Chapter 27

Drug Metabolism

L.A. Stanley
Consultant in Investigative Toxicology, Linlithgow, United Kingdom

Chapter Outline

Learning Objectives

- To understand the role of metabolism in the disposition of drugs and other xenobiotics.
- To know the difference between metabolic activation and detoxification and appreciate the need for an appropriate balance between these processes.
- To be able to explain the phases of xenobiotic metabolism and identify the enzymes and transporters which mediate them.

27.1 INTRODUCTION

Metabolism, the "M" part of ADME (Absorption, Distribution, Metabolism, Excretion) is central to the excretion of lipophilic compounds. Unlike water-soluble polar compounds, which are readily excreted, these tend to be sequestered in body fat. Even if they are excreted into the gut or kidney tubules, they are rapidly reabsorbed. Metabolism to more polar compounds makes them easier to excrete and enhances their elimination from the body.

27.1.1 Clearance

The clearance of a xenobiotic represents the efficiency of its elimination from the body. Clearance can be determined by measuring plasma concentrations at various time points after an intravenous dose and calculating the area under the plasma concentration−time curve (AUC; Fig. 27.1; Eq. (27.1)).

Pharmacognosy. DOI: http://dx.doi.org/10.1016/B978-0-12-802104-0.00027-5

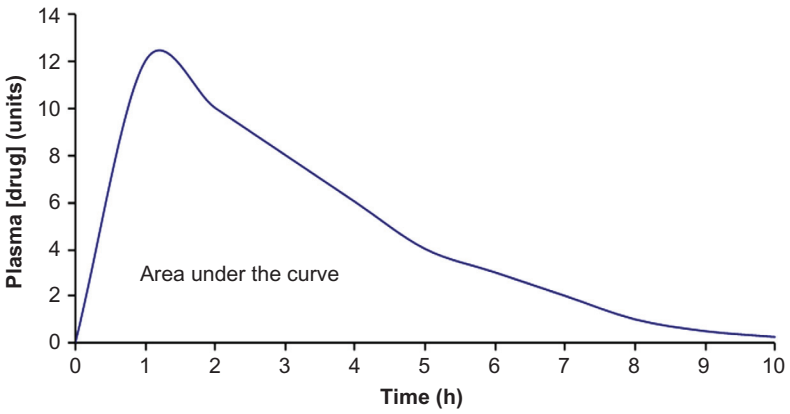

FIGURE 27.1 Representative plasma concentration–time relationship after a single oral dose of a hypothetical drug.

$$\text{Clearance } (L/h) = \frac{\text{Dose (mg)}}{\text{AUC (mg} \times \text{h}/L)} \tag{27.1}$$

The major routes of xenobiotic elimination from the body are excretion as unchanged compound via the kidneys and elimination by metabolism in the liver. The proportion excreted unchanged via the kidneys can be calculated by measuring the amount of intact compound in the urine and can range from near zero (e.g., morphine) to near 1.00 (e.g., penicillin).

Total clearance is the sum of all the clearance processes in the body; it approximates to the sum of the two main processes involved, hepatic clearance (metabolism) and renal clearance (excretion).

27.1.2 First-Pass Metabolism

A xenobiotic which is absorbed via the intestine enters the portal circulation and is delivered straight to the liver. Only after it has passed through the liver does it enter the systemic circulation. Metabolism of the xenobiotic in the liver and intestinal wall before it reaches the systemic circulation is called first-pass metabolism.

It is important to understand first-pass metabolism because it determines the effectiveness of many orally administered drugs; if first-pass metabolism adversely affects therapeutic performance, it may be necessary to create alternative formulations (e.g., skin patches). In addition, variations in first-pass metabolism can affect therapeutic responses and contribute to drug–drug interactions. It is particularly important to be careful when administering oral therapy to patients with compromised liver function because first-pass metabolism may be affected by liver disease.

27.1.3 Bioavailability

When a drug is administered intravenously, it enters the systemic circulation directly. The proportion of an orally administered drug which reaches the systemic circulation is termed its bioavailability and is a function of the opposing processes of absorption and clearance. Oral bioavailability can be calculated from the AUCs of the drug following oral and intravenous administration (Eq. (27.2)):

$$\text{Bioavailability} = \frac{\text{AUC (oral)}}{\text{AUC (intravenous)}} \tag{27.2}$$

The oral absorption and bioavailability of a drug are influenced by its solubility: very hydrophilic drugs are absorbed poorly because they cannot easily cross the plasmalemma. Conversely, very hydrophobic drugs are also absorbed poorly because they are insoluble in aqueous body fluids. Drugs which are unstable at the acid pH of the stomach, or are degraded by digestive enzymes, also tend to have poor oral bioavailability and the absorption and bioavailability of a drug may also be affected by its formulation.

If a drug is absorbed efficiently, first-pass clearance in the liver is likely to be a major determinant of bioavailability. Some drugs undergo extensive metabolism during passage through the liver. Consequently, their bioavailability is near zero (0%): they are called flow limited and this can lead to problems caused by hepatic first-pass metabolism. Others are metabolized very inefficiently by the liver: their hepatic clearance is minimal and their bioavailability is near 1.00 (100%).

27.2 FUNCTION OF XENOBIOTIC-METABOLIZING ENZYMES

The primary function of xenobiotic-metabolizing enzymes is to render hydrophobic chemicals more hydrophilic so that they can be excreted (Fig. 27.2). However, this often involves the generation of highly reactive intermediates. Xenobiotic-metabolizing enzymes can therefore be thought of as double-edged swords, because a single enzyme can mediate both activation and detoxification. If detoxification outweighs metabolic activation, the drug will be removed safely from the body. If metabolic activation outweighs detoxification, the consequence may be protein or DNA binding leading to cytotoxicity, DNA damage, or other toxic effects.

The process of xenobiotic metabolism is conventionally divided into four phases (Fig. 27.3). Of these, strictly speaking only Phases I and II involve xenobiotic-metabolizing "enzymes," although the transporters which mediate Phases "0" and "III" are also often described in terms of substrates and activity.

- "Phase 0": Passage across the plasmalemma and is mediated by influx (uptake) transporters.
- Phase I: Introduction of a reactive group into the molecule.
- Phase II: Transfer of polar groups onto the products of Phase I metabolism.
- "Phase III": Transport of water-soluble Phase I and II metabolites out of the cell and is mediated by efflux transporters.

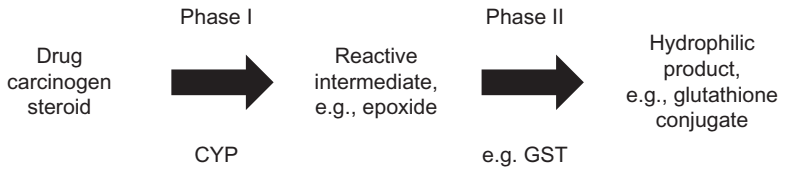

FIGURE 27.2 The role of xenobiotic-metabolizing enzymes in metabolic activation and detoxification.

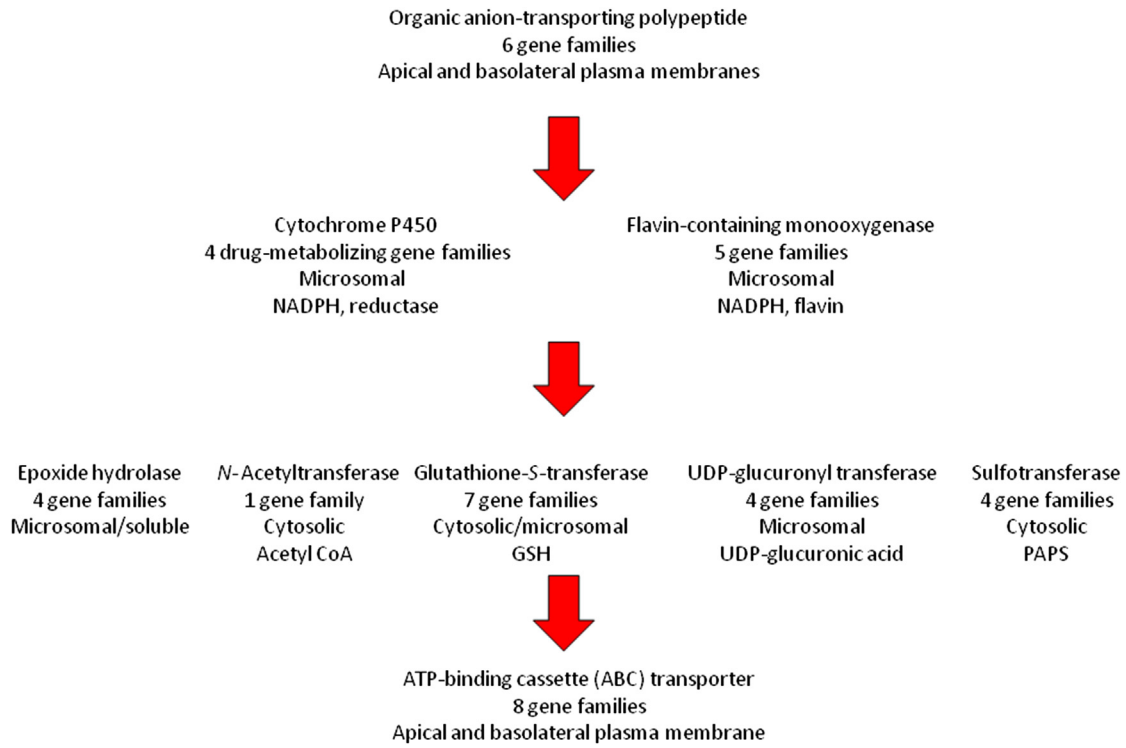

FIGURE 27.3 Key xenobiotic-metabolizing enzyme families. Details (updated January 2015) of the key enzyme/transporter families involved in xenobiotic metabolism.

27.3 PHARMACOGENETIC VARIATION

Interindividual variability is a recurring concept in xenobiotic metabolism. It is often linked to disease susceptibility and differences in drug bioavailability, even between individuals of the same weight who are taking the same dose of drug.

