Biotransformation: Prodrugs and Cytochrome P450 and its Interactions

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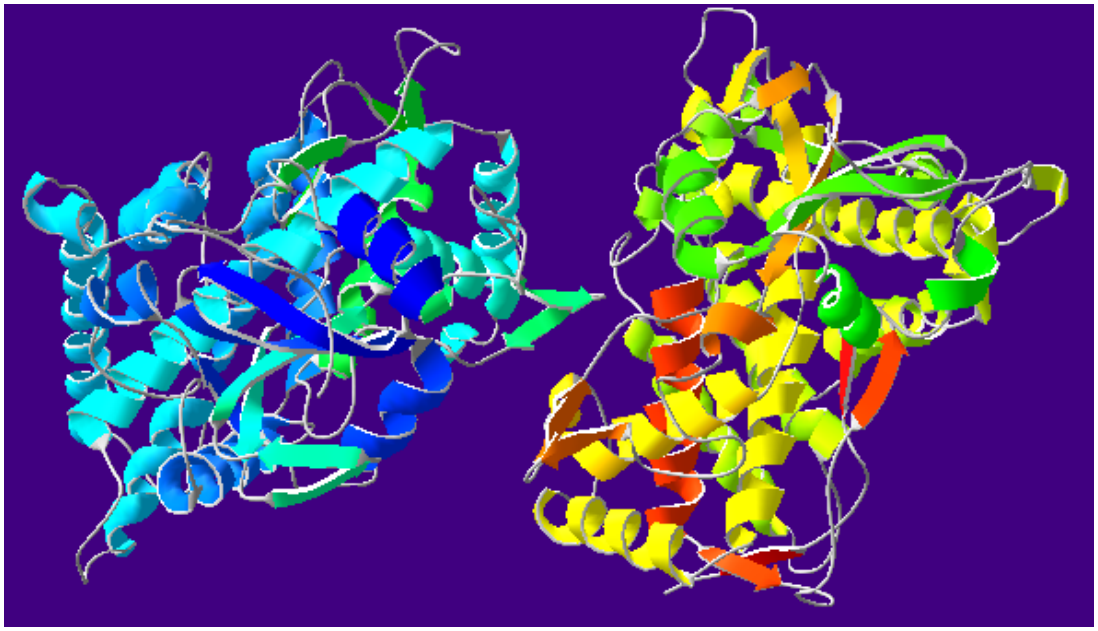
Excretion of the unchanged form of the drug is one way to terminate the action of the drug. Another way is to chemically alter it. This process is called biotransformation and is primarily for lipid-soluble drugs that cannot be eliminated unchanged by the kidney (as they readily cross the cell membranes and have considerable tubular reabsorption). In this article, we will study about various type of metabolic reactions of drugs and factors affecting the biotransformation reactions.



Introduction to Biotransformation

Drugs can be eliminated from the systemic circulation unchanged by **renal excretion** (e.g. benzylpenicillin, aminoglycosides, metformin etc.); fully transformed and excreted only as **metabolites** (e.g. phenothiazines, chloramphenicol etc.); or both unchanged and as metabolites (e.g. salicylates, acetaminophen etc.).
The **primary** site of biotransformation is **the liver**. Other tissues such as intestinal mucosa, colon, placenta, adrenals, etc. also contribute to biotransformation.
Biotransformation can make the drug inactive, but can also produce another pharmacologically active drug that has a more or less or equal activity as the parent drug; it can even produce compounds that are toxic. Some examples are :

- Drugs that become **inactive**: Phenytoin, salicylic acid, phenobarbital, amphetamine
- Drugs that produce **pharmacologically active metabolites**: Amitriptyline

(nortriptyline), imipramine (desipramine), diazepam (temazepam), digitoxin (digoxin)
- Drugs that produce **toxic metabolites**: Paracetamol, isoniazid, valproic acid

The pathways of drug metabolism can be categorized into **phase I and phase II** (and **sometimes phase III**) reactions:

- Phase I reactions involve **attachment (or unmasking) of polar groups** to the drugs.
- Phase II reactions involve **conjugation of compounds such as organic acids to the polar group** to make the compound **highly water soluble**.
- Phase III reactions refer to the **active transport of the compound** into urinary or hepatobiliary system to facilitate elimination.

