Chapter 2
Lipophilic Foreign Compounds

On the basis of solubility, foreign compounds that humans ingest or inhale can be classified into two categories. One class of foreign compounds is soluble in water (hydrophilic), but not in lipid medium. Another class is soluble in lipid medium (lipophilic), but not in water. Lipophilic substances require enzymatic conversion into hydrophilic, polar species before being excreted in the urine. The more lipophilic a foreign compound is, the more difficult it becomes for excretion via the kidney. Hydrophilic compounds can be excreted in the urine without enzymatic conversion to increase their solubility.

A foreign compound needs to move across biological membranes before it can enter the blood stream and distribute throughout the body. Owing to lipid bilayers serving as physical barriers for biomembranes, transport mechanism for lipophilic compounds across biomembranes is distinctive from that of hydrophilic substances. Hydrophilic molecules usually are unable to penetrate cell membranes because of their low lipid solubility. Membrane physical barriers also contribute to different sites of action between lipophilic and hydrophilic substances. Lipophilic molecules are lipid-soluble and generally can diffuse across cell membranes.

The process by which foreign compounds cross cell membranes and enter the blood stream is referred to as absorption. The gastrointestinal tract is one of the most important sites where foreign compounds are absorbed. A major group of foreign compounds that are absorbed by the lungs are gases, vapors, and aerosols. The present chapter discusses the membrane transport, the metabolic conversion, the sites of action, and excretion of foreign compounds. Figure 2.1 briefly describes the entry, absorption, metabolism, and excretion of a foreign compound.

2.1 Lipophiles

Water molecule is polar species, which has a positive end and a negative end. Positive and negative ends of a water molecule display electrostatic attraction. Water owes its great superiority as a solvent for ionic substances partly due to its polarity.

C.-H. Chen, *Activation and Detoxification Enzymes: Functions and Implications*,
DOI 10.1007/978-1-4614-1049-2_2, © Springer Science+Business Media, LLC 2012

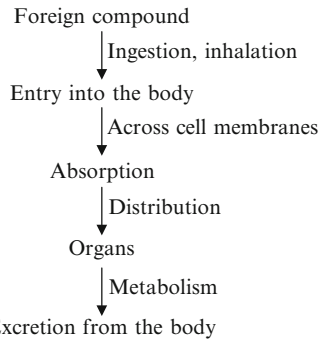

Fig. 2.1 Entry, absorption, metabolism, and excretion of foreign compound

Table 2.1 Solubility of typical lipophilic foreign compounds

Lipophilic foreign compounds	Solubility in water
Menadione	Insoluble
Acetaminophen	Very slightly
Benzo[a]pyrene	Insoluble
Quinone	Slightly
Polychlorinated biphenyls	Insoluble
Diazepam	Slightly
Aniline	Insoluble
Pebbendazole	Insoluble

The polarity of water permits it to solvate ions strongly. Water has a high dielectric constant (78 at room temperature). When an ionic compound (either solid or liquid) dissolves in water, its structural units (ions) become separated from each other and the spaces in between become occupied by water molecules (hydration). Water contains the hydroxyl group (–OH). Compounds containing hydrogen attached to oxygen (hydroxyl group) or nitrogen (amine group) tend to increase solvation powers.

For nonionic compounds, their solubility is dependent on their polarity. The rule of thumb is "Like dissolve like." Nonpolar or weakly polar compounds dissolve in nonpolar or weakly polar solvents (organic solvents). Unlike water, nonpolar solvents have a low dielectric constant (e.g., 2 for benzene). Lipid bilayers serve as physical barriers for biological membranes, where lipid medium has a low dielectric constant (about 5). Nonpolar foreign compounds prefer to dissolve in low dielectric constant lipid medium, and are, therefore, referred to as lipophiles. Some lipophilic foreign compounds are shown in Table 2.1, which reveals poor water solubility for lipophiles.

