Drug Metabolism and Toxicity

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Group Meeting Literature Talk
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Drug discovery and development

- Discovery
- Preclinical development
- Clinical trials
- FDA review

> 5000 compounds
250 compounds
5 compounds
1 compound

Image credit: Ian B. Perry
Reasons drugs fail in the pipeline

- Most common reason for failures in drug discovery attributed to pharmacokinetic properties
- Harmful toxicity another major concern in drug development

**Pharmacokinetics**

The study of the bodily processes that affect the movement of drugs across the body

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**Absorption**
- Route of administration
- Bioavailability

**Distribution**
- Transfer of drug through body by bloodstream
- Sites of accumulation

**Metabolism**
- Biotransformation pathways
- Mechanism of detoxification/active species formation

**Excretion**
- Method and rate of drug clearance from body
**Outline**

*Introduction to drug metabolism*
- Common biotransformations

*Metabolism-induced toxicity*
- Mechanisms of adverse reactions
- Reactive intermediates and structural alerts

*Applications to drug design*
- Designing around metabolism

*Applications to drug design*
- Designing around drug-induced toxicity
- Challenges in predicting adverse effects
Outline

- Introduction to drug metabolism
  - Common biotransformations

- Metabolism-induced toxicity
  - Mechanisms of adverse reactions
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- Applications to drug design
  - Designing around drug-induced toxicity
  - Challenges in predicting adverse effects
Introduction to drug metabolism

Xenobiotic metabolism

The chemical alteration of non-endogenous compounds within the body
The early studies of metabolism

1841-1842
- Ure and Keller independently ingest benzoic acid
- Observed compound in urine in “copious amounts”
- Compound similar to benzoic acid, but contained nitrogen

1845
- Compound identified by Dessaignes as hippuric acid (glycine–benzoic acid conjugate)
- Glycine conjugation first metabolic reaction to be described!

1848-1893
- Methylation, aromatic oxidation, and conjugation reactions characterized
- Mechanisms considered “absolutely puzzling”, a “biochemical curiosity”

1947
- Richard Williams publishes “Detoxification mechanisms”, solidifying drug metabolism as a defined field of study

Early 1900’s
- Metabolism found to occur primarily in liver
- Liver microsome isolated in 1943 by Claude

1945
- Lipmann elucidates role of coenzyme A in acetylation reactions
- Discovery wins Nobel Prize in Medicine in 1953

1960’s
- Discovery and characterization of cytochrome P450’s and their role in drug metabolism

First pass metabolism

The concentration of orally administered drugs is greatly reduced by “first pass metabolism” before reaching systemic circulation.

All orally administered drugs pass through portal vein into liver before distribution to the rest of body.

Metabolism by gastrointestinal enzymes

Phase I and II biotransformations

Fraction of dose enters bloodstream

Orally administered drug

Metabolism by gut bacterial enzymes

Phases of drug metabolism

Phase 1: Modification
Introducing functional handles to drug

Drug molecule
Lipophilic, not excretable

Phase 2: Conjugation
Attaching highly hydrophilic groups

Modified compound
Polar, hydrophilic

Excretion
Bile, urine
Phases of metabolism

**Phase I metabolism**
- Initial modifications to drug molecules
- Adding or uncovering reactive functional groups
- Mostly facilitated by CYP enzymes in liver

**Phase II metabolism**
- Conjugation of polar functional groups, usually resulting in deactivation
- Facilitated by wide variety of transferase enzymes
- Conjugated molecules are recognized by transporters and excreted
Cytochrome P450s

Superfamily of heme-containing monooxygenase enzymes

Heme B

\[
\text{H} + \text{O}_2 + \text{H}^+ + \text{NADPH} \rightarrow \text{R} \rightarrow \text{R} + \text{OH} + \text{H}_2\text{O}
\]

The Cytochrome P450-dependent mixed-function oxidase system

- In human microsomes, P450s found on in mitochondrial and endoplasmic reticulum membranes
- CYP enzymes responsible for 75% of metabolism

Other enzymes that facilitate metabolism
- Flavin-containing monooxygenases (FMO)
  - Alcohol dehydrogenase (ALD)
  - Aldehyde oxidases (AO)
  - Monoamine oxidases (MAO)

**Phase I biotransformations - Oxidations at carbon centers**

**Aromatic hydroxylation**

\[
\begin{align*}
R & \rightarrow R \\
\text{CYP} & \rightarrow \text{OH}
\end{align*}
\]

**Epoxidation**

\[
\begin{align*}
R & \rightarrow R \\
\text{CYP} & \rightarrow \text{O}
\end{align*}
\]

