Drug Metabolism

4

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4.1 Introduction

Through evolution, the body has developed different strategies to dispose metabolic waste and protect itself from exposure to potential toxic chemicals. It is useful to differentiate chemical compounds with a well-defined physiologic role from those that do not have any. The latter will be generically called *xenobiotics* and include most drugs, natural and industrial contaminants, other industrial compounds used for technical purposes (i.e., agrochemicals), cosmetics, food additives, food components with no physiological function, and recreational and social drugs (Testa and Krämer 2006), along with their biotransformation products without physiologic role. There is also a tendency to view as xenobiotics those endogenously produced compounds which are administered at relatively high doses (for medical or nonmedical reasons) (Jenner et al. 1981; Testa and Krämer 2006) and are thus found within the body outside their physiological range.

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► **Definition** The prefix *xeno-* comes from the Ancient Greek and means "alien." As in *xenophobia*, it is used to denote strangeness. Accordingly, the word xenobiotic describes a chemical entity which is found within a living organism but has no physiological function. Xenobiotics can be regarded as potentially toxic, and the body has mechanisms to dispose them.

In contrast, *physiological compounds* are chemicals having essential biological functions, indispensable to the survival or well-being of the body. Among them, we can mention water, nutrients, oxygen, mineral, dietary fiber, or vitamins.

Those mechanisms available within the body to reduce its exposure to nonphysiological compounds will also limit the bioavailability of that particular category of xenobiotic which are therapeutic drugs. Essentially, these mechanisms can be broadly classified as (a) *preventing xenobiotics from entering the bloodstream or sensitive organs* (e.g., the blood–brain barrier limits the distribution of many xenobiotics to the brain); (b) *physically removing the xenobiotic molecules* (mainly through the bile and the urine but also, sometimes, through perspiration and respiration); and (c) *metabolizing xenobiotics (biochemical transformation or biotransformation) to produce drug metabolites whose excretion is faster* than that of the parent (unchanged) compound.

► **Important** Although drug metabolism and drug excretion are presented separately, they are indeed highly integrated phenomena. As will be extensively discussed throughout this and the next chapter, metabolism and excretion work in a concerted manner to provide a more efficient disposal of xenobiotics.

The ultimate objective of all bodily drug elimination systems is to promote excretion of the xenobiotic molecules (either unchanged or as metabolites). The major routes of excretion are bile (those drugs or drug derivatives excreted though bile are eventually, if everything goes well, excreted in feces) and urine. Taken globally, biotransformation processes transform the drug into a considerably more polar biochemical product. Such polar metabolite will not be (re)absorbed in either the intestine or the renal tubules. Furthermore, they can be occasionally subjected to active secretion into the bile or the tubular content.

Some textbooks point to the enhanced water solubility of drug metabolites as the origin of a more efficient excretion. This is only indirectly true, since commonly the drug concentrations in biologic fluids are way below its solubility. Improved hydrophilicity increases the excretion rate of the metabolite (in comparison with the parent compound) because highly polar metabolites are neither efficiently reabsorbed nor distributed (they experience much greater diffusional barriers) and in some occasions are actively secreted into the intestinal, bile canalicular, or tubular lumen.

It emerges from the previous two chapters that to be properly absorbed and distributed most drugs (with the possible exception of those that experience facilitated diffusion or active uptake) require an adequate lipophilic-hydrophilic balance. They must display a certain minimal solubility in physiological media (only soluble molecules are transferred through biological barriers), but they must also possess some lipophilicity so that they can passively permeate to the bloodstream and then move forward to the remaining bodily compartments. Furthermore, lipophilic drugs tend to display higher affinity for their molecular targets. On the one hand, the formation of the drug-target complex implies desolvation; desolvation free energies, which will be higher for polar drugs, oppose the binding event (Kólar et al. 2011). On the other, lipophilic substituents in the drug are often wanted to exploit hydrophobic binding pockets in the target.

The same requisites that a drug must generally fulfill to be absorbed and distributed, and interact with its molecular target conspire against excretion, since lipophilic drugs tend to be extensively reabsorbed. Accordingly, biotransformation to polar metabolites is often required to enhance the excretion of a drug.

4.2 Metabolism: Biotransformation Reactions

► **Definition** Drug biotransformation involves the enzyme-mediated conversion of drugs into drug metabolites (the products of a biotransformation reaction).

A drug is usually metabolized in a sequential manner. The *parent* (unchanged) *drug* (i.e., the pharmacologically active chemical entity that has been administered through a drug delivery system) is thus initially converted to a *primary metabolite*, which may be substrate for a second biotransformation that will produce a *secondary (or sequential) metabolite* (Smith 2008).

Biotransformation reactions can be broadly classified into functionalization reactions (historically known as phase I reactions) and conjugation or synthetic reactions (also known as phase II reactions). Functionalization reactions imply the creation of a functional group or the modification of an existing one. This type of biotransformation introduces a "chemical handle" or anchoring point in its substrate, so that the resulting metabolite is more prone to experience a synthetic reaction (Testa and Krämer 2007; Talevi 2016). They include redox reactions and hydrolyses/hydrations (Testa and Krämer 2006). Synthetic reactions imply coupling the drug or one of its phase I metabolites with a diversity of (endogenous) moieties (e.g., glucuronic acid, glutathione, sulfate, phosphate, amino acids, acetyl, etc.) generally resulting in metabolites with a drastic increase in polarity and also a (moderately) higher molecular weight, which are not likely to be reabsorbed.

Sequential metabolism formed the basis for the common nomenclature of phase I and phase II metabolism, although a phase II reaction may occur to a drug without a precedent phase I reaction if the drug has a suitable anchoring site to which the correspondent conjugation moiety is transferred. In other words, conjugation reactions are able to produce first-generation and later-generation metabolites

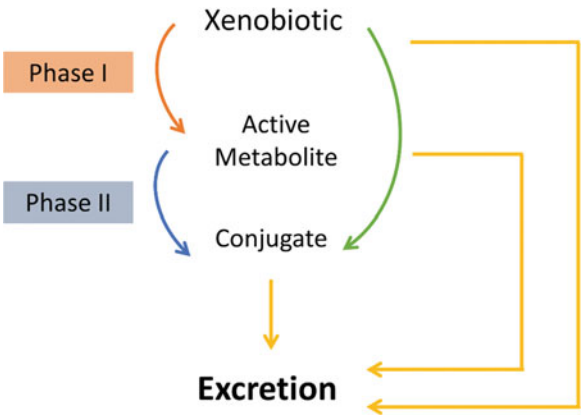

Fig. 4.1 The fate of xenobiotic within the body may depend on different elimination pathways. The unchanged xenobiotic molecule might be directly excreted. Alternatively, it may be excreted as a phase I or phase II metabolite. Synthetic reactions could occur directly on the parent compound or may require a previous phase I reaction to occur and predispose the drug to a conjugation. Often, all these possibilities take place in parallel and contribute to the overall elimination of the drug

(Testa and Krämer 2008). Accordingly, phase I and phase II nomenclature may be misleading in the sense that it may be *wrongly* understood that phase II biotransformations always occur on phase I metabolites. A meta-analysis by Testa et al. on the biotransformation reactions of more than 1100 xenobiotics (Testa et al. 2012) shows that first-generation metabolites are formed mainly (almost 70%) by redox reactions, but about 22% are formed by conjugations. In the second generation, the contribution of redox reactions decreases to about 50%, whereas conjugations increase to 37%. In third and later generations, both redox and conjugation reactions accounted for the same proportion (46%) of metabolites. The proportion of metabolites generated by hydrolysis does not vary significantly from one generation to another, remaining in the 8–12% range. The previous analysis demonstrates the inadequacy of the phase I/phase II classification, which assumes the metabolism of xenobiotics to begin with redox or hydrolysis reactions, followed in subsequent metabolic steps by conjugations.