2.2 Transport Across Cell Membranes

The accumulation of foreign compounds at the site of action is facilitated by absorption and distribution. Absorption is referred to the transfer of a foreign compound from the site of exposure into the general circulation. Lipid solubility of foreign

compounds is usually the most important properties that influence their absorption. While transporters may contribute to the gastrointestinal absorption of some chemicals, a large majority of foreign compounds traverse epithelial barriers and reach blood capillaries by diffusion through the cells.

Lipid bilayers serve as physical barriers that do not favor a spontaneous exchange of foreign compounds between the internal and external cell compartments. Living organisms use cell membranes as hydrophobic permeability barriers to control access to the internal cell compartment. The movement of foreign compounds into or out of the cells is carried out by various transport mechanisms depending on their solubility characteristics. Lipophilic, nonpolar compounds are able to move across cell membranes, while hydrophilic, polar compounds are largely restricted to the extracellular compartments and cannot enter into the cells simply by free diffusion. The uptake of hydrophilic compounds across cell membranes is mediated by channels or transport proteins, which specifically select substrates (solutes) from the extracellular medium.

2.2.1 Major Transport Mechanisms

Major mechanisms for the transport of a foreign compound (solute) across biological membranes include passive diffusion, facilitated diffusion, and active transport. Passive diffusion of solutes across cell membranes is composed of three steps: partition from the external aqueous medium to the membrane lipid phase, diffusion across membrane lipid bilayers, and partition into the internal cellular medium. In passive diffusion, the driving force for solute to move across membrane lipids into the cells is the concentration gradient, in which the concentration of solute in the external cell medium is higher than that in the internal cell medium.

A large number of foreign compounds are transported across biomembranes through facilitated diffusion. In facilitated diffusion, the transport of a foreign compound across biomembranes into the cells is facilitated by transport protein (carrier). Facilitated diffusion involves no input energy and occurs downhill in accordance with the solute concentration gradient. Transport protein selects a specific solute from the extracellular medium. The binding enables the transport protein to carry the solute across cell membranes into the internal cell medium, such as in the case of glucose permeation across cell membranes mediated by glucose transporter protein.

Active transport of a foreign compound across biomembranes into the cells is also mediated by membrane transporters. But, unlike facilitated diffusion, active transport requires energy input as well as the movement of solute against a concentration gradient. Depending on the driving force, active transport can be classified into primary and secondary active transports. Primary active transport is coupled with ATP hydrolysis catalyzed by Na^+, K^+- ATPase, which provides the energy for the uptake of solute against a concentration gradient. The unidirectional movement of a solute across biomembranes in mammalian cells is mediated by transporter proteins such as ATP binding cassette transporters (ABC transporters).

In secondary active transport, the transport of solute across biomembranes uphill against its concentration gradient is coupled with the movement of another solute downhill in accordance with its concentration gradient. In this case, the driving force for the uptake of solute across biomembranes is the electrochemical potential stored in a concentration gradient of another solute. Therefore, secondary active transport takes place at the expense of a preexisting electrochemical gradient of another solute. For example, the uptake of lactose across E. coli membranes is coupled with the movement of H^+ downhill in accordance with proton electrochemical potential. Another example is Na^+-Ca^{++} exchange protein that uses the energy stored in the Na^+ gradient established by Na^+, K^+-ATPase to export cytosolic Ca^{++}.

2.2.2 Channels and Transporters

Facilitating membrane permeation of inorganic ions and organic compounds involves channels and transporters. Channels exist in two primary states: open and close. In the open state, channels act as pores for selected ions, allowing them to permeate across cell membranes, and then channels return to the close state. By contrast, transporter protein forms an intermediate complex with a specific solute (the substrate) on the external membrane, which induces the translocation of the substrate to the internal membrane. Transporter proteins are membrane proteins that control the influx of essential nutrients and ions as well as the efflux of cellular wastes and foreign compounds.

