PHRM 836 September 17, 2015

Cytochromes P450

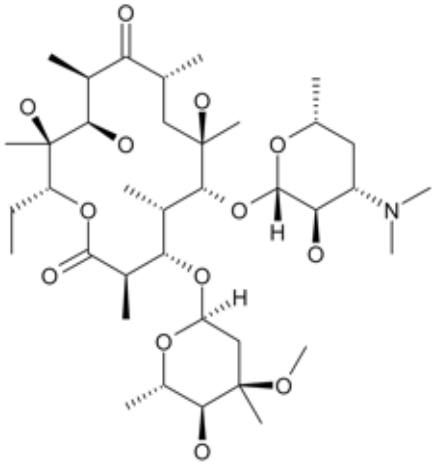
Devlin Chapter 11.1-7

- Catalytic function and structure
- Role in bio-synthesis and drug metabolism
- Inhibition and induction
- Drug interactions

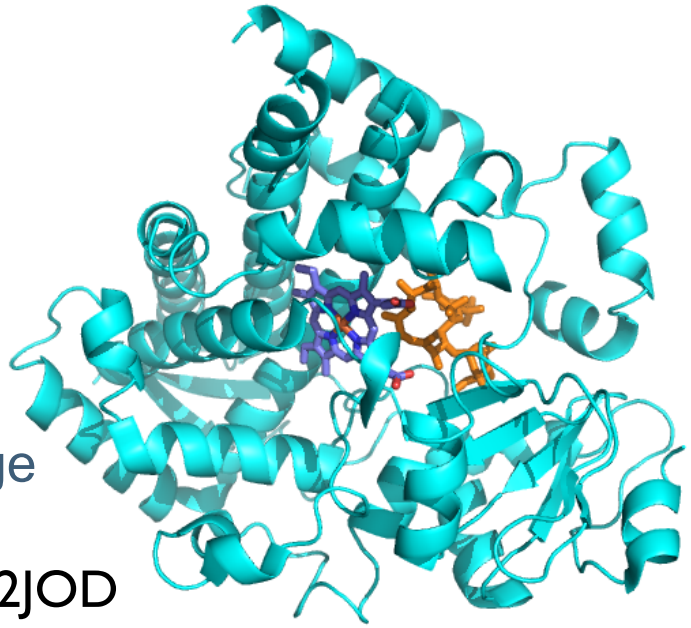
Cytochromes P450

- Introduction:
  - a powerful detoxification system
  - Works on unusual chemicals (drugs, poisonous compounds, carcinogens obtained from eating, breathing)
  - Converts them to a form (by adding oxygen) more readily flushed from the body
  - A first line of defense against toxins
- A family of over 7000 proteins, present in all organisms.
  - Many different forms; act on different selection of molecules
    - Bacteria have ~ 20
    - Humans have ~ 60
    - Plants can have 100s (unusual pigments and toxins for protection)

Cytochromes P450



- Catalysis: monooxygenase.
  - Catalyze Insertion of one atom of molecular oxygen
- Drug interactions of cytochrome P450s
  - Major role in drug detoxification
  - type CYP3A4 estimated to act on ~ 50% of known drugs
  - e.g. the antibiotic erythromycin
  - Some reactions are harmful
    - CYP3A4 catalysis of acetaminophen (Tylenol) generates a highly reactive compound leading to toxicity at high dosage



PDB entry: 2JOD
P450 3A4 in complex
with erythromycin

Cytochromes P450

- Substrates are numerous and diverse compounds.
  - Endogenous – cholesterol, steroid hormones, and fatty acids.
  - Exogenous – drugs, food additives, and environmental contaminants (ex. cigarette smoke).

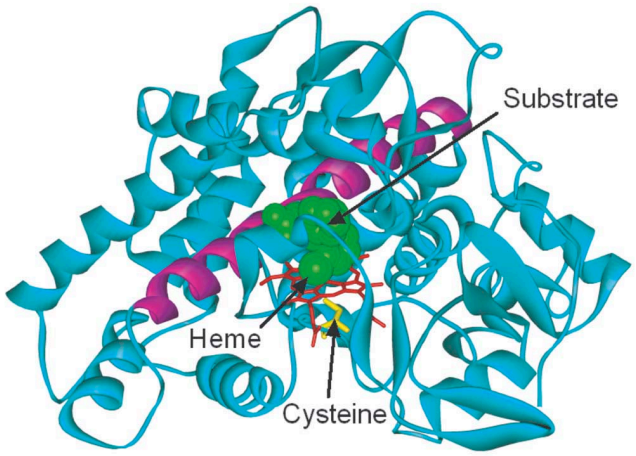
Huge variety of reactions!

