Chapter 4

Drug Biotransformation
Drug Biotransformation

- 1 Why is drug biotransformation necessary
- 2 The role of biotransformation in drug disposition
- 3 Where do drug biotransformation occur
- 4 The enzymes involved in biotransformation
The role of biotransformation

- The polarity of drugs increases after metabolism in the body, which is helpful to the excretion of drugs.

- Alteration or termination of drug activity
The role of biotransformation

- Converts pro-drug to active compound
- Converts less active drug to more active drug
- Produces toxic compound
- Terminates drug actions
Biotransformation process-Phase I

- **Phase 1 reactions usually convert the parent drug to a more polar metabolite by introducing or unmasking a functional group** (-OH, -NH$_2$, -SH).

- **Often these metabolites are inactive, although in some instances activity is only modified or even enhanced**

- **If phase 1 metabolites are sufficiently polar, they may be readily excreted.**
Biotransformation process-Phase II

- Phase 2 reactions: An endogenous substrate such as glucuronic acid, sulfuric acid, acetic acid, or an amino acid combines with the functional group to form a highly polar conjugate.

- Phase 1 reactions was catalyzed by cytochrome P450 in the hepatic microsome and Phase 2 reactions was catalyzed by some transferases.
Biotransformation of Major Functional Groups

- (-OH) oxidation, methylation, glucuronide conjugation, sulfate conjugation.
- (-COOH) oxidation, glucuronide conjugation, glycine conjugation.
- (-NH₂) deamination (and aldehyde formation), glucuronide conjugation, methylation.
The two phases of drug metabolism

Phase 1:
- Oxidation
- Hydroxylation
- Dealkylation
- Deamination
- Hydrolysis

Phase 2:
- Conjugation

Aspirin → Salicylic acid → Glucuronide

Aspirin: COOH
Salicylic acid: COOH with OH group
Glucuronide: COOH with glycosidic bond
WHERE DO DRUG BIOTRANSFORMATIONS OCCUR

Extrahepatic microsomal enzymes (oxidation, conjugation)

Hepatic microsomal enzymes (oxidation, conjugation)

Hepatic non-microsomal enzymes (acetylation, sulfation, GSH, alcohol/aldehyde dehydrogenase, hydrolysis, ox/red)
WHERE DO DRUG BIOTRANSFORMATIONS OCCUR

- Liver is the principal organ of drug metabolism because of its containing most drug-metabolizing enzymes and its high blood flow.
  - Gastrointestinal tract, the lungs, the skin and the kidneys.
- In a cell, the enzymes are localized in the endoplasmic reticulum, mitochondria, cytosol, lysosomes, or even the nuclear envelope or plasma membrane.
MICROSOMAL MIXED FUNCTION OXIDASE SYSTEM & PHASE I REACTIONS

- The enzyme systems involved in phase 1 reactions are located primarily in the endoplasmic reticulum.

- The ER membranes are isolated by homogenization and fractionation of the cell, they reform into vesicles called microsomes.

- The smooth microsomes are relatively rich in enzymes responsible for oxidative drug metabolism.
Phase I Reactions

- Dominated by cytochrome P450 enzymes
This system includes a series of enzymes involved in drug oxidation.

One molecule of oxygen is consumed (reduced) per substrate molecule, with one oxygen atom appearing in the product and the other in water.

In this oxidation-reduction process, two microsomal enzymes play a key role:
- NADPH-cytochrome P450 reductase
- Cytochrome P450
Mechanism of Drug Oxidation in the Cytochrome P450 Cycle
Note the Fe^{2+} / Fe^{3+} valence transitions.

Molecular oxygen

Electrons from NADPH via reductase

Parent drug binds ferric state of enzyme.

