METABOLISM/SAFETY CONSIDERATIONS IN DRUG DEVELOPMENT

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DATE

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Knowledge: organized information which provides insights into contextual relationships

Wisdom: the ability to integrate multiple streams of knowledge & experience towards actionable decisions

Information: structured / organized data which may be useful

Data: signals, numbers & error bars

How Data w/ Help Drives the Department

Change

Creates conviction & reveals direction

Added with insight becomes

When given meaning becomes

Given context becomes
Outline for Today

• **Background**

  **Overview of the drug development process**
  Toxicity as a major source of attrition in drug development
  Drug Metabolism/Toxicity studies (dissecting a multifactorial problem)

• **Active Drug Metabolites**

• **Metabolism-dependent Drug Safety Concerns**
Basic Scheme of the Drug Development Continuum

**Phase II and III (patients)**
- Long-term safety and efficacy studies that form the basis of regulatory filing (NDA)

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

**Postmarketing**
- Pharmacovigilence (adverse event reporting)
For regulatory agencies, the decision whether to approve a new drug for marketing can be distilled down to two fundamental questions:

• Do the results of well-controlled studies provide substantial evidence of drug effectiveness?
• Do the results show the product is safe under the conditions of use in the proposed labeling?
  – Safe, in this context extends beyond assessing a drug’s Therapeutic Index and embraces the notion that the benefits of the drug appear to outweigh its risks.

- **Preclinical:** 67% success (n=1106)
  - Pharmacokinetics/bioavailability: 31.6%
  - Clinical safety: 50.0%
  - Commercial: 19.7%
  - Regulatory: 7.1%
- **Phase I:** 51% success (n=982)
  - Pharmacokinetics/bioavailability: 31.7%
  - Clinical safety: 18.5%
  - Commercial: 12.8%
  - Regulatory: 3.5%
- **Phase II:** 23% success (n=546)
  - Non-clinical toxicology: 53.0%
  - Efficacy: 10.4%
  - Technical: 0.2%
  - Regulatory: 1.2%
- **Phase III:** 55% success (n=193)
  - Efficacy: 45.7%
  - Technical: 8.0%
  - Regulatory: 9.5%
  - Clinical safety: 3.4%
Because attrition rates remain high, it is critical that only the best candidates from Discovery / Lead Optimization efforts are taken forward into Clinical Development.

- Challenges for the DM Scientist beyond PK issues in Drug Discovery
  - Minimizing potential for toxicity (esp. cardiovascular and liver toxicity)
  - Minimizing potential drug-drug interaction potential
  - Dealing prospectively with reactive drug metabolite issues
  - Developing strategies to respond to stable drug metabolites (“MIST”)
Considering the Investment, it’s Clear that Identifying Compound Liabilities Early is Best

Given that attrition rates remain high, it is critical that only the very best candidates from Discovery / Lead Optimization efforts are taken forward into Clinical 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)
  - Minimizing potential drug-drug interaction potential
  - Dealing prospectively with reactive drug metabolite issues
  - Developing strategies to respond to stable drug metabolites (“MIST”)
Drug Metabolism / Toxicity “Touch points” at Various Times Across the Development Process

- Safety Pharmacology (hERG)
- In Vivo Genetic Toxicity
- Chronic (long term) Toxicity Studies
- Carcinogenicity Studies
- In Vitro Genetic Toxicity
- Single/repeat Dose Toxicity Studies
- Reproductive Toxicity (teratogenicity)
- Reproductive Toxicity (fertility/pre/postnatal)

Diagram of the drug development process with stages and touch points:

- Discovery: Lead selection, Optimization
- Preclinical development: Candidate IND, 1st in Man
- Clinical development: Phase I, Phase II, Phase III, NDA Approval

Touch points:
- Soft-spot and reactive metabolite analysis
- ADME in animals using radiolabel
- ADME in humans
- Species comparison in vitro-in vivo correlation
- Tissue distribution
- Definitive in vitro CYP inhibition & induction, reaction phenotyping
- Clinical DDI studies
- Post market DDI studies

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Typical View of The Role of Drug Metabolism in Drug Toxicity

Drug ➔ Reactive Metabolite ➔ Reaction with Proteins

Reaction with DNA ➔ Mutagenicity Carcinogenicity Teratogenicity

Target Organ Toxicity (reproducible or idiosyncratic)