Pharmacogenetic variation is determined by genes, each of which is found at a specific chromosomal locus. The most prevalent genetic variants are known as polymorphisms. A polymorphism is conventionally defined as a sequence variant at a particular locus which is present in $\geq 1\%$ of the population. An understanding of genetic polymorphisms facilitates the evaluation of variability in pharmacological and toxic responses (Fig. 27.4) and may make it possible to personalize therapy and identify/protect susceptible populations.

A polymorphism may alter the amino acid sequence of the protein encoded by a particular gene; alternatively, some polymorphisms affect splicing or the control of transcription. Others have no direct effect on the genes in which they are found but still exert biological consequences, possibly via genetic linkage to functional polymorphisms elsewhere in the genome. The complete absence of a gene (null genotype) may also be classified as a polymorphism. Polymorphisms can always, in principle, be detected at the level of nucleotide sequence and those which affect expressed proteins may also be identified in terms of their effects on amino acid sequence, mRNA/protein expression, and/or biological function.

27.3.1 Genotyping and Phenotyping

An individual's genetic characteristics define his/her genotype; the term phenotype refers to the biological expression of this genotype. Accordingly, genotyping involves examining the altered nucleotide sequences of polymorphic variants in the DNA itself while phenotyping involves measuring biological functions such as enzyme activity.

In the pharmacogenetic context, the principle of phenotyping is to measure the extent of metabolism of a "probe" drug *in vivo* or *in vitro* to identify altered activity due either to the existence of variants of an enzyme which have intrinsic differences in activity or to differential expression of a uniformly active enzyme. If the observed variability in activity is due to differences in expression, this may also be characterized by determining the level of gene expression at the protein or mRNA level.

The detection of polymorphisms at the DNA level is called genotyping. An individual's genotype can be used to predict his/her phenotype if a clear correlation between genotype and phenotype has been demonstrated; however, genotyping can only ever be a surrogate for biological phenotype because it only provides an indirect measure of activity.

27.3.2 Single Nucleotide Polymorphisms

The vast majority (99.5%) of the human genome is common to everybody; most differences between individuals are due to single nucleotide polymorphisms (SNPs), which may account for as much of 90% of human genetic variation [1].

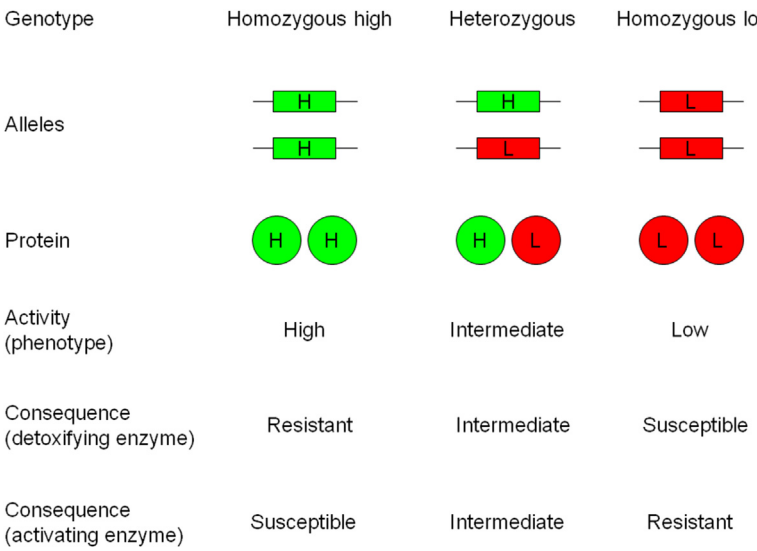

FIGURE 27.4 Genotype and phenotype and their consequences. Possible consequences of polymorphism in a hypothetical xenobiotic-metabolizing enzyme with both high-activity and low-activity variants. *Reprinted from Stanley LA, Toxicogenetics. In: Greim H, Snyder R, editors.* Toxicology and risk assessment: a comprehensive introduction *(ISBN: 978-0-470-86893-5) with the permission of John Wiley and Sons Ltd.*

On average there is about one SNP every 300 bases in the human genome, but they are not evenly distributed: there are SNP hotspots with much higher densities of SNPs than in other regions.

Probably only about 2000 SNPs cause actual amino acid changes. These nonsynonymous SNPs alter the amino acid sequence of the cognate protein and may affect its function. Synonymous SNPs do not affect the coding information, although if located within a regulatory region they can affect responses to xenobiotics. Most SNPs are located in non-coding/nonregulatory regions of the genome and are functionally silent.

27.4 INDUCTION

Many xenobiotic-metabolizing enzymes are subject to induction, i.e., increased expression in response to xenobiotics which may or may not be substrates. This often involves transcriptional activation. Inducing agents activate nuclear receptors either by direct binding or indirectly. Following heterodimerization with additional transcription factors, these bind to specific enhancer or response elements and upregulate gene expression. Inducing agents include flavonoids in cruciferous vegetables and polycyclic aromatic hydrocarbons (PAHs) in barbecued food. Drugs (e.g., phenobarbital) can also be enzyme inducers, as can environmental pollutants (e.g., dioxins), occupational exposures (e.g., acrylonitrile), and herbal remedies (e.g., St. John's Wort).

27.5 NOMENCLATURE

One of the key features of mammalian xenobiotic metabolism is its complexity. This led to complex nomenclature systems which were originally based on the inducibility, substrate specificity, protein chemistry, and antibody cross-reactivity of purified enzymes. The nomenclature is, however, now based on cloned genes, permitting unequivocal classification based on DNA sequence. Luckily for those of us who worked on xenobiotic-metabolizing enzymes in the days before cloning, the two usually correspond!

27.6 PHASE 0

27.6.1 Hepatic Uptake Transporters

The organic anion-transporting polypeptides (OATPs) comprise six families, the most important for drug uptake being the OATP1A, OATP1B, and OATP2B1 families [2]. The substrates of OATP1A include bile acids, estrogen derivatives, peptides, and drugs, while OATP1B1 and OATP1B3 also transport conjugated and unconjugated bilirubin, thyroid hormones, and drugs (e.g., digoxin). The functions of the OATP2B family still require clarification.

27.6.1.1 Example: Role of OATP1B1 in the Disposition of Methotrexate

A 25-fold decrease in hepatic uptake of methotrexate, which is transported by human OATP1B1/murine Oatp1b1, is observed in Oatp1a/1b null mice, while OATP1B1-humanized mice exhibit increased hepatic uptake and decreased plasma concentrations [3]. This suggests a mechanism for the increased plasma methotrexate concentrations and attenuated gastrointestinal toxicity observed in patients carrying low activity variants of OATP1B1: reduced liver uptake and diminished biliary excretion of methotrexate may reduce direct intestinal exposure and hence toxicity.

27.7 PHASE I

Phase I metabolism involves flavin-containing monooxygenases, monoamine oxidases, cyclooxygenases, dihydrodiol dehydrogenases, NAD(P)H:quinone oxidoreductases, alcohol dehydrogenases, and aldehyde dehydrogenases, but this section focuses on polymorphic cytochrome P450 (CYP) enzymes, 50−60 kD heme-thiolate monooxygenases with broad substrate specificity in oxidative xenobiotic metabolism.

CYPs are intrinsic membrane proteins located in the endoplasmic reticulum which mediate the terminal oxidation stage of the microsomal electron transport chain, generating reactive intermediates, such as epoxides [4].

In order to catalyze the monooxygenase reaction, CYPs require the electron-donating proteins cytochrome b_5 and NADPH:CYP oxidoreductase. In contrast to the multiplicity of CYPs, mammals have only one NADPH:CYP oxidoreductase gene, and all murine CYP activity can effectively be abolished by conditional deletion of this single gene [5].

Monooxygenation is essential to the detoxification of many xenobiotics; however, in some cases CYP enzymes generate intermediates that are more toxic than the original substrate and require detoxification by Phase II enzymes.

If this process is inefficient, reactive metabolites can bind to proteins or DNA leading to cytotoxicity and/or mutations.

Not all CYPs are polymorphic but polymorphism is an important feature of this multigene family [6]. Polymorphisms can affect therapeutic responsiveness and influence the consequences of occupational exposure and accidental ingestion, although it is debatable whether they are relevant at low (environmental) exposures.

Mammals have many CYP gene families, each containing numerous genes, but a few enzymes belonging to the CYP1, CYP2, CYP3, and CYP4 families account for the metabolism of most drugs and xenobiotics [6].

27.7.1 The CYP1 Family

The CYP1 family comprises three functional genes organized into two subfamilies, the highly conserved CYP1A subfamily and the more distantly related CYP1B subfamily.

Many PAHs are potent carcinogens which may be either activated or detoxified by CYP1A1. The regulation of CYP1A gene expression is therefore important in determining susceptibility to chemical carcinogenesis, and has been the subject of intense research ever since the discovery that PAHs upregulate their own metabolism [7]. Polychlorinated biphenyls and flavones also induce this activity. The major PAH-inducible CYP, now known as CYP1A1, is highly active in the metabolism of PAHs and model substrates (e.g., 7-ethoxyresorufin).

In rodents, PAHs upregulate Cyp1A gene expression via a regulatory system involving the aryl hydrocarbon receptor (AhR), which mediates the effects of PAHs by a mechanism similar to that by which steroid hormone receptors regulate steroid-responsive gene expression. Briefly, the highly lipophilic PAH molecule crosses the plasmalemma and binds tightly to the AhR, which then forms a heterodimer with the AhR nuclear translocator, moves to the nucleus, binds to regulatory sequences in the 5′ flanking regions of target genes, and activates transcription.

The physiological role of the AhR system is still subject to debate. One possibility is that it arose to deal with combustion products and plant toxins; however, since many naturally occurring compounds are activated to potent carcinogens by AhR-inducible CYPs it is hard to see what overall benefit such a system may confer. Another possibility is that the AhR coordinates aspects of the immune system. Immunologists have taken an interest in this receptor since its role in T-cell differentiation was identified in 2008, leading to the identification of the tryptophan breakdown product kynurenine as the first endogenous AhR ligand [8]. In addition, the AhR may play a role in normal development, since AhR ligands also induce the expression of important enzymes including alcohol dehydrogenases and phospholipase A2.

