Drug Metabolism

S.P. Markey  
Laboratory of Neurotoxicology  
NIMH, NIH  
Nov. 19, 2009

Metabolism vs Drug Action

Drug receptor

Biological response

Excretion through kidney or bile

Drug Metabolism

Extrahepatic microsomal enzymes (oxidation, conjugation)

Hepatic microsomal enzymes (oxidation, conjugation)

Hepatic non-microsomal enzymes (acetylation, sulfation, GSH, alcohol/alddehyde dehydrogenase, hydrolysis, oxid)
Liver Microsomal System

- Oxidative Reactions: Cytochrome P450 mediated
  - Formation of an inactive polar metabolite
    - Phenobarbital

Liver Microsomal System

- Oxidative Reactions: Cytochrome P450 mediated
  - Formation of a toxic metabolite
    - Acetaminophen → NAPQI

Liver Microsomal System

- Oxidative Reactions: Cytochrome P450 mediated
  - Formation of an active metabolite
    - By Design: Purine & pyrimidine chemotherapy
      - Inadvertent: terfenadine → fexofenadine
Evolution of Drug Metabolism As a Science
Post WWII Pioneers

- Richard Tecwyn Williams – Great Britain
  - 1942, worked on the metabolism of TNT with regard to toxicity in munitions workers; due to the war he assembled teams to work on metabolism of sulfonamides, benzene, aniline, acetanilide, phenacetin, and stilbesterol
  - Developed concept of Phase 1 & Phase 2 Reactions.
    - Biotransformation involves metabolic oxygenation, reduction, or hydrolysis; result in changes in biological activity (increased or decreased)
    - Second phase, conjugation, in almost all cases resulted in detoxification.

- Bernard B. Brodie, U.S.
  - NYU and Laboratory of Industrial Hygiene, NYC 1949 – Metabolic fate of acetanilide and phenacetin in man (with Julius Axelrod as pre-doc; later an NIMH Nobel laureate)
  - 1950s, NIH – pioneering studies on all aspects of drug metabolism; esp. reserpine, serotonin; hexobarbital tolerance
  - 1952 – R.T. Williams spent 6 months at NIH; subsequently many students went between both labs (Richard Adamson, James Gillette, and Sidney Udenfriend)
  - 1950s, Brodie lab developed the spectrophotofluorimeter (Robert Bowman)

Sites of drug metabolism – Cytochromes P450 (CYPs)
Liver enriched
Endoplasmic reticulum
Certain transferases also localized to the ER
Cytochrome P450 Isoforms (CYPs) - An Overview

- NADPH + H+ + O2 + Drug → NADP+ + H2O + Oxidized Drug
- Carbon monoxide binds to the reduced Fe(II) heme and absorbs at 450 nm (origin of enzyme family name)
- CYP monooxygenase enzyme family is major catalyst of drug and endogenous compound oxidations in liver, kidney, G.I. tract, skin, lungs
- Oxidative reactions require the CYP heme protein, the reductase, NADPH, phosphatidylcholine and molecular oxygen
- CYPs are in smooth endoplasmic reticulum in close association with NADPH-CYP reductase in 10/1 ratio
- The reductase serves as the electron source for the oxidative reaction cycle

CYP Families

- Multiple CYP gene families have been identified in humans, and the categories are based upon protein sequence homology
- Most of the drug metabolizing enzymes are in CYP 1, 2, & 3 families.
- CYPs have molecular weights of 45-60 kDa.
- Frequently, two or more enzymes can catalyze the same type of oxidation, indicating redundant and broad substrate specificity.
- CYP3A4 is very common to the metabolism of many drugs; its presence in the GI tract is responsible for poor oral availability of many drugs
ROLE OF CYP ENZYMES IN HEPATIC DRUG METABOLISM

