CHAPTER 13

Phase I metabolism

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13.1 *Introduction*

The overriding aim of an organism when exposed to a potential toxicant is to remove it from the body as quickly as possible. Whilst this is a sensible strategy from the point of view of survival of the organism, it represents a major barrier to the scientist seeking to produce an effective medicine. In higher mammals, myriad systems have evolved to facilitate the efficient removal of xenobiotics (i.e. foreign chemicals such as drugs, pesticides, food additives, etc.) of which the drug metabolising enzymes are a major part. The term drug metabolism enzymes may be something of an over simplification since they are often involved primarily in the metabolism of endogenous compounds. Moreover, interference with these endogenous pathways by drug molecules can result in unwanted side-effects which may not have been readily predicted from the primary pharmacology of the molecule. Therefore, a good understanding of the nature of the enzymes involved in drug metabolism can be vital in achieving a balance between efficacy and safety during the optimisation of potential new medicines.

Drug metabolism can be conveniently divided into two areas, Phase I and Phase II. Phase I metabolism, and in particular, the cytochrome P450 system, has traditionally attracted the greatest attention from the drug metabolism community and the purpose of this chapter is to provide a concise introduction to this area.

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TABLE 13.1 *Reaction types catalysed by Phase I enzymes*

Enzyme	Reaction
Cytochrome P450s (CYPs)	oxidation, reduction
Monoamine oxidases (MAO)	oxidation
Flavin-containing monooxygenases (FMO)	oxidation
Alcohol dehydrogenase	oxidation
Aldehyde dehydrogenase	oxidation (reduction)
Xanthine oxidases	oxidation
Epoxide hydrolase	hydrolysis
Carboxylesterase and peptidases	hydrolysis
Carbonyl reductases	reduction

However, it is important to stress that Phase II enzymes also play a vital role in both the detoxification and elimination of drugs and that it is the combination of the two phases that helps to produce such an effective barrier to xenobiotics. Phase 2 enzymes are discussed in detail in Chapter 14. Phase I metabolism includes a range of activities such as oxidation, hydrolysis, reduction and hydration. These are often termed functionalisation reactions since they generally lead to the introduction or uncovering of key functional groups (e.g. OH, COOH, NH_2, SH, etc.) which may facilitate removal from the body, either directly, or via conjugation with the polar co-factors of Phase II metabolising systems. Table 13.1 lists the major classes of Phase I enzymes involved in drug metabolism which are described in more detail in the following sections.

13.2 *Cytochrome P450s*

The cytochrome P450s (CYPs) are a superfamily of haem-thiolate containing enzymes which play a major role in the metabolism of many drugs and other xenobiotics. A number of carcinogens are also metabolised by CYPs and it is often these metabolites which are the ultimate carcinogenic species. It has been estimated that over 50 per cent of the most commonly prescribed drugs are cleared primarily by CYPs. In addition to their role in drug metabolism, CYPs have important endogenous functions such as the synthesis and regulation of steroid hormones, eicosanoids and bile acids. Because of this, CYPs are almost ubiquitous in the human body, although the major site of drug metabolism is the liver. CYPs involved in drug metabolism are predominantly membrane bound within the endoplasmic reticulum of cells, together with the flavoprotein NADPH-cytochrome P450 reductase which is involved in the transfer of electrons from NADPH to CYPs.

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In contrast to most enzymes, the CYPs involved in xenobiotic metabolism have evolved a broad substrate specificity which enables them to metabolise a very wide range of compounds to which an organism may be exposed. Despite differences in the active site architecture between CYPs, the catalytic mechanism is essentially constant across isoforms. CYPs catalyse the oxidation of bound substrates through the redox action of the haem moiety and the activation of molecular oxygen (Figure 13.1). By this mechanism, CYPs are able to carry out a variety of hydroxylations, dealkylations and heteroatom oxidations as shown in Figure 13.2. The nature of the resultant product(s) is governed by the steric interactions between enzyme and substrate, which determine the regions of a molecule that are accessible to the oxidising species, and thermodynamic factors, which can influence the relative rates of competing pathways.

