Drug Metabolism

• Most metabolic products are less pharmacologically active

  Important exceptions:
  • Where the metabolite is more active
    (Prodrugs, e.g. Erythromycin-succinate (less irritation of GI) --> Erythromycin)
  • Where the metabolite is toxic (acetaminophen)
  • Where the metabolite is carcinogenic

• Close relationship between the biotransformation of drugs and normal biochemical processes occurring in the body:
  – Metabolism of drugs involves many pathways associated with the synthesis of endogenous substrates such as steroid hormones, cholesterol and bile acids
  – Many of the enzymes involved in drug metabolism are principally designed for the metabolism of endogenous compounds
  – These enzymes metabolize drugs only because the drugs resemble the natural compound
Phases of Drug Metabolism

• **Phase I Reactions**
  – Convert parent compound into a more polar (=hydrophilic) metabolite by adding or unmasking functional groups (-OH, -SH, -NH₂, -COOH, etc.)
  – Often these metabolites are inactive
  – May be sufficiently polar to be excreted readily

• **Phase II Reactions**
  – Conjugation with endogenous substrate to further increase aqueous solubility
  – Conjugation with glucoronide, sulfate, acetate, amino acid
  – Phase I usually precede phase II reactions

Liver is principal site of drug metabolism:
  – Other sites include the gut, lungs, skin and kidneys
  – For orally administered compounds, there is the “First Pass Effect”
    • Intestinal metabolism
    • Liver metabolism
    • Enterohepatic recycling
    • Gut microorganisms - glucuronidases
Drug Metabolism

- **Hydrophilic drug**
  - Renal excretion

- **Lipophilic drug**
  - Excretion impossible

- **Lipophilic drug**
  - Slow conversion in liver to hydrophilic metabolite
  - Renal excretion of metabolite

- **Lipophilic drug**
  - Rapid and complete conversion in liver to hydrophilic metabolite
  - Renal excretion of metabolite
Drug Metabolism - Phase I

• Phase I Reactions
  – Oxidation
  – Reduction
  – Hydrolytic cleavage
  – Alkylation (Methylation)
  – Dealkylation
  – Ring cyclization
  – N-carboxylation
  – Dimerization
  – Transamidation
  – Isomerization
  – Decarboxylation
Drug Metabolism - Oxidation

Two types of oxidation reactions:
- Oxygen is incorporated into the drug molecule (e.g. hydroxylation)
- Oxidation causes the loss of part of the drug molecule (e.g. oxidative deimination, dealkylation)

Microsomal Mixed Function Oxidases (MFOs)

- “Microsomes”
  form in vitro after cell homogenization and fractionation of ER
  - Rough microsomes are primarily associated with protein synthesis
  - Smooth microsomes contain a class of oxidative enzymes called

- “Mixed Function Oxidases” or “Monooxygenases”
  - These enzymes require a reducing agent (NADPH) and molecular oxygen (one oxygen atom appearing in the product and the other in the form of water)
Drug Metabolism - Oxidation

• **MFO consists of two enzymes:**

  – **Flavoprotein, NADPH-cytochrome c reductase**
    • One mole of this enzyme contains one mole each of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD)
    • Enzyme is also called NADPH-cytochrome P450 reductase

  – **Cytochrome P450**
    • named based on its light absorption at 450 nm when complexed with carbon monoxide
    • is a hemoprotein containing an iron atom which can alternate between the ferrous (Fe++) and ferric (Fe+++ ) states
    • Electron acceptor
    • Serves as terminal oxidase
    • its relative abundance compared to NADPH-cytochrome P450 reductase makes it the rate-limiting step in the oxidation reactions
Drug Metabolism - Oxidation

