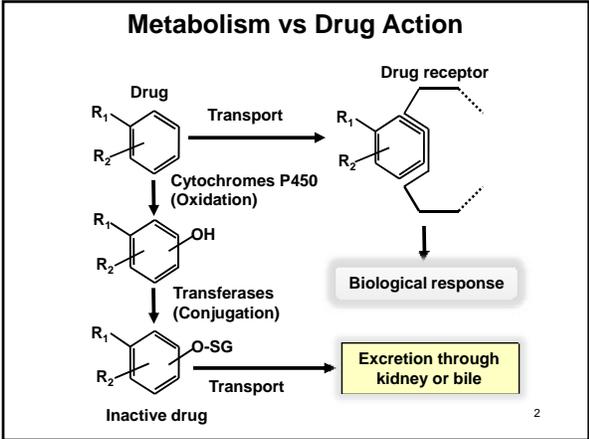
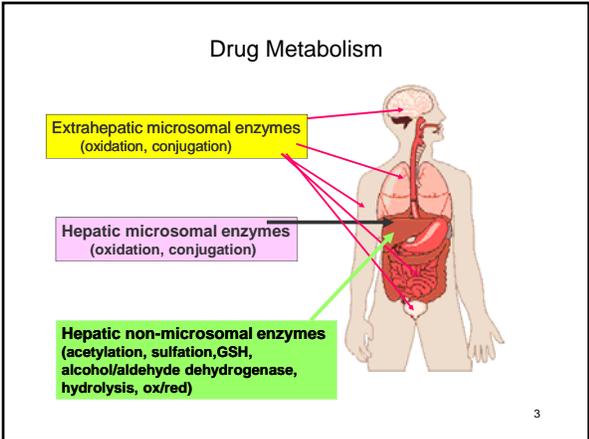


Drug Metabolism

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

1



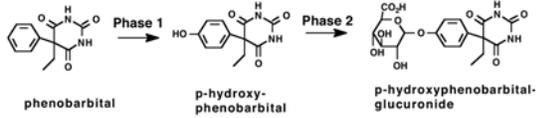


Liver Microsomal System

•Oxidative Reactions: Cytochrome P450 mediated

– Formation of an inactive polar metabolite

• Phenobarbital



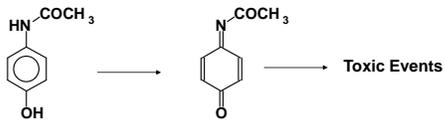
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Liver Microsomal System

•Oxidative Reactions: Cytochrome P450 mediated

– Formation of a toxic metabolite

• Acetaminophen – NAPQI



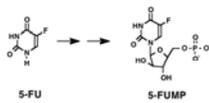
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Liver Microsomal System

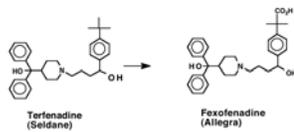
•Oxidative Reactions: Cytochrome P450 mediated

– Formation of an active metabolite

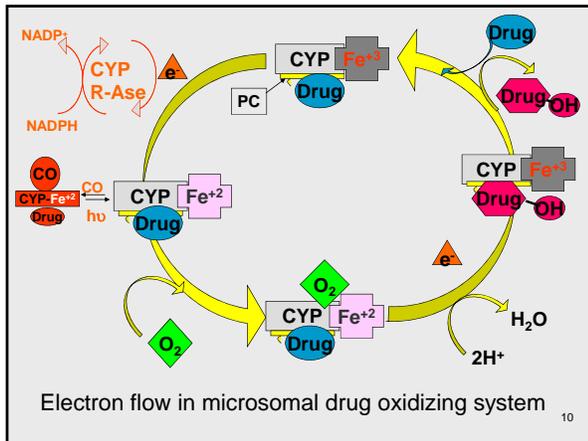
• By Design: Purine & pyrimidine chemotherapy



• Inadvertent: terfenadine – fexofenadine



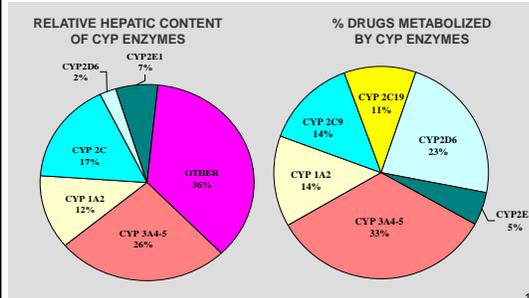
6



- Cytochrome P450 Isoforms (CYPs) - An Overview
- $\text{NADPH} + \text{H}^+ + \text{O}_2 + \text{Drug} \rightarrow \text{NADP}^+ + \text{H}_2\text{O} + \text{Oxidized Drug}$
 - 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
- ¹¹