A systematic nomenclature has been developed to classify the CYP superfamily. This is based on the amino acid sequence of each isoform rather than a particular reaction or substrate of individual CYPs. Isoforms with greater than 40 per cent sequence homology are assigned to the same gene family (e.g. CYP1, CYP2, CYP3, etc.). Isoforms with greater than approximately 60 per cent homology are further classified as belonging to the same subfamily (e.g. CYP2A, CYP2B, etc.). Each member of a subfamily is then given a number to denote the individual isoform (e.g. CYP2A1, CYP2A2, etc. (Table 13.2). This system does not specify in which species a given isoform is found, or provide information on the function of the enzymes. For instance, the metabolic profiles of many substrates in rat mirror those seen in human, although the P450 isoforms involved may differ. This is exemplified by the CYP3A/2C 'cross-talk' between rat and human. Thus, mephenytoin is hydroxylated predominantly by CYP2C in human but by CYP3A in rat; and

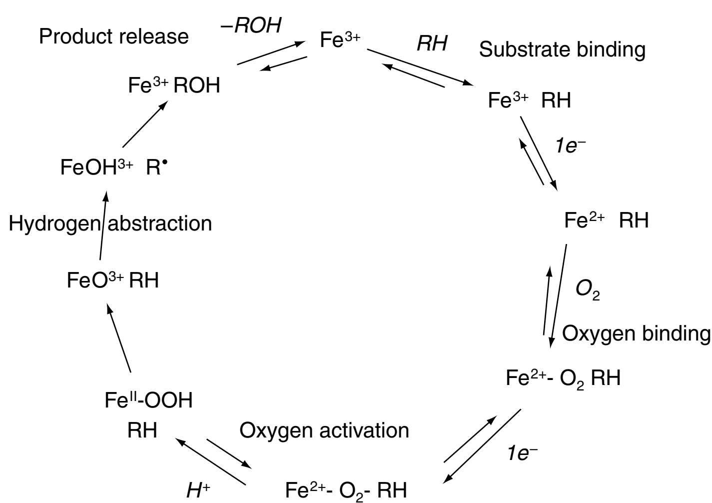

FIGURE 13.1 *Catalytic cycle of cytochrome P450s.*

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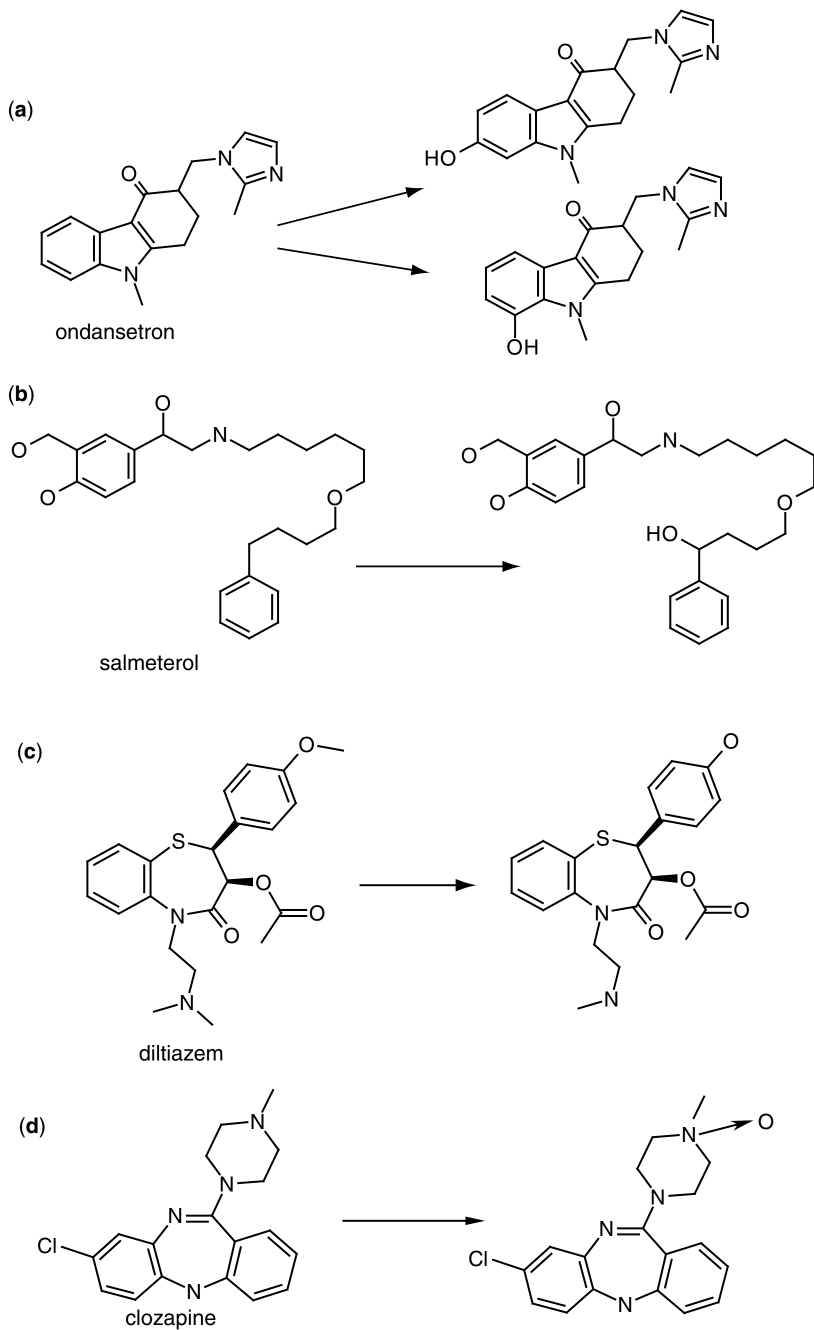