- **Humans have 18 families of cytochrome P450 genes and 43 subfamilies:**
  - CYP1 drug metabolism (3 subfamilies, 3 genes, 1 pseudogene)
  - CYP2 drug and steroid metabolism (13 subfamilies, 16 genes, 16 pseudogenes)
  - **CYP3 drug metabolism** (1 subfamily, 4 genes, 2 pseudogenes)
  - CYP4 arachidonic acid or fatty acid metabolism (5 subfamilies, 11 genes, 10 pseudogenes)
  - CYP5 Thromboxane A2 synthase (1 subfamily, 1 gene)
  - CYP7A bile acid biosynthesis 7-alpha hydroxylase of steroid nucleus (1 subfamily member)
  - CYP7B brain specific form of 7-alpha hydroxylase (1 subfamily member)
  - CYP8A prostacyclin synthase (1 subfamily member)
  - CYP8B bile acid biosynthesis (1 subfamily member)
  - CYP11 steroid biosynthesis (2 subfamilies, 3 genes)
  - CYP17 steroid biosynthesis (1 subfamily, 1 gene) 17-alpha hydroxylase
  - CYP19 steroid biosynthesis (1 subfamily, 1 gene) aromatase forms estrogen
  - CYP20 Unknown function (1 subfamily, 1 gene)
  - CYP21 steroid biosynthesis (1 subfamily, 1 gene, 1 pseudogene)
  - CYP24 vitamin D degradation (1 subfamily, 1 gene)
  - CYP26A retinoic acid hydroxylase important in development (1 subfamily member)
  - CYP26B probable retinoic acid hydroxylase (1 subfamily member)
  - CYP26C probable retinoic acid hydroxylase (1 subfamily member)
  - CYP27A bile acid biosynthesis (1 subfamily member)
  - CYP27B Vitamin D3 1-alpha hydroxylase activates vitamin D3 (1 subfamily member)
  - CYP27C Unknown function (1 subfamily member)
  - CYP39 7 alpha hydroxylation of 24 hydroxy cholesterol (1 subfamily member)
  - CYP46 cholesterol 24-hydroxylase (1 subfamily member)
  - CYP51 cholesterol biosynthesis (1 subfamily, 1 gene, 3 pseudogenes) lanosterol 14-alpha demethylase
Drug Metabolism - Oxidation

• **Induction of P450 enzymes:**
  - PPAR (peroxisome proliferator activated receptor) ligands (e.g. clofibrate)
  - CYP1 family are induced by aromatic hydrocarbons (cigarette smoke; charred food)
  - CYP2E enzymes induced by ethanol
  - CYP2B enzymes induced 40-50 fold by barbiturates

• **Polymorphisms cause differences in drug metabolism:**
  - CYP2C19 has a polymorphism that changes the enzyme's ability to metabolize mephenytoin (a marker drug). In **Caucasians**, the polymorphism for the poor metabolizer phenotype is only seen in 3% of the population. However, it is seen in 20% of the **Asian** population.
    => It is important to be aware of a person's race when drugs are given that are metabolized differently by different populations

• **P450s and drug interactions:**
  - **Barbiturates** induce CYP2B => increased metabolism of other drugs
  - **Antifungals** (e.g. ketoconazole) inhibit fungal CYP51 and unintentionally also human CYP3A4
    => reduced metabolism of other drugs
  - **Grapefruit juice** contains a CYP3A4 inhibitor => 12 fold increase in some drug concentrations

**CYP3A4 Substrates:**
- Acetaminophen (Tylenol)
- Codeine (narcotic)
- Cyclosporin A (immunosuppressant)
- Diazepam (Valium)
- Erythromycin (Antibiotic)
- Lidocaine (local anaesthetic)
- Lovastatin (HMGCoA reductase inhibitor)
- Taxol (cancer drug)
- Warfarin (anticoagulant)
Drug Metabolism - Oxidation

- Drug oxidation requires:
  - Cytochrome P450
  - Cytochrome P450 reductase
  - NADPH
  - Molecular oxygen

- The cycle involves four steps:
  1. Oxidized (Fe3+) cytochrome P-450 combines with a drug substrate to form a binary complex.
  2. NADPH donates an electron to the cytochrome P-450 reductase, which in turn reduces the oxidized cytochrome P-450-drug complex.
  3. A second electron is introduced from NADPH via the same cytochrome P-450 reductase, which serves to reduce molecular oxygen and form an "activated oxygen"-cytochrome P-450-substrate complex.
  4. This complex in turn transfers "activated" oxygen to the drug substrate to form the oxidized product. The potent oxidizing properties of this activated oxygen permit oxidation of a large number of substrates.
Drug Metabolism - Oxidation

Aromatic hydroxylation:

Aliphatic hydroxylation:
Drug Metabolism - Oxidation

Epoxidation:

Dealkylation:
Drug Metabolism - Oxidation

**O-demethylation:**

The O-demethylation of codeine.

**S-demethylation:**

The S-demethylation of S-methylthiopurine.

**N-oxidation:**

The N-oxidation of 3-methylpyridine.