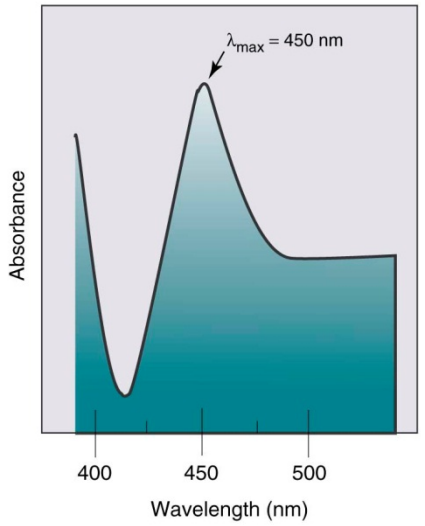
- Biological functions
  - Production of steroid hormones, vitamins A and D, lipid-like eicosanoid molecules involved in signaling
  - Metabolism of fatty acids and eicosanoids
    - e.g. P450 CYP51,
      essential in eukaryotic sterol biosynthesis.
  - Detoxification
  - Many substrates are lipid-soluble; hydroxylation increases solubility

PDB entry: 1EA1
P450 CYP51

Cytochrome P450

- Characteristic absorbance at 450 nm when cyanide is bound.
  - P450 – **P**igment with an absorbance at **450** nm
- Integral membrane protein with a single heme group
- Associated with the membrane by an N-terminal membrane anchoring sequence.
- The structure is well conserved in all known cytochrome P450.
  - Conformational changes can occur upon ligand binding
- The heme iron can form six bonds.
  - Four with porphyrin ring.
  - One with a protein residue.
  - The last one can be open or occupied by O_2 or other ligand.





Nomenclature

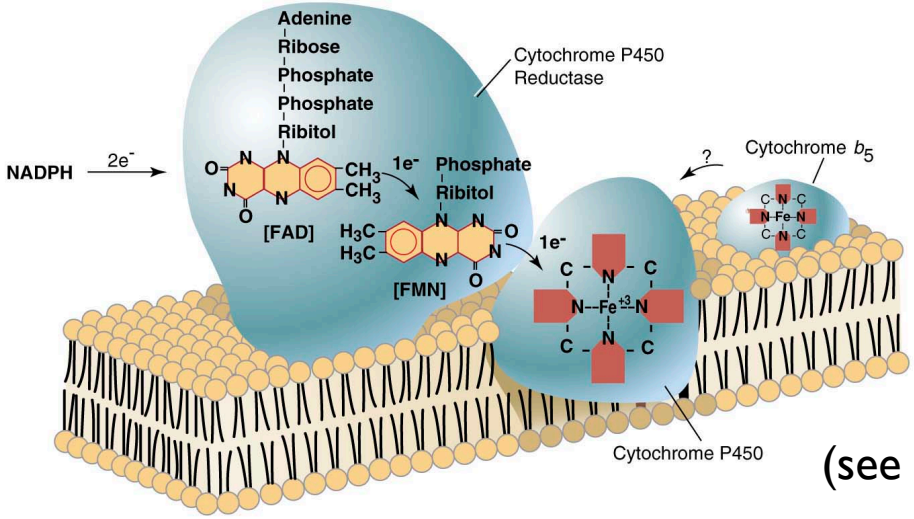
- The superfamily of cytochrome P450 – over 7,000 cytochromes P450 have been identified.
- The superfamily is divided into families: CYP1, CYP2, CY3, etc. (the sequence identity of the members > 40%)
- Each family is divided into subfamilies: CYP1A, CYP1B, CYP1C, etc. (the sequence identity of the members > 55%)
- The individual members of each subfamily are numbered: CYP1A1, CYP1A2, CYP1A3, etc.
- Human has 57 cytochromes P450s, which belong to 18 families and 41 subfamilies.