Immune Hypersensitivity reactions (idiosyncratic)

Safrole

Acetaminophen

Halothane
Putting Drug Safety into Context

...rarely is it a straightforward scenario
Typically there are Multiple Factors Working in Concert which Contribute to Drug Safety

You finally got the big promotion you were waiting for!  
You had a couple extra drinks celebrating with friends  
It was raining hard and roads were slick

You were late for dinner and driving too fast
Integration of Factors Associated with Patient Variability in Drug Metabolism

Clinical trials provide evidence of efficacy and safety at usual doses in controlled populations

Patient (Drug Metabolizing Enzyme)

Environment
- Smoking
- Alcohol
- Diet

Age

Disease

Genetics

Drugs

\[ \sum \]

Drug CL

Plasma [D]

Response
Impact of Inflammation on Drug Metabolism

Plasma concentrations of simvastatin were higher in RA patients than those reported for healthy volunteers. (Actemra (mAb IL-6) reduced significantly the AUC\textsubscript{last} and C\textsubscript{max} of simvastatin on Day 15 by 57% (1 week after Actemra infusion)).

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Fraction of clinically used drugs metabolized by P450 isoforms and factors influencing variability
The Role of BSEP (ABCB11) in Liver Toxicity

BSEP is an efflux transporter expressed on the canalicular membrane of the hepatocyte and it secretes bile acids into bile.

BSEP inhibition shows a correlation but does not always lead to drug-induced liver injury. Additional factors required?
Acetaminophen Metabolism & Toxicity

- Acetaminophen has been approved for OTC use since 1960 and in most cases the drug is remarkably safe, however toxicity can occur at therapeutic doses.
  - At therapeutic doses, 90% of APAP is metabolized in the liver to sulfate and glucuronide conjugates that are then excreted in the urine while the remaining 10% is metabolized via the cytochrome CYP2E1 (P450 2E1) to a toxic, reactive, N-acetylimidoquinone (NAPQI) which is rapidly conjugated with hepatic glutathione to form a nontoxic compound which is excreted.
Acetaminophen Metabolism & Toxicity

However, if you: Increased APAP concentration; Blocked the conjugation pathways; Depleted Glutathione levels & Induced CYP2E1 enzyme activity.
Acetaminophen Metabolism & Toxicity

- Excessive intake of acetaminophen
- Increased CYP2E1 activity due to induction by other drugs or chronic alcohol use
- Competition for conjugation enzymes
- Depletion of glutathione stores due to malnutrition or chronic alcohol ingestion

- Glucuronidation & Sulfation
- Cysteine conjugate (nontoxic)

- Glucuronide & Sulfate conjugates (nontoxic)

- Protein-SH

- Damaged Tissue

- Hepatic Necrosis

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Outline for Today

• **Background**
• **Active Drug Metabolites**
  • Inhibitory Metabolites
  • Pharmacological Active Metabolites
  • Cardiovascular toxicity (QTc effects) caused by DDI

![Metabolism-dependent Drug Safety Concerns](image)

• Metabolism-dependent Drug Safety Concerns
Rationale around Active Metabolites

For metabolite 1, the biotransformation modification occurs on a position not involved in ligand binding, so the metabolite would be predicted to possess reasonable activity. However, in the case of metabolite 2 the modification is on a position that disrupts receptor interaction, so the metabolite would be inactive.

Parent Drug, MeO<sub>D</sub><br>
Verdaxine<br>SR<sub>i</sub> potency: ~100 nM<br>

Metabolite 1, H<sub>2</sub>C<sub>2</sub>N<sub>3</sub><br>Desvenlafaxine<br>SR<sub>i</sub> potency: ~116 nM

Metabolite 2, Cl<br>Zopiclone<br>GABA<sub>a</sub> receptor potency: 50-200 nM

Zopiclone-N-oxide<br>GABA<sub>a</sub> receptor potency ~5000 nM

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That said, while modification on metabolite 2 disrupts receptor interaction, the change may now introduce a binding motif for a different enzyme where metabolite 2 now possesses activity.
Drug-Drug Interactions

Defined: A modification of the effect of a drug when co-administered with another drug. The effect may be an increase or a decrease in the action of either substance, or it may be an adverse effect that is not normally associated with either drug.
The general assumption for DDIs

If Drug X interacts with DM enzyme, and DM enzyme metabolizes Drugs A, B and C, then Drug X alters the metabolism of Drugs A, B and C.

