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An Introduction to Drug Disposition: The Basic Principles of Absorption, Distribution, Metabolism, and Excretion*

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ABSTRACT

A knowledge of the fate of a drug, its disposition (absorption, distribution, metabolism, and excretion, known by the acronym ADME) and pharmacokinetics (the mathematical description of the rates of these processes and of concentration–time relationships), plays a central role throughout pharmaceutical research and development. These studies aid in the discovery and selection of new chemical entities, support safety assessment, and are critical in defining conditions for safe and effective use in patients. ADME studies provide the only basis for critical judgments from situations where the behavior of the drug is understood to those where it is unknown: this is most important in bridging from animal studies to the human situation. This presentation is intended to provide an introductory overview of the life cycle of a drug in the animal body and indicates the significance of such information for a full understanding of mechanisms of action and toxicity.

Keywords. Xenobiotics; human and animal exposure; predictive value

INTRODUCTION

Humans and other animals are exposed on a daily basis to many xenobiotics, that is, compounds that are foreign to the normal energy-yielding metabolism of the body. Exposure to these xenobiotics may occur deliberately, as in the case of drugs and food additives; accidentally, as in the case of food contaminants and pesticides, or coincidentally, as in the case of industrial chemicals and environmental pollutants. In this paper, the terms drug, xenobiotic, and foreign compound will be used interchangeably. In the present context, the importance of ADME (absorption, distribution, metabolism, and excretion) principles in drug development will be emphasized, but it should be appreciated that these have comparable applicability in the safety assessment of all types of chemicals to which humans might be exposed.

To achieve its effect, whether therapeutic or toxic, a drug and/or its metabolites must be present in appropriate concentrations at its sites of action. The concentration of xenobiotic attained will depend on the dose, formulation, and route of administration, the rate and extent of absorption, its distribution through the body and binding to tissues, biotransformation, and excretion. It is the purpose of this presentation to give an overview of these processes and to comment upon the factors influencing them and their biological significance.

ABSORPTION

The processes of absorption are those that lead to the entry of a xenobiotic into the systemic circulation of the body. The most important site of absorption is the gastrointestinal tract, although absorption through the skin, the main barrier between the internal milieu and the external environment, and the respiratory tract, which is important for volatile compounds and materials present in aerosols and dust particles, can also occur. Regardless of the site of absorption, xenobiotics must cross cell membranes to enter the systemic circulation. Mechanistically this can occur in 1 of 2 ways (4). Small, lipophilic compounds can cross the cell membrane by passive diffusion along a concentration gradient. This transfer is directly proportional to the magnitude of the concentration gradient across the

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membrane and the lipid : water partition coefficient of the drug (3). Large, highly polar or charged xenobiotics cannot cross the cell membranes by simple diffusion and, hence, are dependent on the presence of active carrier-mediated transport mechanisms.

The Effect of pH and pKa on Absorption from the Gastrointestinal Tract

Many xenobiotics are weak acids or bases and are thus present in solution in both non-ionized and ionized forms. The non-ionized molecules tend to be lipid-soluble and cross membranes by passive diffusion, whereas the ionized forms have low lipid solubility and cannot cross the cell membrane (3). The partition of weak electrolytes across membranes will thus be a function of the pKa of the xenobiotic and the pH gradient across the membrane.

The low pH in the stomach favors absorption of weak acids. Weak bases are ionized and, thus, generally not absorbed from the stomach. In the intestine, absorption is rapid for weak acids ($pK > 3$) or weak bases ($pK < 7.8$). The longer transit time and increased surface area of the intestine mean that, for the majority of drugs, intestinal absorption is quantitatively more important even if it would be predicted to be less favorable on pH grounds (7).

