Drug Safety Considerations in Drug Development

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Outline

• **Background**
  – Toxicity as a major source of attrition in drug development
  – Toxicity-related drug withdrawals
  – Toxicity studies in drug development

• **Metabolism-dependent drug toxicities**
  – Cardiovascular toxicity (QTc effects) caused by drug interactions
  – Liver injury (chemically reactive drug metabolites)

• **Stable drug metabolites ("MIST")**
  – FDA and ICH Guidances
  – Species differences in drug metabolism & toxicity
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)


- Clinical Efficacy (25%)
- Animal Toxicity (20%)
- Human AEs (11%)
- Financial (8%)
- Formulation (3%)
- Other (5%)
- Human PK (8%)
- Commercial (20%)
Target Organ Contributions to Drug Withdrawals

Summary of target organ contributions to drug withdrawals 1975–2007. Data were compiled from reviews [8–10] and CDER Reports to the Nation [11] as well as the CDER website. Target organs were identified on the basis of the reasons for withdrawal as noted in the references.

Widely Accepted That Attrition Must Occur Earlier!

• Given that attrition rates remain high, it is critical that only the very best candidates from Discovery / Lead Optimization efforts are taken forward into development

• The role of scientists engaged in drug discovery has expanded in recent years such that it is now important to consider many issues beyond organic synthesis, pharmacology, etc, notably:
  – Drug Metabolism and Pharmacokinetics (DMPK)
  – Preclinical Toxicology

• Challenges in Drug Discovery
  – Minimizing potential for toxicity (esp. cardiovascular and liver toxicity)
  – “Dialing-out” drug-drug interaction potential
  – Dealing prospectively with reactive drug metabolite issues
  – Addressing potential issues with stable drug metabolites (“MIST”)
The principal barrier to bringing a continuous stream of innovation to the market place is that converting a chemical with interesting biological properties into a drug involves solving multiple complex issues “simultaneously” (in a single molecule).

The Pharmaceutical Industry Challenge is: Solving Multiple Issues Simultaneously

Figure modified from: Drug Discovery and Development, July 2004
From Sequential to Parallel Data Acquisition
A Paradigm Shift in Drug Discovery

Synthesis of analogs

Test in lead biochemical assay
Test in in vitro toxicology assays
Test in biochemical counterscreen(s)
Test in functional cell culture assay
Test in vivo
Candidate for in vivo toxicological evaluation

Obtain PK and ADME information in preclinical species

ADME = Absorption, Distribution, Metabolism, and Excretion

The Drug Discovery / Development Pipeline

- Target identification
- Hit generation
- Lead generation

Clinical development phase:
- Early-stage research and discovery
- Preclinical studies in animal models
- Phase I: safety; 20-80 healthy individuals; 1-2 years
- Phase II: efficacy, safety; 100-300 patients; 1-2 years
- Phase III: efficacy, safety; 1,000-3,000 patients; 2-3 years
- FDA review and approval; ~1-2 years

Approval phase:
- Post approval

% compounds advanced at each stage:
- ~5%
- ~2%
- ~20%

Cost:
- $335 million
- $467 million
- $95 million
Preclinical Toxicity Studies in Support of Drug Development

• **Mutagenicity** (Ames, chromosomal aberration) and hERG binding

• **General toxicology** – rodent (rat or mouse) and non-rodent (dog or monkey)
  – Single doses to evaluate effects of high acute exposure (significant tox or lethality) and MTD
  – Multiple doses at sub-lethal levels to assess sub-acute tox profile and define NOAEL
  – >2-Week multiple dose data needed to support clinical trials up to 2 weeks in duration
  – *In vivo* studies incorporate toxicokinetic (TK) assays to assess exposure ($C_{\text{max}}$ and AUC) to parent and major metabolites

• **Safety Pharmacology**
  – Panel of receptor binding assays *in vitro*
  – Battery of *in vivo* studies to assess effects on CV, CNS, GI, and respiratory systems

• **Reproduction toxicity**
  – Women of child-bearing potential

• **Carcinogenicity**
  – 2-Year bioassay in rats and mice (starts ~4 years prior to anticipated filing date)

http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/default.htm
Clinical Safety Studies in Support of Drug Development

**Phase I (healthy volunteers)**

- Single ascending dose (SAD) for safety, tolerability, PK
  - Starting dose selected to give ~100-fold lower AUC than NOAEL in most sensitive animal
- Multiple ascending dose (MAD) – duration not to exceed that of animal studies
  - Detailed analysis of side-effect profile; circulating metabolites, drug interaction studies, etc