RELATIVE HEPATIC CONTENT OF CYP ENZYMES

% DRUGS METABOLIZED BY CYP ENZYMES

**Human Liver Drug CYPs**

<table>
<thead>
<tr>
<th>CYP enzyme</th>
<th>Level (%total)</th>
<th>Extent of variability</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A2</td>
<td>~ 13</td>
<td>~40-fold</td>
</tr>
<tr>
<td>1B1</td>
<td>&lt;1</td>
<td>~50-fold</td>
</tr>
<tr>
<td>2A6</td>
<td>~4</td>
<td>~30 - 100-fold</td>
</tr>
<tr>
<td>2B6</td>
<td>~1</td>
<td>~50-fold</td>
</tr>
<tr>
<td>2C2</td>
<td>~18</td>
<td>25-100-fold</td>
</tr>
<tr>
<td>2D6</td>
<td>Up to 2.5</td>
<td>&gt;1000-fold</td>
</tr>
<tr>
<td>2E1</td>
<td>Up to 7</td>
<td>~20-fold</td>
</tr>
<tr>
<td>2F1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2J2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3A4</td>
<td>Up to 28</td>
<td>~20-fold</td>
</tr>
<tr>
<td>4A, 4B</td>
<td></td>
<td>90-fold**</td>
</tr>
</tbody>
</table>

S. Rendic & F.J. DiCarlo, Drug Metab Rev 29:413-80, 1997
*L. Wojnowski, Ther Drug Monit 26: 192-199, 2004

**Participation of the CYP Enzymes in Metabolism of Some Clinically Important Drugs**

<table>
<thead>
<tr>
<th>CYP Enzyme</th>
<th>Examples of substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A1</td>
<td>Caffeine, Testosterone, R-Warfarin</td>
</tr>
<tr>
<td>1A2</td>
<td>Acetaminophen, Caffeine, Phenacetin, R-Warfarin</td>
</tr>
<tr>
<td>2A6</td>
<td>17β-Estradiol, Testosterone</td>
</tr>
<tr>
<td>2B6</td>
<td>Cyclophosphamide, Erythromycin, Testosterone</td>
</tr>
<tr>
<td>2C-family</td>
<td>Acetaminophen, Tobutamide (2C9); Hexobarbital, S-Warfarin (2C8,19); Phenytoin, Testosterone, R-Warfarin, Zidovudine (2C9,19);</td>
</tr>
<tr>
<td>2E1</td>
<td>Acetaminophen, Caffeine, Chlorzoxazone, Halothane</td>
</tr>
<tr>
<td>2D6</td>
<td>Acetaminophen, Codeine, Debrisoquine</td>
</tr>
<tr>
<td>3A4</td>
<td>Acetaminophen, Caffeine, Carbamazepine, Codeine, Cortisol, Erythromycin, Cyclophosphamide, S- and R-Warfarin, Phenytoin, Testosterone, Halothane, Zidovudine</td>
</tr>
</tbody>
</table>

Adapted from: S. Rendic Drug Metab Rev 34: 83-448, 2002
Also D.F.V. Lewis, Current Medicinal Chemistry, 2003, 10, 1955-1972
Drug Metabolism Studies

- Determine the nature of metabolites
  - Stable metabolites → good
  - Electrophiles → bad
    - Bind to cellular nucleophile - DNA, RNA and protein
    - Cause cell death or transformation – cancer
- Which P450s are involved in metabolism of the drug candidate?
  - Several P450s → good
  - Single P450 → bad
    - CYP2D6 - polymorphism
    - CYP3A4 - drug interactions

Factors Influencing Activity and Level of CYP Enzymes

<table>
<thead>
<tr>
<th>Factors</th>
<th>Enzymes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrition</td>
<td>1A1; 1A2; 1B1; 2A6, 2B6, 2C8, 2D6, 3A4, 5</td>
</tr>
<tr>
<td>Smoking</td>
<td>1A1; 1A2, 2E1</td>
</tr>
<tr>
<td>Alcohol</td>
<td>2E1</td>
</tr>
<tr>
<td>Drugs</td>
<td>1A1, 1A2, 2A6, 2B6, 2C8, 2D6, 3A3, 3A4, 5</td>
</tr>
<tr>
<td>Environment</td>
<td>1A1, 1A2, 2A6, 2B6, 2C8, 2D6, 3A3, 3A4, 5</td>
</tr>
<tr>
<td>Genetic Polymorphism</td>
<td>1A; 2A6, 2C9, 2D6, 2E1</td>
</tr>
</tbody>
</table>