The possible fate of a xenobiotic after entering the body is illustrated in Fig. 4.1. Acetaminophen metabolism is used in Fig. 4.2 to provide the reader with a concrete idea of the complexity of elimination processes and how phase I and phase II reactions may be or may be not coupled.

Drug metabolism not only introduces physical-chemical modifications to the drug. Aside from the changes in molecular weight and polarity, the resulting metabolites are usually inactivated from a pharmacological viewpoint. Occasionally, however, they can be pharmacologically active (and even more active than the parent compound), or, if excessively reactive from a chemical perspective, they may be toxic.

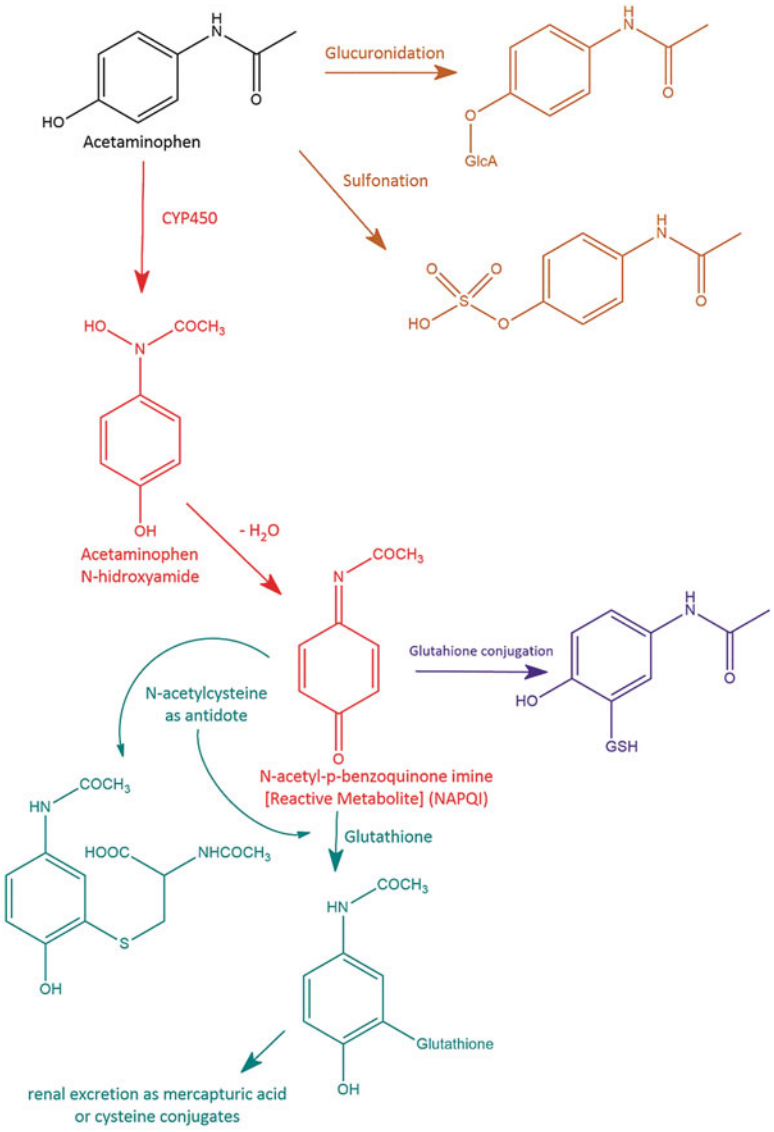

Fig. 4.2 Elimination pathways for acetaminophen/paracetamol. A fraction of the dose is eliminated with no changes in urine. The drug undergoes direct conjugation with glucuronide and sulfate or sequential CYP450-mediated biotransformation to the highly toxic N-acetyl-p-benzoquinone imine (NAPQI) which is inactivated by conjugation with glutathione. If not inactivated, NAPQI reacts with hepatic proteins resulting in acute hepatic failure

4.2.1 Functionalization (Phase I) Reactions

Functionalization reactions expose, as in hydrolysis reactions, or introduce, usually through oxidation reactions, chemically reactive functional groups such as -OH, $-NH_2$, or -SH. The enzymes that catalyze these reactions may be oxygenases/oxidases (such as cytochrome P450 (CYP450) isozymes, flavin-containing monooxygenases, monoamine oxidases, peroxidases, xanthine oxidases, alcohol oxidases, and others), reductases (such as aldo-keto reductases), or hydrolytic enzymes (e.g., esterases, amidases). It is worth mentioning that some of the phase I enzymes can catalyze both oxidations and reductions (Testa and Krämer 2007). It has been estimated that redox reactions account for about 60% of total xenobiotic metabolic reactions (Testa et al. 2012).

Regarding subcellular localization of phase I reactions, a significant fraction of the correspondent enzymes are membrane proteins located in the smooth endoplasmic reticulum (e.g., CYP450, flavin-containing monooxygenases, some esterases, and epoxide hydrolases) (Cribb et al. 2005). The lumen of the endoplasmic reticulum has an oxidizing environment relative to the cytosol (the glutathione/glutathione disulfide ratio is around 3, compared to 100 in the cytosol). This balance seems to be crucial to the process of oxidative protein folding within the reticulum and may contribute to the generation of oxidative stress through the use of oxygen as the terminal electron acceptor in protein folding (Tu and Weissman 2004). It also creates an environment that favors further oxidation of reactive intermediates that enter the endoplasmic reticulum lumen. To a lesser extent, some phase I enzymes are also located in the mitochondria (e.g., monoamine oxidase) and the cytosol (e.g., xanthine oxidase or alcohol dehydrogenase).

► **Important** The liver is, by far, the organ that expresses the highest levels of metabolizing enzymes. However, some metabolism enzymes are also extensively expressed in other organs (and, in some particular cases, at higher levels than in the liver itself) (Jennen et al. 2010). Aside from the liver, other organs that significantly contribute to xenobiotic metabolism are the small intestine, the lungs, and the kidneys. Note that all of them are involved in matter exchange with the environment; accordingly, their involvement in biotransformation of xenobiotics does not lack evolutionary logic. It is also important to note that gut microbiota can also significantly contribute to drug metabolism and have an impact on drug elimination kinetics (Swanson 2015).

Some examples of functionalization reactions can be found in Table 4.1.

