Approaches to Mitigating the Bioactivation Potential of Compounds in Lead Optimization

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Question

Is covalent binding or GSH Adduct Formation a kill shot for a candidate??
A common cause for drug recalls or black box warnings

Unpredicted or Idiosyncratic ADR (IADR) – more problematic

- Normally not observed until phase III or post launch
- Frequency of occurrence – 1 in 10000 to 1 in 100000
- Responsible for drug withdrawal
- Very expensive - law suits etc.

Patients have been deprived of several good drugs
Not easy to predict IADR
- No animal models
- Lack of specific biomarkers

Circumstantial evidence links metabolic activation to IADR

Several examples demonstrate that bioactivation liability and IADR are linked
- Kalgutkar AS and Soglia JR (2005). Exp. Opin. Drug Metab. & Toxicol. 1:91-141)
### Drugs Associated With IADRs

#### Drugs Withdrawn

<table>
<thead>
<tr>
<th>Drug</th>
<th>Temp. Withdrawn or Withdrawn in other Countries</th>
<th>Marketed Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aclclofenac (antiinflammatory)</td>
<td>Aminopyrine (analgesic)</td>
<td>Abacavir (antiretroviral)</td>
</tr>
<tr>
<td>Hepatitis, rash</td>
<td>Hepatitis (fatal)</td>
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<tr>
<td>Alpidem (anxiolytic)</td>
<td>Hepatitis (fatal)</td>
<td>Acetaminophen (analgesic)</td>
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<tr>
<td>Hepatitis (fatal)</td>
<td>Hepatitis (fatal)</td>
<td>Captopril (antihypertensive)</td>
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<tr>
<td>Amodiaquine (antimalarial)</td>
<td>Agranulocytosis</td>
<td>Carbamazepine (anticonvulsant)</td>
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<tr>
<td>Hepatitis, agranulocytosis</td>
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</tr>
<tr>
<td>Aminiptine (antidepressant)</td>
<td>Agranulocytosis</td>
<td>Clozapine (antipsychotic)</td>
</tr>
<tr>
<td>Hepatitis, cutaneous ADRs</td>
<td></td>
<td>Agranulocytosis</td>
</tr>
<tr>
<td>Benoxaprofen (antiinflammatory)</td>
<td>Agranulocytosis</td>
<td>Cyclophosphamide (anticancer)</td>
</tr>
<tr>
<td>Hepatitis, cutaneous ADRs</td>
<td></td>
<td>Agranulocytosis, cutaneous ADRs</td>
</tr>
<tr>
<td>Bromfenac (antiinflammatory)</td>
<td>Trovan (antibacterial)</td>
<td>Dapsone (antibacterial)</td>
</tr>
<tr>
<td>Hepatitis (fatal)</td>
<td>Hepatitis</td>
<td>Agranulocytosis, cutaneous ADRs, aplastic anaemia</td>
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<tr>
<td>Carbutamide (antidiabetic)</td>
<td>Zileuton (antiasthma)</td>
<td>Furosemide (diuretic)</td>
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<tr>
<td>Bone marrow toxicity</td>
<td></td>
<td>Agranulocytosis, cutaneous ADRs, aplastic anaemia</td>
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<tr>
<td>Ibufenac (antiinflammatory)</td>
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<td>(fatal), severe restriction in use</td>
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<td>Nomifensine (antidepressant)</td>
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<td>(fatal), severe restriction in use</td>
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<td>Hepatitis (fatal), anaemia</td>
<td></td>
<td>Furosemide (diuretic)</td>
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<tr>
<td>Practolol (antiarrhythmic)</td>
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<td>Agranulocytosis, cutaneous ADRs, aplastic anaemia</td>
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<tr>
<td>Severe cutaneous ADRs</td>
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<td>Remoxipride (antipsychotic)</td>
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<td>Furosemide (diuretic)</td>
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<td>Aplastic anaemia</td>
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<td>Sudoxicam (antipsychotic)</td>
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<td>Hepatitis (fatal)</td>
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For most of these drugs, bioactivation to reactive metabolites has been demonstrated in vitro or in vivo.