**Aliphatic hydroxylation**

\[
\begin{align*}
R & \rightarrow R \\
\text{CYP} & \rightarrow \text{OH}
\end{align*}
\]

**Alcohol oxidation**

\[
\begin{align*}
R & \text{OH} \\
\text{ADH/ALDH} & \rightarrow \text{COOH}
\end{align*}
\]

**Oxidative dehalogenation**

Halothane

\[
\begin{align*}
\text{CYP} & \rightarrow \text{OH} \\
\text{HBr} & \rightarrow \text{Cl} \\
\text{H}_{2}\text{O} & \rightarrow \text{OH}
\end{align*}
\]

Phase I biotransformations - Oxidations at heteroatoms

**N-dealkylation**

\[ R-NCH_3 \xrightarrow{\text{CYP}} R-NH \]

via

\[ R-N-OH \]

**N-oxidation**

\[ R-N-R \xrightarrow{\text{FMO}} R-N^\ominus \]

**O-dealkylation**

\[ R-OCH_3 \xrightarrow{\text{CYP}} R-\cdot \cdot \cdot \cdot \]

via

\[ R-OH \]

**Deamination**

\[ R-\text{NH}_2 \xrightarrow{\text{MAO}} R-\cdot \cdot \cdot \cdot \]

- Responsible for the inactivation of endogenous neurotransmitters
- Targeted by class of antidepressant and anti-anxiety medications known as “MAO inhibitors”

Phases of metabolism

Phase I metabolism

- Initial modifications to drug molecules
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Phase II metabolism

- Conjugation of polar functional groups, usually resulting in deactivation
- Facilitated by wide variety of transferase enzymes
- Conjugated molecules are recognized by transporters and excreted
Phase II conjugations: glucuronidation

Glucuronidation

\[ R-OH \xrightarrow{\text{UDP-glucuronosyl transferase (UGT)}} O-\text{R} \]

UDP-Glucuronic acid

Phase II conjugations

**Acetylation**

\[
\text{R-NH}_2 \xrightarrow{\text{N-acetyltransferase}} \text{R-CONMe}
\]

**Sulfation**

\[
\text{R-OH} \xrightarrow{\text{Sulfotransferase (SULT)}} \text{R-SO}_3^-
\]

(3′-Phosphoadenosine-5′-phosphosulfate)

**Glutathione conjugation**

\[
\text{R-O} \xrightarrow{\text{Glutathione transferase (GST)}} \text{R-S-S-NH}_2
\]

Reactive electrophile

**Phases of drug metabolism**

**Phase 1: Modification**
- Introducing functional handles to drug
- Drug molecule: Lipophilic, not excretable

**Phase 2: Conjugation**
- Attaching highly hydrophilic groups
- Modified compound: Polar, hydrophilic

**Excretion**
- Bile, urine
Implications of metabolism on drug design

Drugs need to reach their targeted site and remain in the system for a meaningful amount of time in order to elicit the desired pharmacological effects.

**Pharmacokinetic properties which are impacted by metabolism:**

- Clearance
- Half life
- Bioavailability
**Prodrugs**

Prodrugs: a class of drugs administered in a pharmacologically inactive form, which upon administration is enzymatically converted into its active form *in vivo*.

Prodrug strategy can overcome challenges in:
- Absorption
- Route of administration
- Low bioavailability

**Case study:**

Sofosbuvir (Pharmasset/Gilead)
- Approved in 2013 for hepatitis C
- WHO List of Essential Medications
- Nucleotide prodrug (ProTide)
The ProTide approach: Sofosbuvir

Nucleoside analogue drugs tend to suffer from low potency due to slow first phosphorylation.

Nucleosides delivered as the monophosphate suffer from poor absorption and bioavailability due to charged phosphate.

The ProTide approach: Sofosbuvir

- Phosphate group masked
- Effectively distributed to hepatocyte cytosol (cells expressing high levels of CTSA/CES1)
- ProTide hydrolyzed intracellularly (no metabolite detected in plasma)

The ProTide approach: Sofosbuvir

Sofosbuvir triphosphate

Recognized by HCV NS5B polymerase

Incorporated into RNA strand, leading to chain growth termination

UMP-CMP kinase, NDPK

Sofosbuvir triphosphate

Lessons learned from metabolic studies

Information from metabolic studies can be used for rational drug design

Case study:

**Ezetimibe (Schering-Plough/Merck)**
- Approved in 2002 for hypercholesterolemia
- 125\textsuperscript{th} most prescribed medication in 2018