Phase I Reactions

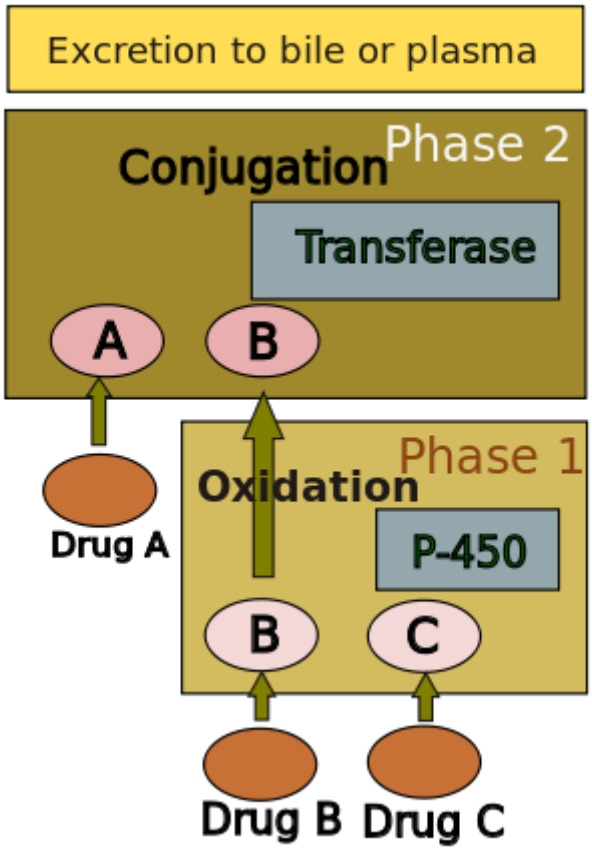


Image: "Drug metabolism in liver. transferases are : glutathione, sulfate, acetate, glucoronic acid. P-450 is cytochrome P-450 enzymes. 3 different pathways are depicted for Drugs A,B and C." by Drriad. License: CC BY-SA 3.0

Phase I reactions, also known as **functionalization reactions**, involve the transformation of lipophilic drugs into a more water-soluble compound. This occurs by introduction of a polar group, such as **hydroxyl (-OH)**, **amine (-NH_2)**, and **sulfhydryl (-SH)** groups.

For example, phenylbutazone is a benzene compound. An -OH group is added to one of the benzene rings, yielding a para-hydroxylated product known as oxyphenbutazone.

Phase I reactions are primarily catalyzed by cytochrome P450 (CYP) enzymes. Other enzymes involved in phase I reactions and examples of their substrates are as follows:

Oxidation

CYP-catalyzed reactions	Drugs
C-hydroxylation	Amphetamines, Phenytoin, Warfarin, Barbiturates, Propranolol, Tolbutamide, Phenylbutazone
N-hydroxylation	Dapsone
N-dealkylation	Morphine, Theophylline, Caffeine, Lidocaine, Amitriptyline
O-dealkylation	Codeine, Dextromethorphan
N-oxidation	Acetaminophen
S-oxidation	Chlorpromazine, Thioridazine
Deamination	Amphetamine, Diazepam
Flavin-containing monooxygenase (FMO)-catalyzed reactions	**Drugs**
S-oxidation	Cimetidine
N-oxidation	Nicotine
Monoamine oxidases (MAO)-catalyzed reactions	Dopamine, Norepinephrine, Serotonin
Molybdenum-containing oxidase-catalyzed reactions	Hypoxanthine, Allopurinol, Benzaldehyde, 6-Mercaptopurine
Alcohol/aldehyde dehydrogenase-catalyzed reactions	Methanol, Ethanol, Ethylene glycol

Reduction

Reaction	Drug
Azo-reduction	Tartrazine, Prontosil
Nitro-reduction	Nitrobenzene, Chloramphenicol, Dantrolene, Clonazepam
Carbonyl-, aldehyde- and aldose-reduction	Chloral hydrate, Methadone