- 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
- ¹²

ROLE OF CYP ENZYMES IN HEPATIC DRUG METABOLISM



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

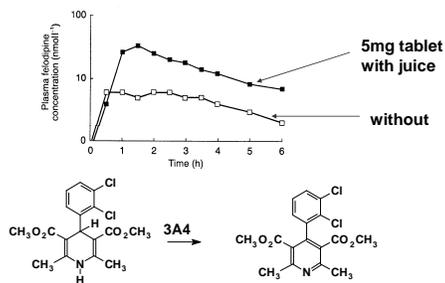
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Overheard Conversation

- 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



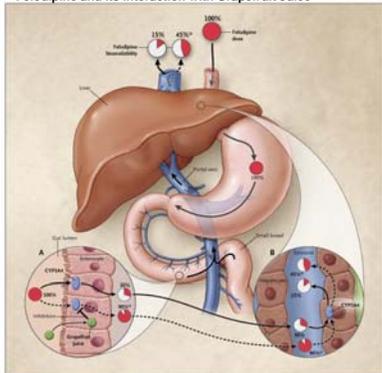
Review- D.G. Bailey, et al.; Br J Clin Pharmacol 1998, 46:101-110 20

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



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 Rxns)**
 - 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.

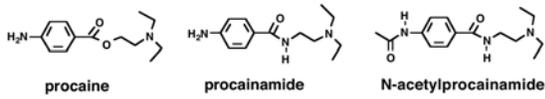
26

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 monooxygenating 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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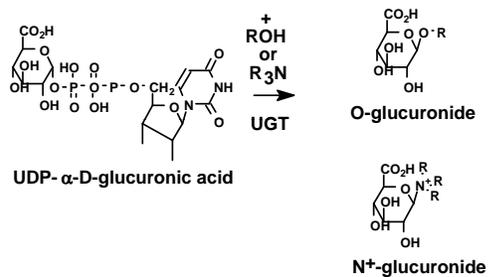
Hydrolysis – Ester or Amide



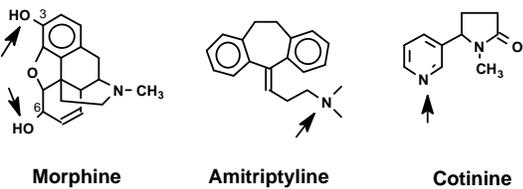
- Procaine – ester, rapidly hydrolyzed
- Procainamide - amide, more slowly hydrolyzed; valuable anti-arrhythmic
- N-acetylprocainamide (NAPA); metabolite with anti-arrhythmic activity, 2.5 x longer elimination half-life (Atkinson et al., 1988, *Angiology*, 39, 655-67)

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Conjugation Reactions Glucuronidation



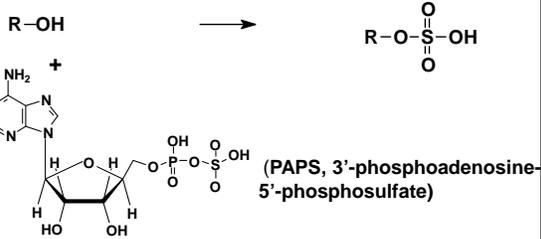
Liver has several soluble UDP-Gluc-transferases 29