Lead compound: SCH-48461

Case study: the development of Ezetimibe

Lead compound: SCH-48461

Initial results with cholesterol-fed hamsters showed promising efficacy

- Control group showed no decrease in plasma cholesterol until dosed with compound in week 3
- Cholesterol-fed hamsters dosed with 1 mg/kg compound displayed a lower total plasma cholesterol level
- Dosed group showed increase in plasma cholesterol when dosing stopped at week 3

Case study: the development of Ezetimibe

Lead compound
ED$_{50}$ = 2.2 mg/kg/day

Compound is rapidly metabolized in vivo, resulting in poor half life

Relatively high dose needed

Hamsters fed $^{14}$C cholesterol

$^{14}$C cholesterol levels in plasma measured

Demethylated metabolite accounted for majority of activity

N OMe

O

ED$_{50}$ = 2.2 mg/kg/day


Case study: the development of Ezetimibe

Lead compound
ED$_{50}$ = 2.2 mg/kg/day

(S)-benzylic hydroxylation
ED$_{50}$ = 0.9 mg/kg/day
2x more potent than lead
5x more potent than (R)-hydroxylation

N-Aryl hydroxylation
-78% LCE @ 50 mg/kg/day
Slight decrease in activity

C3 Aryl hydroxylation
-16% LCE @ 50 mg/kg/day
Large decrease in activity

Case study: the development of Ezetimbe

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- Challenges in predicting adverse effects
Adverse drug reactions (ADRs)

Adverse drug reaction:

- a response to a drug that is noxious, unintended and occurs at doses normally used in man

**Type A**
- Associated with primary pharmacology of drug
- Usually detected in animal models/clinical trials
- Can be mitigated through dose adjustments

**Type B (idiosyncratic ADRs)**
- Not associated with primary pharmacology, mechanism usually not understood
- Does not occur at any dose for most people
- Poor correlation with animal models
- Often not detected until drug has been exposed to a large population of patients

Bioactivation to reactive metabolites

Drug molecule

Detoxified/deactivated metabolite

Metabolism
Bioactivation to reactive metabolites

Bioactivation is usually the initial event in most drug-induced toxicities
Mechanisms of adverse reactions

Drug molecule

Metabolism

Reactive intermediate

Enzyme binding to enzyme deactivation

Covalent binding

DNA
- Mutagenesis, cancer

Essential cell protein
- Cell death, necrosis

Autologous macromolecule
- Immune response, hypersensitivity
Mechanisms of adverse reactions

**Drug molecule**

**Metabolism**

**Reactive intermediate**

- **Covalent binding**
  - **DNA**
    - Mutagenesis, cancer
  - **Essential cell protein**
    - Cell death, necrosis
  - **Autologous macromolecule**
    - Immune response, hypersensitivity

**Enzyme binding**

**Enzyme deactivation**
In some instances, a substrate can escape the oxygen-rebound step, binding CYP and leading to inactivation.

Mechanism-based inhibition

General mechanism of C(sp³)–H hydroxylation

Mechanism-based inhibition

Substrate escapes oxygen rebound step

Reactive intermediate binds enzyme

Enzyme deactivated

Mechanism-based inhibition by paroxetine

Paroxetine

- Approved in 1992 for use as antidepressant
- 74th most prescribed drug in 2018
- Known to cause drug-drug interactions

Strong inhibition of CYP2D6 and CYP2B6

Prescribing guidelines warn that paroxetine is not to be taken with certain other drugs

Mechanisms of adverse reactions

Drug molecule

Metabolism

Reactive intermediate

Enzyme binding

Enzyme deactivation

Covalent binding

DNA

Essential cell protein

Autologous macromolecule

Mutagenesis, cancer

Cell death, necrosis

Immune response, hypersensitivity
Classic example: drug-induced liver damage from acetaminophen

![Chemical structure of acetaminophen, reactive quinone intermediate, and glutathione conjugate]

- Acetaminophen
- Reactive quinone intermediate
- Glutathione conjugate

- Most commonly used medication for pain and fever in the US and Europe
- On WHO List of Essential Medicines
- Leading cause of acute liver failure and drug overdoses in Western countries

Treatment of overdose may involve administering of N-acetylcysteine (glutathione precursor)

Endogenous GSH levels depleted

Covalent binding to hepatic proteins

Cell death, liver damage

Classic example: Toxicity of benzene

Benzene

Quinone redox cycling generates reactive oxygen species

Reactive quinone intermediates

Covalent binding

The structural alert concept

**Structural alerts (toxicophores):** functional groups frequently found in drugs associated with adverse reactions

Acetanilides

Hydrazides

Amines

Halogenated compounds

Phenyl rings

Heteroaromatics

Nitroaromatics

The structural alert concept

**Structural alerts (toxicophores):** functional groups frequently found in drugs associated with adverse reactions

- **Acetanilides**
  - Michael acceptors
- **Hydrazides**
  - Alkylating/acylating agents
- **Amines**
  - Acylating agents, possible fluoride toxicity
- **Halogenated compounds**
- **Phenyl rings**
  - Epoxidation leading to reactive intermediates
- **Heteroaromatics**
- **Nitroaromatics**
  - Reactive oxygen species