A key feature of phase I reactions is that, while intending to contribute to xenobiotic detoxification, they may play a significant role in the etiology of some diseases and metabolic-mediated toxification processes by resulting in the formation of electrophilic intermediates capable of covalently modifying biological

Table 4.1 Some examples of phase I biotransformation reactions

Reaction	Examples
Hydroxylation	Aromatic hydrocarbons (R-Ar)
Sulfo-oxidation	Disulfides (R-S-R)
Dehydrogenation	Alcohols (R-OH)
Reduction	Nitro compounds (R-NO_2)
Hydrolysis	Esters (R-COO-R')

Table 4.2 Examples of bioactivation of halogenated solvents and their associated toxicity

Halogenated solvent	Toxicity
Bromobenzene	Hepatic necrosis
Vinyl chloride	Liver cancer
Carbon tetrachloride	Hepatic necrosis, renal necrosis
Chloroform	Hepatic necrosis, renal necrosis

macromolecules (adduct formation), typically proteins or DNA, a process known as drug bioactivation (Tang and Lu 2010; Matias et al. 2014; Gan et al. 2016) (note, though, that mechanistic pathways of toxicity pathways for drugs that form adducts are no fully understood yet and that adduct formation may coexist with adduct-independent pathways). A relevant example of drug bioactivation will be later discussed when describing the molecular basis of paracetamol toxicity. Furthermore, the responsible moiety for toxification by some halogenated solvents (after oxidation) is presented in Table 4.2. After scrutinizing xenobiotic metabolites, Testa et al. concluded that about 7% are toxic, mostly due to strong electrophilicity (Testa et al. 2012). Toxic compounds usually arise from Csp^2 and Csp^3 oxidations (e.g., resulting in epoxide formation), N- and S-oxidations, and, above all, generation of quinones and analogues (quinonimines, quinonimides, and quinone diimines), which account for around 40% of all toxic and/or reactive metabolites. The major enzyme systems involved in bioactivation are the CYP450 and peroxidase systems (Walsh and Miwa 2011).

Furthermore, it is relatively frequent for phase I metabolites to retain pharmacological activity (occasionally, a metabolite could display more potency than the unchanged drug!) (Talevi 2016). C-hydroxylation and hydrolysis reactions play a predominant role in the generation of pharmacologically active metabolites (Testa et al. 2012). Pharmacologically active metabolites are majorly first-generation ones. The role of ester hydrolyses is not surprising having in mind the current interest in prodrugs (which are defined later). As for the pharmacological significance of alkyl and aryl hydroxylations, this is majorly owing to the hydroxylated metabolite retaining the target affinity and thus pharmacological activity of the parent compound. Note that while phase I metabolites are chemically distinct from the parent drug, they are still chemically similar, and a similar activity profile to the one of the parent compound might be attained if the modification is introduced in a non-pharmacophoric group or whenever it leads to optimization in binding to the molecular target (Fura 2006).

▶ **Definition** A prodrug is a compound that, after administration, is metabolized into a pharmacologically active drug within the body. Prodrugs are often designed to improve bioavailability (enhanced oral absorption, differential distribution) or improve safety and, sometimes, to mask unpleasant organoleptic properties.

▶ **Important** Sometimes, phase I metabolites will retain the pharmacological activity of the parent compound. In some occasions, they may even display more activity than the parent compound. Bioactivation to toxic metabolites by phase I reactions is also possible.

4.2.1.1 Cytochrome P450

CYP450 comprises a superfamily of hemoproteins (i.e., they contain the organic cofactor *heme*, a prosthetic group crucial for their catalytic activity). This enzymatic system is arguably the most important phase I xenobiotic metabolizing system, participating in the elimination of 70–85% of known drugs. Furthermore, CYP450 is also involved in the metabolism of endogenous compounds, including the biosynthesis, bioactivation, and, sometimes, breakdown of steroids (sex hormones, neurosteroids, cholesterol, vitamin D) and fatty acids (Gibbons 2002; Jones et al. 2014; Westphal et al. 2015). Fifty-seven individual P450s have been characterized in humans (Lewis 2004), distributed across 18 families. In mammals, CYP450 members are located either in the endoplasmic reticulum or in the inner mitochondrial membrane. Mutations or other defects in genes encoding CYP450 members (especially, from those families characterized by low redundancy and narrow substrate specificity) result in P450-mediated diseases including those caused by aberrant steroidogenesis; defects in fatty acid, cholesterol, and bile acid pathways; and vitamin D and retinoid dysregulation (Nebert et al. 2013). Furthermore, CYP members from microorganisms provide the basis for the development of anti-infective therapies (e.g., antifungal and antiprotozoal drugs) (Lepesheva and Waterman 2011; Lepesheva et al. 2018).

▶ **Important** Monooxygenation is beyond a doubt the most common reaction catalyzed by CYP450, i.e., insertion of one atom of oxygen into an organic substrate (RH), while the remaining oxygen atom from an oxygen molecule is reduced to water:

$$\mathrm{RH} + \mathrm{O_2} + \mathrm{NADPH} + \mathrm{H^+} \rightarrow \mathrm{ROH} + \mathrm{H_2O} + \mathrm{NADP^+} \qquad (4.1)$$

However, other less common reactions are also catalyzed by CYP450, including reductions, ester cleavage, and ring expansions (Guengerich 2001).

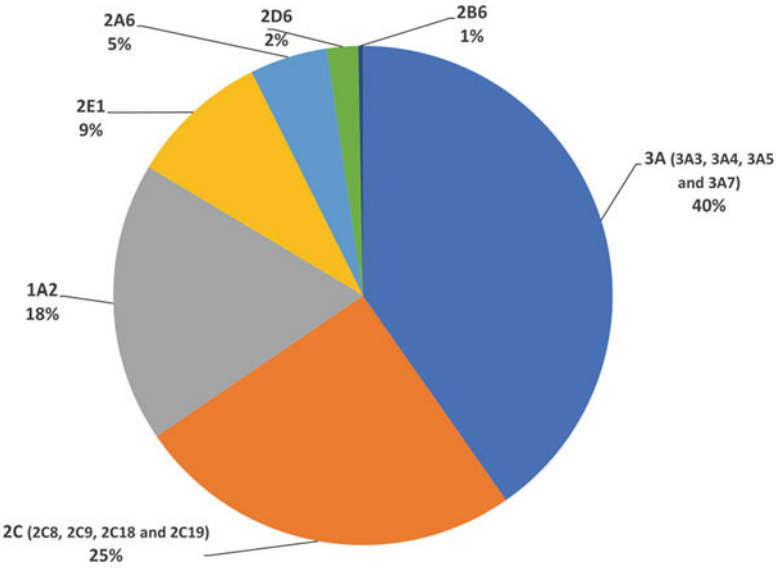

Fig. 4.3 Comparison of expression levels of individual P450 isoforms in the total P450 in human liver microsomes. (Adapted from Kwon 2002)

The liver, kidneys, intestine, lungs, and skin are the organs with the highest expression levels of CYP450 members. Since each CYP450 member has its own substrate specificity, it is not hard to imagine that, as a whole, the superfamily confers great versatility for the biotransformation of a wide range of xenobiotics. Furthermore, some of its members display a wide substrate specificity (i.e., they are polyspecific): in particular, CYP3A4 is the most promiscuous member of the superfamily, catalyzing the phase I biotransformation of hundreds of known drugs (Bu 2006).

Figure 4.3 displays the relative abundance in the liver of different CYP450 isozymes. Figure 4.4 shows the approximate percentage of drugs in the market whose metabolism is mediated by those CYP450 members. Note that the rate at which a given member catalyzes the conversion of a substrate depends on *the turnover number* (maximum number of chemical conversions of substrate molecules per second that a single catalytic site will execute), *the number of available enzyme copies*, and *the affinity of the substrate for the enzyme*. Consequently, there exist isoforms (e.g., CYP2D6) which have a remarkable role in xenobiotic metabolism despite being expressed at comparatively low expression levels.