**Phase II and III (patients)**

- Long-term safety and efficacy studies that form the basis of regulatory filing (NDA)

**Postmarketing**

- Pharmacovigilence (AE reporting)
Toxicity Due to Drug-Drug Interactions at the Level of P450 Inhibition
P450 Inhibitors that Have Caused Life-Threatening DDIs

Ketoconazole
Competitive IC₅₀ <0.1μM for CYP3A
Warnings for serious DDIs

Mibefradil
Withdrawn in 1998 due to DDIs (QTc prolongation)
Competitive IC₅₀ for CYP3A <1μM
Mechanism-based CYP3A4/5 inhibitor (partition ratio 1.7)

http://www.fda.gov/cder/drug/druginteractions
Metabolism by P450 Enzymes – A Key Determinant of Drug Clearance

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 glucuronosyl transferase.
**hERG Binding and QTc Prolongation**

- Adverse cardiovascular effects, often associated with inhibition of hERG (α-subunit of the I_{Kr} potassium channel) leading to QTc prolongation, represent a major source of attrition in drug discovery.

- Drug withdrawals due to QTc effects:

- While the parent compound normally is the culprit, drug-drug interactions (DDIs) that result in *reduced drug clearance* may greatly exacerbate this type of toxicity.

- **Potent inhibitors of CYP3A4** have been most frequently implicated in these DDIs, e.g. ketoconazole (warning label), mibefradil (withdrawn).
Torsades de Pointes Occurring in Association With Terfenadine Use

Brian P. Monahan, MD; Clifford L. Ferguson, MD; Eugene S. Killeavy, MD; Bruce K. Lloyd, MD;
James Troy; Louis R. Cantilena, Jr, MD, PhD

Torsades de pointes is a form of polymorphic ventricular tachycardia that is associated with prolongation of the QT interval. Although found in many clinical settings, torsades de pointes is most often drug induced. This report describes the first association (exclusive of drug overdose) of symptomatic torsades de pointes occurring with the use of terfenadine in a patient who was taking the recommended prescribed dose of this drug in addition to cefaclor, ketoconazole, and medroxyprogesterone. Measured serum concentrations of terfenadine and its main metabolite showed excessive levels of parent terfenadine and proportionately reduced concentrations of metabolite, suggesting inhibition of terfenadine metabolism. We believe that a drug interaction between terfenadine and ketoconazole resulted in the elevated terfenadine levels in plasma and in the cardiotoxicity previously seen only in cases of terfenadine overdose.

(JAMA. 1990;264:2788-2790)

**Terfenadine:**
- Potent inhibitor of hERG channel
- CYP3A4 inhibitors raise terfenadine levels and cause QTc prolongation
- Introduced as Seldane (Marion Merrell Dow) in 1985, withdrawn in 1997

**Fexofenadine:**
- An active metabolite of terfenadine
- Contributes to H1 antagonism, but not to hERG inhibition
- Not subject to metabolism by CYP3A4 inhibitors (minimizes DDI potential)
- Marketed as Allegra (Aventis) in 1996
Mibefradil - Calcium channel blocker, approved as an antihypertensive in 1997

- Exhibited non-linear PK due to self-inactivation of CYP3A4
- Highly potent CYP3A4 inactivator in vitro ($k_{\text{inact}} = 0.5 \text{ min}^{-1}, K_I = 2 \mu\text{M}$)
- Caused serious clinical AEs when dosed with CYP3A4 substrates (eg simvastatin, atorvastatin)
- Fatal cases of DDIs leading to rhabdomyolysis (statins) and QTc prolongation
- Voluntarily withdrawn by the manufacturer in 1998

**hERG Binding – Current Status**

- *In vitro* and *in vivo* screens for CV effects established

- Regulatory guidance for nonclinical (ICH S7B) and clinical (ICH E14) testing strategies published in 2005

- Safety margin based on ratio of:
  \[
  \frac{\text{hERG IC}_{50}}{C_{\text{max}}} \quad \text{or} \quad \frac{\text{NOAEL}}{C_{\text{max}}}
  \]
  at expected top dose should be >30, and preferably >100

- Med Chem strategies to minimize QTc effects include:
  - Formation of zwitterions (e.g. terfenedine to fexofenadine)
  - Modulation of LogP
  - Attenuation of pKa
  - Computational (QSAR models)