For the most part, our thinking is directly linked towards the interplay between the candidate molecule and the enzyme.
Metabolites Contributing to Clinical DDI: Sulfinpyrazone and (S)-Warfarin Clinical Drug Interaction

• (S)-warfarin is the pharmacologically active enantiomer of warfarin and is almost exclusively cleared via CYP2C9 oxidation
• Sulfinpyrazone $K_i$ for CYP2C9 = 230$\mu$M which resulted in an $I/K_i$ ratio of 0.04
• Based on our prediction model these drugs are safe to be coadministered.
Sulfinpyrazone Inhibits (S)-Warfarin Clearance

Although the initial in vitro Ki values were 25 times greater than therapeutic concentrations of Sulfinpyrazone achieved in vivo, coadministration with (S)-Warfarin resulted in a 3-fold decrease in the active enantiomers metabolism.
Pharmacokinetic profiles of sulphinpyrazone and it’s inhibitory metabolites

Plasma concentration-time profiles for sulphinpyrazone (●), sulphide (■) and sulphone (○) after receiving 200 mg oral dose.

Sulfinpyrazone sulfone
CYP2C9 Ki = 73 μM

Sulfinpyrazone
CYP2C9 Ki = 230 μM

Sulfinpyrazone sulfide
CYP2C9 Ki = 17 μM
Additive Effects of an Active Metabolite (Tolterodine & CYP2D6)

The primary metabolic route for tolterodine is via oxidation of the 5-methyl group and is mediated by the CYP2D6 and leads to the formation of a pharmacologically active 5-hydroxymethyl metabolite.
Additive Effects of an Active Metabolite (Tolterodine & CYP2D6)

- Tolterodine is cleared at a slower rate in poor metabolizers than in extensive metabolizers; this results in significantly higher serum concentrations of tolterodine and in negligible concentrations of the 5-hydroxymethyl metabolite.

Despite the effect on pharmacokinetics, the CYP2D6 polymorphism does not appear to be of great importance in the antimuscarinic effect, probably because of the additive action of parent drug and active metabolite.
Terfenadine / Ketoconazole DDI

- Terfenadine – antihistamine drug on market as an ‘over the counter’ remedy for hayfever.
- Found to cause life threatening cardiac arrhythmias when co-administered with medicines such as erythromycin (antibiotic) or ketoconazole (antifungal) via QTc prolongation.
- Caused by inhibition of hepatic P450 enzymes, specifically CYP3A4.
Terfenadine / Ketoconazole DDI

Found that the major metabolite of terfenadine, caused by oxidation of the tert-butyl group, is the pharmacologically active species.

Moreover, the major metabolite, fexofenadine, has little hERG activity as it is a zwitterion, and was developed as a medicine.
Bottom-line:

To characterize an active and/or inhibitory metabolite of significant exposure you essentially need a set of information that looks the same as the set assembled to understand the pharmacology/ADME of the parent drug.

- Target binding potency, as well as a readout of functional activity (i.e., antagonist, agonist, partial agonist, inhibitor, activator, etc.).
- Plasma protein binding in humans and laboratory animal species.
- In some cases, target tissue(s) concentrations and prediction of the free tissue–to–free plasma concentration ratio (i.e., Kp,uu).
- Pharmacokinetics in humans and laboratory animal species, with some level of understanding of underlying clearance pathways for the active metabolite in humans.
Outline for Today

• **Background**

• **Active Drug Metabolites**

• **Metabolism-dependent Drug Safety Concerns**
  • Liver injury (chemically reactive drug metabolites)
  • Species differences in drug metabolism & toxicity
  • FDA and ICH* “MIST” Guidance
Toxicity types associated with drug withdrawals

Toxicity can arise through a variety of mechanisms
- Predictable, dose-dependent toxicities (animal model, clear dose response relationship, etc)
- Metabolism related toxicities account for the majority of observed liver toxicities

