Drug Metabolism

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Hepatic microsomal enzymes
(oxidation, conjugation)

Extrahepatic microsomal enzymes
(oxidation, conjugation)

Hepatic microsomal enzymes
(oxidation, conjugation)

Hepatic non-microsomal enzymes
(acetylation, sulfation, GSH,
alcohol/aldehyde dehydrogenase,
hydrolysis, oxid)

Liver Microsomal System

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

\[
\text{Phenobarbital} \xrightarrow{\text{Phase 1}} \text{p-hydroxy-phenobarbital} \xrightarrow{\text{Phase 2}} \text{p-hydroxyphenobarbital-glucuronide}
\]
Liver Microsomal System

• Oxidative Reactions: Cytochrome P450 mediated
  – Formation of a toxic metabolite
    • Acetaminophen – NAPQI

\[
\text{HN COCH}_3 \rightarrow \text{N COCH}_3 \quad \text{Toxic Events}
\]

Liver Microsomal System

• Oxidative Reactions: Cytochrome P450 mediated
  – Formation of an active metabolite
    • By Design: Purine & pyrimidine chemotherapy prodrugs

\[
\text{5-FU} \rightarrow \text{5-FUMP}
\]

• Inadvertent: terfenadine – fexofenadine

Evolution of Drug Metabolism As a Science

Post WWII Pioneers

• Richard Tecwn Williams – Great Britain
  – 1942, worked on the metabolism on 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.
Evolution of Drug Metabolism As a Science
Post WWII Pioneers

• 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)

Electron flow in microsomal drug oxidizing system

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

CYP Tables

• Human CYPs - variability and importance in drug metabolism
• Isoforms in metabolism of clinically important drugs
• Factors that influence CYP activity
• Non-Nitrogenous CYP inhibitors
• Extrahepatic CYPs

ROLE OF CYP ENZYMES IN HEPATIC DRUG METABOLISM
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>~30-100-fold</td>
</tr>
<tr>
<td>2A6</td>
<td>~4</td>
<td>~50-fold</td>
</tr>
<tr>
<td>2B6</td>
<td>&lt;1</td>
<td>~25-100-fold</td>
</tr>
<tr>
<td>2C2</td>
<td>~18</td>
<td>~1000-fold</td>
</tr>
<tr>
<td>2D6</td>
<td>Up to 2.5</td>
<td>~20-fold</td>
</tr>
<tr>
<td>2E1</td>
<td>Up to 7</td>
<td>~50-fold</td>
</tr>
<tr>
<td>2J2</td>
<td></td>
<td>~20-fold</td>
</tr>
<tr>
<td>3A4</td>
<td>Up to 28</td>
<td>~30-60*</td>
</tr>
<tr>
<td></td>
<td>30-60*</td>
<td>~20-fold*</td>
</tr>
<tr>
<td>4A, 4B</td>
<td></td>
<td>~20-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, Tolbutamide (2C9); Hexobarbital, S-Warfarin (2C9,19); Phenyltoin, Testosterone, R-Warfarin, Zidovudine (2C8,9,19);</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, Phenyltoin, 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

Factors Influencing Activity and Level of CYP Enzymes

<table>
<thead>
<tr>
<th>Factors</th>
<th>1A1, 1A2, 1B1, 2A6, 2B6, 2C6, 2D6, 3A3, 3A4, 3A5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrition</td>
<td></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, 2C, 2D6, 3A3, 3A4, 3A5</td>
</tr>
<tr>
<td>Environment</td>
<td>1A1, 1A2, 2A6, 1B, 2E1, 3A3, 3A4, 3A5</td>
</tr>
<tr>
<td>Genetic Polymorphism</td>
<td>1A, 2A6, 2C9, 19, 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

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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?”