FIGURE 13.2 *Examples of reactions catalysed by CYP: (a) aromatic hydroxylation; (b) aliphatic hydroxylation; (c) N-, O-dealkylation; (d) N-oxidation.*

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TABLE 13.2 *Major CYP forms*

CYP	Tissue	Inducibility	Total P450 (%)
HUMAN			
1A1	Extra hepatic	Inducible	
1A2	Liver	Constitutive/inducible	13
2A6	Liver		4
2B6	Liver	Constitutive	0.2
2C9/19	Liver	Constitutive	20
2D6	Liver	Constitutive	2
2E1	Liver	Constitutive/inducible	7
3A4	Liver/others	Constitutive/inducible	30
RAT			
1A1	All tissues	Inducible	<1
1A2	Liver	Constitutive	2
	All tissues	Inducible	
2A1	Liver	Constitutive	7–30
	Liver	Inducible	(varies according to strain/induced state, etc.)
2B1/2	Lung, testis	Constitutive	5
	All tissues	Inducible	
2C11	Liver	Constitutive	54
2D1	Liver/kidney	Constitutive	
	Inducible		
2E1	Liver/kidney	Constitutive	
	Liver/lung	Inducible	
3A1	Liver/intestine	Constitutive/inducible	17
DOG			
1A	Hepatic	Inducible	similar to human
2B11	Liver	Constitutive	high levels c.f. human
2C21	Liver	Constitutive	minor form
2D	Liver	Constitutive	lower levels c.f. human
3A12	Liver/others	Constitutive	similar to human levels 3A; higher catalytic efficiency

lidocaine and nifedipine are classic CYP3A substrates in human but are preferentially metabolised by CYP2C in rat.

The number of identified CYPs is increasing on a seemingly daily basis. Fortunately, the group of isoforms involved in the majority of human drug metabolism is currently limited to only five or six: CYP1A2, 2C9, 2C19, 2D6 and 3A4/5 (Figure 13.3). Despite the relatively small number of CYPs involved, marked intra- and inter-individual variability exists in both the genotype and phenotype of the

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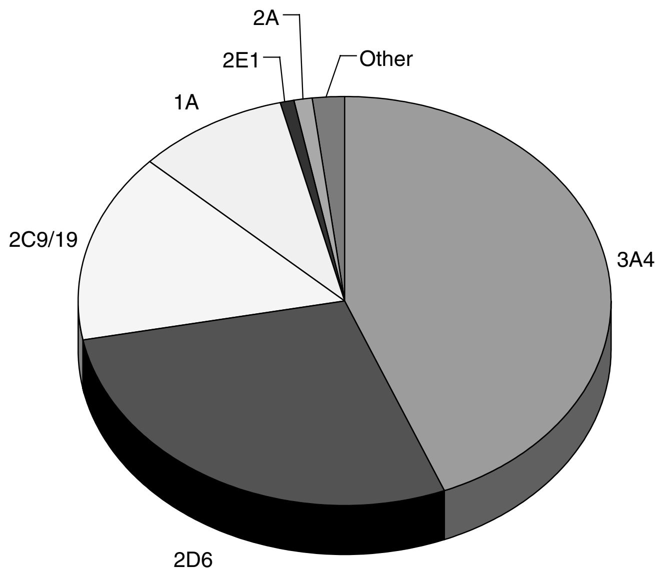

FIGURE 13.3 *Percentage of commonly prescribed drugs metabolised by each CYP isoform.*

human population, which can often be a major cause of the variability in efficacy and/or toxicity of many prescribed medicines observed in patients.

