3 & 4 (high CVB burden) contained the great majority of toxic agents.
Conclusions

• There is compelling evidence that chemically reactive metabolites can mediate the serious adverse reactions to drugs and other foreign compounds.

• However, the frequency and severity of such ADRs will depend upon a complex series of factors related to the host and the environment, as well as the reactive intermediate.

• While “avoidance” strategies continue to be pursued during the lead optimization stage of drug discovery, integrated reactive metabolite hazard assessment strategies are now emerging, based on considerations of reactive metabolite body burden, *in vitro* toxicity markers, and *in vivo* safety testing in animals.

• The formation of reactive metabolites needs to be viewed as only one component of overall risk assessment in the development of new pharmaceuticals.