Cytochrome P450 catalysis

- Overall reaction

  $NADPH + H^+ + O_2 + R\text{-}H \rightarrow NADP^+ + H_2O + R\text{-}OH$

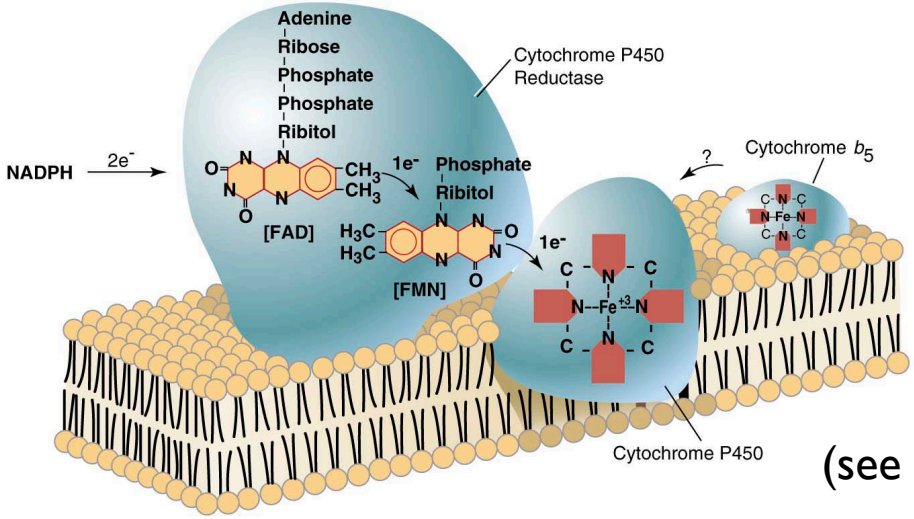
- O_2 is activated and cleaved; one to the product, the other to water.
- Electron transport systems in endoplasmic reticulum (microsomal; 50 of 57 isoforms) and mitochondria (7 of 57 isoforms)



Devlin Figure 11.5
Components for ER system
(see also 11.7 for mitochondrial system)

Cytochrome P450 catalysis

- NADPH is a two-electron donor, but the heme iron can accept only one electron at a time ($Fe^{3+} \rightarrow Fe^{2+}$).
- Electron transfer to cytochrome P450 is by NADPH-cytochrome P450 reductase relays the electron from NADPH to cytochrome P450 one at a time.
- Role of cytochrome b_5 is not understood and varies among the different P450s



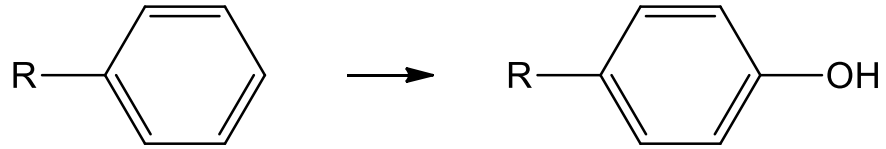
Devlin Figure 11.5
Components for ER system
(see also 11.7 for mitochondrial system)

Common reactions catalyzed by cytochromes P450

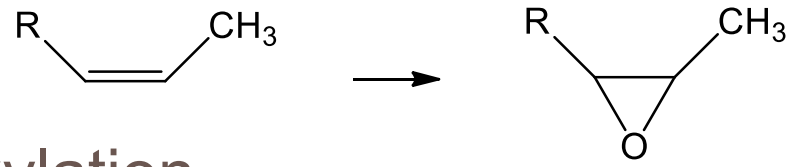
- Aliphatic hydroxylation

  $R\text{-}CH_2\text{-}CH_3 \rightarrow$ _____

- Aromatic hydroxylation



- Epoxidation



- Dealkylation

$R\text{-}CH_2\text{-}NH\text{-}CH_3 \rightarrow R\text{-}CH_2\text{-}NH\text{-}CH_2\text{-}OH \rightarrow R\text{-}CH_2\text{-}NH_2 + HCHO$