Besides the monooxygenases, the CYP450 includes "auxiliary" enzymes that contribute with the electrons required to the redox reaction: NADPH-CYP450 reductase and cytochrome b5, which in turn may be reduced by NADPH-cytochrome reductase and NADH-cytochrome-b5 reductase (Gan et al. 2009).

The most common proposed mechanism of the catalysis involves the following (Meunier et al 2004; Guengerich 2007) (Fig. 4.5): (a) binding or the substrate in the vicinity of the heme group, which induces a change in the conformation of the active

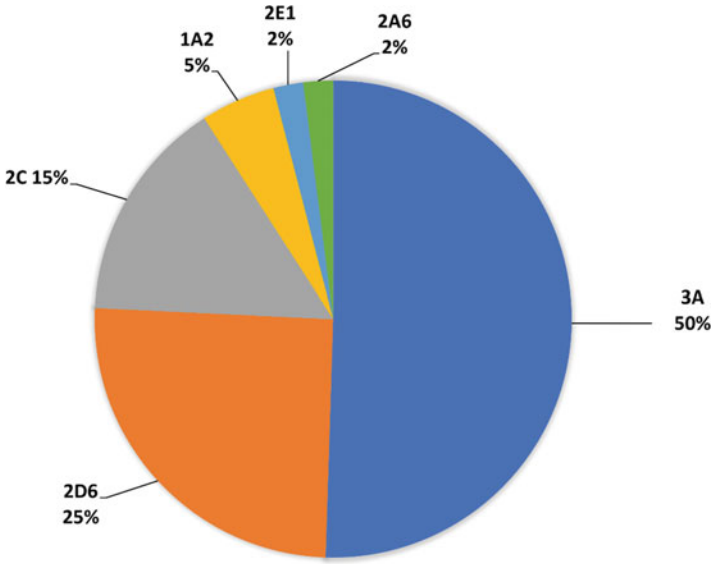

Fig. 4.4 Estimated percent of drugs on the market metabolized by various cytochrome P450 isoforms. (Adapted from Kwon 2002)

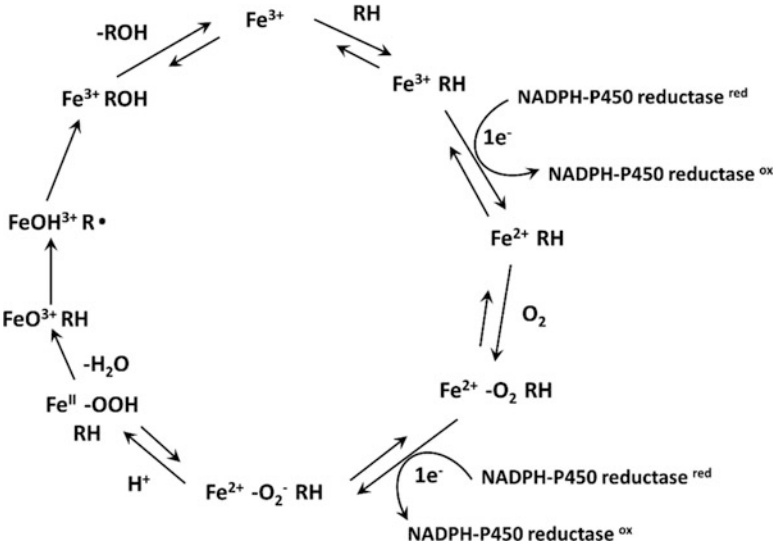

Fig. 4.5 Generalized catalytic cycle for CYP450 reactions. RH denotes the parent compound (substrate for the biotransformation), whereas ROH represents the metabolite (i.e., product of the mono-oxygenation reaction)

site, often displacing a water molecule from the distal axial coordination position of the heme iron; (b) substrate binding induces electron transfer from NADPH via CYP450 reductase or another associated reductase; (c) molecular oxygen binds to the resulting ferrous heme center at the distal axial coordination position, initially giving a dioxygen adduct; (d) a second electron is transferred from either cytochrome P450 reductase, cytochrome b5, or ferredoxins, reducing the Fe-O_2 adduct to give a short-lived peroxo state; (e) the peroxo group formed in step d is rapidly protonated twice, releasing one molecule of water and forming highly reactive species (an iron(IV) oxo or ferryl species with an additional oxidizing equivalent; the remaining oxygen atom is transferred to the substrate. After the product is released from the active site, the enzyme returns to its original state, with a water molecule returning to occupy the distal coordination position of the iron nucleus.

The names of CYP450 enzymes are given with a number-letter-number designation (e.g., CYP3A4) with the CYP abbreviation indicating CYP450 membership, the first number indicating the P450 family based on 40% or greater sequence identity, the letter indicating the P450 subfamily based on 55% or greater sequence identity, and the final number representing the individual P450 within that family/subfamily. In humans, CYP3A4, CYP2D6, CYP2C9, CYP1A2, and CYP2C19 perform the majority of drug metabolism, with some contribution from CYP2E1, CYP2A6, CYP2C8, and CYP2B6 (Furge & Guengerich 2006).

CYP450 activity is frequently studied using microsomes.

► **Definition** Microsomes are vesicle-like artifacts reformed from pieces of the endoplasmic reticulum when eukaryotic cells (frequently, hepatocytes) are broken up in the laboratory. They can be concentrated and separated from other cellular debris by differential centrifugation. Unbroken cells, nuclei, and mitochondria sediment out at 9000–10,000 g, whereas soluble enzymes and fragmented ER, which contains CYP450, remain in solution. At 100,000 g, achieved by faster centrifuge rotation, endoplasmic reticulum components sediment out, and the soluble enzymes remain in the supernatant (called cytosolic fraction). The so-called S9 fraction can be obtained after the first centrifugation step at about 9000 g, hence the name. It contains both cytosolic and microsomal enzymes.

Incubation of test compounds with hepatic microsomal preparations is the primary mean by which phase I biotransformations are determined. For microsomal stability determination, the compound is typically incubated for 30 min in about 1.0 mg/mL microsomal protein, phosphate buffer pH 7.4 at 37 C. Compound stability is then determined by an appropriate analytical method.

Note, however, that microsomes cannot be used to study enzyme induction phenomena (see later in this chapter), since the later requires the whole cell machinery functional.

4.2.2 Synthetic (Phase II) Reactions

In phase II reactions, a chemical compound is conjugated with (generally polar) endogenous moieties (the endogenous conjugating moiety, sometimes abbreviated as the *endocon*), such as glucuronic acid, glutathione, sulfate, or acetate, among others. A marked difference between phase I and phase II reactions is that the latter tend to produce pharmacologically inactive and chemically nonreactive products. Only about 10% of the known toxic metabolites originate in conjugation reactions (Testa et al. 2012). Only exceptionally phase II metabolites are pharmacologically active (a typical counterexample is morphine 6-O-glucuronide). We will then say that, generally, phase II reactions *inactivate* their substrates.

Besides detoxifying reactive molecules, the addition of such polar groups through conjugation produces more hydrophilic and larger metabolites which are not able to easily diffuse across cell membranes, conditioning their reabsorption and distribution. Furthermore, many of the products from phase II metabolism are weak acids which are (a) nearly completely ionized at physiological pH range and (b) prone to interact with albumin, which also conspires against their extravasation (Table 4.3). As we will discuss in Chap. 5, some of the phase II metabolites are so polar that they will need the help of efflux transporters to leave the metabolizing cell.