Glucuronic acid conjugation to phenols, 3^o-amines, aromatic amines

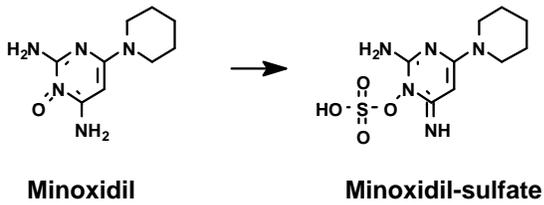
30

Conjugation Reactions Sulfation



Examples: ethanol, p-hydroxyacetanilide, 3-hydroxycoumarin

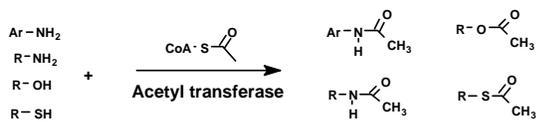
31



Sulfation may produce active metabolite

32

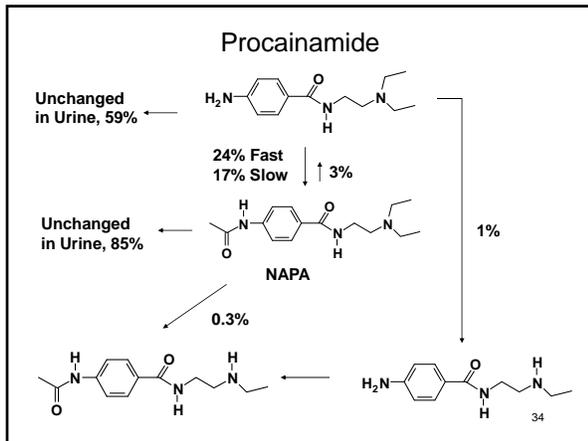
Conjugation Reactions Acetylation

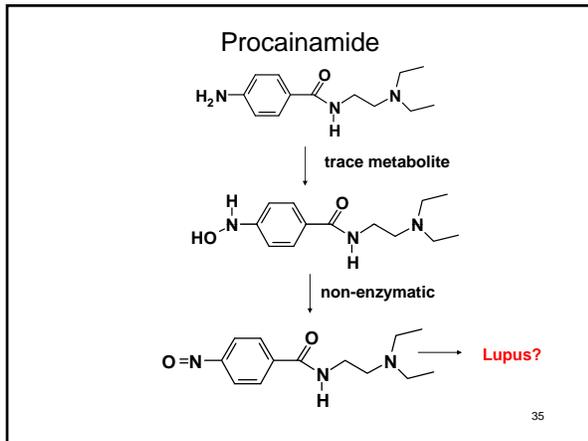


Examples: Procainamide, isoniazid, sulfanilimide, histamine

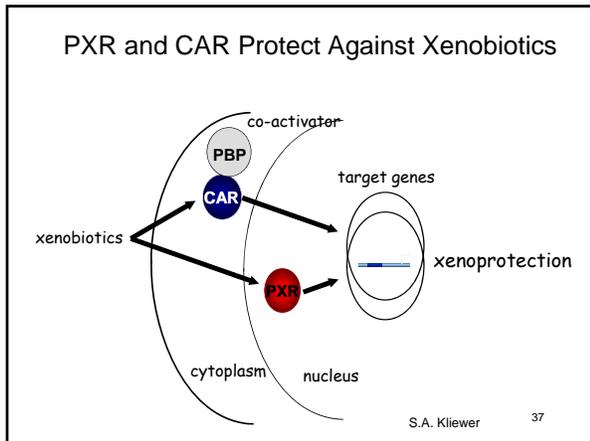
N-acetyl transferase (NAT) enzyme is found in many tissues, including liver

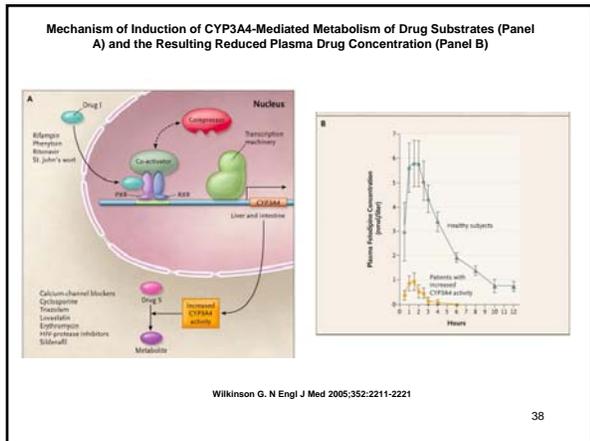
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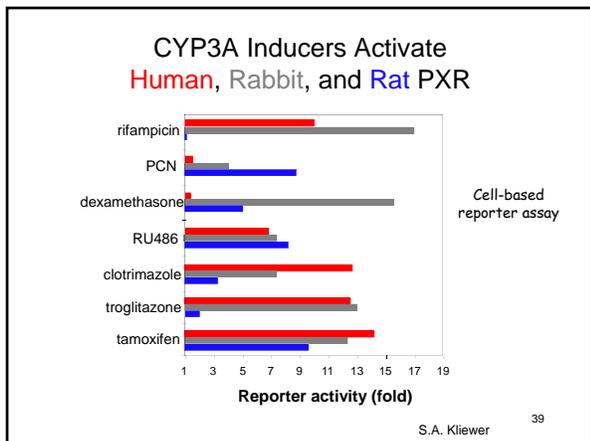




- Additional Effects on Drug Metabolism**
- **Species Differences**
 - Major differences in different species have been recognized for many years (R.T. Williams).
 - Phenylbutazone half-life is 3 h in rabbit, ~6 h in rat, guinea pig, and dog and 3 days in humans.
 - **Induction**
 - Two major categories 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.
 - 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
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Pregnane X Receptor (PXR)

	DNA	Ligand
human PXR	94%	82%
rabbit PXR	96%	77%
mouse PXR	96%	76%

- 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 16 α -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 40

Constitutive Androstane Receptor (CAR)

	DNA	Ligand
CAR	66%	41%
PXR		

S.A. Kliewer

- 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.