Hydrolysis

Reaction	Drug
Alkaline phosphatase	Fosphenytoin, Fospropofol, Clindamycin phosphate (examples of pro-drugs)
Paraoxonase	Statins, Spironolactone
Carboxylesterases/Butyrylcholinesterase	Succinylcholine, Mivacurium, Procaine, Tetracaine, Cocaine, Esmolol, Meperidine, Remifentanil, Enalapril
Microbial hydrolase (in the colon)	Bisacodyl

Phase II Reactions

Many phase I metabolites are not sufficiently polar to be directly excreted by the kidneys. These compounds are then subjected to phase II reactions, also known as **conjugation or substitution reactions**, which involve the conjugation of compounds such as organic acids (glucuronic acid, sulfuric acid, acetic acid, etc.) to the polar group to make the compound highly water soluble and, usually, pharmacologically inactive.

If a drug already possesses a polar group, it may **directly undergo phase II reaction**, thus bypassing the phase I reaction. The reactions are catalyzed by a group of transferase enzymes that are often non-specific. The most dominant enzymes are uridine 5′-diphosphate (UDP)-glucuronosyl transferases (UGTs).

The most important conjugation pathway is **glucuronidation**, and perhaps the most notable example is **morphine**: it undergoes glucuronidation to become **morphine-6-glucuronide**, which is more potent than morphine.

Other pathways and examples are listed in the following table:

Glucuronidation	Acetaminophen, Diazepam, Digoxin, Chloramphenicol, Oxazepam, Phenolphthalein, Propofol, Diethylstilbestrol, Estradiol, Thyroxine, Diclofenac, Furosemide
Sulfation	Acetaminophen, Methyldopa, Minoxidil
Glycine conjugation	Deoxycholic acid, Nicotinic acid (Niacin), Salicylic acid, Benzoic acid
Glutathione conjugation	Ethacrynic acid, reactive phase I metabolite of acetaminophen
Acetylation	Clonazepam, Dapsone, Isoniazid, Mescaline, Sulfonamides
Methylation	Dopamine, Epinephrine, Histamine, Norepinephrine, Thiouracil

Prodrug

Some drugs are **administered in the inactive form and become pharmacologically active after they have undergone phase I or II reaction**. Such drugs are called as prodrugs and are administered to improve the drug's bioavailability.

A typical example is **enalapril**, which gets converted by hydrolysis into its active form **enalaprilat**. In fact, most other **ACE inhibitors** are prodrugs—**Benazepril, fosinopril, moexipril, perindopril, quinapril, ramipril,** and **trandolapril**—and get converted to their active form in the liver via hydrolysis.

Other examples include L-dopa, cyclophosphamide, bacampicillin, bambuterol, chloramphenicol succinate, dipivefrin, fosphenytoin, pralidoxime, etc. In the presence of CYP inhibitors, a prodrug that gets converted to its active form by CYP enzymes will show considerably diminished activity because of reduced amount of conversion.

Toxic Metabolism

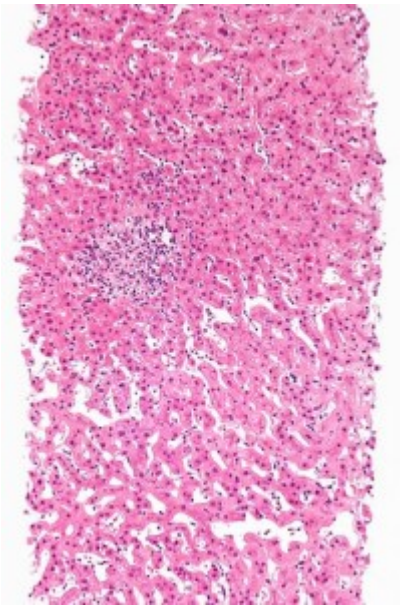


Image: “Low magnification micrograph of an adverse drug reaction leading to a hepatitis, also known as drug-induced hepatitis, with non-caseatinggranulomata. Liver biopsy. H&E stain.” by Nephron. License: CC BY-SA 3.0

CYPs are present mainly in the liver. Metabolism of certain drugs by CYPs leads to **production of toxic metabolites that can cause hepatotoxicity**.

Acetaminophen (also known as paracetamol) and halothane are well-known examples of hepatotoxic drugs, by the production of toxic metabolites N-acetyl-p-benzoquinone imine and 1-chloro-2,2,2-trifluoroethyl radical, respectively. Other **NSAIDs** such as diclofenac also induce hepatotoxicity by CYP-medicated mechanisms.