Conjugations are catalyzed by a series of transferases. As previously mentioned, the substrate must have an appropriate "anchoring point" for the conjugation to occur. Sites on drugs where conjugation reactions occur include carboxyl, hydroxyl, amino, and sulfhydryl groups. An important aspect of phase II reactions is that they require a co-substrate (often called cofactor) to take place (the transferase is sometimes figuratively described as a "nuptial bed"). The co-substrate carries the endogenous conjugating moiety, with the chemical bond linking the cofactor and the endocon being a high-energy one such that the Gibbs energy released upon its cleavage drives the transfer of the endocon to the substrate (Testa and Krämer 2008). The molecular structures of the cofactors of the main phase II reactions are shown in Fig. 4.6. Since there is a limited supply of the co-substrate, its availability (and the rate at which the body can replenish it) will determine the capacity of the reaction. For example, sulfonation is a high affinity but low capacity reaction: it has a fast initial turnover rate that decreases as the limited amount of the co-substrate (3′-phosphoadenosine-5′-phosphosulfate, PAPS) is depleted. In contrast, glucuronidation has comparatively low affinity but high capacity (the correspondent cofactor, uridine-5′-diphospho-a-d-glucuronic acid, is produced endogenously by the C(6) oxidation of UDP-a-d-glucose, with about 5 g synthesized daily in adults). At low doses, for a xenobiotic which is subjected both to sulfonation and glucuronidation, sulfonation is faster and its metabolite predominates; at high doses, the sulfonation capacity is exceeded and glucuronidation predominates. Metaphorically, sulfonation can be thought as a sprinter, whereas glucuronidation may be regarded as a marathon runner.

Some of the phase II conjugates may experience deconjugation. Sulfates and acyl glucuronides can hydrolyze within physiological pH range. Glucuronides are susceptible to β-glucuronidase cleavage in the gut, as will be described in the next

Table 4.3 Increase in molecular weight and pKa values of the resulting products from some common phase II reactions. The capacity and affinity of the correspondent transferases are also listed

Reaction	Transferase	ΔMW	pKa	Affinity	Capacity
Glucuronidation	UDP glucuronosyltransferases (UGTs)	176	3.0–3.5	Low	High
Glycine conjugation	Glycine transferases	57	3.5–4.0	Intermediate	Intermediate
Sulfonation	Sulfotransferases	81	<1	High	Low
Glutathione conjugation	Glutathione S-transferases (GSTs)	289	2.1, 3.5	High	Low
Acetylation	N-acetyl transferases	42	Neutral	Variable	Variable

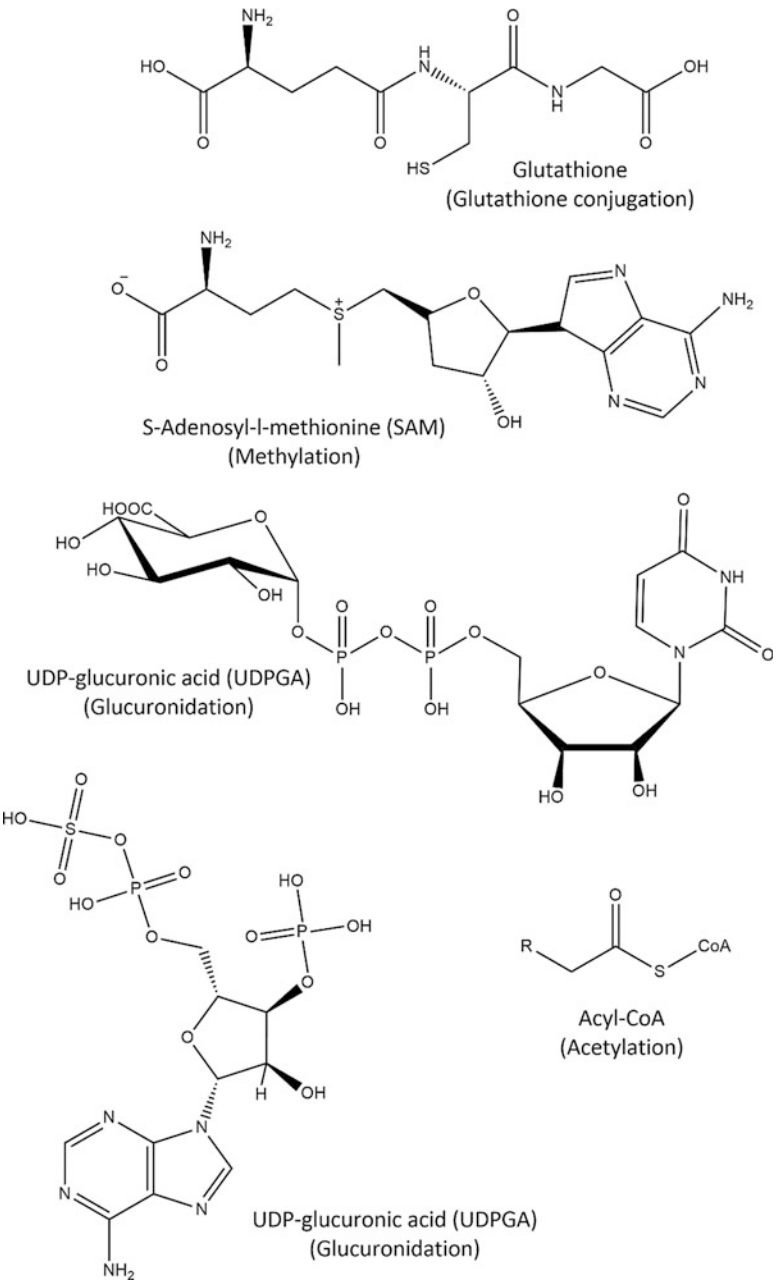

Fig. 4.6 Molecular structures of co-substrates for common phase II reactions

chapter. Glycine conjugates and acetylation products are subject to possible in vivo cleavage by hydrolases/esterases.

Regarding the subcellular localization of the different phase I reactions, sulfonation and N-acetylation are cytosolic reactions; conjugation with amino acids takes place in the cytosol and the mitochondria; glutathione conjugation occurs in the cytosol, the endoplasmic reticulum, mitochondria, and peroxisomes (though glutathione S-transferases are often found in higher levels in the cytosol); and glucuronidation locates in the endoplasmic reticulum (Testa and Krämer 2008; Badenhorst et al. 2013). By far, the most frequent phase II reaction is glucuronidation, followed by glutathione conjugation and sulfonation (Williams et al. 2004; Testa et al. 2012). Note how convenient is that the enzymes that catalyze the most frequent phase II reaction colocalize with those catalyzing the most common phase I biotransformation, increasing the efficiency of the overall process and limiting the exposure of the metabolizing cell and tissue to reactive phase I metabolites (the bioactivated phase I metabolite is rapidly converted to the inactivated phase II product).

Regarding organ distribution, UDP glucuronosyltransferases are the most ubiquitously distributed, having significant expression levels in the liver and bile ducts, kidneys, gastrointestinal tract, reproductive organs, and skin; sulfate conjugation primarily takes place in the liver, the kidneys, and the intestine; glutathione S-transferases are highly expressed in the liver and kidneys; high levels of N-acetyltransferases are found in the liver, kidneys, gastrointestinal tract, lungs, and skeletal muscle (Liston et al. 2001; Testa and Krämer 2008).

