Drug Metabolism

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Drug Metabolism

Drawing of a human male showing internal organs. Labels with directional arrows that identify where in the body certain enzymes exist.

Extrahepatic microsomal enzymes
(oxidation, conjugation)

Hepatic microsomal enzymes
(oxidation, conjugation)

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

Liver Microsomal System

Oxidative Reactions: Cytochrome P450 mediated

-Formation of an inactive polar metabolie

Phenobarbital

Chemical structures of phenobarbital, p-hydroxy-phenobarbital and p-hydroxyphenobarbital-glucuronide

Liver Microsomal System

Oxidative Reactions: Cytochrome P450 mediated

-Formation of a toxic metabolite

Acetaminophen – NAPQI

Chemical structures

Liver Microsomal System
Oxidative Reactions: Cytochrome P450 mediated

Oxidative Reactions: Cytochrome P450 mediated

- Formation of an active metabolite

  By Design: Purine & pyrimidine chemotherapy prodrugs

- Inadvertent: terfenadine – fexofenadine

Evolution of Drug Metabolism As a Science
Post WWII Pioneers

Richard Tecwyn 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)**

Flow chart

Electron flow in microsomal drug oxidizing system

Cytochrome P450 Isoforms (CYPs) - An Overview

$NADPH + H^+ + O_2 + Drug \rightarrow NADP^+ + H_2O + Oxidized\ Drug$

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

Pie chart showing relative hepatic content of CYP Enzymes.

Pie chart showing % of drugs metabolized by CYP enzymes.

A.Atkinson, 2005

Human Liver Drug CYPs

Chart identifying CYP enzymes and their level (% total) and extent of variability

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

Chart showing CYP Enzymes and Examples of their substrates.

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

Nutrition
Smoking
Alcohol
Drugs
Environment
Genetic Polymorphism

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

- Bailey, D.G. et al.; Br J Clin Pharmacol 1998, 46:101-110
- Bailey, D.G et al.; Am J Cardiovasc Drugs 2004, 4:281-97**.**

St John's wort, other herbal products

- Tirona, R.G and Bailey, D.G. ; Br J Clin Pharmacol. 2006,61: 677-81

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

Chart showing that plasma felodipine concentration over time is higher when a 5 mg tablet is given with grapefruit juice.

Chemical structures

Review- D.G. Bailey, et al.; Br J Clin Pharmacol 1998, 46:101-110

Grapefruit Juice Facts

GJ or G, lime, or Sun Drop Citrus soda, Seville OJ(not most OJ) elevates plasma peak drug concentration, not elimination $t_{1/2}$

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 C_{max}) 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

Illustration of process.

Human Drug Metabolizing CYPs Located in Extrahepatic Tissues

Chart showing CYP enzymes and their tissue distribution.

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

Human Drug Metabolizing CYPs Located in Extrahepatic Tissues (cont'd)

Chart showing CYP enzymes and their tissue distribution.

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 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)**
- **Complete structures defined (Review: J. Cashman, 1995, Chem Res Toxicol 8:165-181; Pharmacogenomics 2002; 3:325-39)**
- **Require molecular oxygen, NADPH, flavin adenosine dinucleotide (FAD)**
- **Single point (loose) enzyme-substrate contact with reactive hydroperoxyflavin monoxoygenating 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

Chemical structures of procaine, procainamide, and N-acetylprocainamide

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

Chemical structures

Liver has several soluble UDP-gluc-transferases

Chemical structures of Morphine, Amitriptyline and Cotinine.

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

Conjugation Reactions
Sulfation

Chemical structure

Examples: ethanol, p-hydroxyacetanilide, 3-hydroxycoumarin

Chemical structures of Minoxidil and Minoxidil-sulfate.

Sulfation may produce active metabolite

Conjugation Reactions
Acetylation

Examples: Procainamide, isoniazid, sulfanilimide, histamine

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

Procainamide

Procainamide

Unchanged in urine, 59%

NAPA unchanged in urine, 85%

Chemical structures

Procainamide

Chemical structure of procainamide

Chemical Structure of trace metabolite

Chemical structure of reactive metabolite that may cause lupus.

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.

  Polycylic 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

Illustration of this process (nuclear receptors PXR and CAR and their target genes).

S.A. Kliewer

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

Illustrations of this mechanism.

Wilkinson G. N Engl J Med 2005;352:2211-2221

CYP3A Inducers Activate Human, Rabbit, and Rat PXR

Chart for

Rifampicin
PCN
Dexamethasone
RU486
clotrimazole
troglitazone
tamoxifen

Reporter activity (fold)

S.A. Kliewer

Pregnane X Receptor (PXR)

Chart

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

Chart for phase I and phase II enzymes

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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

Chemical structures

Acetanilide (synthesized in 1886)
Phenacetin (synthesized in 1887)
Acetaminophen (synthesized in1893)
Metabolic pathway quantified (Brodie and Axelrod, 1948)

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]

Management of acetaminophen overdose [Trends Pharm Sci 24:154-157, 2003

Poisoning Fatalities U.S. 2006

Categories associated with largest numbers of fatalities

Chart showing different drug classes and the number of fatalities attributed to them.

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

Acetominophen Metabolism

Chemical structures

N-acetyl-p-benzoquinone imine (toxic metabolite)

Acetaminophen Protein Adducts

Chemical structures

S.D. Nelson, *Drug Metab. Rev*. 27: 147-177 (1995)
K.D. Welch et al., *Chem Res Toxicol* 18:924-33 (2005)

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

G. Guo et al. 2004, Toxicol Sci 82(2):374-80

Chemical structures –

Possible oxidative stress mechanism

Drug Metabolism - Web Information Resources

http://en.wikipedia.org/wiki/Cytochrome_P450_oxidase

- **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**

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

- **Site contains many useful documents regarding drug metabolism and FDA recommendations including "Drug Metabolism/Drug Interaction Studies in the Drug Development Process: Studies in Vitro", FDA Guidance for Industry**

http://www.sigmaaldrich.com/Area_of_Interest/Biochemicals/Enzyme_Explorer.html

- **Site has many commercially available drug metabolizing enzymes and useful links to multiple drug metabolism resources**