**N-hydroxylation:**

The N-hydroxylation of 2-acetylaminofluorene.
Drug Metabolism - Oxidation

Oxidation reactions NOT catalyzed by Cytochrome P450:

Flavin containing monoxygenase system
- Present mainly in liver but some is expressed in gut and lung
- Located in smooth endoplasmic reticulum
- Oxidizes compounds containing sulfur and nitrogen
- Uses NADH and NADPH as cofactors

- Alcohol dehydrogenase (cytosol)
- Aldehyde oxidation (cytosol)
- Xanthine oxidase
- Amine oxidases
  - Monoamine oxidase (nerve terminals, mitochondria)
  - Diamine oxidase found in liver microsomes
    - Primarily endogenous metabolism
Monoamine Oxidases (MAO):

- Catalyze oxidative deamination of endogenous catecholamines (epinephrine)
- Located in nerve terminals and peripheral tissues
- Substrates for catecholamine metabolism found in foods (tyramine) can cause a drug/food interaction

- Inhibited by class of antidepressants called MAO inhibitors
  (Inhibition of MAO isoforms in the CNS also effects levels of serotonin - Tranylcypromine)

These drugs can cause severe or fatal drug/drug interactions with drugs that increase release of catecholamines or inhibit their reuptake in nerve terminals
  (Meperidine, pentazocine, dextromethorphan, SSRI antidepressants)
Drug Metabolism - Reduction

Azo-reduction:

Nitro-reduction:

Dehalogenation:

Reductive defluorination of halothane.
Drug Metabolism - Reduction

Hydrolysis reactions

Ester hydrolysis:

\[
\text{CO.O.(CH}_2\text{)_2.N(C}_2\text{H}_5\text{)_2} + \text{H}_2\text{O} \rightarrow \text{COOH} + \text{HO.(CH}_2\text{)_2.N(C}_2\text{H}_5\text{)_2}
\]

Hydrolysis of procaine.

Amide hydrolysis:

\[
\text{CO.NH.NH}_2 + \text{H}_2\text{O} \rightarrow \text{COOH} + \text{H}_2\text{N}-\text{NH}_2
\]

Hydrolysis of isoniazid.
Drug Metabolism - Phase I

- Almost any drug can undergo modifications by drug-metabolizing enzyme systems.
- Drugs can be subject to several Phase I pathways.
- These reactions create functional groups that place the drugs in a correct chemical state to be acted upon by Phase II conjugative mechanisms.
- Main function of phase I reactions is to prepare chemicals for phase II metabolism and subsequent excretion.
- Phase II is the true “detoxification” step in the metabolism process.
Drug Metabolism - Phase II

- **Conjugation reactions**
  - **Glucuronidation** by UDP-Glucuronosyltransferase:  
    (on -OH, -COOH, -NH₂, -SH groups)
  - Sulfation by Sulfotransferase:  
    (on -NH₂, -SO₂NH₂, -OH groups)
  - Acetylation by acetyltransferase:  
    (on -NH₂, -SO₂NH₂, -OH groups)
  - Amino acid conjugation  
    (on -COOH groups)
  - Glutathione conjugation by Glutathione-S-transferase:  
    (to epoxides or organic halides)
  - Fatty acid conjugation  
    (on -OH groups)
  - Condensation reactions
Drug Metabolism - Glucuronidation

- **Glucuronidation** (= conjugation to $\alpha$-d-glucuronic acid)
  - Quantitatively the **most important phase II pathway** for drugs and endogenous compounds
  - Products are often excreted in the bile.
  - Enterohepatic recycling may occur due to gut glucuronidases
  - Requires enzyme **UDP-glucuronosyltransferase (UGT)**:
    - Genetic family of enzymes
      - Metabolizes a broad range of structurally diverse endogenous and exogenous compounds
      - Structurally related family with approximately 16 isoforms in man

<table>
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<tr>
<th>Substrate</th>
<th>UGT activity$^a$</th>
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<tr>
<td></td>
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<tr>
<td>Simple phenols</td>
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<td>Opioids</td>
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$^a$Data are maximum specific enzyme activities (pmol/min/mg protein). nd, not determined. Adapted from Tukey and Strassburg (2000) *Ann. Rev. Pharmacol. Toxicol.* **40**, 581–616. Note that these substrate specificities have yet to be further refined (see text).
Drug Metabolism - Glucuronidation

