DRUG BIOTRANSFORMATION REVIEW

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The body is exposed to a wide variety of foreign compounds, called xenobiotics. Exposure to some such compounds is unintentional (e.g., environmental or food substances), while others are deliberately used as drugs. The following discussion of drug biotransformation is applicable to all xenobiotics, and to some endogenous compounds (e.g., steroids) as well.

The kidneys are capable of eliminating drugs which are low in molecular weight, or which are polar and fully ionized at physiologic pH. Most drugs do not fit these criteria, but rather are fairly large, unionized or partially ionized, lipophilic molecules. The general goal of drug metabolism is to transform such compounds into more polar (i.e., more readily excretable) water soluble products. For example, were it not for biotransformation to more water-soluble products, thiopental, a short-acting, lipophilic anesthetic, would have a half-life of more than 100 years!

Most products of drug metabolism are less active than the parent compound. In some cases, however, metabolites may be responsible for toxic, mutagenic, teratogenic or carcinogenic effects. For example, overdoses of acetaminophen owe their hepatotoxicity to a minor metabolite which reacts with liver proteins. In some cases, with metabolism of so-called prodrugs, metabolites are actually the active therapeutic compounds. The best example of a prodrug is cyclophosphamide, an inert compound which is metabolized by the liver into a highly active anticancer drug.

A. Sites of drug metabolism

1. At the **organ level**

   The liver is the primary organ of drug metabolism. The gastrointestinal tract is the most important extrahepatic site. Some orally administered drugs (e.g., isoproterenol) are conjugated extensively in the intestinal epithelium, resulting in decreased bioavailability. The lung, kidney, intestine, skin and placenta can also carry out drug metabolizing reactions. Because of its enormous perfusion rate and its anatomic location with regard to the circulatory system, the lungs may exert a first-pass effect for drugs administered IV.

2. At the **cellular level**

   Most enzymes involved in drug metabolism are located within the lipophilic membranes of the smooth endoplasmic reticulum (SER). When the SER is isolated in the laboratory by tissue homogenation and centrifugation, the SER membranes re-form into vesicles called microsomes. Since most of the enzymes carry out oxidation reactions, this SER complex is referred to as the microsomal mixed function oxidase (MFO) system.

3. At the **biochemical level**

   Phase I reactions refer to those which convert a drug to a more polar compound by introducing or unmasking polar functional groups such as - OH, -NH_2, or -SH. Some Phase I products are still not eliminated rapidly, and hence undergo Phase II reactions involving conjugation of the newly established polar group with endogenous compounds such as glucuronic acid, sulfuric acid, acetic acid, or amino acids (typically glycine). Glucuronide formation is the most common phase II reaction. Sometimes, the parent drug may undergo phase II conjugation directly. In some cases,

a drug may undergo a series of consecutive reactions resulting in the formation of dozens of metabolites.

Most phase I MFO biotransformation reactions are oxidative in nature and require a reducing agent (NADPH), molecular oxygen, and a complex of microsomal enzymes; the terminal oxidizing enzyme is called cytochrome P450, a hemoprotein so named because its carbon monoxide derivative absorbs light at 450 nm. We now know that cytochrome P450 is actually a family of enzymes which differ primarily with regard to their substrate specificities. Advances in molecular biology have led to the identification of more than 70 distinct P450 genes in various species.

The nomenclature of the P450 reductase gene products has become complex. Based upon their amino acid homologies, the P450 reductases have been grouped into families such that a cytochrome P450 from one family exhibits < 40% amino acid sequence identity to a cytochrome P450 in another gene family. Several of the gene families are further divided into subfamilies, denoted by letters A, B, C, etc. Ten major mammalian gene families have been defined (see Table 1).