First-Pass Elimination

Following absorption, drugs can be metabolized in the gut wall, prior to being transported to the liver via the hepatic portal vein (78). The hepatocytes of the liver are the major site of metabolism for the majority of drugs, and compounds can be extensively metabolized in the liver before reaching the systemic circulation. That portion of the dose that is absorbed from the lumen of the gastrointestinal tract but eliminated by metabolism in the gut wall and/or the liver on the way to the heart is said to have undergone first-pass elimination (3). The extent to which xenobiotics undergo first-pass elimination will have a major influence on the exposure to the compound following oral administration. The enzymes contributing to the metabolism of xenobiotics are also found in organs other than the liver, such as the lung and skin, albeit usually at a lower level. Thus, xenobiotics entering the body by routes other than the gastrointestinal tract can also be subject to first-pass metabolism.

Distribution

Following entry of a xenobiotic to the systemic circulation, its distribution into the various tissues of the body will be influenced by tissue hemodynamics, passive diffusion across lipid membranes, the presence of carrier-mediated active transport processes recognizing the xenobiotic, and protein binding in the blood and tissues.

The majority of tissue membranes behave as typical lipid barriers allowing small lipophilic molecules to cross cell membranes. Equilibrium drug concentration ratios are maintained by diffusion of drugs into and out of tissues. Drugs can accumulate in tissues at a higher concentration than predicted by simple diffusion under the influence of pH gradients, binding to intracellular constituents, or partitioning into lipid depots. Larger or more polar substances do not cross lipid membranes by passive diffusion and require specific transporters to enter the tissues (44). If a drug does enter a tissue by an active transport mechanism, its concentration in the tissue may be many times greater than its plasma concentration.

Active uptake processes tend to show stereoselectivity and can be particularly important for xenobiotics that may be analogs of nutrients (51). The operation of specific uptake mechanisms for xenobiotics may play an important role in the toxicity of some compounds. For example, amantanide and phalloidin are toxic cyclopeptides of the fungus *Amanita phalloides* (21). The toxins enter the liver via an active transport system involved in the transport of bile acids (23). Once inside the cells, the toxins bind to microfilamentous F-actin and destroy the mechanical stability of the liver cell membrane. This results in hemorrhagic liver swelling and animals die within 2–3 hr of intravenous dosing with the peptides (22). Co-administration of bile salts with the toxins reduces their hepatic uptake by this active transport mechanism and thereby limits the toxicity of the compounds. Distribution of xenobiotics can also be limited by binding to plasma proteins. Acidic drugs tend to bind to albumin, and basic drugs tend to bind to α_1-acid glycoprotein. As only unbound drug is in equilibrium across membranes, a drug that is extensively and strongly bound to plasma proteins has only limited access to the tissues.

Drug Reservoirs

Accumulation of a drug within a tissue can act as a reservoir serving to prolong its duration of action. If the stored xenobiotic is in equilibrium with that in plasma and is released as its plasma concentration falls, then the concentration of xenobiotic in plasma will be sustained and the pharmacological effect of the xenobiotic will be prolonged (3). Thus, the storage of a drug can prolong its action either within the tissue where the drug is held or at a distant site reached following rediffusion into the systemic circulation (29).

The concepts of drug reservoirs and how they influence the concentration of a xenobiotic at its

target tissue are well illustrated by the behavior of the lipophilic anesthetic thiopental, which is given by bolus intravenous injection (2). As a consequence of the high blood flow to the brain and its lipid solubility, thiopental reaches its maximum concentration in its target tissue within 1 min of intravenous injection. When the injection is stopped, the plasma concentration falls as the drug distributes into tissues such as muscle. As thiopental is not tightly bound to brain lipid, its concentration in the brain changes in parallel with changes in the plasma concentration, leading to a rapid termination of anesthesia by redistribution rather than elimination. A third distributive phase for thiopental occurs as the result of a slow, blood flow–limited uptake into poorly perfused tissues such as fat (3).

On repeated administration, fat and other poorly perfused tissues can accumulate large amounts of thiopental. These reservoirs are then capable of maintaining plasma and, hence, brain concentrations of thiopental at levels above those needed for anesthesia. Thus, a compound whose duration of action is limited by rapid redistribution from its site of action to storage sites can become long acting if storage deposits of sufficient size are established. At this point, termination of drug action becomes dependent on biotransformation and excretion of drug. The pharmacological consequence of these changes in tissue distribution is that the sleeping time after dosing of thiopental is changed from a few minutes following a single administration to a few hours following multiple dosing (29).