Kalutkar AS and Soglia JR (2005). *Exp. Opin. Drug Metab. & Toxicol.* 1:91-141)
Identify toxicophores – “Structural Alerts” early on
- Especially those undergoing ‘metabolic activation’

One approach adopted by many pharmaceutical companies
- Eliminate metabolic activation of a candidate
  - Screen for ability to form reactive metabolites very early
- Avoid chemical functionaliies that undergo bioactivation
  - No guarantee that this will make compounds safer
  - But, preventing reactive metabolite formation \( \downarrow \) chances of IADR

TALL ORDER BUT AVOIDS RISK!
- Saves $$
- Saves time and effort
Methods to Assess Bioactivation Potential

Two Methods Commonly Used

Covalent Binding to Proteins

✦ Most definitive
✦ Useful in quantitation of the reactive metabolite
✦ Helps to detect all reactive metabolites
✦ Limited by availability of radiolabel

Trapping with nucleophiles

✦ Most popular in a discovery setting
✦ Nucleophiles used to trap are:
  • Glutathione
  • N-Acetylcysteine
  • Other nucleophiles - cyanide
✦ Acts as a surrogate marker of covalent binding

Drug (10 – 50 µM) + Human Liver Microsomes + GSH (3 mM)

Incubation at 37° C for 1.0 hr

NADPH regenerating system (+/-)

Samples analyzed for GSH adducts using various LC-MS methods
Some Known Facts:

- No assay predicts potential of a new drug to cause IADR

Important to Weigh the Benefits versus Risks

- Is it a prototype or a back up?
- Indication
- Is the drug being developed for an unmet medical need?
  - First in class?
- Dosing regimen of the drug
  - Acute or chronic?
  - Drugs that are used chronically are more prone to IADRs
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Relationship Between Dose and Frequency of Idiosyncratic Reactions

- **Dose**
  - A lower dose reduces the risk

### Issue:
- **Dose is unknown in the early stages**
Other Factors from a Drug Metabolism Perspective

- Contribution of pathways that detoxify reactive metabolites
- Contribution of competing metabolic routes

“After all it is all about body burden of RM”
Case Studies

- Paroxetine – an antidepressant SSRI
- Raloxifene – A selective estrogen receptor modulator (SERM)
- A Case Study from Pfizer
Paroxetine contains the methylenedioxy group - a toxicophore

- Results in inactivation of CYP2D6
  
  - clinical pharmacokinetic interactions with substrates well established

Paroxetine Also Undergoes Metabolic Activation

Incubation with HLM in the presence of GSH and NADPH

Paroxetine → CYP2D6 → Catechol → o-Benzquinone

bis-Adduct → [GSH] → mono-Adduct

A Novel Cyclic Adduct

Incubation of Radiolabeled Paroxetine with HLM

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Inference?

- Paroxetine is prone to bioactivation
  - Covalently binds to microsomal protein

- Would one nominate Paroxetine if it was to be developed as an antidepressant to date?

- Paroxetine is a commonly prescribed antidepressant
  - IADRs (especially hepatotoxicity) are extremely rare
Effect of GSH on Covalent Binding of Paroxetine

Incubation with Human Liver Microsomes

![Graph showing covalent binding with and without GSH and UDPGA.](image)

GSH and UDPGA Mops Reactive Intermediate Reduces covalent binding!

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Covalent Binding of Paroxetine in Human S9

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Analysis of the Data

- Paroxetine was bioactivated but:
  - o-Quinone is detoxified by Glutathione
  - Catechol detoxified by methylation and glucuronidation

- Additional factors that may contribute to less IADRs with paroxetine
  - Low daily dose (20 mg QD)
    - Reactive metabolite burden readily handled by endogenous glutathione pool in the mammal
  - CYP2D6 inactivation by paroxetine following chronic dosing may inhibit its own metabolism
    - Decreases the body burden of the catechol and o-benzoquinone
Lesson

- Thorough knowledge of **ALL** metabolism pathways is important

- GSH conjugate detection needs to be put into right context
  - GSH is a detoxication pathway!
  - Acts as a electrophile mopping agent in the body

- Early assessment of dose can help
Raloxifene Case

- A second generation SERM approved for osteoporosis
- **Bioactivated** by CYP3A4 to quinonoid intermediates
- A **mechanism-based inactivator** of CYP3A4
- No IADRs or DDIs reported

![Raloxifene Chemical Structure](image1)

**Incubation with HLM/NADPH and GSH**

![Graph showing absorbance over time](image2)

So would one nominate this compound for further development?
Why is Raloxifene is Devoid of any IADRs or DDI

- Primary route of raloxifene metabolism – Glucuronidation

Glucuronidation of raloxifene

50% reduction in covalent binding observed in the presence of GSH and UDPGA!

Impact of Intestinal Metabolism on Bioactivation of Raloxifene

- Glucuronidation primarily catalyzed by 1A8 and 1A10
  - UGT1A1 also catalyzes the glucuronidation but to a minor extent
  - UGT 1A8 and 1A10 are exclusively present in the small intestine

Preincubation Experiment

Preincubation with Human Intestinal Microsomes results in significant decrease in covalent binding!
Efficiency of Raloxifene Glucuronidation versus Oxidation

Efficiency of detoxification pathway explains the impact of glucuronidation on hepatic bioactivation

<table>
<thead>
<tr>
<th>Glucuronidation</th>
<th>Oxidation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Cl_{\text{int \ glu}}$</td>
<td>$Cl_{\text{int \ oxi}}$</td>
</tr>
<tr>
<td>$\mu$L/min/mg protein</td>
<td>$\mu$L/min/mg protein</td>
</tr>
<tr>
<td>HIM</td>
<td>397 ± 14</td>
</tr>
<tr>
<td>HLM</td>
<td>37 ± 1.6</td>
</tr>
</tbody>
</table>

What does this data tell us!