Table 1: Major Cytochrome P450 Gene Families

P450 Gene Family/Subfamily	**Characteristic Substrates**	**Characteristic Inhibitor**	**Characteristic Inducers**
CYP 1A2	Acetaminophen Estradiol Theophylline Caffeine	Cimetidine Ciprofloxacin Amiodarone Ticlopidine	Omeprazole Tobacco Char-Grilled Meats Insulin
CYP 2B6	Cyclophosphamide Methadone	Orphenadrine Thiotepa	Rifampin Phenobarbital
CYP 2C8	Carbamazepine Diazepam	Cimetidine Verapamil	Rifampin Phenobarbital
CYP 2C9/10	Tolbutamide Warfarin, Ibuprofen Fluoxetine	Cimetidine Fluvastatin, Lovastatin Isoniazid, Amiodarone	Rifampin Secobarbital
CYP 2C19	Diazepam. Mephenytoin Omeprazole Progesterone	Cimetidine, Omeprazole Ketoconazole Fluconazole	Prednisone Rifampin Artemisinim
CYP 2D6	Codeine, Debrisoquine Dextromethorphan Metoprolol, Ondansetron Amphetamine	Cimetidine Fluoxetine Methadone Quinidine, Ritonavir	Dexamethasone? Rifampin?
CYP 2E1	Chlorzoxazone Ethanol, Benzene Halothane	Disulfiram Water Cress	Ethanol Isoniazid
CYP 3A4	Midazolam Erythromycin breath test	Erythromycin Cimetidine, Ketoconazole Grape fruit juice	Carbamazepine Rifampin. Phenytoin St. John's Wort
CYP 3A5	Caffeine Midazolam	Dexamethasone	Troleandomycin
CYP 3A7	Midazolam	Unknown	Unknown

B. Enzyme Induction

An interesting and important feature of the cytochrome P_{450} mixed function oxidase system is the ability of some xenobiotics to induce the synthesis of new enzymes. Microsomal enzyme induction is a complex and poorly understood process associated with an increase in liver weight, proliferation of the SER, and synthesis of P_{450} enzymes. For example, phenobarbital induces the P_{450}IIB subfamily, while polycyclic aromatic hydrocarbons (e.g., found in cigarette smoke or charcoal broiled foods) induce the P_{450}IA subfamily; these and other inducers are listed in Table 1, above. The dose and frequency of drug administration required to achieve therapeutic drug concentrations in blood can vary enormously from person to person, and this is often dependent upon the degree of exposure to microsomal inducers.

For example, consider patients who routinely ingest barbiturates or tranquilizers (P_{450} inducers) who must, for medical reasons, be treated with warfarin or dicumarol (oral anticoagulants). Because of a faster rate of drug metabolism, the dose of warfarin will need to be high. If the patient should for some reason discontinue the barbiturates, the blood level of warfarin will rise, perhaps leading to a bleeding disorder.

C. Enzyme Inhibition

Relatively few xenobiotics are known to inhibit microsomal enzymes. Some drugs are used therapeutically because they inhibit specific enzyme systems (e.g., monoamine oxidase inhibitors for depression, xanthine oxidase inhibitors for gout, etc.). Sometimes such drugs are not totally specific and inhibit other enzyme systems to some extent. However, cimetidine, a widely used anti-ulcer drug, is an important, potent inhibitor of microsomal drug metabolism which retards the metabolism of many other drugs, including warfarin and similar anticoagulants, theophylline and caffeine, phenobarbital, phenytoin, carbamazepine, propranolol, diazepam, and chlordiazepoxide. Other inhibitors are erythromycin and ketoconazole. Grapefruit juice also inhibits cytochrome P_{450}.

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HEPATIC CLEARANCE REVIEW

Clearance Concepts

Drug clearance can be defined as the proportionally factor between drug concentration and the rate of elimination of the drug from the body. When we talk about clearance, we are typically talking about clearance from plasma, since we most commonly measure drug concentrations in plasma.