Toxicity testing is often performed using much higher doses of xenobiotics than humans are exposed to. As well as leading to saturation of metabolic pathways, it must be appreciated that these high doses can lead to changes in tissue distribution similar to those seen following multiple dosing of thiopentone.

METABOLISM

Drugs and other xenobiotics that gain access to the body may undergo 1 or more of 4 distinct fates, as follows (12):

1. Elimination unchanged
2. Retention unchanged
3. Spontaneous chemical transformation
4. Enzymic metabolism

Each of these fates are of importance but, in quantitative terms it is enzymic metabolism, often also referred to as biotransformation, that predominates.

The main site of metabolism of foreign compounds is the liver, although extrahepatic tissues, frequently the site of entry to or excretion from the body (e.g., lungs, kidneys, gastrointestinal mucosa), also play a role in the metabolism of xenobiotics (24 and references therein).

Compounds eliminated unchanged are generally either (a) highly polar such as strong carboxylic or sulfonic acids (e.g., sodium cromoglycate) or quaternary amines (e.g., pancuronium), which if absorbed are rapidly cleared into the urine or bile, or (b) volatile and hence readily lost via the lungs. In contrast, nonpolar, highly lipophilic compounds may be retained for long periods in tissue lipids, as occurs with chlorophenothane and many polyhalogenated aromatics. For a small number of compounds, spontaneous chemical transformation within the tissues of the body can be important: this may involve hydrolysis at the appropriate pH (e.g., thalidomide with its numerous breakdown products) or reaction with nucleophilic or electrophilic centers in tissue macromolecules, most notably the nucleophilic −SH of glutathione (71).

The scope of drug metabolism is immense, and this is reflected in the range of chemical reactions that are involved in the metabolism of substrates, including oxidation, reduction, hydrolysis, hydration, conjugation, and condensation. Typically, the process of metabolism of xenobiotics is biphasic, whereby the compound first undergoes a functionalization reaction (oxidation, reduction, or hydrolysis), which introduces or uncovers a functional group ($-OH, -NH_2, -SH$) suitable for subsequent conjugation with an endogenous conjugating agent.

By far the most important enzyme system involved in Phase 1 metabolism is cytochrome P-450, the terminal oxidase component of the microsomal electron transfer system, which is responsible for the oxidation of many xenobiotics. The required electrons are supplied by the closely associated enzyme NADPH cytochrome P-450 reductase, a flavoprotein that transfers 2 electrons to cytochrome P-450 from NAD(P)H.

The cytochromes P-450 are an enzyme superfamily consisting of a number of related isoenzymes, all of which possess an iron protoporphyrin IX as prosthetic group. The enzymes are named for the Soret band around 450 nm exhibited by the CO complex of the reduced form. The P-450 enzymes have been grouped together into families that share sequence identity. There are 10 mammalian gene families comprised of 18 subfamilies (52, 53). The most important enzymes involved in xenobiotic metabolism belong to the 1A, 2B, 2C, 2D, and 3A subfamilies. Although the individual enzymes are thought to metabolize substrates via the same catalytic mechanism (27), they tend to show selectivity toward substrates. For individual isoforms of P-450, the extent of this selectivity is highly variable with overlap of substrates and regio- and stereospecificities being

observed (26). In addition, substrates are often metabolized at more than 1 position, as in the case of testosterone. This is believed to be a function of both the binding characteristics of the enzyme and the ease with which the functional groups of the substrate undergo oxidation (66). A number of active site models have been proposed to explain the different substrate specificities of various P-450 isoenzymes.

