Drug Metabolism

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Dec. 2, 2010

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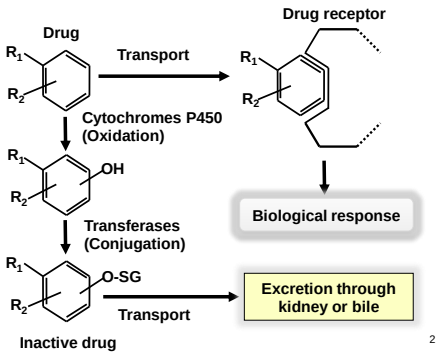
Metabolism vs Drug Action



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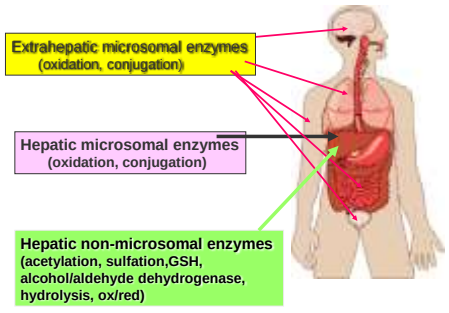
Drug Metabolism



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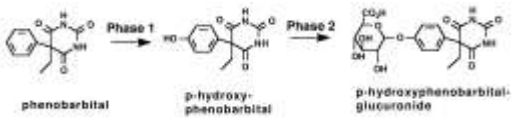
Liver Microsomal System

•Oxidative Reactions: Cytochrome P450 mediated

– **Formation of an inactive polar metabolite**
  • **Phenobarbital**



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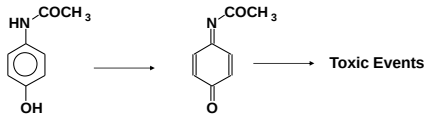
Liver Microsomal System

•Oxidative Reactions: Cytochrome P450 mediated

– **Formation of a toxic metabolite**
  • **Acetaminophen – NAPQI**



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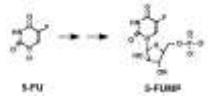
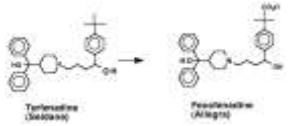
Liver Microsomal System

•Oxidative Reactions: Cytochrome P450 mediated

– **Formation of an active metabolite**
  • **By Design: Purine & pyrimidine chemotherapy**



  • **Inadvertent: terfenadine – fexofenadine**



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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.**

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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)**

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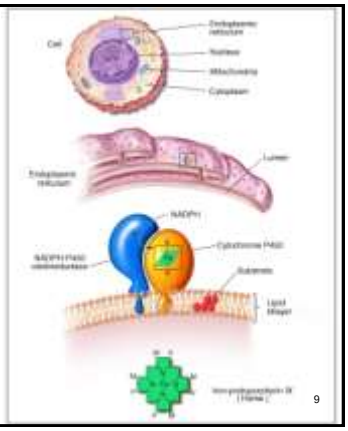
Sites of drug metabolism – Cytochromes P450 (CYPs)

Liver enriched Endoplasmic reticulum

Certain transferases also localized to the ER



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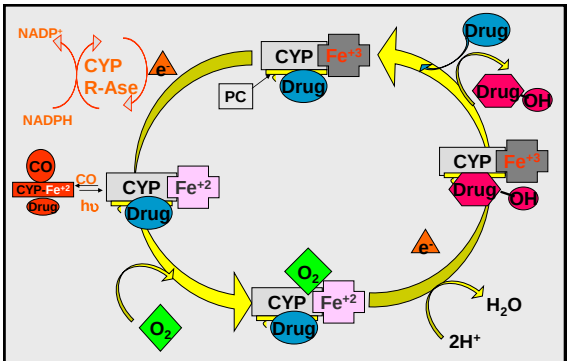

Electron flow in microsomal drug oxidizing system

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Cytochrome P450 Isoforms (CYPs) - An Overview

- **NADPH + H^+ + O_2 + Drug → $NADP^+$ + 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**

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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 availabilty of many drugs**

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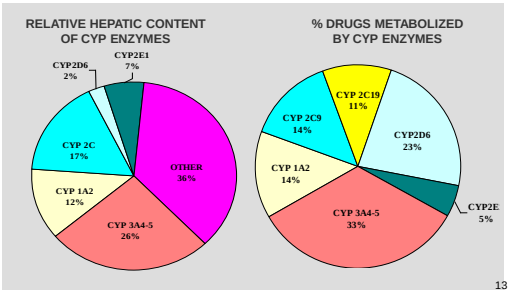
ROLE OF CYP ENZYMES IN HEPATIC DRUG METABOLISM