*Curr. Topics Med Chem., 8(13), 2008*
A P450 Inhibitor that is Therapeutically Valuable

Ritonavir (HIV Protease Inhibitor)

Potent mechanism-based CYP3A4 inhibitor
Mechanism of CYP inhibition likely associated with thiazole ring(s)
Used clinically to "boost" exposure of other anti-HIV agents

Other HIV protease inhibitors that lack a thiazole functionality do not serve as mechanism-based inhibitors of CYP3A4

T. Koudriakova et al., Drug Metab. Dispos., 26, 552-561 (1998)
Drug-Induced Liver Toxicity
Drug-Induced Liver Toxicity

Several forms, including:

- Predictable, dose-dependent toxicities (animal model, clear dose-response relationship, etc)

- “Idiosyncratic” toxicities (rare, not predictable, no animal model)

- Occur only after prolonged dosing (carcinogenicity, teratology)

- Evidence suggests that reactive metabolites may play a causative role in each of the above forms of liver toxicity

- Idiosyncratic toxicities of greatest concern in drug development

Bioactivation and Liver Toxicity

• A wide range of therapeutic agents have been withdrawn from use due to an unacceptably high incidence of hepatotoxicity:
  - Aclofenac, alpidem, amodiaquine, amineptine, benoxaprofen, bromfenac, ibufenac, iproniazid, nefazodone, nomifensine, sudoxicam, tienilic acid, tolrestat, troglitazone, trovafloxacin, zileuton, zomepirac

• Many other marketed drugs have warnings for a risk of liver toxicity, or severe restrictions in their use

• For most of these agents, bioactivation to reactive metabolites has been demonstrated to occur either in vitro (human hepatic tissue) or in vivo (characterization of downstream stable metabolites)

• High dose drugs (>100mg/day) tend to be the ones which most frequently cause liver toxicity

Bioactivation and Liver Toxicity
Acetaminophen

Acetaminophen (APAP) → NAPQI (Quinone imine) → APAP-GSH

APAP Sulfate APAP Glucuronide → Covalent Binding to Proteins Oxidative Stress Depletion of GSH Pools → Liver Toxicity

I. M. Copple et al., Hepatology, 48, 1292-1301 (2008)
Acetaminophen-Induced Liver Toxicity

Figure 2 | Current concepts of experimental acetaminophen (APAP) hepatotoxicity. Upstream events in hepatocytes lead to exposure to NAPQI which undergoes covalent binding after preferential depletion of glutathione (GSH). A protective response mediated by the transcription factor Nrf2 modulates the toxic threshold. Upstream events promote intracellular stress and mild injury activate the downstream innate immune system, which represents a balance of pro- and anti-inflammatory responses, the interplay of which determine progression to severe injury or no injury. APAP, acetaminophen; FeaL, FeaL ligand; GSH, glutathione; GST, GSH S-transferase; IFN, interferon; MCP1, monocyte chemotactic protein 1; MIP2, macrophage inflammatory protein 2; NK, natural killer; TNF, tumour-necrosis factor.

D. P. Williams, Toxicology 226, 1-11 (2006)
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"
Quinoid Precursors as Structural Alerts

For ROS formation, $X = O$ or $N$, and $Y = O$)
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!
Assessing Formation of / Exposure to Reactive Drug Metabolites

(A) *In vitro* “trapping” experiments (eg with GSH, CN⁻), or *in vivo* metabolic profiling studies:
- Invaluable in enabling rational structural re-design

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

*Nucleophilic trapping experiments and covalent binding studies employ different end-points and serve different purposes!*

Minimizing Metabolic Activation: (1) Block Site of Metabolism


Minimizing Metabolic Activation: (2) Introduce Steric Hindrance

Steric hindrance from the phenylsulfone reduces oxidative metabolism on the thiazole S atom

Minimizing Metabolic Activation: (2) Introduce Steric Hindrance

W. Tang et al., Xenobiotica 38, 1437-1451 (2008)
Minimizing Metabolic Activation: (3) Introduce Electronic Changes

ADDRESSING METABOLIC ACTIVATION IN DRUG DISCOVERY

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

![Chemical structures](image)

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

Minimizing Metabolic Activation: (4) Redirect Metabolism to “Soft Spot”


Sudoxicam (Withdrawn during Phase III trials)

Meloxicam (Non-hepatotoxic)