Valproic acid also shows hepatotoxicity when its main metabolite (2-propyl-4-pentenoic acid) undergoes further metabolism. Compounds such as ethanol, carbon tetrachloride, chloroform, N-nitrosodimethylamine also involve CYP-mediated hepatotoxicity (by generation of free radicals). **CYP2E1** is involved in anti-tubercular drug hepatotoxicity.

Factors Affecting Drug Metabolism

In addition to drug distribution and elimination, drug metabolism also affects the dose and frequency of drug administration. Many factors can affect the rate of biotransformation, including biological factors relating to variation among individuals. The study of interindividual variation in drug response is known as **pharmacogenetics**. Some of the inter- and intra-individual factors are as follows:

- Demographics factors such as age (gender is not an important factor)
- Diet
- Physiological states such as pregnancy, hormones
- Diseases
- Ethnicity
- Temporal factors such as circadian rhythm
- Genetic factors

Genetic factors

Gene influence is known as **genetic polymorphism**. A variant allele of a gene can affect the enzyme levels or the functional activity of the enzyme, or both, thereby leading to variation in the expected response of the drug (in terms of efficacy and/or adverse effects). Obviously, this requires titration of the dose, especially for drugs with narrow therapeutic windows.

Depending on the allele an individual may be a poor or slow metabolizer of a drug (e.g. **slow acetylators of isoniazid**). Such individuals may be hyperreactive to the drug. On the other hand, higher levels of the enzymes may cause individuals to be a fast metabolizers (e.g. **fast acetylators of isoniazid**), sometimes leading to non-reactivity to the drug. This genetic variation also shows **ethnic diversity**: while almost equal number of whites and blacks are slow and fast acetylators, slow acetylators are predominant in Japanese and Eskimo populations.

Succinylcholine is usually rapidly metabolized by pseudocholinesterase, and this activity lasts for 5 min. In rare cases, this is metabolized very slowly, to the extent that the duration of action of one dose can last for a few hours.

Another example is **codeine**, which is metabolized by the CYP2D6 enzyme into its active metabolite morphine. However, some individuals carry two inactive copies of the CYP2D6 gene (**poor metabolizers**); therefore, they are unable to derive any pharmacological effect from codeine. On the other hand, there are individuals who are **ultrarapid metabolizers** of codeine (because they carry more than two copies), thereby leading to symptoms of drug overdose even from a usual dose of codeine. (Note that most individuals are **extensive metabolizers**, with two CYP2D6 copies, and some are **intermediate metabolizers**, with one copy).

Other factors include physical and chemical properties of the drug as well as factors that inhibit or enhance drug metabolizing enzymes.

Cytochrome P450

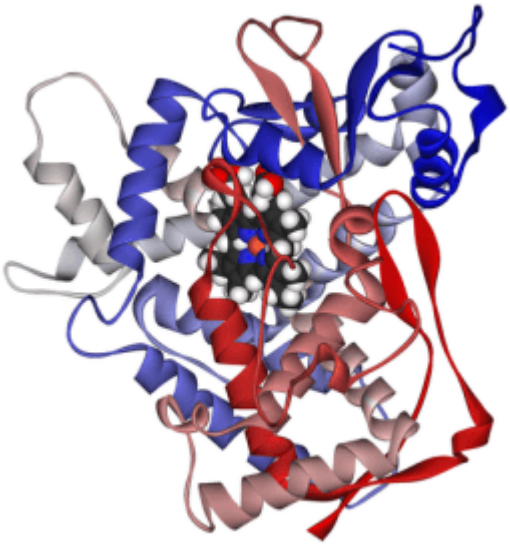


Image: “Ribbon diagram of human cytochrome P450 isozyme 2C9” by Fvasconcellos. License: Public Domain

Cytochrome P450 (or CYP450) are a group of proteins. Although there are many enzymes in this family, about six of them (CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2D6, CYP2E1 and CYP3A4) metabolize most of the drugs. In fact, **CYP3A4 alone metabolizes around 50 %** of all drugs metabolized by the liver.