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A. Atkinson, 2005

Human Liver Drug CYPs

CYP enzyme	Level (%total)	Extent of variability
1A2	~ 13	~40-fold
1B1	<1	
2A6	~4	~30 - 100-fold
2B6	<1	~50-fold
2C	~18	25-100-fold
2D6	Up to 2.5	>1000-fold
2E1	Up to 7	~20-fold
2F1		
2J2		
3A4	Up to 28 30-60*	~20-fold 90-fold*
4A, 4B		

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

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

CYP Enzyme	Examples of substrates
1A1	Caffeine, Testosterone, **R-Warfarin**
1A2	Acetaminophen, Caffeine, Phenacetin, **R-Warfarin**
2A6	17β-Estradiol, Testosterone
2B6	Cyclophosphamide, Erythromycin, Testosterone
2C-family	Acetaminophen, Tolbutamide (2C9); Hexobarbital, S-Warfarin (2C9,19); Phenytoin, Testosterone, **R- Warfarin**, Zidovudine (2C8,9,19);
2E1	Acetaminophen, Caffeine, Chlorzoxazone, Halothane
2D6	Acetaminophen, Codeine, Debrisoquine
3A4	Acetaminophen, Caffeine, Carbamazepine, Codeine, Cortisol, Erythromycin, Cyclophosphamide, S- and **R-Warfarin**, Phenytoin, Testosterone, Halothane, Zidovudine

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

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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**

F. Gonzalez, 2009 16

Factors Influencing Activity and Level of CYP Enzymes

Nutrition	**1A1;1A2; 1B1, 2A6, 2B6, 2C8,9,19; 2D6, 3A4,5**
Smoking	**1A1;1A2, 2E1**
Alcohol	**2E1**
Drugs	**1A1,1A2; 2A6; 2B6; 2C; 2D6; 3A3, 3A4,5**
Environment	**1A1,1A2; 2A6; 1B; 2E1; 3A3, 3A4,5**
Genetic Polymorphism	**1A; 2A6; 2C9,19; 2D6; 2E1**

Red indicates enzymes important in drug metabolism

Adapted from: *S. Rendic Drug Metab Rev 34: 83-448, 2002* 17

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

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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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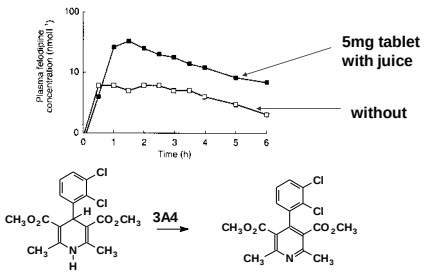
Effect of Grapefruit Juice on Felodipine Plasma Concentration



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

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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**

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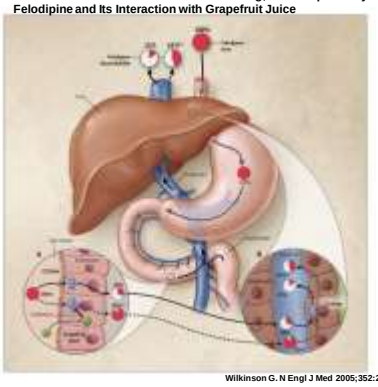
First-Pass Metabolism after Oral Administration of a Drug, as Exemplified by Felodipine and Its Interaction with Grapefruit Juice



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

Limited Expression of Human Drug Metabolizing CYPs in Extrahepatic Tissues

CYP Enzyme	Tissue
1A1	Lung, kidney, GI tract, skin, placenta, others
1B1	Skin, kidney, prostate, mammary,others
2A6	Lung, nasal membrane, others
2B6	GI tract, lung
2C	GI tract (small intestine mucosa) larynx, lung
2D6	GI tract
2E1	Lung, placenta, others
2F1	Lung, placenta
2J2	Heart
3A4	GI tract, lung, placenta, fetus, uterus, kidney
4B1	Lung, placenta
4A11	Kidney

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

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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

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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**

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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.**

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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**

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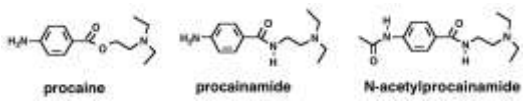
Hydrolysis – Ester or Amide



- 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)