27.7.1.1 Example: CYP1A1 and Dioxin

Dioxin is a persistent environmental pollutant produced during combustion and industrial processes. It induces CYP1A1 via the AhR and is a potent carcinogen in rodents; however, its carcinogenicity in humans remains the subject of intense debate. The only toxic effect to be unequivocally linked with dioxin in humans is the skin condition chloracne. The best known example of this is Ukrainian president Viktor Yushchenko, who became ill during the 2004 election campaign and was diagnosed with dioxin poisoning. Even 2 years later, disfiguration due to chloracne could still be clearly seen (Fig. 27.5). Ironically, Mr. Yushchenko has made a significant contribution to dioxin research because the well-documented nature of his poisoning and the high dose he received have allowed researchers to characterize the toxicokinetics of this long-lived compound in detail [9]. Fortunately, however, Mr. Yushchenko and other people exposed to high levels of dioxin show no evidence of increased risk of cancer, although the WHO classifies dioxin as a known human carcinogen.

The second, closely related, member of the CYP1A gene family, CYP1A2, is expressed constitutively in human liver. Several nongenetic factors influence its hepatic expression and/or *in vivo* activity: it is inducible by β-naphthoflavone and isosafrole in rodents and, in humans, by cigarette smoke [10].

Unlike CYP1A1, which prefers planar aromatic hydrocarbons, CYP1A2 metabolizes aromatic amines and heterocyclic compounds, mediating the metabolic activation of industrial combustion products and carcinogens found in chargrilled food. Polymorphisms in the CYP1A family have limited impact on xenobiotic metabolism, although interindividual variability in CYP1A2 may affect susceptibility to colorectal cancer.

The third member of the CYP1 superfamily, CYP1B1, mediates the 4-hydroxylation of PAHs, aromatic amines, and steroid hormones. It can also mediate the metabolic inactivation of structurally diverse anticancer drugs. Like CYP1A1 and CYP1A2, CYP1B1 is regulated by the AhR.

FIGURE 27.5 Viktor Yushchenko with chloracne from dioxin poisoning. *"Viktor Yuschenko" by Muumi. Licensed under CC BY-SA 3.0 via Wikimedia Commons.*

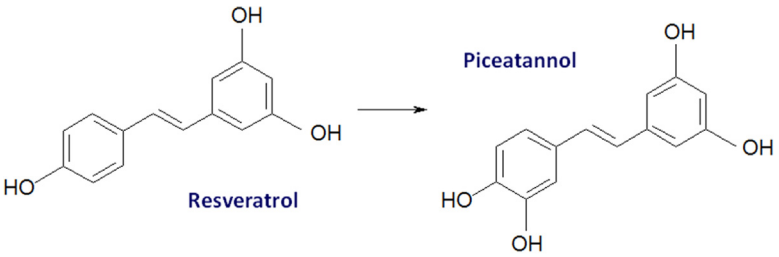

FIGURE 27.6 Conversion of resveratrol to piceatannol.

27.7.1.2 Example: Chemoprevention by CYP1B1

Solid human tumors express high levels of CYP1B1 which contrasts with a lack of expression in histologically normal tissues [11]. This observation is now being exploited in oncology; although most published studies assume that CYP1B1 acts as a carcinogen-activating enzyme like CYP1A1, it can activate the phytoalexin resveratrol to the potent protein kinase inhibitor piceatannol (Fig. 27.6) [12], with potential implications for cancer chemoprevention and therapy.

27.7.2 The CYP2 Family

Of the several CYP2 subfamilies, the CYP2B subfamily contains genes which are highly inducible by barbiturates, whereas others (CYP2A and CYP2C) exhibit higher constitutive expression but are only marginally inducible. Many pharmacologically important CYP2 genes are highly polymorphic.

The CYP2A enzyme family is expressed constitutively in liver. It mediates testosterone 7α-hydroxylation in the rat but metabolizes only a few drugs (e.g., coumarin, nicotine) in humans. Little is known about its physiological function, although human CYP2A6 is the major isoform involved in the oxidative inactivation of nicotine [13]. Polymorphism at the CYP2A6 locus can be associated with either increased or decreased expression and activity.

The major barbiturate-inducible CYP in rat liver, CYP2B1, is regulated via the constitutive androstane receptor (CAR), which is related to the pregnane-X-receptor (PXR) and the steroid, retinoid, and thyroid hormone receptors. The basic mechanism by which CAR and PXR activate transcription is a conventional one, involving xenobiotic binding, heterodimerization (with the retinoid-X-receptor), and interactions with the 5′ regulatory sequences of target genes, but the ligand responsiveness of CAR is complex. This constitutively active receptor is regulated both by agonists which upregulate its activity (e.g., 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene, TCPOBOP) and inverse agonists which downregulate it (e.g., androstanol) [14].

Members of the CYP2B family catalyze benzphetamine *N*-demethylation, 7-pentoxyresorufin depentylation, and aldrin epoxidation. The main human isoform is CYP2B6, which metabolizes environmental chemicals, chemotherapeutic agents, antiretrovirals, antidepressants, anesthetics, the antimalarial artemisinin, and the μ-opioid agonist methadone.

Polymorphisms in CYP2B6 can be clinically important; e.g., CYP2B6 4-hydroxylates the prodrug cyclophosphamide, ultimately generating the cytotoxic metabolites phosphoramide mustard and acrolein, and polymorphic variation can have a marked impact on the success of cyclophosphamide chemotherapy [15].

Encoded on chromosome 10, the human CYP2C subfamily comprises four highly homologous enzymes: CYP2C8, CYP2C9, CYP2C19, and the inefficiently translated CYP2C18, which does not contribute significantly to xenobiotic metabolism.

The polymorphic enzyme CYP2C8 metabolizes antidiabetic agents, antimalarials, the antiarrhythmic amiodarone, and the natural product anticancer drug paclitaxel. The consequences of polymorphism in CYP2C8 are currently under investigation [16].

Diclofenac and tolbutamide are commonly used to phenotype CYP2C9, which accepts weakly acidic substances including the anticoagulant warfarin, anticonvulsants, angiotensin receptor blockers, oral antidiabetic agents, and most nonsteroidal antiinflammatory drugs.

The first CYP2C enzyme to be discovered was actually CYP2C19, which is responsible for the inactivating metabolism of proton pump inhibitors and metabolic activation of the anticoagulant clopidogrel. It has also a prominent role in antidepressant metabolism, while its endogenous substrates include progesterone and melatonin [6]. Like CYP2C9, CYP2C19 is highly polymorphic, resulting in the *S*-mephenytoin poor and extensive metabolizer phenotypes identified in the early 1980s [17].

As indicated above, the CYP2C subfamily is highly polymorphic and, since all its members are involved in the metabolism of key therapeutic agents, this is often clinically significant.

27.7.2.1 Example: CYP2C9 and Warfarin Metabolism

Warfarin, a natural product anticoagulant administered as racemic mixture of *R*- and *S*-stereoisomers, acts by antagonizing the vitamin K cycle [18]. Individuals' responses to treatment with warfarin vary enormously and it can sometimes take months to identify an appropriate dose. Excessive bleeding occurs if the initiating dose is too high, while control of clotting is ineffective if the dose is too low. *S*-warfarin is 3−5 times more potent than *R*-warfarin and is metabolized in the human liver via CYP2C9, generating harmless 6- and 7-hydroxy metabolites which are excreted in the urine. Approximately 40% of the Caucasian population carry one or both of the functionally defective variants CYP2C9*2 and CYP2C9*3; they are likely to need lower doses of warfarin and have an increased risk of bleeding complications during therapy. It is now possible to use demographic characteristics, CYP2C9 genotype, and control of the vitamin K cycle for warfarin dose setting [19], and the FDA recommends dose reduction in individuals known to have a variant CYP2C9 allele.

The only known member of the human CYP2D subfamily is CYP2D6, originally identified as the polymorphic enzyme debrisoquine 4-hydroxylase which, when defective, causes a dramatic hypotensive response to debrisoquine [20]. Despite its poor expression in the liver, CYP2D6 is responsible for the metabolism of 15−25% of all drugs, including antiarrhythmics, antidepressants, antipsychotics, β-blockers, opioid analgesics, and anticancer drugs [21].

Thanks to its wide spectrum of genetic variants (from null alleles to several-fold gene amplification), variable expression at the protein level, and extraordinarily broad substrate selectivity, CYP2D6 shows the greatest impact of genetic polymorphism among all major xenobiotic-metabolizing CYPs. Individuals are now classified as poor, intermediate, efficient, or ultrarapid metabolizers [21]. Poor metabolizers (5−10% of the Caucasian population) have significantly altered metabolism of several major drug classes. This can lead to failure of detoxification and adverse drug reactions, while extensive and ultrarapid metabolizers may exhibit poor therapeutic responsiveness to CYP2D6 substrates because of rapid clearance. As a consequence, the development of drug candidates which are metabolized by CYP2D6 is usually discontinued.

The major ethanol-inducible CYP, CYP2E1, encoded on chromosome 10, is the only member of the CYP2E subfamily. Its expression is induced by ethanol and other small organic molecules via complex mechanisms involving transcriptional, translational, and posttranslational effects and is modulated in conditions including diabetes and nonalcoholic liver disease (in which it is believed to play a pathophysiological role). The substrate preference of CYP2E1 is for low molecular weight molecules, including industrial chemicals, environmental toxicants, procarcinogens anesthetics, and drugs (e.g., acetaminophen). There is little evidence that polymorphic variation in CYP2E1 has any clinical significance.

27.7.3 The CYP3A Family

The human CYP3 subfamily, located on chromosome 7, comprises four genes (CYP3A4, CYP3A5, CYP3A7, and CYP3A43). These are inducible by glucocorticoids via PXR, whose mechanism of action is similar to that of conventional nuclear receptors except that, instead of binding a narrow range of ligands with very high affinity, it binds a wide range of ligands with relatively low affinity. This pattern of ligand responsiveness, which exhibits marked species differences between humans and rodents, may have evolved in order to deal with the vast number of exogenous chemicals to which organisms are exposed.