CYPs are the important targets for drug interactions. Knowledge of these interactions is crucial when more than one drug is being administered to a patient, especially, for example, in older people who are often subjected to polypharmacy.

Some drugs are metabolized largely or exclusively by only one CYP. E.g. phenytoin (by CYP2C9) and halothane (by CYP2E). Some other drugs can be metabolized by several CYPs. E.g. acetaminophen (by CYP2E1, CYP1A2, and CYP3A4) and dextromethorphan (by CYP2D6 and CYP3A4).

Interactions of CYP450: Induction and Inhibition

Interaction with CYP450 can be through one of the following mechanisms:

- Two drugs can compete for the same enzyme, thereby leading to slight prolongation of the duration of action of both drugs.
- One drug can inhibit the metabolism of the other.
- One drug can induce the metabolism of the other.

Induction

Consequences of increased drug metabolism are as follows:

- Concentration of the drug in the blood decreases
- Activity of the drug decreases or increases (depending on whether the metabolite is inactive or active, respectively)
- Pharmacological effect, and the duration of action, of the drug is diminished

E.g., rifampicin, an antitubercular drug, induces the metabolism of the anti-HIV drug class protease inhibitors; this considerably reduces their efficacy. **As mentioned, many drugs are metabolized by CYP3A4. Therefore, it is crucial to know at least the most common inducers of this enzyme. These are barbiturates, carbamazepine, corticosteroids, efavirenz, phenytoin, rifampin, pioglitazone, St. John's wort.**

Other examples of CYP inducers are as follows:

CYP1A2	Carbamazepine, Phenobarbital, Rifampin, Omeprazole, St John's wort
CYP2E1	Ethanol, Isoniazid
CYP2C9	Barbiturates, especially Phenobarbital, Phenytoin, Primidone, Rifampin
CYP2B6	Phenobarbital, Cyclophosphamide

Inhibition

Contrary to CYP induction, inhibition of CYP enzymes would lead to increase in the drug's concentration, pharmacological activity, and duration of action (of course, if the drug is activated by CYP enzymes, then CYP inhibition will reduce the drug's activity). Inhibition is usually due to competition; however, certain drugs such as ketoconazole inhibit CYP without being metabolized by it.

Erythromycin, ketoconazole, and ritovarin are important inhibitors as they inhibit several

isoenzymes. Natural substances can also inhibit CYP enzymes, e.g. furanocoumarins present in grapefruit inhibit CYP3A4. The inhibitors of CYP3A4 and other CYP enzymes are indicated in the following table:

CYP3A4	Itraconazole, Clarithromycin, Ketoconazole, Erythromycin, Diltiazem
CYP1A2	Ciprofloxacin, Fluvoxamine
CYP2E1	4-Methylpyrazole, Disulfiram
CYP2C9	Tienilic acid, Sulfaphenazole
CYP2B6	Ticlopidine, Clopidogrel
CYP2D6	Fluoxetine, Paroxetine, Methadone, Quinidine

Certain drugs (or their metabolites) bind irreversibly to the CYP enzymes, thereby inhibiting them. Such drugs are known as **suicide inhibitors**. Important examples of suicide inhibitors are spironolactone, ethinyl estradiol, allopurinol, propylthiouracil, fluroxene, and secobarbital.

P-glycoprotein (MDR-1) Inhibitors

P-glycoprotein (P-gp), also known as **multidrug resistance protein 1** (MDR1) or **ATP-binding cassette sub-family B member 1** (ABCB1) or **cluster of differentiation 243** (CD243), is an important modulator of intestinal drug transport, i.e. its function is to pump drugs out of the cells in the intestinal mucosa back into the lumen. In this way, it participates in presystemic elimination. Therefore, if a drug inhibits intestinal P-gp, it would act like a drug metabolism inhibitor and increase the drug activity and bioavailability.

P-gp inhibitors include verapamil and grapefruit (furanocoumarin). Important drugs that are normally expelled by P-gp (and are therefore potentially more toxic when given with a P-gp inhibitor) include digoxin, cyclosporine, and saquinavir. If P-gp inhibitors are administered along with a CYP (or other enzyme) inhibitor, then the concentration of the drug may reach toxic levels.

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Notes