Red indicates enzymes important in drug metabolism

Adapted from: S. Rendic Drug Metab Rev 34: 83-448, 2002

Non-nitrogenous Substances that Affect Drug Metabolism

- **Grapefruit juice** - CYP 3A4 inhibitor; highly variable effects; fucocoumarins
- **St John’s wort, other herbal products**
- **Isosafrole, safrole**
  - CYP1A1, CYP1A2 inhibitor; found in root beer, perfume
Overheard Conversation

• At a B&B breakfast table, after grapefruit juice was served, someone remarked "A friend read the package insert with her prescription and the fine print warned against drinking grapefruit juice...is this true? Should it be avoided with all medications? How about grapefruit itself? How about orange juice?"

Effect of Grapefruit Juice on Felodipine Plasma Concentration

Grapefruit Juice Facts

• GJ or G, lime, or Sun Drop Citrus soda, Seville OJ (not most OJ) elevates plasma peak drug concentration, not elimination t1/2
• GJ reduced metabolite/parent drug AUC ratio
• GJ caused 62% reduction in small bowel enterocyte 3A4 and 3A5 protein; liver not as markedly affected (i.v. pharmacokinetics unchanged)
• GJ effects last ~4 h, require new enzyme synthesis
• Effect cumulative (up to 5x Cmax) and highly variable among individuals depending upon 3A4 small bowel basal levels
First-Pass Metabolism after Oral Administration of a Drug, as Exemplified by Felodipine and Its Interaction with Grapefruit Juice

Limited Expression of Human Drug Metabolizing CYPs in Extrahepatic Tissues

<table>
<thead>
<tr>
<th>CYP Enzyme</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A1</td>
<td>Lung, kidney, GI tract, skin, placenta, others</td>
</tr>
<tr>
<td>1B1</td>
<td>Skin, kidney, prostate, mammary, others</td>
</tr>
<tr>
<td>2A6</td>
<td>Lung, nasal membrane, others</td>
</tr>
<tr>
<td>2C</td>
<td>GI tract (small intestine mucosa) larynx, lung</td>
</tr>
<tr>
<td>2C9</td>
<td>GI tract</td>
</tr>
<tr>
<td>2C10</td>
<td>Lung, placenta, others</td>
</tr>
<tr>
<td>2D1</td>
<td>Lung, placenta</td>
</tr>
<tr>
<td>2D2</td>
<td>Larynx, lung</td>
</tr>
<tr>
<td>3A4</td>
<td>GI tract, lung, placenta, fetus, uterus, kidney</td>
</tr>
<tr>
<td>4B1</td>
<td>Lung, placenta</td>
</tr>
<tr>
<td>4A11</td>
<td>Kidney</td>
</tr>
</tbody>
</table>

S. Rendic & F.J. DiCarlo, Drug Metab Rev 29:413-80, 1997

CYP Biotransformations - Summary

- Chemically diverse small molecules are converted, generally to more polar compounds
- Reactions include:
  - Aliphatic hydroxylation, aromatic hydroxylation
  - Dealkylation (N-, O-, S-)
  - N-oxidation, S-oxidation
  - Deamination
  - Dehalogenation
- Examples - see *Principles of Clinical Pharmacology*, Chapter 11
Non-CYP Drug Biotransformations

- Oxidations
- Hydrolyses
- Conjugation (Phase 2 Rx's)
  - Major Conjugation Reactions
    - Glucuronidation (high capacity)
    - Sulfation (low capacity)
    - Acetylation (variable capacity)
      - Examples: Procainamide, Isoniazid
  - Other Conjugation Reactions: O-Methylation, S-Methylation, Amino Acid Conjugation (glycine, taurine, glutathione)
  - Many conjugation enzymes exhibit polymorphism

Non-CYP Drug oxidations (1)