Example

Paracetamol/acetaminophen is a widely used over-the-counter analgesic and antipyretic drug. When properly used it is generally safe (in fact, it is regarded as the safest analgesic and antipyretic for pregnant women); it is, though, the leading cause of acute liver failure in the Western world, including developed countries (Lauretti 2012) and large doses (such as those observed in intentional or unintentional overdose) may lead to severe hepatic necrosis and fatal hepatic failure (Mahadevan et al. 2006).

Now we have enough tools to perform a deeper analysis of Fig. 4.2 and understand the mechanism of paracetamol toxicity, risk factors, and its antidote. Note that the elimination of paracetamol follows different parallel competing processes: a small proportion of the unchanged drug is excreted in urine, whereas most of the ingested drug is metabolized before excretion. The drug might be directly biotransformed through sulfonation and glucuronidation (thus generating a first-generation sulfonate or glucuronide conjugate, respectively). Alternatively, it may be oxidized through CYP450 (CYP2E1) resulting in the toxic N-acetyl-p-benzoquinone imine, which attacks cellular components in the main metabolizing organ and produces the liver failure. At therapeutic doses, though, this toxic metabolite is rapidly inactivated by glutathione conjugation. In contrast, overdose leads to depletion of the co-substrates of the sulfonation and glutathione

conjugation reactions. Depletion of the co-substrate in sulfonation reactions, 3′-phosphoadenosine-5′-phosphosulfate (PAPS) implies overloading the remaining biotansformation pathways (including the CYP450-mediated one, thus leading to higher NAPQI levels). Depletion of glutathione impedes NAPQI inactivation.

Several variables (such as chronic ethanol misuse, concomitant use of CYP450 inducing drugs or UDP glucuronosyltransferase inhibitors, and prolonged fasting or malnutrition) may increase the risk of hepatic injury (Bray et al. 1992; Castellano et al. 2001; Daly et al. 2008; Liu et al. 2011), though the influence of such factors is source of controversy and some authors claim that none of them have so far been unequivocally linked to paracetamol toxicity (Caparrotta et al. 2018). Aside from several case reports suggesting the previously mentioned factors increase the risk of toxicity from paracetamol (sometimes, even at therapeutic doses), there are reasonable grounds to suspect from them. Both CYP450 inducers and glucuronosyltransferase inhibitors would favor the generation of NAPQI. On the other hand, a malnourished state could deplete hepatic glutathione reserves and thus reduce the ability to detoxify NAPQI. In fact, many guidelines for the management of paracetamol poisoning have taken the risk factors into consideration (Daly et al. 2008).

Activated charcoal administered within 1–2 h of ingestion reduces the absorbed paracetamol dose and the likelihood of hepatic failure. If activated charcoal cannot be administered within that time frame, infusion of N-acetylcysteine is an effective antidote which guarantees survival if administered within 8 h of paracetamol ingestion. Beyond 8–10 h after ingestion, efficacy decreases. N-acetylcysteine is a glutathione precursor that acts to replenish depleted glutathione reserves in the liver.

Case Study

A 53-year-old woman with concurrent squamous cell carcinoma of the anus and renal cell carcinoma developed severe hepatotoxicity while receiving paracetamol at recommended dosage (4 g daily) under medical supervision and without concomitant administration of enzyme-inducing agents. Her appetite had been poor for several months, during which time she received chemotherapy and radiotherapy and lost 8 kg in bodyweight. She was hospitalized for insertion of brachytherapy rods to treat the anal malignancy. On admission, she was severely malnourished based on standards of weight for height. Liver span, enzyme levels, and standard functional parameters were normal. Brachytherapy rods were inserted, and she received morphine for postoperative analgesia. Administration of paracetamol started postoperatively, after a period of fasting of about 20 h. She remained fasting for other 18 h after starting administration of this drug. Four days later, the alanine

(continued)

aminotransferase level had increased to 269 IU/L (normal range 4–51 IU/L) and the aspartate aminotransferase level to 252 IU/L (normal range 15–45 IU/L). The next day, she developed severe nausea, right upper quadrant discomfort, and tender hepatomegaly. Severe paracetamol-related hepatotoxicity was diagnosed. Paracetamol was suspended. N-acetylcysteine and glucose were administered via intravenously. Serological and virological testing excluded acute infection with hepatitis viruses A, B, C, and E, cytomegalovirus, Epstein–Barr virus, herpes simplex virus, varicella–zoster virus, enteroviruses, and adenovirus. Testing for metabolic, immune-mediated, and metastatic liver disease proved negative. The blood glucose and bilirubin levels and prothrombin time normalized after 2 days of treatment with N-acetylcysteine. Liver enzyme and albumin levels normalized after 4 weeks. Over the next 3 months, the patient underwent nephrectomy and abdominoperineal resection of the anal tumor. Her appetite had remained poor, and she had lost additional 4 kg in bodyweight. She received paracetamol again, ranging from 1.3 g to 2.6 g daily up to three times a week, over several weeks following the anal surgery. Nonetheless, liver enzymes, bilirubin, prothrombin time, and blood glucose values remained normal during this second period of exposure.

Do you concur with the diagnosis? Why do you think liver toxicity was not observed the second time the patient received paracetamol? Would you suggest limiting the daily dose of paracetamol to malnourished or fasting patients, or do you think you lack evidence to formulate such recommendation?

Based on a case report by Kurtovic and Riordan (2003).

4.2.3 Further Drug Metabolism: Phase III Reactions

After phase II reactions, the xenobiotic conjugates may be further biotransformed. For example, glutathione conjugates may be processed to acetylcysteine (mercapturic acid) conjugates (the glutamate and glycine residues in the glutathione molecule are removed by gamma-glutamyl transpeptidase and dipeptidases). In the final step, the cystine residue in the conjugate is acetylated.

Some authors also include within the phase III the transporter-mediated excretion of conjugates, with the anionic groups in most conjugates acting as affinity tags for a variety of membrane efflux pumps. Whereas this process is certainly articulated with metabolism, we will restrict the idea of metabolism to chemical modifications catalyzed by enzymes, and we will consider the previously mentioned transport processes separately in subsequent chapters (Homolya et al. 2003).

4.3 First-Pass Effect

► **Definition** We will define first-pass effect (or first-pass metabolism or pre-systemic metabolism) as any biotransformation suffered by drug molecules before reaching systemic circulation. While first-pass effect might be present in any administration route (except, maybe, intra-arterial administration), it will be considerably more significant for the oral route, since there the drug will face organs expressing high levels of biotransformation enzymes before reaching systemic circulation.

Orally administered drugs will be majorly absorbed in the small intestine and transported to the portal vein (which accounts for approximately 75% of the total liver blood flow) through the mesenteric vessels. Blood passes from branches of the portal vein through liver sinusoids between "plates" of hepatocytes. Blood also flows from branches of the hepatic artery and mixes in the sinusoids to supply the hepatocytes with oxygen. This mixture percolates through the sinusoids and collects in a central vein which drains into the hepatic vein. The hepatic vein subsequently drains into the inferior vena cava, which carries deoxygenated blood from the lower and middle body into the right atrium of the heart. This blood will later go to the lungs before finally reaching systemic circulation.