- **Hepatocellular injury, immunoallergic**: phenytoin, sulfonamides, allopurinol, halothane, diclofenac, quinolones, telithromycin,
- **Hepatocellular injury, metabolic**: INH, troglitazone, ximelagatran, bromfenac
- **Cholestatic**: estrogens, 17a androgens, chlorpromazine, clavulinic acid, piroxicam
- **Bile duct injury**: carbamazepine, chlorpromazine, chlorpropamide, cyproheptadine, thiabendazole, haloperidol
- **Microvesicular steatosis**: valproate, tetracycline, didanosine
- **Phospholipidosis & pseudoalcoholic hepatitis**: amiodarone, perhexiline maleate
- **Chronic autoimmune-like hepatitis**: dantrolene, methyldopa, nifurantoin, oxyphenisatin, propylthiouracil, tienilic acid

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Anticipating Drug Activation & Toxicity

One means to minimize metabolism related toxicity is to avoid the formation of reactive metabolites is to identify functional groups that are known to form reactive metabolites and avoid these functional groups in the structure of drug candidates.

So regarding the question around whether a drug candidates that can form reactive metabolites must be totally avoided. There really is no simple answer to this question. That said, two major factors seem to be important: the dose of the drug and the fraction of the metabolic pathways that lead to covalent binding. As mentioned previously, drug that is taken at a total dose of 10 mg/day or less is unlikely to be associated with a high incidence of idiosyncratic drug reactions in humans.
Screening for Metabolic activation via GSH Trapping

One of the methods to detect the formation of electrophilic intermediates is to look for GSH conjugates of the drug. Basically, formation of GSH conjugates can be detected by mass spectrometry, which, in turn, provides insight into the reactive metabolite structure.

It is noteworthy to point out that not all reactive metabolites can be trapped with GSH. Hard electrophiles including DNA-reactive metabolites (e.g., electrophilic carbonyl compounds) will preferentially react with hard nucleophiles such as amines (e.g., semicarbazide and methoxylamine), amino acids (e.g., lysine), and DNA bases (e.g., guanine and cytosine) affording the corresponding Schiff bases.
Anticipating Metabolism Based Drug Toxicity

There are ways to minimize the metabolic liability associated with reactive metabolite formation: Substituting the structural alert with substituents that are resistant to metabolism; (b) Incorporating moieties which change the electron withdrawing characteristics of the structure alert; (c) Incorporating a bulky substituent close to the site of metabolism to block the site of metabolic activation.

Of course elimination of reactive metabolite formation will be of no benefit if it also eliminates the therapeutic effects of the drug and, therefore, it is essential that the pharmacological effects of drug candidates be tested at each step in the optimization of the structure.
Raloxifene Oxidation to Reactive Intermediates

Raloxifene (Evista) is selective estrogen receptor modulator used in the treatment of osteoporosis and for chemoprevention of breast cancer. In vitro raloxifene is bioactivated to reactive intermediates, which covalently bind to proteins and form GSH conjugates upon incubation with NADPH and GSH-supplemented human microsomes.

Despite these in vitro findings, no major raloxifene-related toxic events have been reported upon its oral administration to humans.
Raloxifene Metabolism via Glucuronidation vs Oxidation Pathways

- UGT1A1 and 1A8 were found to catalyze the formation of both the 6-beta- and 4'-beta-glucuronides.
- Raloxifene is rapidly absorbed from the gastrointestinal tract and undergoes extensive first-pass glucuronidation.
  - Approximately 60% of an oral dose is absorbed; however, because of extensive presystemic glucuronide conjugation, absolute bioavailability is only 2%.
Rationale for Conducting Preclinical Safety Studies

Safety or secondary pharmacology studies are generally more standardized animal studies using mainly physiological monitoring of vital organs or organ systems.

Toxicology studies have been standardized by GLP guidelines that embody daily dosing of animals, general clinical examination and monitoring, and clinical pathology testing of blood and urine, followed by extensive histopathological examination of tissues after detailed NECROPSY.

- Serves as basis for calculating a safe starting for clinical studies
- Provides understanding the potential for reversibility of toxic effects
Bottom-line Question: “Are human metabolites of a drug candidate, as well as the parent compound, adequately evaluated for safety during preclinical toxicology studies?”