- Monoamine Oxidase (MAO), Diamine Oxidase (DAO)
  - MAO (mitochondrial) oxidatively deaminates endogenous substrates including neurotransmitters (dopamine, serotonin, norepinephrine, epinephrine); drugs designed to inhibit MAO used to affect balance of CNS neurotransmitters (L-DOPA, MPTP converted to toxin MPP+ through MAO-B). DAO substrates include histamine and polyamines.
- Alcohol & Aldehyde Dehydrogenase - non-specific enzymes found in soluble fraction of liver; ethanol metabolism
- Xanthine Oxidase - converts hypoxanthine to xanthine, and then to uric acid. Drug substrates include theophylline, 6-mercaptopurine. Allopurinol is substrate and inhibitor of xanthine oxidase; delays metabolism of other substrates; effective for treatment of gout.

Non-CYP Drug oxidations (2)

- Flavin Monooxygenases
  - Family of enzymes that catalyze oxygenation of nitrogen, phosphorus, sulfur – particularly facile formation of N-oxides
  - Different FMO isoforms have been isolated from liver, lung (S.K. Krueger, et al. Drug Metab Rev 2002; 34:523-32)
  - Require molecular oxygen, NADPH, flavin adenine dinucleotide (FAD)
  - Single point (loose) enzyme-substrate contact with reactive hydroperoxyflavin monooxygenating agent
  - FMOs are heat labile and metal-free, unlike CYPs
  - Factors affecting FMOs (diet, drugs, sex) not as highly studied as CYPs
Hydrolysis – Ester or Amide

- Procaine – ester, rapidly hydrolyzed
- Procainamide – amide, more slowly hydrolyzed, valuable anti-arrhythmic
- N-acetylprocainamide (NAPA); metabolite with anti-arrhythmic activity, 2.5 x longer elimination half-life (Atkinson et al., 1988, Angiology, 39, 655-67)

Conjugation Reactions

Glucuronidation

Liver has several soluble UDP-Gluc-transferases

Glucuronic acid conjugation to phenols, 3°-amines, aromatic amines
Conjugation Reactions

Sulfation

\[
R-OH + \text{(PAPS, 3'-phosphoadenosine-5'-phosphosulfate)} \rightarrow R-O-S-OH
\]

Examples: ethanol, p-hydroxyacetanilide, 3-hydroxycoumarin

Minoxidil

\[\text{H}_2\text{N}-\text{N}-\text{N} \rightarrow \text{H}_2\text{N}-\text{N}-\text{N} \quad \text{Sulfation may produce active metabolite}\]

Minoxidil-sulfate

Conjugation Reactions

Acetylation

\[
\text{Ar} - \text{NH}_2 + \text{Acetyl transferase} \rightarrow \text{Ar} - \text{N}^0\text{CH}_3
\]

Examples: Procainamide, isoniazid, sulfanilimide, histamine

N-acetyl transferase (NAT) enzyme is found in many tissues, including liver
Procainamide

Unchanged in Urine, 59%

Unchanged in Urine, 85%

NAPA

0.3%

Additional Effects on Drug Metabolism

- Species Differences
  - Major differences in different species have been recognized for many years (R.T. Williams).
    - Phenylbutazone half-life is 3 h in rabbit, ~6 h in rat, guinea pig, and dog and 3 days in humans.
- Induction
  - Two major categories of CYP inducers
    - Phenobarbital is prototype of one group - enhances metabolism of wide variety of substrates by causing proliferation of SER and CYP in liver cells.
    - Polycyclic aromatic hydrocarbons are second type of inducer (ex: benzo[a]pyrene).
  - Induction appears to be environmental adaptive response of organism
  - Orphan Nuclear Receptors (PXR, CAR) are regulators of drug metabolizing gene expression
PXR and CAR Protect Against Xenobiotics

Mechanism of Induction of CYP3A4-Mediated Metabolism of Drug Substrates (Panel A) and the Resulting Reduced Plasma Drug Concentration (Panel B)

CYP3A Inducers Activate Human, Rabbit, and Rat PXR

S.A. Kliewer
Pregnane X Receptor (PXR)