CYP1A1

The substrate binding site of CYP1A1 has been proposed to consist of a hydrophobic cleft asymmetrically disposed to the heme iron atom. The asymmetric position of the binding site restricts the number of faces of the substrate that can be exposed to the active oxygen species (32). CYP1A1 has been implicated in the metabolism of a number of polycyclic aromatic hydrocarbon (PAH) compounds such as benzo(a)pyrene. The substrates tend to be large, rigid, planar molecules containing fused (hetero)aromatic rings that are good electron acceptors. Lewis et al (42) proposed that the binding site of CYP1A1 contains a number of aromatic amino acids that form a planar pocket to complement the (hetero)aromatic rings of the substrates. The metabolism of benzo(a)pyrene results in the preferential production of the bay region 7,8-diol-9,10-epoxide, which is a potent DNA-reactive ultimate carcinogen (33). In addition to PAH metabolism, CYP1A1 can metabolize a number of smaller non-PAH compounds in a regio- and stereoselective manner (62). It has been suggested that these substrates are positioned in the active site via hydrogen-bonding interactions between the substrate and an active site residue of CYP1A1 (38).

CYP2B1/2

The P-450 2B isozymes are involved in a number of biotransformations in the rat and are induced by phenobarbital. The substrates for CYP2B tend to be bulky, nonplanar molecules with greater conformational flexibility than CYP1A substrates (39). The substrates tend to have functional groups of similar size and hydrophobicity to isopropyl and to be poor electron acceptors (30, 39). It has been proposed that the binding site of CYP2B contains hydrophilic amino acids that are capable of forming hydrogen bonds with carbonyl and/or amine groupings of the substrate and hydrophobic nonaromatic residues that complement the isopropyl function (42).

CYP2C

The CYP2C subfamily appears to be important in metabolizing a number of xenobiotics particularly in humans (66). CYP2C8 effects the aromatic hydroxylation of warfarin and phenytoin, whereas CYP2C9 is involved in the metabolism of tolbutamide and a number of acidic nonsteroidal anti-inflammatory drugs and is potently inhibited by sulfaphenazole. CYP2C18 is subject to a genetic polymorphism manifest in the hydroxylation of *S*-mephenytoin and is not inhibited by sulfaphenazole (26). CYP2C substrates tend to have areas of strong hydrogen bond–forming potential positioned 5–10 Å from the site of oxidation, and a number are also charged at physiological pH (66). This has lead to the suggestion that hydrogen-bonding potential and possibly ion pair interactions are important in determining the substrate structure activity relationships of the P-4502C isozymes (66).

CYP2D

The CYP2D isozymes have been extensively investigated, as they are involved in the genetic polymorphic metabolism of debrisoquine, sparteine, and some 30 other substrates (17, 48). CYP2D1 (rat) and 2D6 (human) have similar substrate selectivities, but inhibition studies with quinidine (more potent in humans than rats) and its diastereoisomer quinine (more potent in rats than humans) demonstrate that differences in the enzyme active site must exist (65).

Substrates for CYP2D enzymes possess a basic nitrogen grouping that is mainly ionized at physiological pH, a hydrophobic region and a functional group capable of P-450 oxidation 5–7 Å from the basic nitrogen (69). Reactions catalyzed include aromatic hydroxylation (propranolol), aliphatic hydroxylation (metoprolol), and *N*-dealkylation (amiflamine) (17). The substrate binding site of CYP2D appears to contain a carboxyl group that binds and neutralizes the basic nitrogen of the substrate and a hydrophobic domain. The carboxylate group is assumed to serve as an anchoring site on the protein. Substrates can interact with either of the oxygen atoms of the carboxylate group (which are 2.2 Å apart), explaining why for some substrates the distance between basic nitrogen and site of oxidation is 5 Å, typified by debrisoquine, and for other substrates it is 7 Å, typified by dextromethorphan (38). The ionic bonding between substrate and enzyme means that the enzyme tends to have a high affinity for substrates and, thus, a low K_m (65). Many substrates also exhibit a coplanar conformation near the oxidation site and have a negative molecular electrostatic potential in a part of this planar domain approximately 3 Å away from the oxidation site (38).

The predictive value of the model was assessed by measuring the CYP2D6-mediated metabolism of 4 compounds, showing among them at least 14

TABLE I.—The 8 classical conjugation reactions.