The predominant CYP isoform in human liver is CYP3A4, which can account for up to 50% of total hepatic CYP expression and metabolizes immunosuppressants, macrolide antibiotics, benzodiazepines, statins, antidepressants, opioids, and anticancer drugs. It is also involved in endogenous steroid catabolism. Midazolam, erythromycin, alprazolam, and dextromethorphan are commonly used to phenotype human CYP3A activity while its *in vitro* model substrates include testosterone, 7-benzyloxyresorufin, and benzoquinoline.

The expression of CYP3A4 is highly variable between individuals, but the question of whether this is due to adventitious exposure to inducing agents or to genetic regulation remains unresolved. Some drugs (e.g., ketoconazole) and dietary components (e.g., bergamottins found in grapefruit juice) inhibit CYP3A4, while others (e.g., rifampicin) induce its expression. Both induction and inhibition can lead to drug−drug interactions.

In individuals who express appreciable amounts of CYP3A5 (only 5−10% of Caucasians, but $\geq$60% of Africans and African-Americans), CYP3A5 could contribute significantly to the metabolism of xenobiotics, particularly those which are better substrates for CYP3A5 than CYP3A4 (e.g., tacrolimus).

The CYP3A7 isoform is preferentially expressed in fetal liver and, although expression shifts to CYP3A4 after birth, it continues to be expressed in some adult livers and in the intestine. The clinical significance of the CYP3A7 polymorphism remains unclear.

27.7.4 The CYP4A Family

Peroxisome proliferators, so-called because they induce a spectacular increase in the number of peroxisomes within hepatocytes, include phthalate plasticizers and fibrate-based hypolipidemic agents. They represent a unique class of nongenotoxic hepatocarcinogens in rodents [22] and also induce a family of CYP enzymes, the CYP4A family, which catalyzes ω-hydroxylation of lauric acid and important eicosanoids such as the arachidonic acid metabolite 20-hydroxyeicosatetraenoic acid.

Expression of CYP4A family members is induced via peroxisome proliferator-activated receptor α (PPARα), which mediates diverse responses to peroxisome proliferators and, along with the related receptors PPARγ and PPARδ, plays a key role in lipid homeostasis [23]. Important toxicological questions surround PPARα because it mediates peroxisome proliferator-induced hepatocarcinogenesis, at least in rodents. The relevance of this phenomenon, and the role therein of PPARα, has been the subject of intense debate for many years because human cells appear to be resistant to the *in vitro* effects of PPARα ligands, raising questions regarding the validity of species−species extrapolation when assessing potential risks due to peroxisome proliferators.

27.8 PHASE II

Phase II xenobiotic metabolism involves conjugation to hydrophilic moieties, typically leading to more water-soluble and therefore more readily excretable compounds. Phase II enzyme families include the glutathione *S*-transferases (GSTs), UDP-glucuronosyltransferases (UGTs), sulfotransferases (STs), and *N*-acetyltransferases (NATs). Epoxide hydrolases (EHs), which convert epoxides to dihydrodiols, are also classified as Phase II enzymes since they act on the products of CYP-mediated Phase I metabolism.

27.8.1 Glutathione *S*-transferases

Many reactive electrophiles form conjugates with the tripeptide glutathione (GSH: Fig. 27.7) which is present in cells at concentrations up to 10 mM. These conjugation reactions are catalyzed by cytosolic GSTs found mainly in the liver, lung, and kidney, although strong electrophiles may also react nonenzymatically with GSH. The resulting conjugates are degraded to *N*-acetylated cysteine thioethers (mercapturic acids), which are subsequently excreted. Generation of

diversity among GSTs is achieved by the existence of homo- or heterodimers of 25−27 kD subunits, which leads to a wide range of specificities.

The generic model substrate for GSTs is 1-chloro-2,4-dinitrobenzene; they also metabolize carcinogens, anticancer drugs, and organophosphorous insecticides. The peroxidase activity of GSTs helps to protect cells against oxidative stress, but GSTs can also mediate the metabolic activation of some compounds (e.g., hexachlorobutadiene), leading to toxicity.

The cytosolic GSTs were originally named using the Greek symbols α, μ, ο, π, σ, τ, and ζ. They are now classified using the superfamily code GST and the same initial as the original Greek family name, i.e., GSTA (human members GSTA1−5); GSTM (GSTM1−5); GSTO (GSTO1, GSTO2); GSTP (one member, GSTP1, in humans); GSTS (one member, GSTS1, in humans); GSTT (GSTT1, GSTT2); and GSTZ (GSTZ1) [24].

The microsomal GSTs (MGST1, MGST2, and MGST3) are distinct from the cytosolic forms, with which they have low sequence homology, suggesting that this is the result of convergent evolution. Microsomal GSTs metabolize α-hexachlorocyclohexane and are activated by *N*-ethylmaleimide.

27.8.1.1 Example: GST Gene Expression and Cancer

The distribution of GST expression can determine the tissue specificity of carcinogens; e.g., arylmethylsulfate metabolites of arylmethanols are substrates for rodent Gstt2 (formerly $Y_{rs}-Y_{rs}$), which is expressed in liver but not skin; they are, correspondingly, carcinogenic in rat skin but not liver [25].

27.8.1.2 Example: GSTP as a Tumor Marker

The GSTP isoenzyme is normally a fetal form, but when cells become transformed they start to express it again, possibly due to upregulation by the *fos/jun* oncogene complex. In rodent hepatocarcinogenicity studies, GSTP-positive foci (Fig. 27.8) are considered to be preneoplastic lesions; they indicate that initiation has occurred and the promotion stage of carcinogenesis is under way. In clinical medicine, GSTP can be used as an immunohistochemical tumor marker [26], although it cannot be used as a blood test because it is expressed in red blood cells.

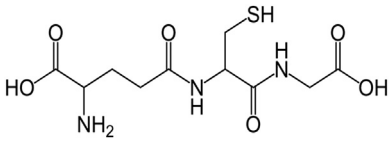

FIGURE 27.7 Structure of glutathione.

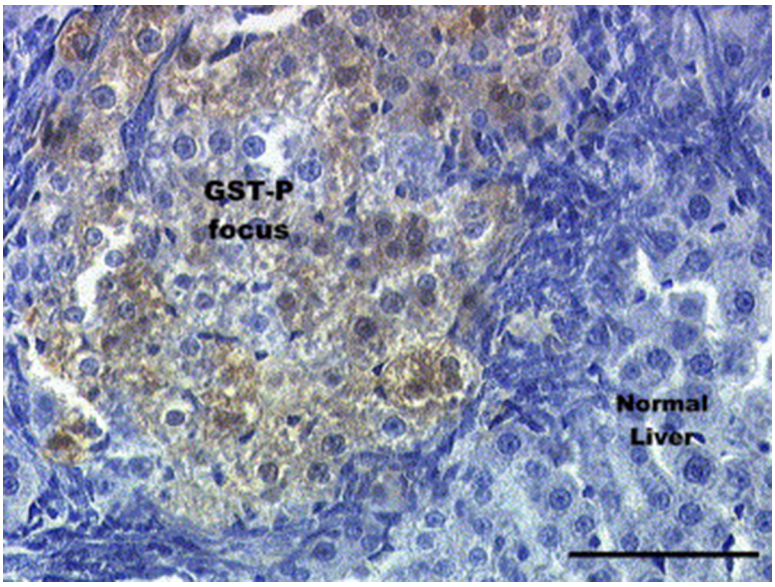

FIGURE 27.8 Photomicrograph of a GST-P positive preneoplastic nodule. *Reprinted from Gonzalez de Mejia et al. Inhibition of liver carcinogenesis in Wistar rats by consumption of an aqueous extract from leaves of* Ardisia compressa*. Food Chem Toxicol 2004;42:509−16 with permission from Elsevier.*

27.8.2 UDP-Glucuronosyltransferases

These membrane-bound 50−56 kD isoenzymes catalyze the transfer of D-glucuronic acid from Uridine 5′-diphospho (UDP)-glucuronic acid to aliphatic and aromatic alcohols, carboxylic acids, amines, hydroxylamines, amides, and thiols. They generate *O*-, *N*-, *S*-, and *C*-glucuronides by forming a β-glycosidic bond between the xenobiotic and the glucuronic acid and are essential in endogenous homeostasis for the glucuronidation of bilirubin, steroids, and thyroid hormone. Xenobiotic UGT substrates include phenols, anthraquinones, carcinogen metabolites, and synthetic steroids.

Xenobiotic-metabolizing UGTs comprise two subfamilies: UGT1 contains a single gene whereas UGT2 is a multigene family. They exemplify different ways of generating diversity [27]: in the case of UGT2, diversity is generated by the conventional mechanism of having multiple individual genes, but diversity in the UGT1 family is generated by an unusual mechanism involving alternative mRNA splicing. There is only one UGT1 gene (*UGT1A1*, located on chromosome 2), but this encodes both phenol and bilirubin UGTs. The specificity-determining region of the gene is encoded by exon 1. There are many different exon 1 sequences, any of which can be spliced to exons 2−5, thus generating enzymes with different specificities from a single gene.

27.8.2.1 Example: Crigler-Najjar Syndrome

Crigler-Najjar (CN) Syndrome is a congenital metabolic disorder caused by UGT1A1 deficiency, which leads to defective glucuronidation and severe hyperbilirubinemia. It has two forms, Types 1 and 2 (Arias Syndrome). A milder condition, Gilbert's Syndrome, also results from *UGT1A1* mutations. The commonest defect found in CN Type 1 sufferers is a 13 bp deletion in exon 2 [28]. Since there is only one copy of each of exons 2−5, this cannot be compensated for by redundancy, unlike defects in exon 1. Treatment for CN Type 1 requires regular phototherapy throughout life, although liver transplantation has been successful in some patients. Taking advantage of the phenomenon of induction, upregulation of UGT1A1 by treatment with phenobarbital is sometimes used to manage the milder form, Arias Syndrome.

27.8.3 Sulfotransferases

Sulfotransferases and UGTs frequently metabolize the same substrates and may cooperate in generating conjugates which are excreted from the liver into the blood, making them available for renal clearance. The human genome contains four Sulfotransferase gene families (SULT1, SULT2, SULT4, and SULT6) with ≥ 14 members encoding cytosolic 32−36 kD enzymes which use 3′-phosphoadenosine-5′-phosphosulfate as a sulfate donor [29]. They metabolize endogenous biogenic amines and mediate the sulfation of dehydroepiandrosterone and progesterone in the adrenals, after which the sulfated forms are secreted and transported to sites of estrogen/androgen synthesis. This may regulate hormonal activity in target tissues since the sulfated forms are less potent than the unconjugated forms, presumably due to weaker receptor binding properties. Synthetic steroids (e.g., 4-hydroxytamoxifen) are also Sulfotransferase substrates; this may influence the susceptibility of estrogen-receptor positive breast tumors to antiestrogen therapy [30].