In addition to the effects of age and gender, the range and extent of CYP activity are influenced by both genetic and environmental factors. Inherited mutations in the coding and/or regulatory regions of CYP genes may prevent synthesis of a particular CYP or result in a protein that has reduced catalytic activity. The impact of polymorphism amongst CYPs on human drug metabolism has been most clearly demonstrated for CYP2D6, CYP2C9 and CYP2C19. These isoforms are an important part of the human complement of drug-metabolising enzymes and thus deficiencies in their activities can have important consequences for the disposition of compounds which are dependent on them for clearance. Significant proportions of various ethnic populations have been identified as poor metabolisers of drugs such as debrisoquine (a substrate for CYP2D6) and *S*-mephenytoin (CYP2C19) (Table 13.3) and many other examples have been observed. From a clinical perspective, the appearance of a poor metaboliser phenotype for a given drug is dependent largely on the fraction of the administered dose that is cleared via the affected isoform and the availability of alternative or competing clearance mechanisms. For example, the 5-HT_3 receptor antagonists, ondansetron and tropisetron can both be metabolised *in vitro* by the polymorphic CYP2D6. However, whilst this is the major pathway for tropisetron, metabolism by CYP3A4 is the predominant route of clearance for ondansetron. Thus, in patients who are deficient in CYP2D6, the plasma concentration half-life of tropisetron increases from approximately 9 to 30 hours whereas no change in the half-life of ondansetron is observed. Whether the existence of polymorphism in drug clearance

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TABLE 13.3 *Distribution of polymorphic CYPs in human populations*

Enzyme	Variant allele	Enzyme function	Allele frequency (%)		
			Caucasian	Asian	Black African
CYP2A6	CYP2A6 × 2	Inactive	1–3	0	–
	CYP2A6del	Enzyme absent	1	15	–
CYP2C9	CYP2C9 × 2	Reduced affinity for reductase	8–13	0	–
	CYP2C9 × 3	Altered substrate specificity	6–9	2–3	–
CYP2C19	CYP2C19 × 2	Inactive	13	23–32	13
	CYP2C19 × 3	Inactive	0	6–10	–
CYP2D6	CYP2D6 × 2 × n	Increased activity	1–5	0–2	2
	CYP2D6 × 4	Inactive	12–21	1	2
	CYP2D6 × 5	Enzyme absent	2–7	6	4
	CYP2D6 × 10	Unstable enzyme	1–2	51	6
	CYPD6 × 17	Reduced	0	–	34

has any adverse clinical consequences is in turn dependent on the therapeutic margin of the drug and any impact its elevated concentrations may have on co-administered medications.

Environmental agents such as drugs, other xenobiotics or disease can also cause a (usually) more transient attenuation of CYP activity, either through the prevention of gene expression or by inhibition of the subsequently expressed protein. Inhibition of CYP activity by drug molecules is an area of intense interest for drug metabolism scientists. The occurrence of adverse drug–drug interactions and/or the disruption of endogenous biochemical pathways as a result of CYP inhibition can severely compromise or even preclude the clinical viability of a drug candidate. Several well-documented examples of this exist, including the interaction between the antihistamine, terfenadine, and the antifungal, ketoconazole, in which potent inhibition of the CYP3A4-mediated metabolism of terfenadine by ketoconazole resulted in cardiac toxicity and, in some cases, death, in patients taking both agents concurrently. Despite the increased awareness of the occurrence of CYP inhibition and the development of an array of *in vivo* and *in vitro* methodologies to investigate potential interactions, the recent withdrawal of mibefradil (Posicor^{TM}) from the market, due to a plethora of unforeseen drug–drug interactions, shows that this can still be a serious problem in drug development.