A significant fraction of the absorbed drug amount might be subjected to pre-systemic loss due to biotransformation in the intestinal walls, biotransformation and/or biliary excretion in the liver, or biotransformation in the lungs (note that these three organs are highly relevant in terms of drug metabolism). This first-pass effect can be clinically relevant when the metabolized fraction is high or when it varies significantly from individual to individual or within the same individual over time, resulting in variable or erratic absorption. Note that all the substances absorbed in the stomach and the intestines will have to pass through the liver before reaching systemic circulation, with the exception of lipids, which form chylomicrons that are not absorbed directly into capillary blood but transported first into the lymphatic vessel that penetrates each intestinal villus. Chylomicron-rich lymph then drains into the lymphatic system and only then into blood, without participation of the portal system.

Substances absorbed through the sublingual mucosa also evade hepatic first-pass effect, since the veins originating there do not join the portal system. For its part, about two thirds of the drug absorbed through the rectal route bypasses the hepatic first-pass metabolism as the rectum's venous drainage is two thirds systemic (middle and inferior rectal vein) and only one third hepatic portal system (superior rectal vein).

► **Important** Have in mind that, since enzymatic systems are saturable systems (and generally, but, not always, described through the Michaelis–Menten kinetics discussed in Chap. 2), the fraction of the dose whose absorption will be affected by the first-pass effect will largely depend on the drug flux to the metabolizing organ. If the metabolizing system is exposed to a large quantity of drug per unit of time, it could get saturated (this is particularly true for drugs administered in large doses).

The higher the amount of drug above the saturation condition, the higher the fraction of the dose that will survive (unchanged) the first-pass effect.

The fraction of the dose absorbed (F) when the drug is given by any route of administration can be estimated by comparing the area under the plasma concentration-time curve (AUC) after administering the drug for that route with the area under the plasma concentration-time curve after administering the drug intravenously. Remember that the AUC is proportional to the amount of drug that has reached systemic circulation.

For example, if one wants to estimate the fraction of the dose absorbed for a drug given orally, we should compute

$$F = \frac{\mathrm{AUC}_{\mathrm{oral}}}{\mathrm{AUC}_{\mathrm{iv}}} \times \frac{D_{\mathrm{iv}}}{D_{\mathrm{oral}}} \tag{4.2}$$

An F below 1 suggests incomplete absorption of the drug, due to inappropriate release of the drug from the dosage form, drug degradation in gastric media, intestinal low permeability, and/or first-pass metabolism in the gut and/or liver.

Example

Recently, Fathi et al. studied the bioavailability of paracetamol tablet, capsule, and effervescent dosage forms (each one containing 500 mg of the drug) in healthy subjects (Fathi et al. 2018). Thirty volunteers were divided into three gender-balanced groups of ten subjects. Participants displayed a mean (±SD) age of 21 (±2) years. They were prohibited to use acetaminophen or other analgesics 1 day before the experiment.

The effervescent dosage form had a quicker absorption (greater Cmax and lower Tmax), whereas the area under the plasma concentration curve was significantly higher for the effervescent dosage form than for the two other formulations (data is shown in Table 4.4). In other words, the effervescent dosage form displayed better bioavailability, both in quantitative and kinetic terms.

Table 4.4 Results obtained by Fathi et al. when comparing three paracetamol dosage forms. The table shows mean data for the ten volunteers in each group

Plasma concentration (μg/mL) – mean (SD)			
Tablet	Capsule	Effervescent	Time (min)
0	0	0	0
6.61 (2.41)	11.29 (3.94)	15.25 (2.54)	60
8.74 (2.49)	7.43 (1.37)	9.94 (1.63)	120
7.35 (2.80)	4.67 (1.49)	6.67 (1.81)	240
2.64 (0.85)	2.08 (1.70)	1.49 (0.64)	480
47.04	40.62	53.11	∑AUC (μg min/mL)

Data extracted from Fathi et al. (2018)

Can you provide any possible explanations for the observations? Do you think that the results could have been confounded by other factors asides the differences between the dosage forms under comparison?

4.4 Inter- and Intra-Individual Factors Affecting Drug Metabolism: Intrinsic and Extrinsic Sources of Variability

Factors affecting drug metabolism are among the most important sources of inter- and intra-individual variability in drug pharmacokinetics and in the pharmacological response to medications.

► **Definition** Inter-individual factors are factors which differ between individuals but are constant throughout the life of an organism. This applies to factors dependent on the genetic makeup of an individual (Krämer and Testa 2008). Variations in metabolism which are not based on genetic differences will be called intra-individual factors and mostly depend on environmental factors and physiological or pathological states (in some cases it could be argued that some of these factors have mixed origin: e.g., disease conditions often impact on drug pharmacokinetics, and usually disease is the result of a combination of intrinsic and extrinsic factors).

It is worth underlining, though, that two individuals of the same species may differ, at a given time point, in their drug metabolism capacity due to either constitutive or environmental factors.

Regarding inter-individual factors, within a particular species there might be stable (i.e., at significant frequencies) genetic variations called polymorphisms. Genetic variations in metabolizing enzymes (usually arising from point mutations) can result in differences in enzyme expression levels, substrate specificity or activity. Carriers of variant alleles can be at risk of experimenting toxic effects or therapy failure when treated with a substrate of the respective enzyme. Other variations of genetic origin may result from gene duplication/multiplication events (leading to enhanced expression levels of the gene products and, in the long term, to divergence of duplicates). We may also include here sex-related differences in drug metabolism, though in general they have a minor impact in humans (Krämer and Testa 2008).

Personalized pharmacotherapy considering the genetic makeup of an individual will be specially required when the therapeutic range is narrow, and the altered enzyme represents the major elimination pathway of the administered drug. Although the relationship between pharmacokinetics and pharmacogenomics is abundantly addressed in Chaps. 8 and 9 of the present volume, here we will briefly discuss some key points on the matter.

► **Definition** Precision medicine is an emerging approach for disease treatment and prevention that considers individual variability in genes, environment, and lifestyle for each person. It is closely related to personalized and stratified medicine, and these terms are often used interchangeably, though subtle distinctions between them have been realized (Day et al. 2017; Talevi 2018).

Particular mutations may be unique for a certain population or show differences in their frequency when comparing ethnicities, leading to population-specific responses to xenobiotics. For instance, a frequent polymorphism in the aldehyde dehydrogenase ALDH2 gene in Asians is responsible for facial flushing and unpleasant sensations after alcohol intake, with this variant being infrequent in Caucasians. Realization of such population differences has even impacted on regulatory status. For instance, the Japanese regulatory authority requires clinical trials on Japanese volunteers to consider new drug applications and, for multi-regional clinical trials, confirmation of similarities in the dose-response curve and pharmacokinetic data between Japanese and non-Japanese subjects. Note that adaptation to different environments and differences in nutrition constitute additional reasons for ethnic differences in drug metabolism.

Before the genetic background of polymorphic behavior in drug metabolism was known, the consequences of polymorphism were described in a purely phenotypic manner. Patients were assigned to categories such as poor, intermediate, extensive, and ultrarapid metabolizers, according to the frequency distribution of individual metabolic rates. In the case of acetylation, the phenotypes are called slow and rapid acetylators. At present, patients are sometimes classified in these categories based on their genotype rather than on the observed metabolic rates (Krämer and Testa 2008). The drug metabolism enzymes with the clinically most relevant polymorphisms are CYP2D6, CYP2C19, CYP2C9, UDP glucuronosyltransferase 1A1, N-acetyltransferase, dihydropyrimidine dehydrogenase, cholinesterase, and thiopurine methyltransferase.