Mechanistic difference between channels and transporters results in a marked difference in their turnover rates. The turnover rate constants of typical channels are much larger than those of transporter proteins. ATP binding cassette (ABC transporter) and solute carrier (SLC transporter) are two major families of membrane transporters for drug and other xenobiotics. Membrane transporters work in concert with foreign compound-metabolizing enzymes to mediate the uptake and efflux of xenobiotics and their metabolites. Generally, SLC transporters mediate either influx or efflux of drug, while ABC transporters mediate unidirectional efflux.

2.3 Sites of Action

The liver encounters foreign compounds such as food, drugs, and environment chemicals after they are absorbed in the intestinal tract. The liver is the major organ where foreign compounds are metabolized and eventually excreted, chemically active intermediates are produced, and toxicities are manifested. The liver is the main metabolic site for drugs. The metabolic site for drugs depends upon the presence of foreign compound metabolizing enzymes, and most of these enzymes are present in the liver. Hepatocytes contain phase I enzymes that have the capacity to generate metabolic intermediates as well as phase II enzymes that catalyze the

addition of polar groups to lipophilic compounds and target the formed conjugates to transport carriers for excretion.

Although metabolites or metabolic intermediates may react at the site where they are generated, the liver is not necessarily the target organ of toxicity. The metabolites may diffuse away and react with other targets. Metabolic intermediates may be transported to other organs where they exert toxic effects. Meanwhile, before transported to other organs, metabolic intermediates may potentially cause toxicity in the liver. Besides the liver, the kidney is a frequent target organ of toxicity and is also a main site for drug metabolism. The kidney, which receives a large amount of blood, also contains a variety of foreign compound-metabolizing enzymes. The breast, lung, and colon are frequent target sites in spite of their limited metabolic ability. Metabolites of foreign compounds such as aromatic amines are also transported to the bladder, where they are released and converted to carcinogenic species.

2.4 Excretion of Foreign Compounds

Foreign compounds excreted from the body include waste products from the digestion of foods, drugs accumulated in the body, chemical substances in the environment, and industrial chemicals in the household. These compounds are excreted from the body either unchanged or being converted to metabolites. Owing to high solubility in water, hydrophilic species generally can be excreted from the body through urine or bile without chemical modification. Conversely, because of limited solubility in water, lipophilic foreign compounds require metabolic conversion into hydrophilic metabolites before they are excreted through urine.

Metabolic intermediates formed in phase I reactions can overwhelm the body's defense mechanism if they are not removed quickly. Solute metabolites are often products resulted from phase II reactions. Before excreted from the body, foreign compounds transport from the internal to the external cell compartment carried out by transport proteins in phase III metabolism (see below). Figure 2.2 illustrates different metabolic processes that foreign compounds may precede before being excreted from the body. In addition, foreign compounds may proceed from phase I directly to phase III before their excretion.

Urinary and biliary systems are two primary routes for the excretion of foreign compounds and their metabolites from the body. Accordingly, renal and hepatic excretions are the two major pathways. Vectorial transport, i.e., asymmetrical transport of solute across cell membranes, plays a major role in urinary and hepatobiliary excretion of drugs from the blood. ABC transporters are able to achieve vectorial transport by extruding lipophilic xenobiotics to the exterior compartment of cells.

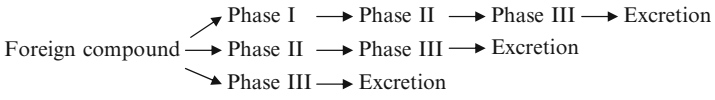

Fig. 2.2 Metabolic processes preceding foreign compound excretion

2.4.1 Renal Excretion

More foreign compounds are eliminated from the body by the kidney than by other organs. The kidney is, therefore, the most important organ for the excretion of foreign compounds including drugs, toxins, carcinogens, and their metabolites, The kidney is very efficient in the elimination of toxicants from the body and is critical in the body's defense against foreign compounds. Renal excretion plays an important role in eliminating conjugation products resulting from phase II reactions. Renal excretion involves glomerular filtration, active tubular secretion, and passive tubular readsorption. Small compounds (<60 kD) are usually able to pass the glomerular filtration barrier, while larger metabolites are excreted via other pathways. After glomerular filtration, a foreign compound or its metabolite may remain in the tubular lumen and be excreted in urine. Transport carriers located on the basolateral membrane of proximal tubule cells can transport foreign compounds from the blood into the epithelial cells for excretion.