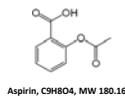
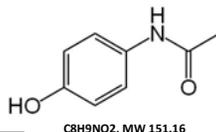
Co-administration:

Sedative
Antihistamine
Vasoconstrictants
Expectorants
Antitussive
Analgesics



Tylenol

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



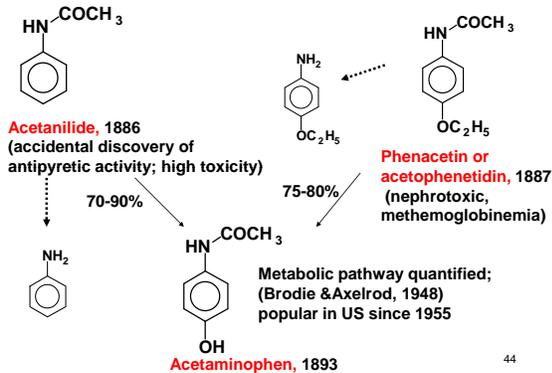
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Acetaminophen (Paracetamol)

- 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



44

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

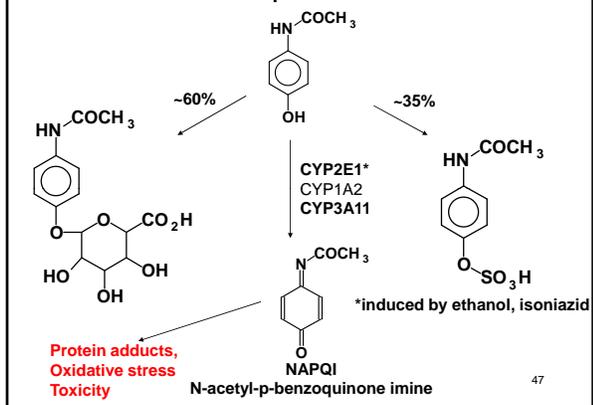
Categories associated with largest numbers of fatalities

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>

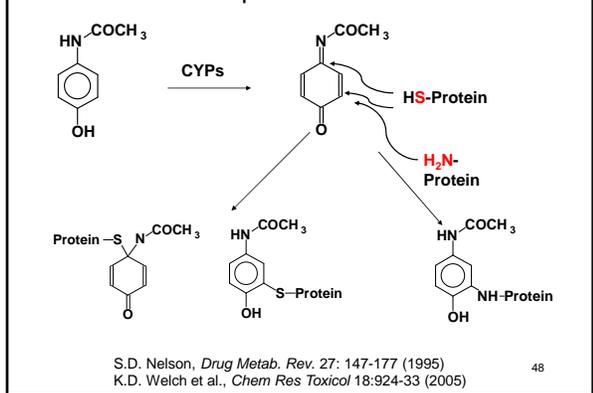
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Acetaminophen Metabolism



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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)

48

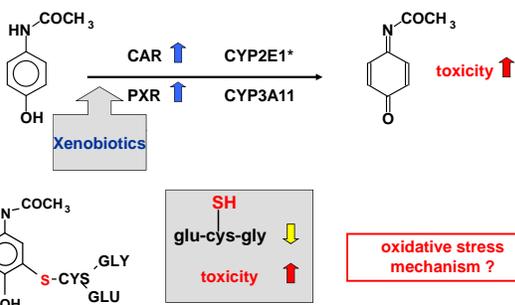
Acetaminophen toxicity mechanism

- 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)

49

NAPQI toxicity linked to PXR activation

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



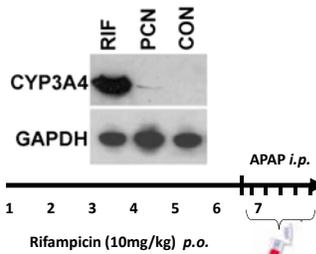
50

Experimental Design



Human PXR and rifampicin
Antibiotic, specific ligand
for human PXR

Curr Drug Metab, 3(5):481-90, 2002.



APAP *i.p.*
1, 2, 4, 8hr 24hr
Serum Urine

Cheng et al. Drug Metab Dispos. 2009 37(8):1611-21.