Conversely, increases in CYP activity can also lead to variability in the disposition of drugs. The presence of multiple copies of a CYP gene can give rise to higher constitutive levels of active enzyme than in individuals possessing only a single copy of the gene. More commonly, certain CYP levels can be induced by exposure to a variety of xenobiotics, typically as drugs, dietary components or cigarette smoke (Table 13.4).

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TABLE 13.4 *Inducers of human liver CYPs*

CYP1A2	CYP2C9	CYP2C19	CYP2E1	CYP3A4
Cruciferous Vegetables	Rifampicin	Rifampicin	Ethanol	Rifampacin
β-Naphthoflavone			Isoniazid	Dexamethasone
Omeprazole				Carbamazepine
Tobacco smoke				Phenobarbital
3-methyl Cholanthrene				Phenytoin
				Troleandomycin
				Troglitazone

The exact mechanisms by which CYP induction occurs are in some cases only partially understood at present. The role of receptors such as the aryl hydrocarbon (Ah), pregnane X (PXR) and constitutive androstane (CAR) receptors has been characterised in the induction of CYP1A, CYP3A and CYP2B, respectively. However, given the importance of the CYP system in so many biochemical processes within the body, it is likely that the regulation of these enzymes is under a complex control system with multiple inputs which are yet to be identified. Induction of CYPs is generally triggered by a degree of prolonged exposure to a given chemical, either through multiple dosing of a drug or the persistence of a longer-lived acute therapy. Induction of a specific CYP isoform may manifest itself clinically through a reduction in the systemic exposure of the inducing agent itself on repeat administration ('autoinduction') and/or that of co-administered drugs and thus result in a significant decrease in the efficacy of drug treatment. For instance, induction of CYP3A4 by the antibiotic, rifampacin, causes a decrease in the systemic exposure of synthetic steroids such as ethynyloestradiol which are metabolised by this isoform, with subsequent loss of contraceptive activity.

13.3 *Monoamine oxidases (MAO)*

MAO enzymes are present in humans as two forms, MAO-A and MAO-B. The enzymes are located in the mitochondria and are present in a range of tissues such as liver, kidney, intestine and brain. MAO-A preferentially oxidises serotonin (5-hydroxytryptamine) whereas MAO-B substrates include arylalkylamines such as phenylethylamine and benzylamine. Substrates of MAO undergo oxidative deamination but, unlike CYPs, the oxygen incorporated into the metabolite is derived from water rather than molecular oxygen. The initial step of the reaction appears to be abstraction of hydrogen from the α-carbon adjacent to the nitrogen atom.

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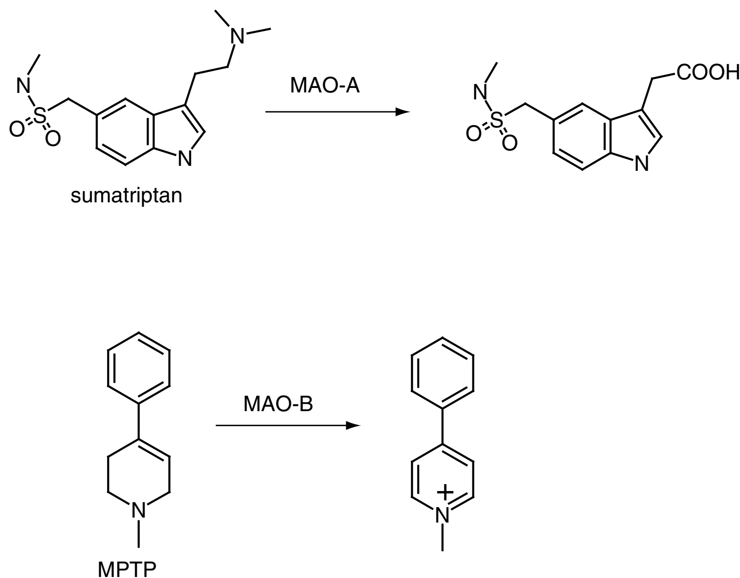

FIGURE 13.4 *The metabolism of sumatriptan and MPTP (1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine) by MAO-A and MAO-B.*

Pharmaceutical agents such as the anti-migraine drugs (5-$HT_{1B/1D}$ receptor agonists) sumatriptan and zolmitriptan are known to be metabolised by MAO-A (Figure 13.4). More recent compounds of this class are substituted at the α-carbon atom, making them resistant to metabolic attack by MAO-A.