Organic anion transporters and organic cation transporters are two major classes of secretory transporters in the mammalian kidney. Structurally diversified organic cations and anions, including positively and negatively charged drugs and their metabolites, are secreted in the proximal tubule of the kidney. Transporters in the kidney mediate the secretion or reabsorption of many foreign compounds, thereby influencing the plasma levels of their substrates. SLC transporters and ABC transporters are two major classes of secretory transporters in the mammalian kidney.

SLC transporters are involved in moving organic cations across the basolateral membrane. They are also implicated in a variety of organic anions that are secreted in the proximal tubule. Major physiological functions of ABC transporters include the transport of toxic foreign compounds, and ABC transporters are also involved in the secretion of organic cations. They translocate a variety of compounds through cell membranes against concentration gradients at the expense of ATP hydrolysis. Besides SLC and ABC transporters, other transporters also participate in the excretion of organic ions. Transporters such as multidrug-resistance-associated proteins localized in the apical brush-border membrane are responsible for the excretion of conjugated metabolites.

2.4.2 Hepatic Excretion

In addition to being the main site for biotransformation of foreign compounds, the liver also plays an important role in removing xenobiotics from blood after they are absorbed in the gastrointestinal tract. Depending on molecular weights, foreign compounds or their conjugates can be excreted into bile in substantial quantities. SLC transporters mediate either influx or efflux of foreign compounds (e.g., drugs). Hepatic uptake of organic anions, cations, and bile salts can be carried out by SLC

transporters in the basolateral membrane of hepatocytes by either facilitated diffusion or secondary active transport. Moreover, ABC transporters in the canalicular membrane of hepatocytes mediate unidirectional efflux of foreign compounds. The excretion of drugs and their metabolites mediated by ABC transporters is carried out uphill against a concentration gradient, where ATP hydrolysis provides the driving force for the transport.

2.4.3 Reabsorption in the Kidney

Transporters in the kidney mediate the secretion or reabsorption of many foreign compounds and thereby influence the plasma levels of their substrates. Kidney excretion of hydrophilic compounds is more efficient than lipophilic compounds. Hydrophilic compounds and ions are readily excreted in the urine. While, lipophilic compounds that have high lipid/water partition coefficients can be readsorbed efficiently across the kidney tubules back into the bloodstream. Tubular reabsorption in the kidney is a major factor contributing to the difficulty in the excretion of lipophilic compounds. Poor solubility in water is another critical factor. Lipophilic compounds are not readily eliminated before they are converted into water soluble polar compounds. Living organisms, therefore, develop metabolic mechanisms with enzyme systems that are proficient in the conversion of lipophilic into hydrophilic compounds.

2.5 Major Metabolic Pathways

Foreign compounds including hydrophilic and lipophilic species are not produced in vivo. They are brought into the body via ingestion and inhalation and are subsequently metabolized by the body. The capacity of removing a wide range of lipophilic species is the major challenge of metabolic pathways for foreign compounds. This challenge is achieved by a combination of low specificity enzymatic systems and physical barriers of biomembranes. Low specificity of enzymatic systems makes it possible to metabolize a wide range of lipophilic and hydrophilic compounds. Physical barriers of biomembranes attribute to the role of membrane permeability and hydrophobicity in the metabolism of foreign compounds.