- PXR is one of Nuclear Receptor (NR) family of ligand-activated transcription factors.
- Named on basis of activation by natural and synthetic C21 steroids (pregnanes), including pregnenolone 16α-carbonitrile (PCN)
- Cloned due to homology with other nuclear receptors
- Highly active in liver and intestine
- Binds as heterodimer with retinoic acid receptor (RXR)

S.A. Kliewer

Constitutive Androstane Receptor (CAR)

- Highly expressed in liver and intestine
- Sequestered in cytoplasm
- Co-factor complex required for activation; anchored by PPAR-binding protein (PBP)
- Binds response elements as RXR heterodimer
- High basal transcriptional activity without ligand
- Activated by xenobiotics
  - phenobarbital, TCPOBOP (1,4-bis[2-(3,5-dichloropyridyloxy)]benzene)

S.A. Kliewer

Acetaminophen (APAP)

Over-the-counter drug; relieving pain, reducing fever, relieving the symptoms of allergies, cold, cough, and flu.

Co-administration:
- Sedative
- Antihistamine
- Vasoconstrictants
- Expectorants
- Antitussive
- Analgesics

Tylenol (Top seller, controlling 35% of the pain killer market in North America)
Acetaminophen (Paracetamol)

- Acetanilide – 1886 – accidentally discovered antipyretic; excessively toxic (methemoglobinemia); para-aminophenol and derivatives were tested.
- Phenacetin introduced in 1887, and extensively used in analgesic mixtures until implicated in analgesic abuse nephropathy.
- Acetaminophen recognized as metabolite in 1899.
- 1948-49 Brodie and Axelrod recognized methemoglobinemia due to acetanilide and analgesia to acetaminophen.
- 1955 acetaminophen introduced in US.

Acetaminophen and p-Aminophenols

Acetanilide, 1886 (accidental discovery of antipyretic activity; high toxicity)

Phenacetin or acetophenetidin, 1887 (nephrotoxic, methemoglobinemia)

Acetaminophen, 1893 (Metabolic pathway quantified; (Brodie & Axelrod, 1948) popular in US since 1955)

Acetaminophen Toxicity

- Acetaminophen overdose results in more calls to poison control centers in the United States than overdose with any other pharmacologic substance.
- The American Liver Foundation reports that 35% of cases of severe liver failure are caused by acetaminophen poisoning which may require organ transplantation.
- N-acetyl cysteine is an effective antidote, especially if administered within 10 h of ingestion [NEJM 319:1557-1562, 1988]
Poisoning Fatalities U.S. 2006
Categories associated with largest numbers of fatalities

<table>
<thead>
<tr>
<th>Substance</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedative/hypnotics/antipsychotics</td>
<td>382</td>
</tr>
<tr>
<td>Opioids</td>
<td>307</td>
</tr>
<tr>
<td>Cardiovascular Drugs</td>
<td>252</td>
</tr>
<tr>
<td>Acetaminophen in combination</td>
<td>214</td>
</tr>
<tr>
<td>Antidepressants</td>
<td>210</td>
</tr>
<tr>
<td>Stimulants and street drugs</td>
<td>203</td>
</tr>
<tr>
<td>Alcohols</td>
<td>139</td>
</tr>
<tr>
<td>Acetaminophen only</td>
<td>138</td>
</tr>
</tbody>
</table>

Excerpt from Table 18
"2006 Annual Report of the American Association of Poison Control Centers’ National Poison Data System"
http://dx.doi.org/10.1080/15563650701754763

Acetaminophen Metabolism

Acetaminophen Protein Adducts

Acetaminophen toxicity mechanism
• N-acetyl cysteine is an effective agent to block GSH depletion and rescue from liver damaging toxicity
• CAR and PXR modulate acetaminophen toxicity (2002, 2004)
• CAR-null mice are resistant to acetaminophen toxicity
  – hepatic GSH lowered in wild type (but not in KO) after acetaminophen
  – CAR-humanized mice demonstrate same toxicity response
• Activation of PXR induces CYP3A11 and markedly enhances acetaminophen toxicity in wild type mice
• CAR transcription co-activator KO blocks toxicity (2005)

NAPQI toxicity linked to PXR activation

Experimental Design

Human PXR and rifampicin
Antibiotic, specific ligand for human PXR