In general, people who cannot metabolize a drug will require a much lower dose than is recommended by the manufacturer; those who metabolize it quickly may require a higher dose. Note, however, that the pharmacological consequences of limited or enhanced drug metabolic rates depend on whether the metabolite is or is not more active than the parent compound (think, e.g., of a prodrug).

Regarding intra-individual factors influencing drug metabolism, the most common ones are physiological factors (diurnal cycle, age, pregnancy, sex hormones levels), pathological conditions (e.g., celiac disease, liver disease, diabetes, inflammation, infection, heart failure), and interactions of the drug with physiological compounds or with other xenobiotics (drugs, drinks, tobacco, pollutants) (Krämer and Testa 2009). The major mechanisms that mediate drug-drug interactions (or interaction between a drug and any other type of xenobiotic) are enzyme induction and enzyme inhibition.

► **Definition** In enzyme induction, a ligand (the *inducer*) activates a transcription factor, which consequently dimerizes with another transcription factor (usually belonging to the class of nuclear receptor), and the dimer binds to a regulatory sequence on the DNA. DNA binding of the transcription factor dimer generally results in an increase in transcription of the controlled gene and consequently increased levels of the transcribed enzyme. When a xenobiotic induces its own metabolism, we will speak of *auto-induction*; if the xenobiotic induces the metabolism of other xenobiotic, we will speak of *hetero-induction*. Some typical enzyme inducers include phenobarbital, phenytoin, rifampin, and dexamethasone.

Enzyme inhibition, on the other hand, involves the interaction of an inhibitor with a biotransformation enzyme, decreasing its activity in a reversible or irreversible manner. Typical examples of inhibitors include ketoconazole, ritonavir, and amiodarone.

Example

In the package insert of a well-known carbamazepine brand, it reads:

"Because carbamazepine induces its own metabolism, the half-life is also variable. Autoinduction is completed after 3–5 weeks of a fixed dosing regimen. Initial half-life values range from 25 h to 65 h, decreasing to 12–17 h on repeated doses. (. . .) Carbamazepine is a potent inducer of hepatic 3A4 and is also known to be an inducer of CYP1A2, 2B6, 2C9/19 and may therefore reduce plasma concentrations of co-medications mainly metabolized by CYP 1A2, 2B6, 2C9/19 and 3A4, through induction of their metabolism. When used concomitantly with carbamazepine, monitoring of concentrations or dosage adjustment of these agents may be necessary. . ."

Note that, due to the induction phenomenon, the pharmacokinetics of carbamazepine will be time-dependent, showing a two- to fivefold decrease in the half-life of the drug, whose pharmacokinetics will require weeks to stabilize. Also note that carbamazepine (auto) induces its metabolism and (hetero) induces the metabolism of other CYP450 substrates.

Example

Back in 2010, Xiang et al. studied the effect of CYP2D6 variants on risperidone bioavailability. Twenty-three healthy Chinese subjects took part in the study. Table 4.5 shows the AUC for CYP2D6 *1/*1, *1/*10, and *10/*10 carriers.

How would you characterize *1/*10 and *10/*10 carriers according to the results? Would you expect lower or higher risperidone pharmacological effect for these subjects (tricky question: some research required!)? In the light of the results obtained for *1/*1 and *10/*10 subjects, are those obtained for *1/*10 subjects reasonable?

It is interesting to note that the fold change in expression levels and activity for CYP450 enzymes (either due to polymorphism or induction) tends to be much more pronounced than for phase II enzymes (Krämer and Testa 2008; Krämer and Testa

Table 4.5 Mean risperidone AUC for Chinese subjects displaying different CYP2D6 genotypes. Data extracted from Xiang et al. 2010. CYP2D6*1 is the most common form, considered "fully functional," also known as wild type. Data for homozygous and heterozygous carriers of the *10 variant is shown

Genotype	*n*	Risperidone – mean (95% confidence interval) (ng h/mL)
1*/1*	5	30.6 (22.9, 38.3)
1*/10*	12	64.8 (45.2, 84.4)
*10/*10	6	248.2 (77.2, 419.2)

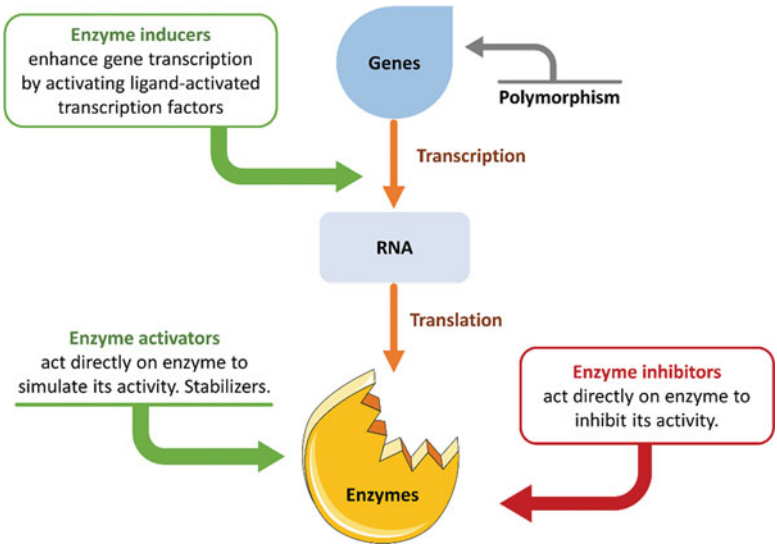

Fig. 4.7 Diagram of the different levels at which a factor of variability in drug metabolism might act

2009). Adjusting treatment with certain CYP450 substrates based on their genotype and concomitant medication would thus be desirable (Lynch and Price 2007). Genotype testing may predict people who are poor metabolizers or nonresponsive to drugs metabolized by CYP450 enzymes. CYP450 genetic variants should be considered when patients exhibit unusual sensitivity or resistance to drug effects at normal doses. Patients should be monitored carefully for the development of adverse drug effects or therapeutic failures when a potent CYP450 enzyme inhibitor or inducer is added to drugs metabolized by one or more CYP450 isozymes. Because they are known to cause clinically significant CYP450 drug interactions, caution must be used when adding the following substances: amiodarone, antiepileptic drugs, antidepressants, antitubercular drugs, grapefruit juice, macrolide and ketolide antibiotics, non-dihydropyridine calcium channel blockers, and protease inhibitors (the list is not exhaustive!). Further examples may be found in Chap. 12 of the present volume.

Fig. 4.7 shows at what level do some of the factors that influence drug metabolism impact

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Further Reading

Drug metabolism is a vast topic, and entire books have been written about it. Here, we intended to provide a summary of the more relevant points of drug biotransformation, but the chapter is far from exhaustive. For a much deeper insight on the topic, the reader is advised to Pearson and Wienkers's *Handbook of Drug Metabolism* (currently in its 3rd edition by CRC Press); the *Drug Metabolism Handbook* edited by Nassar, Hollenberg, and Scatina (2008, Wiley & Sons); and the extensive and unbelievably comprehensive series of articles by Testa and Krämer, many of which have been included in the reference list of the present chapter.