An example of an MAO-B substrate is the experimental compound MPTP (1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine) which causes symptoms of Parkinson's disease in primates. The ultimate metabolite MPP^+ is a neurotoxin which selectively degrades cells in the substantia nigra region of the brain which generates the neurotransmitter dopamine.

13.4 *Flavin monooxygenases (FMO)*

The FMO are a family of microsomal enzymes which complement the CYPs in that they typically carry out oxidation by nucleophilic attack at heteroatoms such as N-, S- and P-CYP substrates are metabolised by electrophilic attack, more usually at carbon atoms. However, both CYPs and FMOs have the same cofactor requirements, NADPH and molecular oxygen, although the mechanism of the reaction is very different. For FMO, the reaction involves formation of a peroxide intermediate which then oxygenates the substrate (Figure 13.5). The activation of the enzyme/

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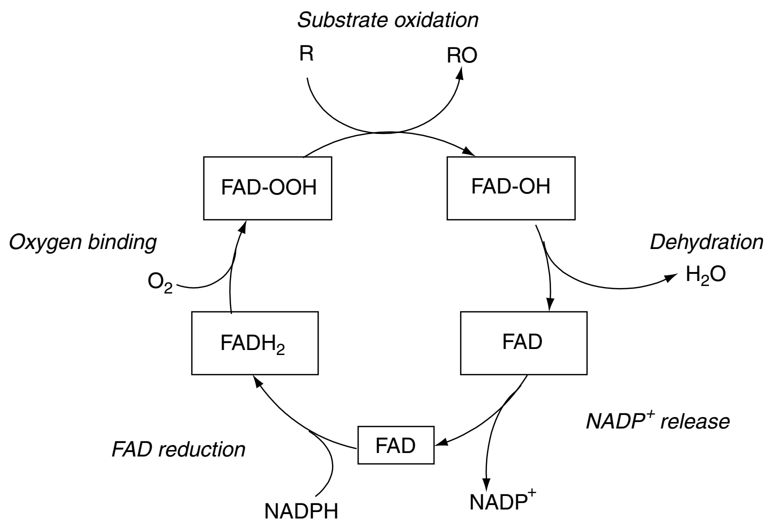

FIGURE 13.5 *Catalytic cycle of FMO.*

cofactor complex prior to interaction with a substrate has led FMOs to be likened to a loaded gun awaiting a target!

As for CYPs, the FMOs are an enzyme superfamily which consists of isoforms FMO1–FMO5. There are also species differences in the occurrence of these isoforms – for example, FMO1 is prevalent in the liver of rat, pig and rabbit but FMO3 is the major human hepatic isoform.

Substrates for FMO3 include nicotine and the H_2-antagonist ranitidine (Figure 13.6).

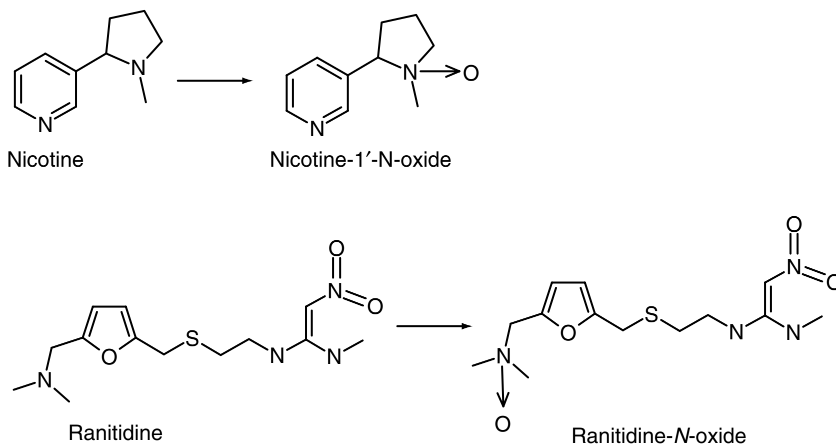

FIGURE 13.6 *Examples of metabolism by FMO.*

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13.5 *Alcohol dehydrogenases (ADH) and aldehyde dehydrogenases (ALDH)*