Reaction product

Figure 1–3. Cytochrome P450 mechanism of oxygen activation and drug oxidation.
NADPH

Reductase

Cytochrome P450

Figure 4-3. Cytochrome P-450 cycle in drug oxidations. (RH = parent drug; ROH = oxidized metabolite; Fp = flavoprotein; e\(^-\) = electron.)
Human Liver P450 Enzymes

- Twelve CYP gene families have been identified in humans, and the categories are based upon protein sequence homology
  - Families - CYP plus arabic numeral (>40% homology of amino acid sequence, eg. CYP1)
  - Subfamily - 40-55% homology of amino acid sequence; eg. CYP1A
  - Subfamily - additional arabic numeral when more than 1 subfamily has been identified; eg. CYP1A2
CYP Families

- Frequently, two or more enzymes can catalyze the same type of oxidation, indicating redundant and broad substrate specificity.
- Most of the drug metabolizing enzymes are in CYP 1, 2, & 3 families.
- 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.
Drug Oxidation — Major Route of Drug Metabolism

Family of Enzymes (CYPs) in Liver

Proportion of Pharmaceuticals Metabolized by Individual Cytochrome P450’s

Major P450 Content of Human Liver

Alastair J. J. Wood

Some Drugs Stimulate and Some Inhibit the MFO System

- **Stimulation** is via induction, which means an increase in enzyme **synthesis**.

- **Inhibition** is via a decrease in the activity of existing enzyme.

**Stimulate**: Sedatives, tranquilizers, analgesics, some antihistamines.

**Inhibit**: The antihistamine cimetidine (Tagamet®); secobarbital (Seconal®; suicide inactivator).
Enzyme Inhibition

- **Competitive inhibition**
  - Imidazole-containing drugs such as cimetidine and ketoconazole
  - Bind tightly to the P450 heme iron
- **Forming complex with P450**
  - Macrolide antibiotics
  - The metabolites complex the P450 heme-iron
- **Irreversibly inhibit P450s via covalent**
  - Chloramphenicol, suicide inhibitors
  - The metabolite modifies its protein and inactivates
Phase II Conjugation Reactions

- Glucuronidation (UDP glucuronosyltransferases)
- Glutathione (glutathione S-transferases)
- Amino acid (glycine, glutamine)
- Sulfate (sulfotransferases)
- Acetylation (N-acetyltransferases)
- Methylation (methyltransferases)

Many conjugation enzymes exhibit polymorphism
PHASE II REACTIONS

- **Glucuronidation** is the most important conjugation reaction
  - Enzyme: uridine diphosphate glucuronyl transferases, localized in the microsome

- **Sulfation** also is an important conjugation reaction
  - Enzyme: sulfotransferases, localized in the cytosol
Several compounds have been shown to be metabolically transformed to reactive intermediates that are toxic to various organs

- Alternative detoxification mechanisms
- Endogenous detoxifying co-substrates

The number of specific examples of such drug-induced toxicity is expanding rapidly.
Acetaminophen Metabolism

CYP2E1* induced by ethanol, isoniazid

Protein adducts, Oxidative stress, Toxicity
Protective effect. Liver cells die (pale areas) when exposed to high doses of acetaminophen (*left*), but a CAR inhibitor protects against such damage (*right*).
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
• Addition of N-acetyl cysteine to acetaminophen tablets proposed to prevent liver toxicity.
Clinical Relevance of Drug Metabolism

- Individual differences
- Genetic factors
- Diet & environmental factors
- Age & sex
- Drug-drug interactions during metabolism
- Interactions between drugs & endogenous compounds
- Disease affecting drug metabolism
Age and sex

- very young
  - less developed enzyme system
  - less developed blood brain barrier
- very old
  - decreased GI absorption
  - decreased renal clearance
Disease

- altered liver enzymes
  - liver disease
    - most decrease enzymes
    - some may increase
  - other diseases that decreased liver enzymes
    - hypothyroid
    - hypoxemia
    - malnutrition
Genetic variations

- genetic alterations or defects in enzymes
  - metabolize drug more slowly or more rapidly
Drug-drug interactions

- Enzyme inducer
- Enzyme inhibitor
- Compete for same metabolic enzyme
Diet and environmental factors

- Food components
- Cigarette
- Alcohol