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**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

Human Drug Metabolizing CYPs Located 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>2B6</td>
<td>GI tract, lung</td>
</tr>
<tr>
<td>2C</td>
<td>GI tract (small intestine mucosa) larynx, lung</td>
</tr>
</tbody>
</table>

S. Rendic & F.J. DiCarlo, Drug Metab Rev 29:413-80, 1997
Human Drug Metabolizing CYPs Located in Extrahepatic Tissues (cont'd)

<table>
<thead>
<tr>
<th>CYP Enzyme</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>2E1</td>
<td>Lung, placenta, others</td>
</tr>
<tr>
<td>2F1</td>
<td>Lung, placenta</td>
</tr>
<tr>
<td>2J2</td>
<td>Heart</td>
</tr>
<tr>
<td>3A</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

- 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 Rxs)**
  - 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 Monoxygenases
  – 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 adenosine dinucleotide (FAD)
  – Single point (loose) enzyme-substrate contact with reactive hydroperoxyflavin monoxygenating 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

Sulfation

Examples: ethanol, p-hydroxyacetanilide, 3-hydroxycoumarin
Conjugation Reactions

Acetylation

Examples: Procainamide, isoniazid, sulfanilimide, histamine

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

Procainamide

Unchanged in Urine, 59% 

24% Fast 17% Slow 3%

Unchanged in Urine, 85% 

0.3%

NAPA
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

S.A. Kleweer
**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**

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Human PXR</th>
<th>Rabbit PXR</th>
<th>Mouse PXR</th>
<th>Rat PXR</th>
</tr>
</thead>
<tbody>
<tr>
<td>rifampicin</td>
<td>96%</td>
<td>96%</td>
<td>82%</td>
<td>82%</td>
</tr>
<tr>
<td>PCN</td>
<td>96%</td>
<td>96%</td>
<td>77%</td>
<td>77%</td>
</tr>
<tr>
<td>dexamethasone</td>
<td>77%</td>
<td>77%</td>
<td>77%</td>
<td>77%</td>
</tr>
<tr>
<td>RU486</td>
<td>77%</td>
<td>77%</td>
<td>77%</td>
<td>77%</td>
</tr>
<tr>
<td>clotrimazole</td>
<td>77%</td>
<td>77%</td>
<td>77%</td>
<td>77%</td>
</tr>
<tr>
<td>troglitazone</td>
<td>77%</td>
<td>77%</td>
<td>77%</td>
<td>77%</td>
</tr>
<tr>
<td>tamoxifen</td>
<td>77%</td>
<td>77%</td>
<td>77%</td>
<td>77%</td>
</tr>
</tbody>
</table>

**Report activity (fold)**

- 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)

PXR and CAR Regulate Overlapping Genes

<table>
<thead>
<tr>
<th>Liver RNA</th>
<th>PCN (PXN)</th>
<th>TCPOBOP (CAR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I enzymes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyp3a11</td>
<td>3.5x</td>
<td>3.4x</td>
</tr>
<tr>
<td>Cyp2b10</td>
<td>12x</td>
<td>110x</td>
</tr>
<tr>
<td>Aldh1a1</td>
<td>2.1x</td>
<td>1.9x</td>
</tr>
<tr>
<td>Aldh1a7</td>
<td>1.6x</td>
<td>1.9x</td>
</tr>
<tr>
<td>Phase II enzymes</td>
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<tr>
<td>Ugt1a1</td>
<td>2.8x</td>
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<tr>
<td>G6p-d</td>
<td>16x</td>
<td>15x</td>
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<tr>
<td>Transporters</td>
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<td></td>
</tr>
<tr>
<td>Mdp2</td>
<td>3.0x</td>
<td>2.0x</td>
</tr>
<tr>
<td>Mdp3</td>
<td>9.2x</td>
<td>15x</td>
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<tr>
<td>Oatp2</td>
<td></td>
<td>15x</td>
</tr>
</tbody>
</table>

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

70-90%
75-80%

HN COCH₃

OH

HN COCH₃

OC₂H₅

NH₂

OC₂H₅

HN COCH₃

NH₂

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]


Acetaminophen Toxicity

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>Sedatives/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

- N-acetyl-p-benzoquinone imine
- Protein adducts, oxidative stress, toxicity

Acetaminophen Protein Adducts


Acetaminophen toxicity mechanism

- N-acetyl cysteine is an effective agent to block GSH depletion and rescue from liver damaging toxicity
- 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

Drug Metabolism - Web Information Resources
  - General web site regarding all aspects of chemical structure (sequence and 3D) of P450 proteins from multiple species; links to related sites including leading researchers on P450
  - Site has many commercially available drug metabolizing enzymes and useful links to multiple drug metabolism resources