  - N or O or S-dealkylation

- N-oxidation

  $R\text{-}CH_2\text{-}CH_2\text{-}NH_2 \rightarrow R\text{-}CH_2\text{-}CH_2\text{-}NH\text{-}OH$

Cytochromes P450: oxygenation of endogenous compounds

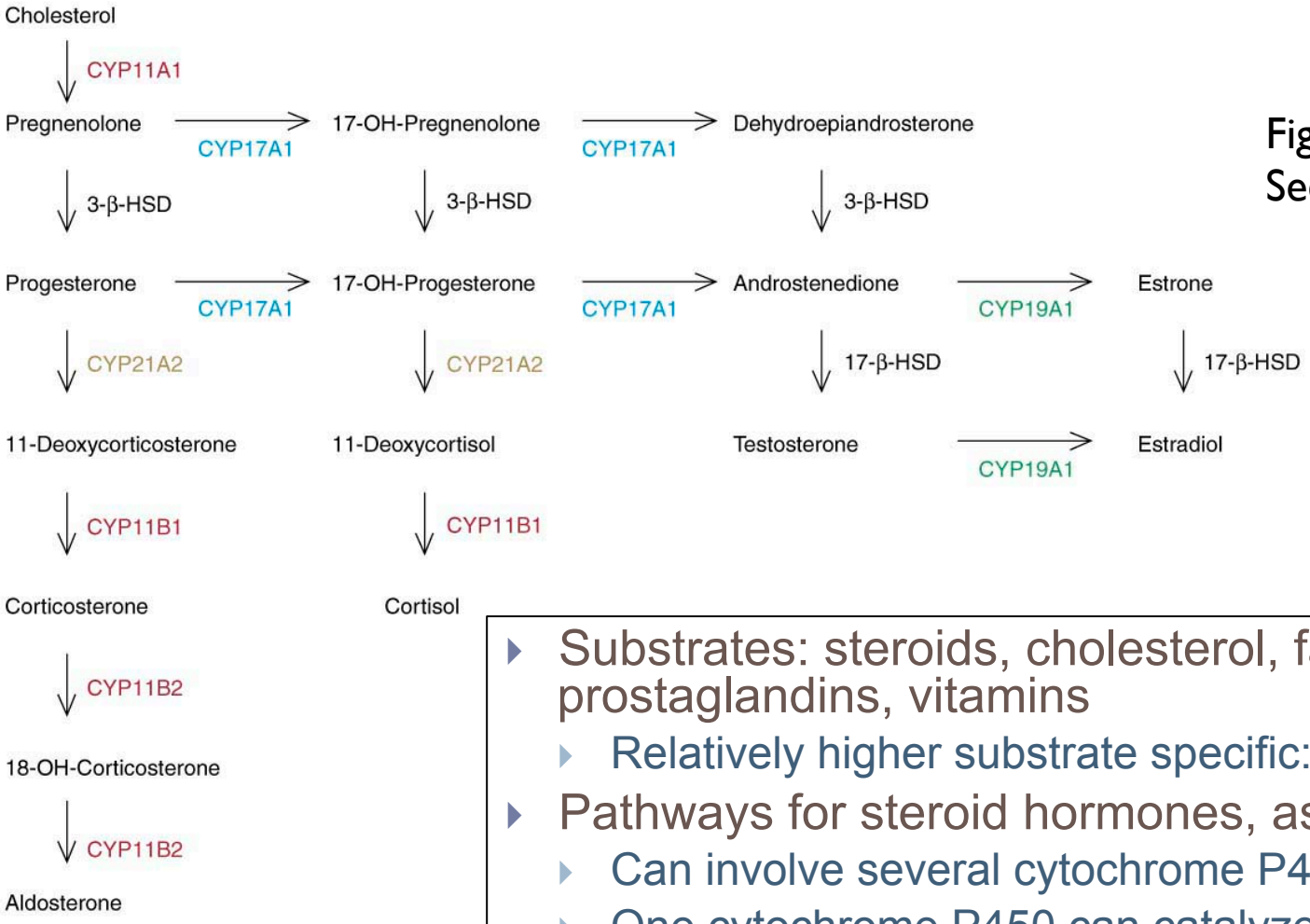


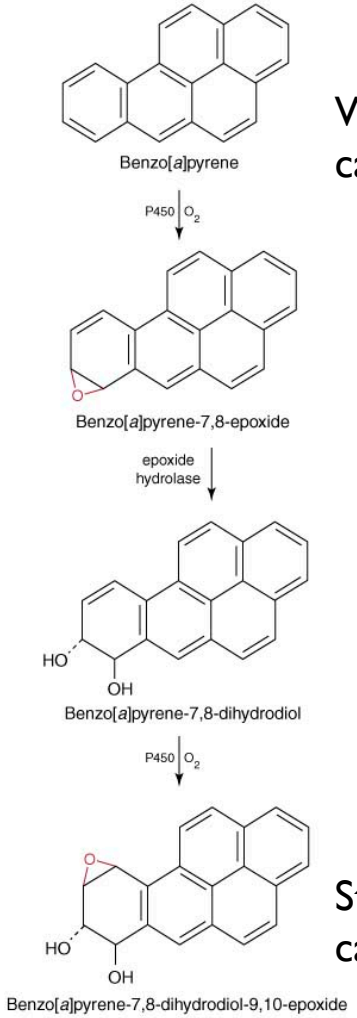
Figure 11.9
See Table 11.2

- Substrates: steroids, cholesterol, fatty acids, prostaglandins, vitamins
  - Relatively higher substrate specific: regio and stereo
- Pathways for steroid hormones, as an example
  - Can involve several cytochrome P450s
  - One cytochrome P450 can catalyze multiple steps

Cytochromes P450: oxidize exogenous compounds, i.e. xenobiotics

- Lipophilic xenobiotics ("foreign to life"): therapeutic drugs, food additives, and environmental contaminants.
  - Promotes elimination
  - 1 of 2 phases for metabolizing xenobiotics; 2nd is biosynthetic rxs such as linking to glutathione, sulfate, etc
  - Cyp3A4 present in gastrointestinal tract and liver; responsible for poor oral bioavailability of some drugs
- P450 isoforms are less discriminating
  - Variety of lipophilic substrates
  - Multiple sites of oxidation (lower regioselectivity)
- Metabolism of xenobiotics and drugs has three possible outcomes.
  - Inactivation (e.g. drug metabolism)
  - Activation (e.g. Prodrug conversion)
  - Formation of a highly toxic metabolite (e.g. Benzo[a]pyrene from coal burning, cigarettes, charcoal briquettes)

Figure 11.14



Weak carcinogen

Strong carcinogen

Cytochrome P450: induction and inhibition

- Role in metabolism of drugs means sensitivity to level of enzymatic activity of cytochrome P450s
  - Xenobiotics/drugs induce expression of the cytochrome P450 that metabolizes that compound
  - Particular xenobiotics/drugs can also inhibit certain cytochrome P450s
  - Unintended effects on one drug can occur due to another drug inducing/ inhibiting P450 levels