2.5.1 Phase I Metabolism and Phase II Metabolism

The metabolism of lipophilic foreign compounds involves a set of metabolic pathways. Among them are two major enzyme-catalyzed pathways: the functionalization

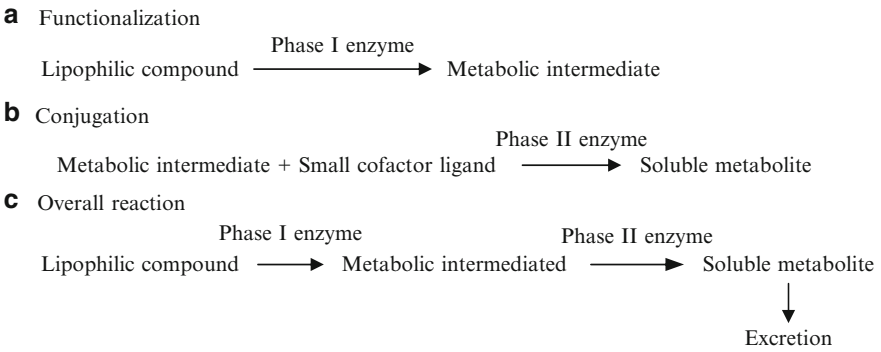

Fig. 2.3 Enzymatic conversion of lipophilic compounds

in phase I reactions involving the addition of a functional group to a xenobiotic and the conjugation in phase II reactions involving the coupling of an endogenous small cofactor molecule to a functionalized xenobiotic. These two pathways convert lipophilic substances into hydrophilic species by the introduction of a functional group and the conjugation with an endogenous molecule. A phase I reaction generally proceeds before a phase II reaction. However, in some cases, a phase II reaction may occur without a preceding phase I reaction or prior to a phase I reaction. Foreign compound-metabolizing enzymes are produced based on the information stored within the genes and are present in the liver at much higher concentrations than in other organs.

Phase I enzyme catalyzes a functionalization reaction by means of oxidation, reduction or hydrolysis, where a functional group is introduced to the structure of a lipophilic foreign compound. While the addition of a functional polar group results in a moderate increase in the water solubility of the parent compound, the functionalization also activates the foreign compound, often leading to the formation of a metabolic intermediate. The functionalized compound then undergoes a conjugation reaction catalyzed by a phase II enzyme, which combines a small cofactor molecule with the functionalized compound to form a conjugate. These two pathways are responsible for the metabolism of foreign compounds, but generally are not implicated in the elimination of endogenous metabolites derived from normal cellular constituents.

Many foreign compounds are converted to metabolic intermediates through biotransformation catalyzed by phase I enzymes. Many metabolic intermediates of chemical compounds are reactive and are ultimately responsible for their toxicities. The conjugation reaction catalyzed by a phase II enzyme detoxifies metabolic intermediate, rendering it less harmful and largely increasing its water solubility, thus facilitating the excretion of foreign compound from the body. Figure 2.3 illustrates the two major steps in the conversion of a lipophilic foreign compound into a water soluble species: step a (phase I metabolism) and step b (phase II metabolism). Step c represents the overall reaction.

2.5.2 Phase III Metabolism

Conjugates produced in phase II metabolism are hydrophilic, polar substances. Preceding their excretion, conjugates require transmembrane movement from the internal to the extracellular cell compartment. Because membrane lipids act as the physical barriers, the transport of conjugates out of the cells cannot be carried out by free diffusion. Instead, the transmembrane movement of conjugates across biomembranes requires transport proteins called export pumps. The process that facilitates the transport of conjugates and other metabolites from the internal to the external cell compartment is referred to as phase III metabolism. Phase III metabolism of foreign compounds is the step that occurs after metabolic conversion and before their excretion from the body. Conjugates may be further processed before being recognized by transport proteins and prior to moving out of the cells. A number of ATP-dependent transport proteins or export pumps have been identified in the liver. An example of ATP-binding transporters is the family of multidrug resistance proteins. The topic of phase III metabolism is not within the scope of this book and is, therefore, not further addressed in the following chapters.

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http://www.springer.com/978-1-4614-1048-5

Activation and Detoxification Enzymes
Functions and Implications
Chen, C.-H.
2012, XIV, 182 p., Hardcover
ISBN: 978-1-4614-1048-5