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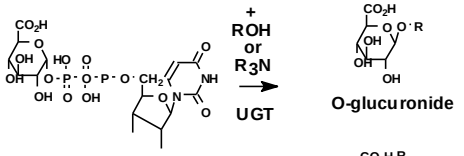
Conjugation Reactions
Glucuronidation



UDP-α-D-glucuronic acid

O-glucuronide

N^+-glucuronide

Liver has several soluble UDP-Gluc-transferases

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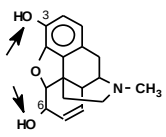
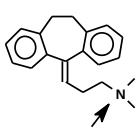
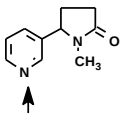


Morphine



Amitriptyline



Cotinine

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

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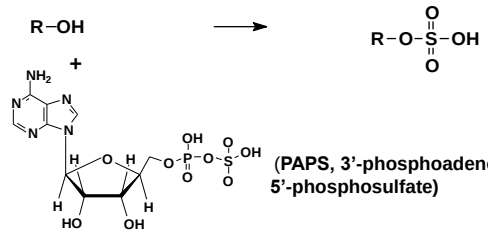
Conjugation Reactions
Sulfation



Examples: ethanol, p-hydroxyacetanilide, 3-hydroxycoumarin

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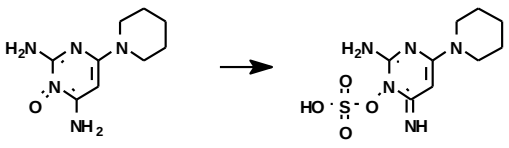

Sulfation may produce active metabolite

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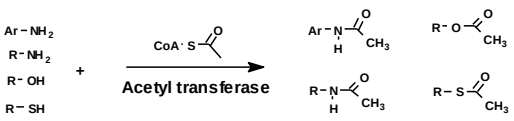
Conjugation Reactions
Acetylation



Examples: Procainamide, isoniazid, sulfanilimide, histamine

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

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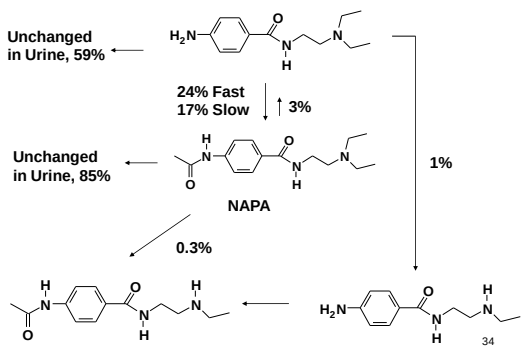
Procainamide



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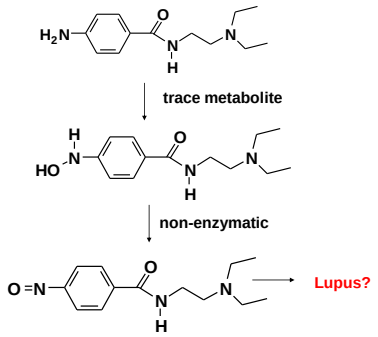
Procainamide



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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**

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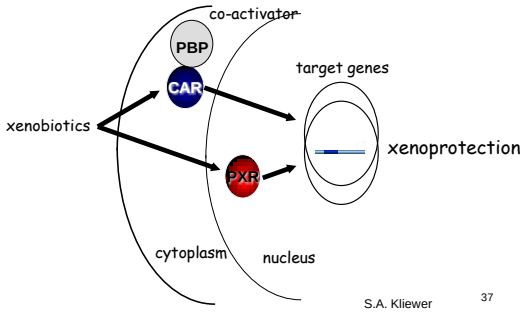
PXR and CAR Protect Against Xenobiotics



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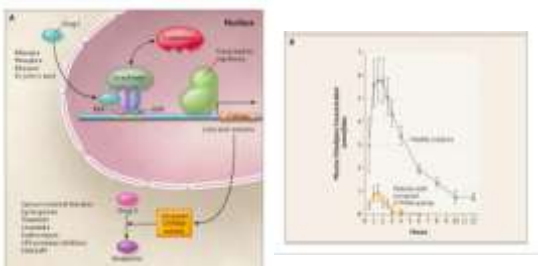
Mechanism of Induction of CYP3A4-Mediated Metabolism of Drug Substrates (Panel A) and the Resulting Reduced Plasma Drug Concentration (Panel B)



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

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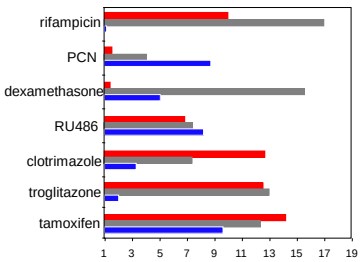
CYP3A Inducers Activate Human, Rabbit, and Rat PXR