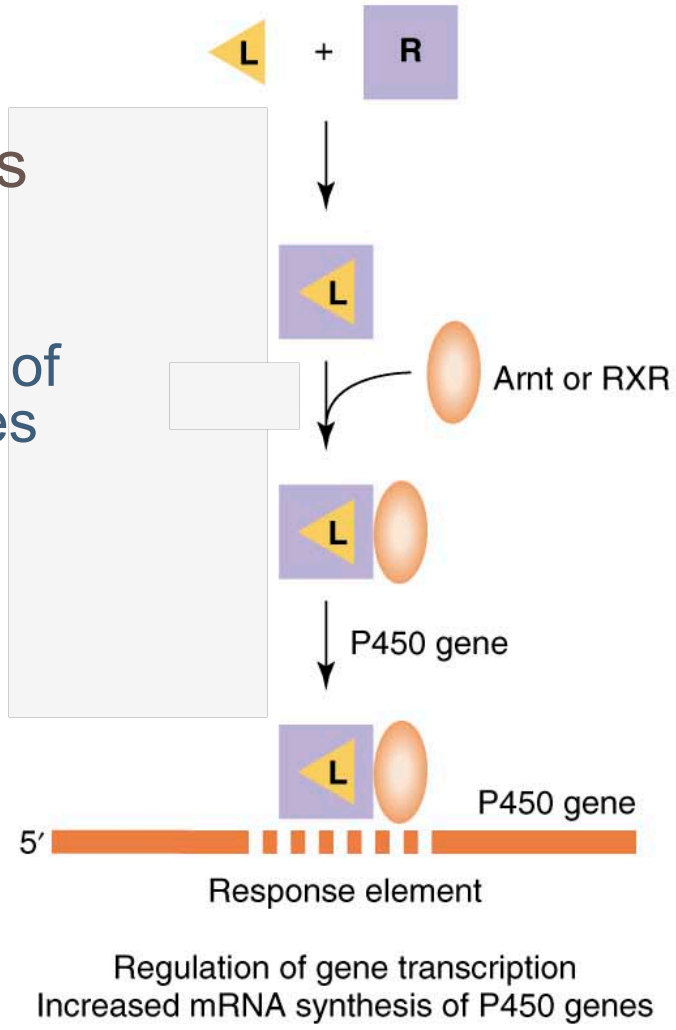
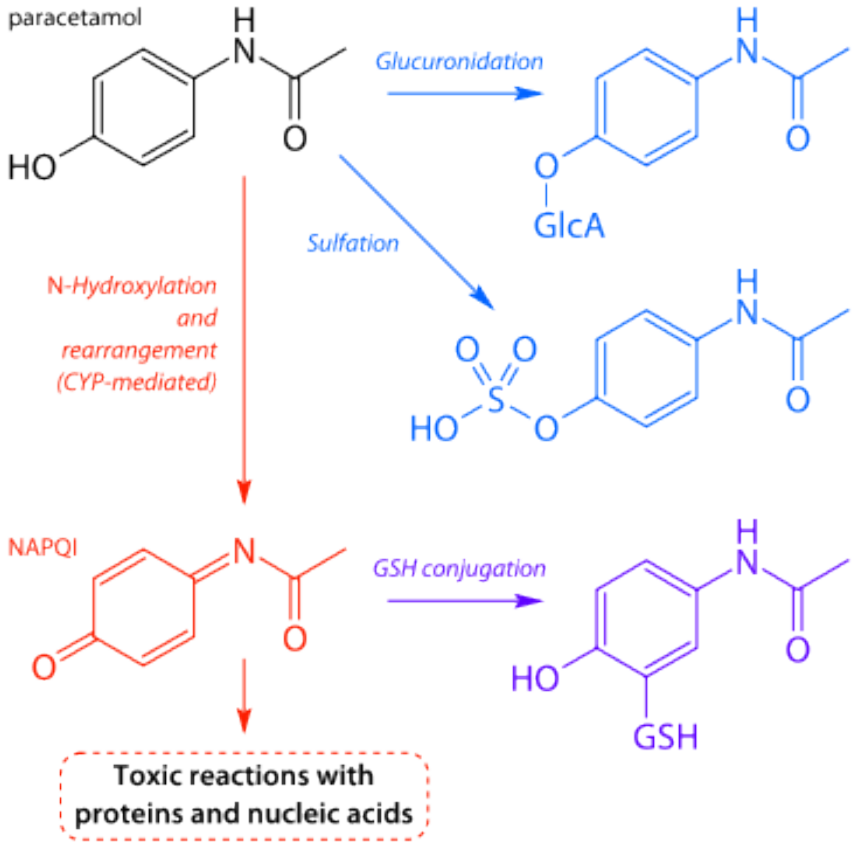


Figure 11.16

Clinical correlation 11.4: Acetaminophen-induced liver toxicity

(other drug-interaction examples p. 438-441 incl CC 11.3, 11.5)