The large hydrophobic binding sites of Sulfotransferases, which can accommodate up to three aromatic rings, confer broad substrate specificity. This is another means by which diversity in xenobiotic metabolism is generated. The sulfonates produced tend to be ionized at physiological pH, increasing water solubility and resulting in detoxification, although Sulfotransferases can mediate the metabolic activation of some mutagens and procarginogens [31].

27.8.4 *N*-Acetyltransferases

Both detoxification via *N*-acetylation (e.g., of aromatic amines) and metabolic activation by *O*-acetylation (e.g., of hydroxylamines) are mediated by NATs. The substrates of these 31 kD cytosolic enzymes include aromatic amines, hydrazines, hydrazides, sulfonamides, some aliphatic primary amines, and hydroxylamines.

Humans have two functional NAT genes (*NAT*1 and *NAT*2) found, along with the pseudogene *NAT*p, on chromosome 8 [32]. Human NATs were among the first xenobiotic-metabolizing enzymes to be identified as polymorphic [33,34].

Human NAT1 was originally known as the "monomorphic" NAT, although it is actually highly polymorphic. It metabolizes *p*-aminobenzoic acid, 4-aminosalicylic acid, and 2-aminofluorene, as well as endogenous substrates such as *p*-aminobenzoyl glutamate. Like its murine homologue (confusingly called Nat2), it has a wide tissue distribution, being expressed in all epithelial tissues and in red blood cells. It can catalyze the direct hydrolysis of acetyl Coenzyme A in the presence of folate [35] and may play a role in homeostasis.

Human NAT2 (previously known as "polymorphic" NAT) behaves more like a "typical" xenobiotic-metabolizing enzyme, being expressed mainly in the liver and small intestine. Its substrates include sulfamethazine, isoniazid, caffeine, and 2-aminofluorene. Humans are classified as fast or slow acetylators according to their NAT2 phenotype (some studies also identify an intermediate group).

While individuals can readily be classified as rapid or slow acetylators according to their *NAT*2 genotype/phenotype, the genotype/phenotype correlation for *NAT*1 is less clear-cut. Part of the problem is that this gene is both genetically polymorphic, having around 30 allelic variants, and subject to regulation at the protein level. Interestingly, cell biology studies have shown that some of the variant alleles fail to fold correctly and accumulate in aggresomes, where they are targeted for ubiquitinylation and proteolysis [36].

27.8.5 Epoxide Hydrolases

Xenobiotic-derived epoxides are formed by the monooxygenation of carbon−carbon double bonds in olefins or aromatic ring systems. These very reactive species are converted to dihydrodiols by EHs, 49 kD enzymes which thereby protect cells from the formation of both DNA and protein adducts. The hydrolysis of an epoxide usually results in increased water solubility and elimination of toxic potential; however, this is not always the case.

27.8.5.1 Example: Metabolic Activation of Benzo(a)pyrene

EHs usually contribute to the detoxification of reactive epoxide intermediates, including PAH metabolites. However, they can also participate in metabolic activation, as exemplified by the metabolism of benzo(*a*)pyrene [37] (Fig. 27.9). While many CYP-derived PAH epoxides are good substrates for hydrolysis, bay region diol epoxides display potent genotoxicity resulting from two-stage metabolic activation. Benzo(*a*)pyrene is converted to benzo(*a*)pyrene-7,8-epoxide by CYP1A1 and/or CYP1B1; this then undergoes regioselective EH-mediated hydrolysis. This would normally lead to detoxification, but benzo(*a*)pyrene-7,8-dihydrodiol is a good substrate for secondary epoxidation at the 9,10-position, yielding the ultimate carcinogen benzo(*a*)pyrene-7,8-dihydrodiol-9,10-epoxide.

Microsomal EH (mEH; EPHX1) and soluble EH (sEH; EPXH2) are the most important isoforms involved in xenobiotic metabolism [38,39]. Additional isoforms also exist, but these do not generally metabolize xenobiotics.

The human mEH gene is located on the long arm of chromosome 1. Two coding region polymorphisms (Tyr113His and His139Arg) have been identified; the resulting protein variants have different stabilities, suggesting a potential impact on mEH activity *in vivo*. Human mEH expression is inducible via the transcription factors *Nrf*2 and CAR by compounds including phenobarbital and *N*-acetylaminofluorene, and alternative noncoding exon 1 sequences found in mammalian genomes permit tissue and cell type-specific expression.

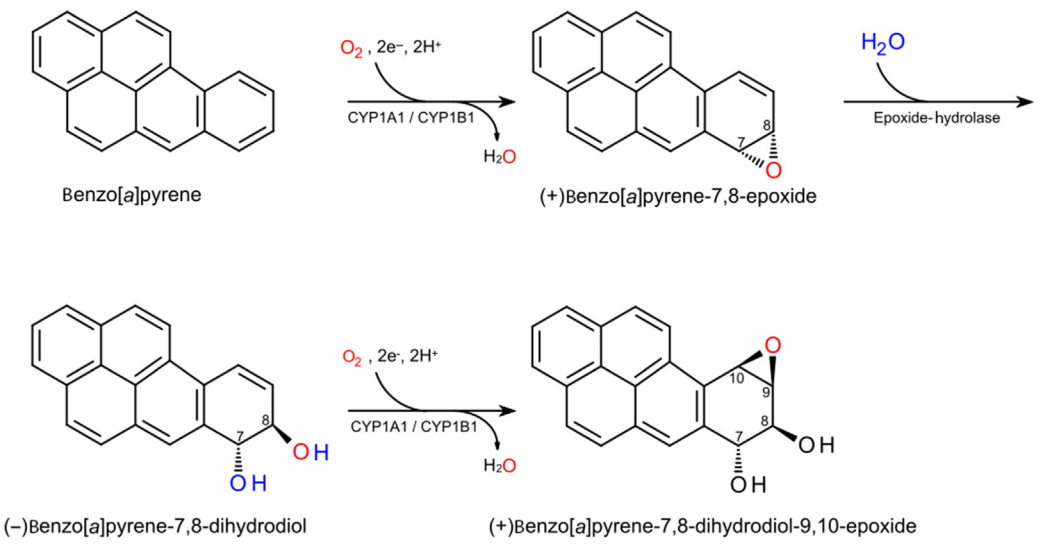

FIGURE 27.9 Metabolism of benzo(*a*)pyrene yielding benzo(*a*)pyren-7,8-diol-9,10-epoxide.

Many of the xenobiotic epoxides degraded by mEH are toxins, mutagens, and carcinogens, while others are drug metabolites; the broad substrate selectivity and distribution of mEH provides systemic defense against such compounds, so inhibition of mEH by therapeutic agents is considered to be undesirable.

The human sEH (EPHX2) locus on chromosome 8 exhibits various polymorphisms which affect its coding sequence and/or enzymatic activity. The substrate selectivity of sEH complements that of mEH in that it hydrolyzes trans-substituted epoxides. Its main function seems to be the turnover of lipid-derived epoxides, which have diverse functions in the control of blood pressure, inflammation, cell proliferation, and nociception. In recent years, sEH has been identified as a promising therapeutic target for the treatment of hypertension and other diseases.

Many organs express sEH, mainly in the cytosol, although peroxisomal expression does occur in some organs. In rodents, peroxisome proliferators (e.g., fibrates, glitazones) induce sEH expression via both PPARα and PPARγ [40].

27.9 POLYMORPHISMS IN XENOBIOTIC-METABOLIZING ENZYMES

There is now overwhelming evidence that polymorphic xenobiotic metabolism is associated with differential responses to drugs, carcinogens, and industrial chemicals.

27.9.1 Metabolic Polymorphisms and Acute Toxicity

27.9.1.1 Example: Occupational exposure to acrylonitrile

Acrylonitrile is both acutely toxic and carcinogenic. It is metabolized by CYP2E1 forming a mutagenic epoxide, cyanoethylene oxide [41] (Fig. 27.10). Further metabolism generates the acute toxin cyanide, either via EH or by rearrangement and hydride transfer. Both acrylonitrile itself and cyanoethylene oxide also undergo GST-mediated reactions with tissue thiols. The acute toxicity of acrylonitrile is considered to be due to a combination of GSH depletion and cyanide generation.

Two cases of acrylonitrile intoxication illustrate this point [42]. Two individuals, one with low and one with high GST activity, were accidentally exposed to acrylonitrile. The person with low activity experienced headache, nausea, and vomiting; furthermore, the level of hydrocyanic acid in his blood entered the lethal range, although he fortunately recovered following treatment with the antidote, *N*-acetylcysteine. This substantiated the hypothesis that, particularly in individuals with low GST activity, acrylonitrile toxicity is gated by GSH depletion. If insufficient GSH is available for conjugation (or the activity of GST is too low), free acrylonitrile can enter the CYP2E1-mediated oxidative pathway, leading to toxicity.