- Intestinal glucuronidation limits the amount of raloxifene undergoing bioactivation

- Dose of raloxifene – 60 mg QD
- Other pathways of clearance are responsible for reducing the body burden
Lesson!

- The compound would have been “withdrawn from development” in the absence of all the relevant metabolism data to date.
- Understand the impact/contribution of other metabolic pathways.
- Identify enzymes responsible – maybe non-hepatic.
  - Especially the intestine.

**Bottomline**

Understand the Metabolism of the candidate thoroughly prior to any Major Decisions.
A Pfizer Case

Mitigating the Bioactivation Risk of 11-β-HSD-1 Inhibitor PF-915275
**Project Goal**

- Develop an $\text{11}\beta\text{HSD1}$ (11β-HydroxySteroid Dehydrogenase type-1) inhibitor for the treatment of Type II diabetes

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$\text{11}\beta\text{HSD1 inhibitor inhibits conversion of cortisone to cortisol}$

- **Cortisone (E)**
  - Inactive glucocorticoid

- **Cortisol (F)**
  - Active glucocorticoid

\[ \text{Cortisol (F)} \rightarrow \uparrow \text{Hepatic Glucose} \]
Lead Candidate in the 11-\(\beta\)HSD1 Program

- Has great physicochemical properties
- Good potency
- Pharmacokinetic Attributes

Key Issues from a metabolism perspective
- Risk of bioactivation
- No Structural Alerts – yet +ve signal in RM assay

PF-915275

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Natilie Hosea
Sajiv Nair
Metabolism of PF-915275

HLM/NADPH

Site of bioactivation and adduct formation

GSH

No metabolism at this site

Bis-conjugate Adduct

Unique GSH Adduct

Reactive metabolites trapped as Novel GSH Adducts
**Risk Assessment for PF-915275**

- Preliminary dose prediction was 30 – 50 mg
  - Uncertainties in clearance (low) and Ceff

- Oxidation leading to reactive metabolites
  - A primary route

- Question?
  - Do other competing metabolic pathways or detoxication of the reactive metabolite play a role?
No oxidative metabolites observed in the absence of GSH or UDPGA!

- 14C-PF-915275 synthesis accelerated to see the impact on covalent binding
  - Assess the covalent binding in vitro and in vivo
Covalent Binding Studies with $^{14}$C-PF-915275 Using Human Liver S9

915275 – was bioactivated but:

- Metabolites were detoxified by GSH, sulfation and glucuronidation
- In vitro covalent binding near background when other co-factors are used
Further De-risking Factors to Move PF-915275 into Development

- Rat studies performed
  - Very low binding to liver protein in vivo (0.21 pmol/mg; <0.05% of dose)
  - No GSH conjugates or related metabolites observed

- Proper PK/PD studies helped to refine the dose
  - Refined Dose = 0.3 to 3 mg using monkey PK/PD data

- Investigative toxicology studies performed
  - Compound dosed to GSH depleted rats
Overall Conclusions From the 3 Cases

- Covalent binding to microsomal proteins may not be predictive of toxicity
  - Some refs by Obach et. al. further confirm this

- Thorough assessment of metabolism is pivotal in early discovery

- Important to consider the contribution of other metabolic pathways (hepatic or extrahepatic) in light of a positive signal

- Some idea of total daily dose/exposure helps
  - Get a range to see if the dose is lower than 20 – 50 mg
    - Use in vitro potency and clearance from HLM as first cut
    - Refine the dose prediction from the efficacy model

- Estimate the body burden of the reactive metabolite:
  - \[ D_{RM} = D \times f_a \times f_m \times f_{RM} \]
Is Covalent Binding or GSH Adduct Formation A Kill Shot??

- Clearly, a positive signal by itself ‘is not’ a kill shot
  - Not good predictors of IADR
  - Needs to be considered as a ‘flag’ to trigger additional studies
  - GSH conjugation is a detoxication pathway – need to put in right context
  - However, helps to elucidate mechanisms of bioactivation
    - Useful in circumventing the liability through iterative design

- Liver microsomal assays do not always address all metabolic pathways for a compound
  - Use of hepatocytes or S-9 – supplemented with co-factors gives a better picture of all metabolic pathways