Reaction	Conjugating agent
A. Reactions involving activated conjugating agents	
Glucuronidation	UDP glucuronic acid
Glucose conjugation	UDP-glucose
Sulfation	3′-Phosphoadenosine-5′-phosphosulfate
Methylation	*S*-adenosyl methionine
Acetylation	Acetyl coenzyme A
Cyanide detoxication	Sulfane sulfur
B. Reactions involving activated foreign compounds	
Glutathione conjugation	Glutathione
Amino acid conjugation	Glycine, ornithine, taurine

oxidative metabolic routes. From the model, 4 routes were predicted to be 2D6-mediated. *In vivo* and *in vitro* data from humans demonstrated that 3 of the 4 predicted metabolic routes were in fact mediated by CYP2D6 (40).

CYP3A

The CYP3A family tends to be involved in the metabolism of large, structurally diverse, fairly lipophilic compounds. Although substrates are bulky, metabolism tends to occur in small exposed functional groups that undergo reactions such as *N*-dealkylation and aliphatic hydroxylation. Substrates include the immunosuppressant cyclosporin A, nifedipine, and verapamil (66).

It has been suggested that the binding site for CYP3A is dominated by hydrophobic interactions and that, in contrast to CYP2D, which is governed by ionic bonding, this allows for a degree of flexibility in the position of substrate binding (67).

Conjugation Reactions

Phase 2 conjugation reactions may be divided into 2 distinct groups, depending on the source of energy for the process (10). In most instances, the energy is derived from the activated endogenous conjugating agent, as is the case for the glucuronic acid, sulfate, methylation, and acetylation reactions. In other examples, the energy is derived by prior metabolic activation of the xenobiotic, as is the case for glutathione and amino acid conjugations. Of the Phase 2 conjugation reactions listed in Table I, glucuronic acid conjugation ranks as highest importance, and many drugs (e.g., indomethacin, paracetamol, dapsone, clofibrate, morphine) are metabolized via this pathway. The conjugations are performed by a family of glucuronyl transferase enzymes located within the endoplasmic reticulum of the cells of the liver, intestine and kidney. These enzymes catalyze the conjugation of uridine diphosphate-α-1-glucuronic acid with nucleophilic O, N, C, and S atoms: during the reaction, C-1 of the sugar ring is inverted so that the products are 1-0-substituted B-D-glucopyranosiduronic acids. The enzymes have a molecular weight of between 50 and 60 kDa and exist as oligomers of between 1 and 4 subunits *in vivo* (60). At least 9 different isozymes in 2 different subfamilies are known to exist (8). Glucuronidation occurs in most mammalian species with the cat and related felines and the Gunn rat being notable exceptions.

Glutathione-*S*-transferases catalyze the conjugation of a number of functional groups (aryl and alkyl halides, lactones, epoxides, and quinones) with glutathione, the tripeptide γ-glutamylcysteinylglycine. The glutathione-*S*-transferases have a very extensive tissue distribution and are principally found in the cytosol of the cell. The proteins have a molecular weight of 24–28 kDa and exist as dimers *in vivo* (77). The dimeric proteins possess binding sites for glutathione and the electrophilic substrate, which brings the reactants close together (47). The mammalian glutathione transferase enzymes have been divided into 5 evolutionary classes: α, μ, π, θ, and microsomal (77). Typical substrates include parathion, urethane, ethacrynic acid, and 1-chloro-2,4-dinitrobenzene. The glutathione transferase enzymes are also very abundant in the liver cytosol (4–5% of total cytosolic protein). Thus, as well as having major significance in drug metabolism, these enzymes are also important in intracellular binding. This is particularly true for glutathione-*S*-transferase B (Ligandin). Compounds that bind to glutathione-*S*-transferases include bilirubin, estradiol, cortisol, testosterone, tetracycline, penicillin, and indocyanine green (43).

A number of catechol, phenol, and alcohol compounds are excreted as sulfate conjugates. This reaction between substrate and sulfate donor, 3′-phosphoadenosine-5′-phosphosulfate, is catalyzed by a family of sulfotransferase enzymes (18). The sulfotransferases have a cytosolic location and are found in many tissues including the liver, adrenals, lung, brain, jejenum, and blood platelets. The proteins

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