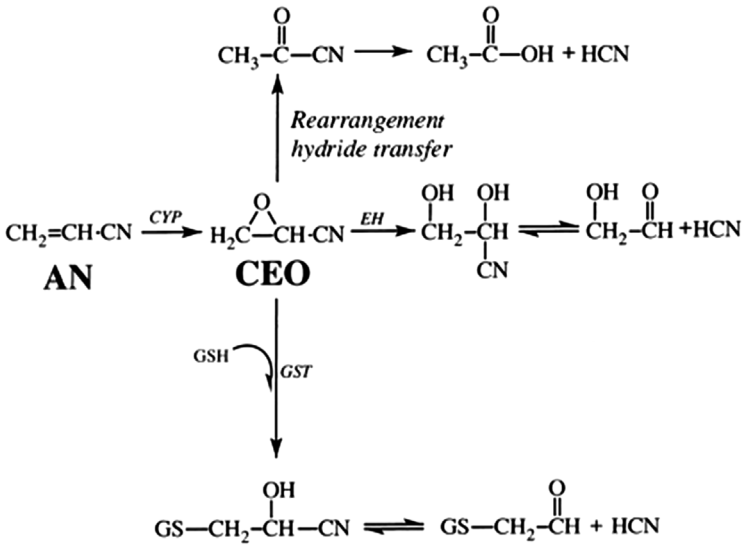

FIGURE 27.10 A proposed scheme showing the role of CYP and epoxide hydrolase enzymes in the metabolism of acrylonitrile to cyanide. *Reprinted from Wang et al. Cytochrome P450 2E1 (CYP2E1) is essential for acrylonitrile metabolism to cyanide: comparative studies using CYP2E1-null and wild-type mice. Drug Metab Dispos 2002;30:911–7 with permission from ASPET.*

27.9.2 Polymorphic Xenobiotic-Metabolizing Enzymes and Cancer

Over the last 30 years a huge amount of effort has been devoted to evaluating the impact of polymorphic xenobiotic metabolism on cancer susceptibility with a view to identifying potentially vulnerable individuals. The simplest paradigm for this approach is aromatic amine-induced bladder cancer.

27.9.2.1 Example: Metabolic Polymorphisms and Bladder Cancer

Bladder cancer is associated with occupational exposure to aromatic amines. A 1982 study found that 22/23 (95.7%) dye factory employees (or ex-employees) with bladder cancer were slow acetylators, compared with only ~60% of controls [43]. Although subsequent studies found lower slow acetylator frequencies among bladder cancer patients [44], the observation that slow acetylators have an increased risk of bladder cancer is now well established, and has been confirmed by meta-analysis [45]. This effect seems to be specific to smokers.

The role of NAT2 should not be considered in isolation, since other activating and detoxifying enzymes in the liver contribute to susceptibility by competing for the aromatic amine substrate, leading to the generation of metabolites which are subsequently delivered to the bladder [46] (Fig. 27.11). Low levels of NAT2-mediated *N*-acetylation allow hepatic CYP1A2-mediated *N*-hydroxylation to predominate. Following further metabolism by UGTs and transport to the bladder, hydrolysis regenerates a reactive *N*-hydroxylamine which can be *O*-acetylated by NAT1 leading to the initiation of carcinogenesis. There is evidence that individuals with both slow NAT2 and rapid CYP1A2 phenotypes have a further increase in their risk of bladder cancer if they smoke [47]. Meta-analysis does not, however, support a key role for NAT1 genotype [45], even though it is NAT1 rather than NAT2 which is expressed in urothelial cells.

It is unusual for studies on toxicogenetic factors in cancer to yield such clear-cut results. Attempts to elucidate the role of metabolic polymorphisms in colorectal cancer illustrate some of the difficulties in obtaining definitive answers.

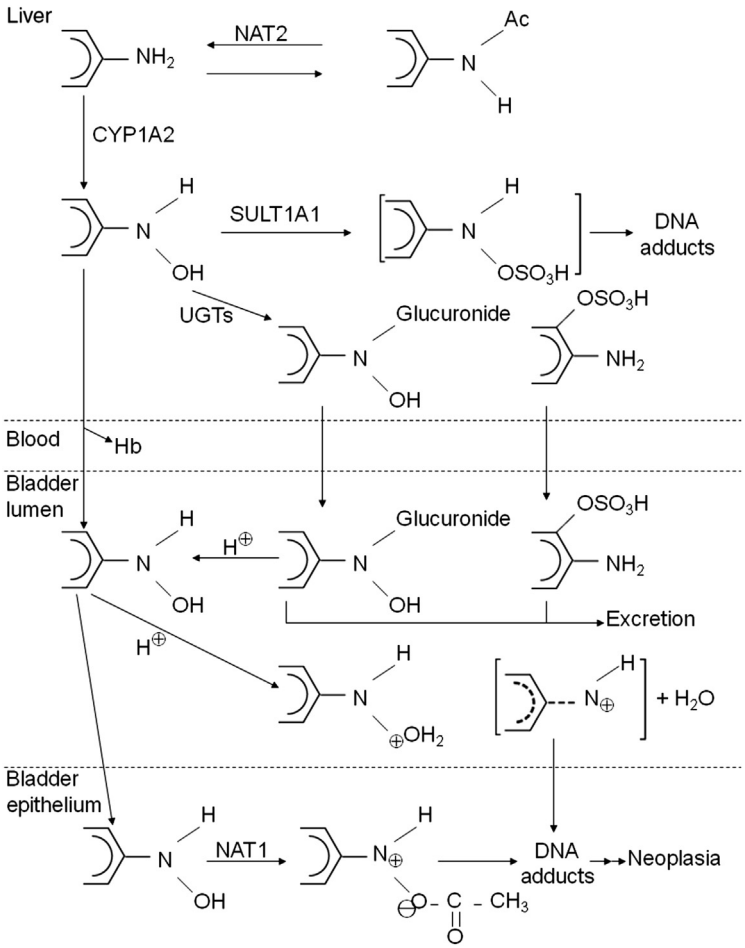

FIGURE 27.11 Pathways of aromatic amine metabolism in the liver and bladder. *Reprinted from Stanley LA. Toxicogenetics. In: Greim H, Snyder R, editors. Toxicology and risk assessment: a comprehensive introduction (ISBN: 978-0-470-86893-5) with the permission of John Wiley and Sons Ltd.*

27.9.2.2 Example: Heterocyclic Amine-Induced Colorectal Cancer

An individual's risk of colorectal cancer is modified by numerous lifestyle and hereditary factors. In particular, people who frequently consume well-done red meat are regularly exposed to heterocyclic amines generated during cooking; these are thought to mediate carcinogenesis in the colon. The carcinogen 2-amino-1-methyl-6-phenylimidazo-[4,5-*b*]-pyridine is particularly significant in this context [48].

The pathway of heterocyclic amine metabolism [48] (Fig. 27.12) suggests that the rapid NAT2 phenotype might confer increased colorectal cancer risk, especially when combined with the rapid CYP1A2 phenotype and regular consumption of well-done red meat. Early phenotyping studies suggested that this was the case, at least in smokers [49], but the results of genotyping studies have been contradictory [50–52] and meta-analysis does not clearly link NAT2 genotype with colorectal cancer susceptibility [53].

27.10 CONSEQUENCES OF PHASES I AND II METABOLISM

The main consequence of Phase I metabolism is the generation of reactive intermediates. In the absence of adequate Phase II metabolism these may bind to DNA (forming nucleotide adducts and possibly acting as carcinogens) or to proteins (forming peptide adducts and possibly acting as immunogens). In the presence of active Phase II enzymes the consequences may be further activation (e.g., formation of diol epoxides), which can lead to toxicity, or formation of hydrophilic products ready to be excreted by efflux transporters—"Phase III."

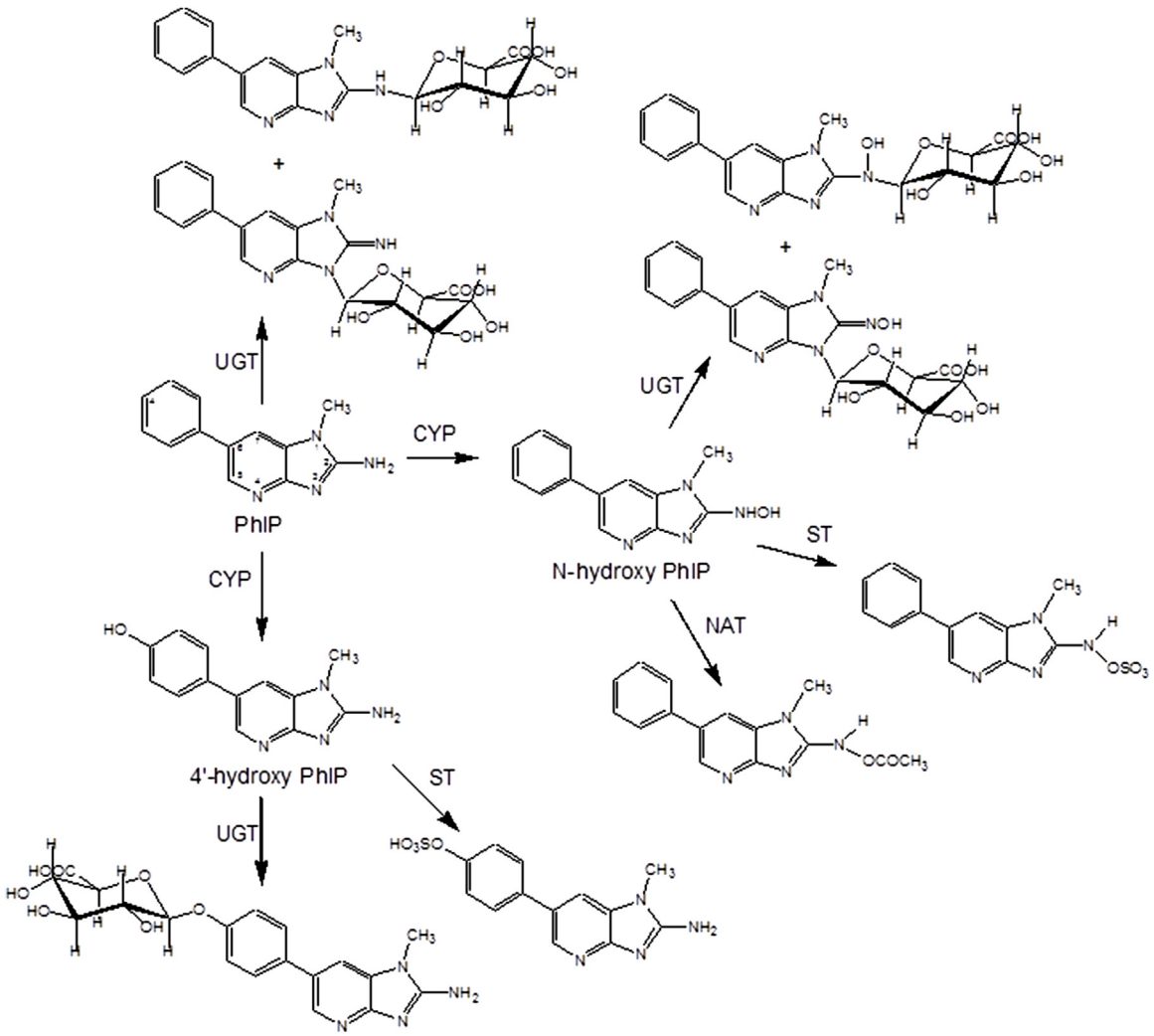

FIGURE 27.12 Pathways of heterocyclic amine metabolism. *Reprinted from Stanley LA. Toxicogenetics. In: Greim H, Snyder R. Toxicology and risk assessment: a comprehensive introduction (ISBN: 978-0-470-86893-5) with the permission of John Wiley and Sons Ltd.*

27.11 PHASE III

In xenobiotic metabolism, "Phase III" is the export of compounds from cells by energy-dependent transporters [54]. However, calling this "Phase III metabolism" is, strictly speaking, erroneous because it describes the transport of molecules across membranes without altering their chemical structure; also, it implies that this is towards the end of the ADME process whereas drug transporters play a key role in intestinal absorption, which is a function of the balance between transporter-mediated influx and efflux in intestinal epithelial cells. Drugs that diffuse passively across the apical membranes of intestinal epithelial cells are often substrates for transporters which extrude them back into the intestinal lumen. These transporters can also mediate active transport of compounds from the blood to the intestinal lumen. If, however, compounds get past this line of defense, hepatic and renal extraction becomes significant, so expression of transporters in the liver and kidney is also important.