Cell-based reporter assay

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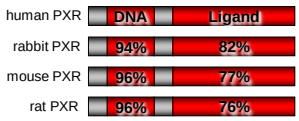
Pregnane X Receptor (PXR)



- 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)

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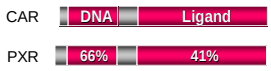
Constitutive Androstane Receptor (CAR)



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- 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)

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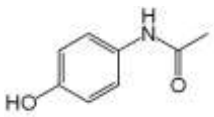
Acetaminophen (APAP)

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



C8H9NO2, MW 151.16

Aspirin, C9H8O4, MW 180.16

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

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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**

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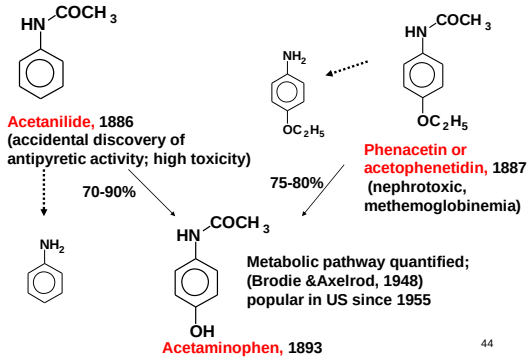
Acetaminophen and p-Aminophenols



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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

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Poisoning Fatalities U.S. 2006

Categories associated with largest numbers of fatalities

Substance	Number
Sedative/hypnotics/antipsychotics	382
Opioids	307
Cardiovascular Drugs	252
Acetaminophen in combination	214
Antidepressants	210
Stimulants and street drugs	203
Alcohols	139
Acetaminophen only	138

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

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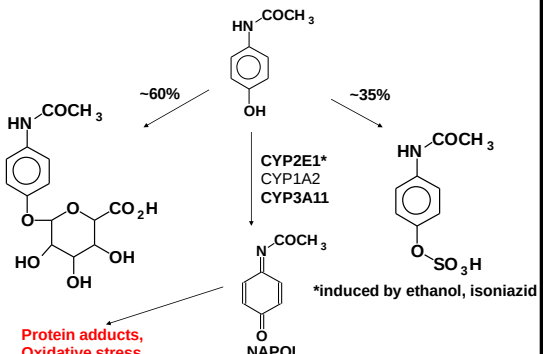
Acetominophen Metabolism



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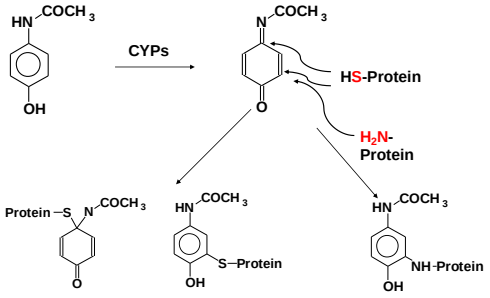
Acetaminophen Protein Adducts



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

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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**)**

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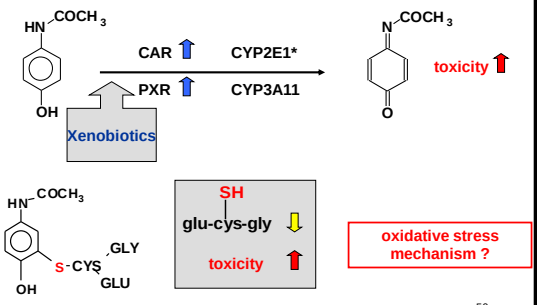
NAPQI toxicity linked to PXR activation

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



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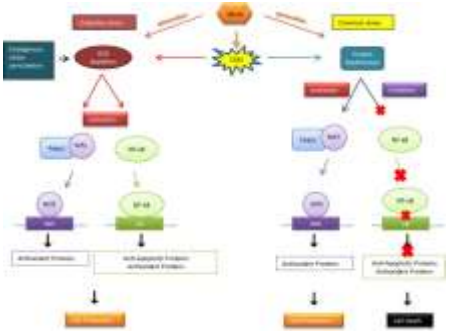
Differential effect of covalent protein modification and GSH depletion on the transcriptional response of Nrf2 and NF-κB



Chia et al., Biochem Pharm. 80 (2010)410-421

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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**
- **http://elearn.pharmacy.ac.uk/flash/view/Cytochrome_P450.html**
  - **Animation of mechanism of Cytochrome P450**

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