- Glucuronidation – requires creation of high energy intermediate: **UDP-Glucuronic Acid:**

\[
\text{Glucose-1-(P) + UTP} \rightarrow \text{UDP-Glucose} \rightarrow \text{Glycogen + PP} \\
+ \text{NAD}^+ \\
\rightarrow \text{UDP-Glucuronic acid} + \text{NADH} + \text{H}^+
\]

Synthesis of UDP-glucuronic acid.
Drug Metabolism - Glucuronidation

- Glucuronidation Pathway and Enterohepatic Recirculation
Drug Metabolism - Glucuronidation

- **N-glucuronidation:**
  - Occurs with *amines* (mainly aromatic)
  - Occurs with *amides* and *sulfonamides*

The glucuronidation of (a) sulfanilamide and (b) cyproheptidine.
Drug Metabolism - Glucuronidation

- **O-glucuronidation:**
  - Occurs by ester linkages with carboxylic acids
  - Occurs by ether linkages with phenols and alcohols

The glucuronidation of (a) morphine, (b) chloramphenicol and (c) salicylic acid.
Drug Metabolism - Sulfation

Sulfation:
- Major pathway for phenols but also occurs for alcohols, amines and thiols
- Energy rich donor required: PAPS (3’-Phosphoadenosine-5’-phosphosulfate)

Sulfation and glucuronidation are competing pathways:
- Sulfation predominates at low substrate concentrations
- Glucuronidation predominates at higher concentrations
- There is relatively less PAPS in cell cytosol compared to UDPGA

Sulfotransferases (=SULTs) catalyze transfer of sulfate to substrates:
- Phenol, alcohol and arylamine sulfotransferases are fairly non-specific
- Steroid sulfotransferases are very specific
Drug Metabolism - Acylation

Acetylation:
- Common reaction for aromatic amines and sulfonamides
- Requires co-factor acetyl-CoA
- Responsible enzyme is N-acetyltransferase
- Takes place mainly in the liver
- Important in sulfonamide metabolism because acetyl-sulfonamides are less soluble than the parent compound and may cause renal toxicity due to precipitation in the kidney

Fatty Acid Conjugation:
- Stearic and palmitic acids are conjugated to drug by esterification reaction
- Occurs in liver microsomal fraction

  (Cannabinols are metabolized in this fashion => long half-life)
Drug Metabolism - Other conjugations

Amino Acid Conjugation:
• ATP-dependent acid:CoA ligase forms active CoA-amino acid conjugates which then react with drugs by N-Acetylation:
  – Usual amino acids involved are:
    • Glycine, Glutamine, Ornithine, Arginine

Glutathione Conjugation:
• Tripeptide Gly-Cys-Glu; conjugated by glutathione-S-transferase (GST)
• Glutathione is a protective factor for removal of potentially toxic compounds
• Conjugated compounds can subsequently be attacked by \( \gamma \)-glutamyltranspeptidase and a peptidase to yield the cysteine conjugate => product can be further acetylated to N-acetylcysteine conjugate

![The further metabolism of a glutathione conjugate.](image)
Drug Metabolism - Phase I & II

Phase I and II - Summary:

- Products are generally more water soluble
- These reactions products are ready for (renal) excretion
- There are many complementary, sequential and competing pathways
- Phase I and Phase II metabolism are a coupled interactive system interfacing with endogenous metabolic pathways
Drug Action: Receptor Theory

Many drugs act by binding to receptors (see Lecture 4) where they either provoke or inhibit a biological response.

**Agonists:**
- Can be **drugs** or **endogenous ligands** for the receptor
- Increasing concentrations of the agonist will produce an increase in the biological response:
  - **Full Agonist:** Evokes 100% of the maximum possible effect
  - **Partial Agonist:** Produces the same type of biological response, but cannot achieve 100% even at very high doses

![Graph showing full and partial agonists](image)
Drug Action: Receptor Theory

Antagonists:

- Block or reverse the effects of agonists. They have no effects on their own
  - **Competitive Antagonists:** Compete with agonist for receptor binding => Agonist appears less potent, but can still achieve 100% effect (but at higher concentrations)
  - **Non-competitive Antagonists:** Bind to receptor at different site and either prevent agonist binding or the agonist effect => maximal achievable response reduced
  - **Inverse Agonists:** Not the same as antagonists! Inverse agonists trigger a negative response (= reduce baseline) (e.g. diazepam = full agonist = anticonvulsant BUT inverse agonists of benzodiazepin receptor are convulsants)