27.11.1 The ABC Superfamily

The ATP-binding cassette (ABC) superfamily includes a variety of membrane transporters, receptors, and ion channels.

27.11.1.1 P-Glycoprotein/MDR1

As its name suggests, the first drug transporter identified, multidrug resistance protein 1 (MDR1), was discovered as a result of its ability to confer drug resistance on cancer cells. This key member of the ABCB1 family will be referred to here by its original name, P-glycoprotein, for reasons which will become apparent when we discuss its murine homologues. It is found in the apical membranes of various normal polarized cell types including intestinal mucosal cells (facing the intestinal lumen), hepatocytes (bile duct-facing membrane), renal proximal tubular cells (brush borders), and capillary endothelial cells of the brain and testis; in other words, in key locations involved the uptake and excretion of xenobiotics. For example, expression at the tip of the intestinal villus means that P-glycoprotein is ideally placed to control xenobiotic absorption from the intestinal lumen.

The pattern of P-glycoprotein expression and its known role in drug resistance are consistent with its normal function in the removal of potentially toxic substances from susceptible tissues. It also has postulated functions in hormone and lipid transport, reproduction, cellular immunity, and the regulation of cell volume. In addition, its presence at the maternal−fetal interface of the placenta suggests a role in protection of the fetus.

The substrate selectivity of P-glycoprotein encompasses molecules ranging in size from 300 to 2000 Da. It seems to have a preference for molecules containing a basic N atom and two planar aromatic domains, though this is not an absolute requirement, and it mediates the ATP-dependent transport of lipophilic amphipathic drugs, particularly cations.

Mice have two *Mdr* genes, *Mdr*1a and *Mdr*1b, which, between them, seem to cover the same functions as the single human *MDR1* gene. In particular, Mdr1a shares many of the functions of human MDR1. The functions of Mdr1b seem to be more limited, but it must have some importance as it has been conserved during evolution. *Mdr*1a and *Mdr*1b null mouse models have been used to study murine P-glycoprotein function.

27.11.1.2 MDR-Associated Proteins: MRP1 and MRP2

The second important family of ABC transporters, ABCC1, includes MRP1 and MRP2.

The MRP1 protein is expressed at high levels on the basolateral membranes of polarized epithelial cells in the kidney (distal tubule and glomeruli), intestine, brain, lung, testis, placental endothelial cells, and liver (lower levels). It is thought to protect cells by extruding substances into the blood for subsequent elimination via the intestine or kidneys and is the primary transporter for endogenous GSH, glucuronide, and sulfate conjugates. It can also transport unconjugated anticancer drugs via a cotransport mechanism with GSH.

The ATP-dependent transport of anionic drugs and metabolites, including sulfates, glucuronides (including bilirubin glucuronides), and GSH conjugates is mediated by MRP2, which is located on the apical membranes of hepatocytes, intestinal mucosal cells, kidney proximal tubular cells, and syncytiotrophoblast cells. This orientation allows MRP2 to drive xenobiotic excretion into bile, feces, and urine and limit xenobiotic intake into protected compartments.

Humans and mice with deficiencies in MRP2/Mrp2, including the inherited disorder Dubin-Johnson syndrome, exhibit impaired biliary secretion of GSH, GSH conjugates, and bilirubin glucuronides associated with conjugated hyperbilirubinemia and pigment deposition in the liver.

27.11.2 Consequences of Altered Drug Transporter Function

One of the drivers of research into the effects of drug transporters on systemic pharmacokinetics is the need to improve plasma concentrations of potent drugs which have very poor bioavailability. This is important in veterinary settings as well as in human medicine: P-glycoprotein substrates have been reported to cause problems in Collies and other canine breeds that carry *MDR1* mutations (http://www.vetmed.wsu.edu/depts-vcpl/drugs.aspx).

Transporter null and humanized mice have been used to study the consequences of altered drug transporter function in various contexts [54]. The loss of *Mdr*1a in mice often has its most striking effects in terms of changes in the permeability of the blood−brain barrier (BBB), the most important barrier between the central nervous system and the systemic circulation. The BBB comprises a monolayer of endothelial cells connected by complex tight junctions which form a physical barrier to large hydrophilic compounds, though it may be crossed by small lipophilic compounds which can enter cells by passive diffusion. Its endothelial cells express an array of metabolic enzymes and efflux transporters, thus forming a biochemical barrier to compound uptake.

27.11.2.1 Example: Altered Susceptibility to Ivermectin in Mdr*1a Null Mice*

Interest in the susceptibility to xenobiotics of mice lacking a functional *Mdr*1 gene arose when naturally Mdr1-deficient CF-1 mice were sprayed with ivermectin to treat a mite infestation. The null mice were 50−100 times more sensitive to the adverse effects of ivermectin, which is normally nontoxic, and the concentration of ivermectin in their brains following oral dosing was 90 times higher than in wild-type littermates [55]. The mice also exhibited slower clearance and increased tissue concentrations when dosed with the anticancer drug vinblastine.

This generated considerable interest because it suggested the possibility of manipulating the access of drugs to the brain. Further studies [56] supported the concept that many relatively hydrophobic drugs are excluded from the brain via secretion back into the blood by P-glycoprotein at the BBB.

27.12 IMPORTANCE OF RECENT DEVELOPMENTS

Until recently we depended on results from animal experiments to understand xenobiotic metabolism; however, numerous *in vitro* and *in silico* methods are now available for predicting xenobiotic disposition. These cannot, individually, provide a complete picture of this complex process; however, by integrating data from *in vitro* studies and *in silico* models effective predictions can be made.

Furthermore, advances in molecular biology and the development of humanized models now enable us to study the *in vivo* functions of human xenobiotic-metabolizing enzymes directly. This is important because there are many differences in xenobiotic metabolism between animals and humans. As better humanized models become available, their use is likely to extend both backwards into the process of drug discovery (verifying bioavailability prior to candidate selection) and forwards into the clinical phase (explaining observations made during clinical trials). These models will enable us to predict responses to drugs and xenobiotics more confidently, but they must be characterized carefully and used judiciously: the traditionalist would still argue that the problem with a humanized mouse is that it is neither a human nor a mouse!

27.12.1 The Future

The term “drug-metabolizing enzyme” conventionally refers to the enzymes which metabolize aromatic and aliphatic organic compounds; however, as the range of substances used as “drugs” is extended, many more enzymes need to be considered. What additional enzyme families should be included in order to allow us to understand the disposition of the many novel plant products which are now being developed for therapeutic use?

27.13 REVIEW QUESTIONS

1. How might changes in the rate of hepatic xenobiotic metabolism affect the bioavailability of an orally administered drug?
2. What are the possible consequences of CYP-mediated metabolism of an aromatic organic chemical?
3. Can you rationalize the fact that NAT2 fast acetylators appear to have an increased risk of colon cancer but be protected from bladder cancer?

REFERENCES

[1] Velasquez JL, Lipkin SM. What are SNPs and haplotypes and how will they help us manage the prevention of adult cancer? Curr Oncol Rep 2005;7:475–9.

[2] Fenner KS, Jones HM, Ullah M, Kempshall S, Dickins M, Lai Y, et al. The evolution of the OATP hepatic uptake transport protein family in DMPK sciences: from obscure liver transporters to key determinants of hepatobiliary clearance. Xenobiotica. 2012;42:28–45.

[3] Iusuf D, van de Steeg E, Schinkel AH. Functions of OATP1A and 1B transporters *in vivo*: insights from mouse models. Trends Pharmacol Sci 2012;33:100–8.

[4] Munro AW, Girvan HM, Mason AE, Dunford AJ, McLean KJ. What makes a P450 tick? Trends Biochem Sci 2013;38:140–50.

[5] Henderson CJ, Otto DME, Carrie D, Magnuson MA, McLaren AW, Rosewell I, et al. Inactivation of the hepatic cytochrome P450 system by conditional deletion of hepatic cytochrome P450 reductase. J Biol Chem 2003;278:13480–6.

[6] Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol Ther 2013;138:103–41.

[7] Conney AH, Miller EC, Miller JA. The metabolism of methylated aminoazo dyes. V. Evidence for induction of enzyme synthesis in the rat by 3-methylcholanthrene. Cancer Res 1956;16:450–9.

[8] Julliard W, Fechner JH, Mezrich JD. The aryl hydrocarbon receptor meets immunology: friend or foe? A little of both. Front Immunol 2014;5:458.

[9] Sorg O, Zennegg M, Schmid P, Fedosyuk R, Valikhnovskyi R, Gaide O, et al. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) poisoning in Victor Yushchenko: identification and measurement of TCDD metabolites. Lancet 2009;374:1179–85.