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

- **The Key FDA recommendations:**
  - A stable metabolite whose AUCp at steady-state is <10% that of parent needs no further study.
  - If AUCp is >10% of parent, “coverage” (i.e. exposure margin >1) needs to be demonstrated in at least one tox species otherwise, human metabolite is “disproportionate” and may require testing.

- **ICH Topic M3 (R2) difference:**
  - Only those human metabolites observed at levels >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.

- **Potential resource and time implications for drug development:**
  - Types of toxicology studies that may be required include general tox (3 months), genotoxicity, embryo-fetal development tox, carcinogenicity.
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.
Metabolism and Lead Optimization

- Drug metabolism plays a defining role in safety and efficacy
- Optimizing metabolism has become a routine in drug discovery
  - Addresses drug metabolism issues early on

The objective is to attenuate CYP-mediated clearance
Reducing CYP-mediated clearance has helped improve PK but has also introduced new problems

- **Strategy used:**
  - Reduce the Log P of new candidates
  - Introduce azaheterocycles in the molecule

- Enables advancement of compounds with minimal P450 metabolism
- Has steered new compounds into non-CYP space for clearance

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Selecting the most relevant nonclinical species for toxicological evaluation

Background: (SGX523) was an orally bioavailable, potent, and selective small molecule inhibitor of c-MET, and was one of the first selective c-MET inhibitors to be evaluated in patients. Because the microsomal metabolism profile of SGX523 was similar among preclinical species and human, investigational new drug-enabling studies were conducted in rats and dogs.

Problem: The SGX523 Phase I study was started at a dose of 40 mg in patients. After escalating to doses 80 mg of SGX523 in patients, acute renal failure was observed as evidenced by increased serum creatinine. The analysis of samples from the discontinued clinical trial revealed a metabolism profile different from that of the preclinical species studied.
Selecting the most relevant nonclinical species for toxicological evaluation

The selection of an appropriate safety species for a molecule which is a substrate of AO, is difficult as the relative order of AO activity in animal species relative to human is highly dependent on the substrate. That said the general dogma regarding species differences is that AO activity is high in monkeys and humans, low in rats and deficient in dogs, such that dog is not an appropriate species to evaluate the safety of an AO-derived metabolite.
Selecting the most relevant nonclinical species for toxicological evaluation

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

Summary, SGX523 is metabolized by AO in a species-specific manner to a markedly less-soluble metabolite, M11 which was likely involved in the observed obstructive nephropathy reported in clinical studies.
A systematic and integrated presentation of the findings from preclinical studies are required such that an informed and experienced expert would reasonably understand and consider possible signals of human risk.
Key Messages Regarding Understanding the Role of Metabolites & Safety in Drug Development

- Circulating metabolites can...
  - Enhance therapeutic response
  - Lead to new off target affects (QT elongation and CYP inhibition)

- Provides improved patent protection for novel molecular structures

- Increase the resource required for safety testing if exposure is extremely high (pro-drug approach)

- Understanding metabolism of lead can improved diagnosis of at risk populations
It doesn’t matter what company you work for, we, for all intent and purposes, are all after the same goal: New advances in pharmacotherapy that are safe and effective; provide acceptable benefit-to-risk ratios for the disease; and are brought to patients who need them with a sense of urgency and diligence. So in all cases features which we need to keep as part of the project conversation include:

• Is the drug intended to address a previously unmet medical need or a life threatening disease?
• Is the drug candidate intended to provide proof-of-mechanism for a novel target (first in class)?
• Is the drug intended for acute or chronic use?
• Is the clinical dose predicted to be low?
• What is the intended patient population (e.g., would it be given to immuneocomprised patients or patients with impaired liver functions)?
• Are there alternate chemical series with comparable pharmacologic and pharmacokinetic attributes, wherein bioactivation liability is minimized or eliminated?
• Is there an alternative (higher affinity but innocuous) route of metabolism within the drug candidate that minimizes bioactivation liability associated with the compound?
• Is metabolism the exclusive route of elimination? What is the likelihood of nonmetabolic elimination processes (e.g., renal and/or biliary excretion of unchanged parent) in humans?

The goal of Drug Metabolism scientist in this particular undertaking is to answer the right question with the correct experiment at the appropriate time.