ADH and ALDH are cytosolic enzymes which convert alcohols to aldehydes (by the action of ADH), and the aldehyde products are subsequently metabolised to carboxylic acids by ALDH enzymes. Both ADHs and ALDHs are found mainly in the liver but are also found in the kidney, lung and gastrointestinal tract and require NAD^+ as cofactor. One form of an atypical ADH is prevalent in Japanese (85 per cent of the population) which results in unusually rapid conversion of ethanol to acetaldehyde. However, the ALDH enzyme responsible for the further oxidation of acetaldehyde to acetic acid is atypical in that about 50 per cent of Japanese are poor metabolisers of acetaldehyde. Thus the unwanted effects of acetaldehyde (flushing, nausea) explain why the Japanese are notoriously intolerant to alcohol.

ADH and ALDH are not widely involved in the metabolism of drugs although the antiviral agent abacavir is an example of a compound which is metabolised by the enzymes.

13.6 *Molybdenum hydroxylases*

13.6.1 Xanthine oxidase (XO)

XO catalyses the sequential oxidation of hypoxanthine to xanthine and uric acid. Monomethylated xanthines can also be oxidised to the corresponding derivatives of uric acid, but dimethylxanthines (theophylline, theobromine) and 1,3,7-trimethylxanthine (caffeine) are oxidised to these types of metabolite by CYP. A classic inhibitor of xanthine oxidase is allopurinol, a drug used to treat gout, a condition due to the over production of uric acid which results in crystal deposits of the poorly soluble acid in the joints.

The prodrug, 6-deoxyaciclovir, is efficiently converted to the anti-viral agent acyclovir by the action of xanthine oxidase (Figure 13.7).

13.6.2 Aldehyde oxidase (AO)

Like XO, AO is a cytosolic molybdenum containing oxidase which functions as a true oxidase, that is the enzyme is first reduced and then re-oxidised by molecular oxygen. However, the oxygen atom incorporated in the metabolite is from water and not molecular oxygen.

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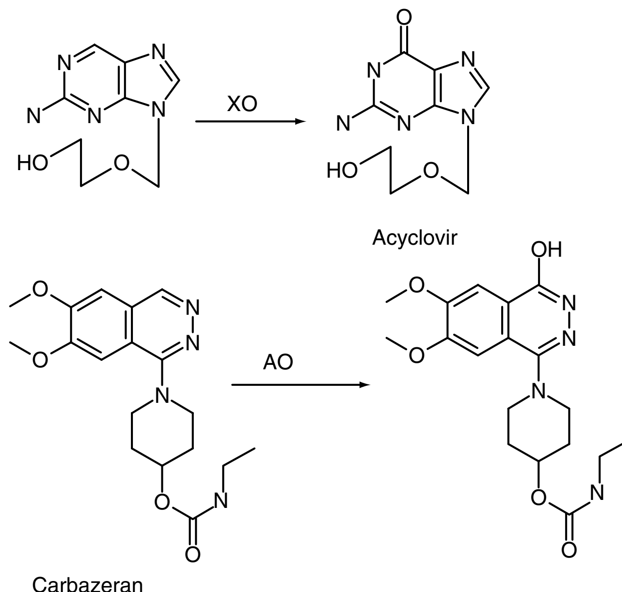

FIGURE 13.7 *Examples of reactions catalysed by xanthine oxidase and aldehyde oxidase.*

The substrates for these enzymes are typically aromatic azaheterocycles such as pyrimidines and purines. Oxidation takes place at a carbon which is electron deficient, typically adjacent to the N heteroatom (which renders the carbon electron deficient). A number of drugs are substrates for aldehyde oxidase including the antiviral prodrug molecule famciclovir which undergoes C-6 oxidation and ester hydrolysis to generate the active antiviral molecule, penciclovir. Phthalazines such as carbazeran are also oxidised by aldehyde oxidase (Figure 13.7).

13.6.3 Epoxide hydrolase

Epoxide hydrolase (EH) catalyses the conversion of epoxides and arene oxides to diols through the addition of water. These potentially toxic electrophilic epoxides are often formed as the result of oxidation of alkenes and aromatic hydrocarbons by CYP. The importance of this role is reflected in the widespread distribution of EH throughout the body in a manner which closely parallels that of the CYPs. Similarly, many known inducers of CYP are also able to induce EH.