[10] Dobrinas M, Cornuz J, Eap CB. Pharmacogenetics of CYP1A2 activity and inducibility in smokers and exsmokers. Pharmacogenet Genom 2013;23:286–92.

[11] Murray GI, Taylor MC, McFadyen MC, McKay JA, Greenlee WF, Burke MD, et al. Tumor-specific expression of cytochrome P450 CYP1B1. Cancer Res 1997;57:3026–31.

[12] Potter GA, Patterson LH, Wanogho E, Perry PJ, Butler PC, Ijaz T, et al. The cancer preventative agent resveratrol is converted to the anticancer agent piceatannol by the cytochrome P450 enzyme CYP1B1. Br J Cancer 2002;86:774–8.

[13] Raunio H, Rahnasto-Rilla M. CYP2A6: genetics, structure, regulation, and function. Drug Metabol Drug Interact 2012;27:73–88.

[14] Tzameli I, Moore DD. Role reversal: new insights from new ligands for the xenobiotic receptor CAR. Trends Endocrinol Metab 2001;12:7–10.

[15] Geisler CH. Cyclophosphamide for CLL: to be or not CYP2B activated? Blood 2013;122:4156–7.

[16] Naraharisetti SB, Lin YS, Rieder MJ, Marciante KD, Psaty BM, Thummel KE, et al. Human liver expression of CYP2C8: gender, age, and genotype effects. Drug Metab Dispos 2010;38:889–93.

[17] Kalow W. The genetic defect of mephenytoin hydroxylation. Xenobiotica 1986;16:379–89.

[18] Cavallari LH. Tailoring drug therapy based on genotype. J Pharm Pract 2012;25:413–16.

[19] Klein TE, Altman RB, Eriksson N, Gage BF, Kimmel SE, Lee MT, et al. Estimation of the warfarin dose with clinical and pharmacogenetic data. N Engl J Med 2009;360:753–64.

[20] Idle JR, Mahgoub A, Lancaster R, Smith RL. Hypotensive response to debrisoquine and hydroxylation phenotype. Life Sci 1978;22:979–83.

[21] Ingelman-Sundberg M. Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. Pharmacogenom J 2005;5:6–13.

[22] Reddy JK, Azarnoff DL, Hignite CE. Hypolipidaemic hepatic peroxisome proliferators form a novel class of chemical carcinogens. Nature 1980;283:397–8.

[23] Wahli W, Michalik L. PPARs at the crossroads of lipid signaling and inflammation. Trends Endocrinol Metab 2012;23:351–63.

[24] Board PG, Menon D. Glutathione transferases, regulators of cellular metabolism and physiology. Biochim Biophys Acta 2013;1830:3267–88.

[25] Hiratsuka A, Sebata N, Kawashima K, Okuda H, Ogura K, Watabe T, et al. A new class of rat glutathione *S*-transferase Yrs-Yrs inactivating reactive sulfate esters as metabolites of carcinogenic arylmethanols. J Biol Chem 1990;265:11973–81.

[26] Kantor RR, Giardina SL, Bartolazzi A, Townsend AJ, Myers CE, Cowan KH, et al. Monoclonal antibodies to glutathione *S*-transferase pi-immunohistochemical analysis of human tissues and cancers. Int J Cancer 1991;47:193–201.

[27] Owens IS, Basu NK, Banerjee R. UDP-glucuronosyltransferases: gene structures of UGT1 and UGT2 families. Methods Enzymol 2005;400:1–22.

[28] Owens IS, Ritter JK, Yeatman MT, Chen F. The novel UGT1 gene complex links bilirubin, xenobiotics, and therapeutic drug metabolism by encoding UDP-glucuronosyltransferase isozymes with a common carboxyl terminus. J Pharmacokinet Biopharm 1996;24:491–508.

[29] Gamage N, Barnett A, Hempel N, Duggleby RG, Windmill KF, Martin JL, et al. Human sulfotransferases and their role in chemical metabolism. Toxicol Sci 2006;90:5–22.

[30] Falany JL, Pilloff DE, Leyh TS, Falany CN. Sulfation of raloxifene and 4-hydroxytamoxifen by human cytosolic sulfotransferases. Drug Metab Dispos 2006;34:361–8.

[31] Banoglu E. Current status of the cytosolic sulfotransferases in the metabolic activation of promutagens and procarcinogens. Curr Drug Metab 2000;1:1–30.

[32] Sim E, Abuhammad A, Ryan A. Arylamine *N*-acetyltransferases: from drug metabolism and pharmacogenetics to drug discovery. Br J Pharmacol 2014;171:2705–25.

[33] Evans DA, Manley KA, Mc KV. Genetic control of isoniazid metabolism in man. Br Med J 1960;2:485–91.

[34] Drayer DE, Reidenberg MM. Clinical consequences of polymorphic acetylation of basic drugs. Clin Pharmacol Ther 1977;22:251–8.

[35] Laurieri N, Dairou J, Egleton JE, Stanley LA, Russell AJ, Dupret JM, et al. From arylamine *N*-acetyltransferase to folate-dependent acetyl CoA hydrolase: impact of folic acid on the activity of (HUMAN)NAT1 and its homologue (MOUSE)NAT2. PLoS One 2014;9:e96370.
[36] Butcher NJ, Arulpragasam A, Minchin RF. Proteasomal degradation of *N*-acetyltransferase 1 is prevented by acetylation of the active site cysteine: a mechanism for the slow acetylator phenotype and substrate-dependent down-regulation. J Biol Chem 2004;279:22131−7.
[37] Shou M, Gonzalez FJ, Gelboin HV. Stereoselective epoxidation and hydration at the *K*-region of polycyclic aromatic hydrocarbons by cDNA-expressed cytochromes P450 1A1, 1A2, and epoxide hydrolase. Biochemistry 1996;35:15807−13.
[38] Decker M, Arand M, Cronin A. Mammalian epoxide hydrolases in xenobiotic metabolism and signalling. Arch Toxicol 2009;83:297−318.
[39] Morisseau C, Hammock BD. Impact of soluble epoxide hydrolase and epoxyeicosanoids on human health. Annu Rev Pharmacol Toxicol 2013;53:37−58.
[40] Tanaka H, Kamita SG, Wolf NM, Harris TR, Wu Z, Morisseau C, et al. Transcriptional regulation of the human soluble epoxide hydrolase gene EPHX2. Biochim Biophys Acta 2008;1779:17−27.
[41] Wang H, Chanas B, Ghanayem BI. Cytochrome P450 2E1 (CYP2E1) is essential for acrylonitrile metabolism to cyanide: comparative studies using CYP2E1-null and wild-type mice. Drug Metabol Disposit 2002;30:911−17.
[42] Thier R, Lewalter J, Bolt HM. Species differences in acrylonitrile metabolism and toxicity between experimental animals and humans based on observations in human accidental poisonings. Arch Toxicol 2000;74:184−9.
[43] Cartwright RA, Glashan RW, Rogers HJ, Ahmad RA, Barham-Hall D, Higgins E, et al. Role of *N*-acetyltransferase phenotypes in bladder carcinogenesis: a pharmacogenetic epidemiological approach to bladder cancer. Lancet 1982;2:842−5.
[44] Risch A, Wallace DM, Bathers S, Sim E. Slow *N*-acetylation genotype is a susceptibility factor in occupational and smoking related bladder cancer. Hum Mol Genet 1995;4:231−6.
[45] Garcia-Closas M, Malats N, Silverman D, Dosemeci M, Kogevinas M, Hein DW, et al. NAT2 slow acetylation, GSTM1 null genotype, and risk of bladder cancer: results from the Spanish Bladder Cancer Study and meta-analyses. Lancet 2005;366:649−59.
[46] Kadlubar FF, Badawi AF. Genetic susceptibility and carcinogen-DNA adduct formation in human urinary bladder carcinogenesis. Toxicol Lett. 1995;82-83:627−32.
[47] Tao L, Xiang YB, Chan KK, Wang R, Gao YT, Yu MC, et al. Cytochrome P4501A2 phenotype and bladder cancer risk: the Shanghai bladder cancer study. Int J Cancer 2012;130:1174−83.
[48] Gooderham NJ, Murray S, Lynch AM, Yadollahi-Farsani M, Zhao K, Boobis AR, et al. Food-derived heterocyclic amine mutagens: variable metabolism and significance to humans. Drug Metab Dispos 2001;29:529−34.
[49] Le Marchand L, Hankin JH, Wilkens LR, Pierce LM, Franke A, Kolonel LN, et al. Combined effects of well-done red meat, smoking, and rapid *N*-acetyltransferase 2 and CYP1A2 phenotypes in increasing colorectal cancer risk. Cancer Epidemiol Biomarkers Prev 2001;10:1259−66.
[50] Brockton N, Little J, Sharp L, Cotton SC. *N*-acetyltransferase polymorphisms and colorectal cancer: a HuGE review. Am J Epidemiol 2000;151:846−61.
[51] Cleary SP, Cotterchio M, Shi E, Gallinger S, Harper P. Cigarette smoking, genetic variants in carcinogen-metabolizing enzymes, and colorectal cancer risk. Am J Epidemiol 2010;172:1000−14.
[52] da Silva TD, Felipe AV, de Lima JM, Oshima CT, Forones NM. *N*-Acetyltransferase 2 genetic polymorphisms and risk of colorectal cancer. World J Gastroenterol 2011;17:760−5.
[53] Zhang L, Zhou J, Wang J, Liang G, Li J, Zhu Y, et al. Absence of association between *N*-acetyltransferase 2 acetylator status and colorectal cancer susceptibility: based on evidence from 40 studies. PLoS One 2012;7:e32425.
[54] Stanley LA, Horsburgh BC, Ross J, Scheer N, Wolf CR. Drug transporters: gatekeepers controlling access of xenobiotics to the cellular interior. Drug Metab Rev 2009;41:27−65.
[55] Kwei GY, Alvaro RF, Chen Q, Jenkins HJ, Hop CE, Keohane CA, et al. Disposition of ivermectin and cyclosporin A in CF-1 mice deficient in mdr1a P-glycoprotein. Drug Metab Dispos 1999;27:581−7.
[56] Schinkel AH. The physiological function of drug-transporting P-glycoproteins. Semin Cancer Biol 1997;8:161−70.