Rate of elimination = Clearance from plasma x Concentration in plasma

We sometimes want to talk about clearance from whole blood. Whole blood contains plasma, as well as red blood cells, white blood cells, and platelets. We know that the rate of elimination of a drug from the body is the same regardless of whether you measure drug concentrations in plasma or blood.
Therefore:

$$\textit{Rate of elimination} = CL \times C = CL_b \times C_b$$

$$CL_b = CL \times (C / C_b)$$

Where CL is clearance of the drug from plasma, C is plasma concentration, CL_b is clearance of the drug from blood and C_b is blood concentration. C/C_b is called the plasma-to-blood ratio. To obtain this value, we determine the concentration of the drug in a sample of whole blood (C_b), centrifuge the sample to obtain plasma, and then determine the concentration of drug in that plasma (C).

Hepatic Clearance

The liver is largely responsible for elimination of most drugs from the body. Hepatic metabolism, in which the drug molecule is transformed through oxidation, reduction, and/or conjugation reactions, is the primary method by which the liver eliminates drugs. The liver can also eliminate drugs through excretion of the drug molecules into the bile.
We can conceptualize the hepatic clearance of drug from blood ($CL_{b,H}$) in terms of hepatic extraction ratio (E_H) and hepatic blood flow (Q_H).

$$CL_{b,H} = Q_H \times E_H$$

E_H is equal to the proportion of drug extracted from blood during a single pass through the liver. Hepatic blood flow, which is equal to approximately 1.35 L/min in a healthy adult, represents the maximum volume from which drug can be extracted per unit of time. If all of the drug is extracted from the blood in a single pass through the liver ($E_H = 1$), then 1.35 L of blood can be cleared of the drug per minute. Since the liver can only clear drug from the volume of blood presented to it, Q_H represents the upper limit of hepatic blood clearance ($CL_{b,H}$). The lower limit occurs when the liver simply doesn't extract drug from the blood ($E_H = 0$, $CL_{b,H} = 0$) such as might be observed with drugs eliminated solely by renal excretion.

Well-stirred Model of Hepatic Clearance

Hepatic clearance involves multiple processes, including presentation of drug to the liver through hepatic blood flow, dissociation of drug from proteins/cells to which it is bound, uptake of drug into hepatocytes, and metabolism or biliary excretion of drug within hepatocytes. The rates of these processes influence the magnitude of hepatic clearance. The "well-stirred model" of hepatic blood clearance aids in understanding how these different processes influence hepatic clearance. The formulas for hepatic blood clearance and extraction ratio for this model are:

$$CL_{b,H} = Q_H \times E_H = Q_H \times (f_{ub} \times CL_{int}) / (Q_H + f_{ub} \times CL_{int})$$

$$E_H = (f_{ub} \times CL_{int}) / (Q_H + f_{ub} \times CL_{int})$$

CL_{intt} is intrinsic clearance; this is the clearance of unbound drug within the hepatocyte and thus is a measure of the intrinsic metabolic/excretory activity of the liver for a given drug. The term fu_b represents the unbound fraction in blood, and is equal to:

$$fu_b = \frac{\textit{Unbound concentration in plasma}}{\textit{Concentration in whole blood}} = f_u \times (C / C_b)$$

Where C/C_b is the plasma-to-blood concentration previously defined. However, for most drugs, $f\underline{u}$ and fu_b are sufficiently similar that f_u can be substituted for fu_b in hepatic clearance formulas.
The well-stirred model describes hepatic clearance in terms of hepatic blood flow, the fraction of unbound drug, and the intrinsic clearance of the drug. The hepatic clearance formula for this model can be used to predict how changes in these factors will influence hepatic clearance. Most drugs appear to be low extraction ratio ($E_H < 0.3$) or high extraction ratio ($E_H > 0.7$) drugs. The well-stirred model formula is best interpreted in the context of these two categories.

This a summary of information from Ch. 5 of Rowland, M. & Tozer, T.N. (2011). Clinical pharmacokinetics and pharmacodynamics : concepts and applications. Philadelphia : Wolters Kluwer Health/Lippincott William & Wilkins.