Reactive metabolites of thiazole ring oxidation, thiourea formation
Minimizing Metabolic Activation: (5) Replacement of Structural Element

Orexin receptor antagonist lead

6-Fluoroquinazoline series

6-Chlorobenzoxazole series MK-4305

No evidence of metabolic activation

Minimizing Metabolic Activation: (6) Combination of Steric and Electronic Changes

Minimizing Metabolic Activation: (6) Combination of Steric and Electronic Changes

Original Lead
Potent, selective, good PK
High degree of metabolic activation

Chloro analog
Potent, selective, good PK
Low degree of metabolic activation

Safety Evaluation of Stable Drug Metabolites

Metabolites in Safety Testing ("MIST")
Metabolites in Safety Testing (“MIST”)

• Central Question
  “Are human metabolites of a drug candidate, as well as the parent compound, adequately evaluated for safety during preclinical toxicology studies?”

• PhRMA “White Paper” on best practices published in 2002

  http://www.fda.gov/cder/guidance/index.htm

• Numerous commentaries on MIST published during past 5 years

Key concern for industry: Resource and time implications for development
FDA Guidance on “Safety Testing of Drug Metabolites”

• Applies only to small molecule non-biologic drug products

• Excludes:
  - anti-cancer agents
  - drug conjugates (other than acylglucuronides)
  - reactive intermediates

• Focuses on:
  - stable metabolites circulating in human plasma
  - unique or “disproportionate” metabolites in humans

• Key recommendations:
  - metabolites whose $\text{AUC}_p$ at steady-state is $<10\%$ that of parent need no further study
  - if $\text{AUC}_p$ is $>10\%$ of parent, require “coverage” (exposure margin $\geq 1$) in at least 1 tox species
  - otherwise, human metabolite is “disproportionate” and may require testing

• Types of toxicology studies that may be required:
  - general tox (3 months), genotoxicity, embryo-fetal development tox, carcinogenicity

http://www.fda.gov/cder/guidance/index.htm
ICH Topic M3 (R2)
Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals

• Key recommendations:

- Only those human metabolites observed at levels \( \geq 10\% \) of total drug-related exposure require nonclinical characterization, if they circulate at “significantly greater” levels in humans than the maximum exposure in animal toxicology studies.

- For drugs dosed at <10mg / day, a larger % of the total drug-related material might be appropriate before safety testing is needed.

- Some metabolites do not warrant testing (eg “most GSH conjugates”)

- “Unique human metabolites” should be considered on a case-by-case basis.

http://www.emea.europa.eu
Practical Issues with MIST Guidances

• **FDA Guidance**
  - How to assess those metabolites in human plasma that circulate at \( >10\% \text{ AUC of parent drug} \) under **steady-state** dosing conditions?
  
  - Multiple ascending dose (safety/tolerability) study with “cold” drug and LC-MS/MS analysis? Availability of validated assay for metabolite(s)?

• **ICH Guidance**
  - How to assess “**total drug-related exposure**” in plasma?
  - Radioactive dose (with required GMP synthesis, dosimetry, etc)?

As of January, 2010, where the FDA and ICH guidances differ, the ICH guidance supersedes the FDA guidance

http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/default.htm
Species Differences in Drug Metabolism

CGRP Receptor Antagonist Telcagepant

Telcagepant (MK-0974)

Rat oral F = 22%, Cl = 9 ml/min/kg
Rhesus oral F = 6%, Cl = 20 ml/min/kg

Species Differences in Drug Metabolism
c-MET Inhibitor SGX 523

Urinary metabolite profiles

Solubility in monkey urine (pH 8.4):  SGX 523 – 13µg/ml  M11 – 0.37µg/ml

S. Diamond et al., Drug Metab. Dispos., 38: 1277-1285 (2010)
Conclusions

• Drug metabolism now plays an integral role in the safety evaluation of new drug candidates and their circulating metabolites.

• Both stable and chemically reactive drug metabolites need to be taken into consideration, and the identities of likely human metabolites established, at the lead optimization stage of preclinical development.

• Preliminary studies on circulating human metabolites need to be conducted during early clinical development (Phase I / II) such that “disproportionate” metabolites can be identified and addressed preclinically.

• The implementation of regulatory guidances from the FDA and ICH requires that a detailed understanding of the metabolic fate of a new drug candidate be established, both in humans and in the animal species used for toxicology studies, prior to the start of large-scale (Phase III) clinical trials.