Survey of withdrawn drugs found structural alerts in 55 out of 68 drugs (80.8%) 

The propensity to form reactive metabolites was observed in 36 out of the 55 drugs (65%)
Drugs recalled due to idiosyncratic drug-induced liver injury

Bromfenac
Structural alerts: Aniline, bromobenzene
Withdrawn in: 1998

Nefazodone
Structural alerts: N-aryl piperazine
Withdrawn in: 2004

Hepatotoxicity is leading cause of idiosyncratic adverse drug reactions

Troglitazone
Structural alerts: Thiazolidinedione, phenol
Withdrawn in: 2000
Linked to 63 cases of liver-failure deaths

Sitaxentan
Structural alerts: 1,3-benzodioxole
Withdrawn in: 2010

Assaying formation of reactive metabolites

**Reactive metabolite trapping assays**

- Incubation of drug in liver microsome
- Addition of appropriate nucleophile (Glutathione, KCN)
- LC-MS analysis of adducts

**Radiolabeling assays**

- Administration of radio labeled ($^3$H / $^{14}$C) compounds
- Isolate and quantify radioactive covalently bound proteins and DNA

Strategies for mitigating effects of structural alerts

Reports of hepatotoxicity, agranulocytosis

Amodiaquine

Analogues with improved safety profile

- Reduce electron density of aromatic rings
- Steric blocking of metabolically active sites

Strategies for mitigating effects of structural alerts

5-HT\textsubscript{2c} (serotonin) receptor agonist (Pfizer)

Ames positive (mutagenic)

Redesigned compound

Ames negative

Investigated as bradykinin B₁ antagonist (Merck)

Strategies for mitigating effects of structural alerts

2,3-diaminopyridine moiety determined to be safety liability

Irreversible binding to liver microsomal proteins

Bioisosteric replacement of structural alerts

**Lead compound**

**Design principle:**
Replace 2,3-DAP with nonaromatic linker

Simplify to ethylene diamine

Add conformational constraint and hydrogen bonding capability

**Structural modification**

hBK B$_1$ $K_i$ = 0.012 μM
(Human bradykinin B$_1$ inhibitor constant)

hBK B$_1$ $K_i$ = 52 μM
Inactive

Bioisosteric replacement of structural alerts

- Thorpe-Ingolde effect allows for bioisosteric mimicry of 2,3-diaminopyridine bond angle
- Large improvement in rat pharmacokinetic profile due to heightened metabolic stability

Challenges in predicting adverse effects

The ability to predict \textit{a priori} whether drug bioactivation will lead to toxicity in humans is \textit{very limited}. 
Challenges in predicting adverse effects

Acetaminophen (APAP)

Dose normalized to give comparable levels of *in vivo* covalent binding in mice liver

Adduction of "critical proteins" is required to cause organ toxicity

Not all protein binding is deleterious

Hepatotoxic

Nonhepatotoxic

Shortcomings of the structural alert concept

Not every structural alert results in toxicity

- 53% of 108 of the most prescribed drugs in 2009 possessed structural alerts
- Evidence of reactive metabolite formation in 41% of those cases

Clopidogrel
Thiophene structural alert

Shortcomings of the structural alert concept

Not every structural alert results in toxicity

- 53% of 108 of the most prescribed drugs in 2009 possessed structural alerts
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Clopidogrel
Thiophene structural alert

Not every IADR associated with a structural alert

Ximelagatran
Does not contain any known structural alerts

Rejected for FDA approval in 2009 after clinical trials due to idiosyncratic drug-induced liver injury

Cannot completely eliminate structural alerts

“If medicinal chemists abstained from the synthesis of phenyl ring-containing compounds because the phenyl ring is considered as a structural alert, humanity would be deprived of countless life-saving drugs”

Challenges in predicting adverse effects

- Lack of thorough characterization distinguishing critical and noncritical proteins
- Structural alert analysis often inaccurate
- Inter- and intra-species variation in drug metabolism

The ability to predict a priori whether drug bioactivation will lead to organ or immune toxicity is very limited.
Drug discovery and development

- Discovery
- Preclinical development
- Clinical trials
- FDA review

- Higher accuracy of testing results
- Increasing stakes and cost of test subjects

- In-vitro studies
- In-vivo studies
- Animal trials
- Human trials

General population

Image credit: Ian B. Perry
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