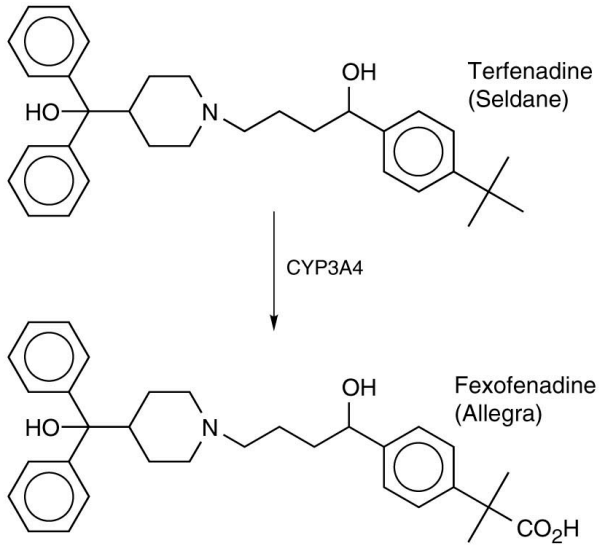
- Normally fraction of acetaminophen metabolized by CYP2E1 is small.
- Large doses of acetaminophen increase NAPQI and liver damage
  - 35% of cases involving liver failure are caused by acetaminophen poisoning.
- NAPQI is normally conjugated by glutathione (GSH) to a nontoxic form, but high doses of acetaminophen can deplete glutathione pool.
- Alcohol induces CYP2E1 and also leads to increased NAPQI.
  - Effects depend on timing of consumption of alcohol and acetaminophen; alcohol is also a substrate of CYP2E1 and so inhibits its metabolism of other drugs



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

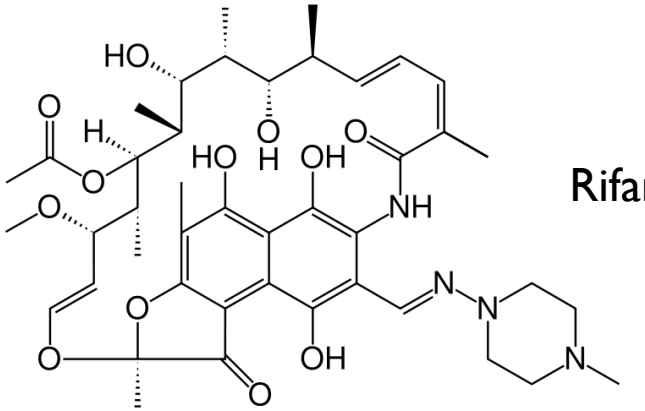
CYP3A4: induction and inhibition

- Terfenadine (Seldane®)
  - H1 antihistamine used to treat seasonal allergies.
  - Prodrug – rapidly metabolized by CYP3A4 to fexofenadine, which is the active compound.
  - Other drugs that inhibit CYP3A4 may increase plasma levels of terfenadine.
    - Erythromycin – antibiotic
    - Ketoconazole – antifungal
  - Replaced by the nontoxic metabolite, fexofenadine (Allegra®).

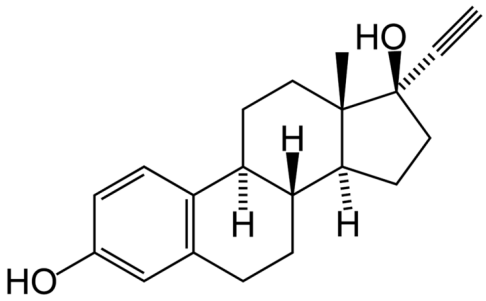


CYP3A4: induction and inhibition

- Rifampin
  - Antituberculosis drug
  - upregulates CYP3A4 increases rate of metabolism of many drugs cleared through the liver
  - Increases the elimination of warfarin and may increase the risk of undertreating anticoagulation
- St. John's wort
  - Herbal medicine for mild depression, which can be purchased without a prescription
  - Induces CYP3A4



Rifampin



Ethynyl estradiol

Summary: Cytochromes P450 and Nitric Oxid Synthases

- P450s are a large class of heme proteins with absorbance at 450 nm, divided into families and subfamilies
- P450s play many biological roles
- P450s are involved in numerous drug interactions, and their gene expression is affected by xenobiotics/drugs
- P450s catalyze a wide range of chemical reactions on a large set of substrates. The reaction involves O_2 and electron transfer from a second enzyme called NADPH-cytochrome P450 reductase
- P450 reactions of endogenous substrates are diverse and involved in different metabolic processes e.g. sterol biosynthesis
- P450 reactions of exogenous substrates have 3 types of outcome
- NOS has two enzymes: one flavin-containing with reductase activity and one heme-containing with the oxygenase activity
- Three NOS isoforms exist with different biological roles