Hydrolysis of epoxides by EH is effected by abstraction of a proton from a water molecule to form a nucleophilic hydroxide ion which then attacks one of the carbon atoms of the epoxide. This attack usually occurs at the less-hindered carbon atom, with the subsequent protonation of the alkoxide ion intermediate preferentially occurring on the opposite side of the molecule to give the *trans* configuration. Substrates for EH include the epoxide metabolites of carbamazepine and several

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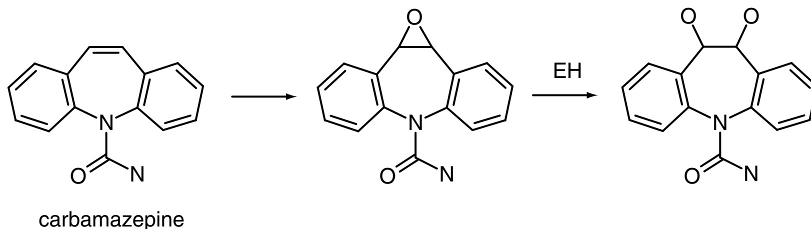

FIGURE 13.8 *Metabolism of carbazepine by epoxide hydrolase.*

polycyclic aromatic hydrocarbons. Metabolism of carbazepine by epoxide hydrolase is illustrated in Figure 13.8.

13.6.4 CARBOXYLESTERASES AND PEPTIDASES

The carboxylesterases comprise a family of enzymes that are able to hydrolyse various drugs and other xenobiotics containing acid, amide or thioester functions. Some carboxylesterases play an important role in the detoxification of organophosphate pesticides (OPs) and it is this activity which is commonly used to classify the enzymes; A-esterases are able to hydrolyse OPs, B-esterases are inhibited by OPs and C-esterases do not interact with OPs. Many A-esterases contain a cysteine residue in the active site whilst the majority of B-esterases contain a serine atom which may explain the difference in behaviour towards OPs of the two groups.

The peptidases are a related group of enzymes found extensively in the blood, liver and other tissues where they are responsible for the hydrolysis of peptides and peptide-mimetics through nucleophilic attack on the carbonyl function of a peptide bond.

13.6.5 CARBONYL REDUCTASES

Carbonyl reductases are enzymes which reduce aldehydes and ketones to primary and secondary alcohols. These enzymes require NADPH as cofactor in contrast to ADH and ALDH. Typical drug substrates are ketones rather than aldehydes since very few therapeutic agents contain an aldehyde function due to its reactive nature. A number of therapeutic agents such as haloperidol, daunorubicin, warfarin and nafimidone undergo metabolism by these enzymes. The smoking cessation drug buproprion is reduced to 494U73 by this class of enzyme (Figure 13.9).

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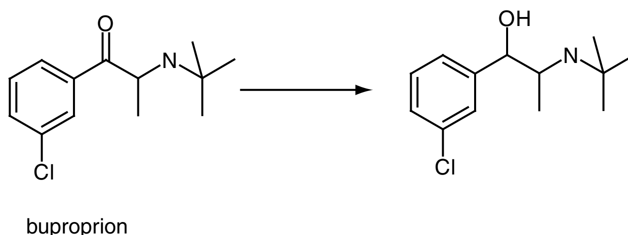

FIGURE 13.9 *Metabolism of bupropion by carbonyl reductases.*

13.7 *Conclusions*

It is clear from even the brief overview presented here that Phase I metabolising enzymes represent a formidable challenge for drug discovery scientists. It has become increasingly clear that the successful development of safe and effective medicines requires a detailed understanding of the way in which drug molecules interact with the drug-metabolising enzymes. This is true not only in terms of achieving optimal pharmacokinetics for a drug candidate itself but also in ensuring that the impact on other co-administered therapies is negligible. To this end, considerable effort continues to be directed towards characterising all aspects of the structure, function and regulation of the major Phase I enzyme systems. Of particular note in this regard is the impact of computational modelling and data analysis methods on improving our ability to rationalise and, ultimately, predict the metabolic disposition of drug molecules in humans. This increase in knowledge of the fundamental principles underlying Phase I metabolism will contribute greatly to a more rational, hollistic approach to drug design.

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