CHAPTER 14

Phase II enzymes

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14.1 *Introduction*

Phase II enzymes are becoming increasingly important in drug discovery and drug development. Although Phase I oxidations are recognised as providing the major rate-limiting processes to metabolic clearance, there are a number of pharmaceutical agents which are primarily cleared metabolically by Phase II enzymes, for example, NSAIDs, tricyclic anti-depressants, β_2-agonists and some anti-HIV drugs (Miners and Mackenzie, 1991). The recognition of this fact is driving the need for more detailed and comprehensive information on the nature of the individual Phase II enzymes and their catalytic properties. This knowledge lags behind that available for CYPs, but it is important to identify specific isoforms within enzyme families that are involved in the Phase II metabolism of new chemical entities (NCEs), particularly with a view to their use in multidrug therapies in patient populations.

There are a variety of Phase II conjugating enzyme systems that attack at functional groups such as –OH, –COOH, $–NH_2$, –SH, which are either present naturally on the target molecules or which have been generated by Phase I oxidative metabolism. Whilst Phase II metabolic reactions usually generate hydrophilic

metabolites which are readily excreted in urine or bile, it should be noted that such metabolism may also give rise to reactive, potentially toxic metabolites which may bind covalently to tissue macromolecules.

When considering the scope of Phase II enzymes, it is also of importance to remember their role in the metabolism of endogenous substrates. Whereas a number of CYPs carry out highly selective, specific reactions (e.g. CYPs involved in cholesterol biosynthesis), the same Phase II enzymes involved in xenobiotic metabolism frequently metabolise a number of endogenous substrates such as bilirubin, steroids and biogenic amines. Hence it is feasible to envisage situations in which xenobiotics and endogenous compounds may compete for the same Phase II metabolic pathway.

This chapter will describe Phase II reactions, nomenclature and the emerging information, which concentrates on the use and nomenclature of Phase II enzyme systems, and focuses on some issues which may be relevant in the development of new medicines such as polymorphisms, induction and drug–drug interactions.

14.2 *Phase II enzyme reactions*

Xenobiotic and endogenous compounds undergo metabolism by Phase I (functionalisation) and Phase II (conjugation) enzymes. For some compounds, Phase I metabolism occurs before a Phase II reaction is possible, and in some cases Phase II products undergo further Phase I metabolism (sulphated steroids). Table 14.1 details the Phase II enzymes. Phase II reactions generally result in the generation of more polar, more easily excretable compounds which are largely devoid of significant pharmacological or toxicological activity.

TABLE 14.1 *Phase II enzymes and their reaction with functional groups*

Enzyme	Reaction	Functional group
UDP-glucuronosyltransferase	Glucuronidation	–OH, –COOH, –NH, –NOH, $–NH_2$, –SH, ring N
UDP-glycosyltransferase	Glycosidation	–OH, –COOH, –SH
Sulphotransferase	Sulphation	–OH, –NH, –NOH, $–NH_2$,
Methyltransferase	Methylation	–OH, $–NH_2$
Acetyltransferase	Acetylation	–OH, $–NH_2$, $–SO_2\,NH_2$
Amino acid conjugation		–COOH
Glutathione-S-transferase	Glutathione conjugation	Epoxide, organic halide
Fatty acid conjugation		–OH

Adapted from Gibson and Skett, 1994.

14.2.1 GLUCURONIDATION

Glucuronidation is a major pathway of Phase II metabolism and involves the transfer of the sugar acid, D-glucuronic acid with the acceptor substrate. Such conjugation reactions are catalysed by a family of enzymes called uridine diphosphate (UDP)-glucuronosyltransferases or UGTs (Tephly and Burchell, 1990). For glucuronidation the cosubstrate UDP-glucuronic acid (UDPGA) is required and this is synthesised from glucose-1-phosphate and UTP in a two-stage reaction (Figure 14.1). The product of the initial reaction is UDP-glucose (UDPG) which is then oxidised by the UDPG dehydrogenase enzyme to produce UDPGA (Dutton, 1966).

The UDP-glucuronosyltransferases are membrane-bound proteins located on the inside of the endoplasmic reticulum (ER) and nuclear membrane of cells. They are found in highest concentration in the liver but are also present in many other tissues including small intestine, kidney, lung and skin. Most UGTs are capable of glucuronidating a wide range of xenobiotics but show a greater specificity for endogenous compounds (e.g. estrogens, androgens, bilirubin) which generally react with one specific form of UDPGT (Tephly and Burchell, 1990). Some examples of the glucuronidation of xenobiotic compounds are shown in Figure 14.2.

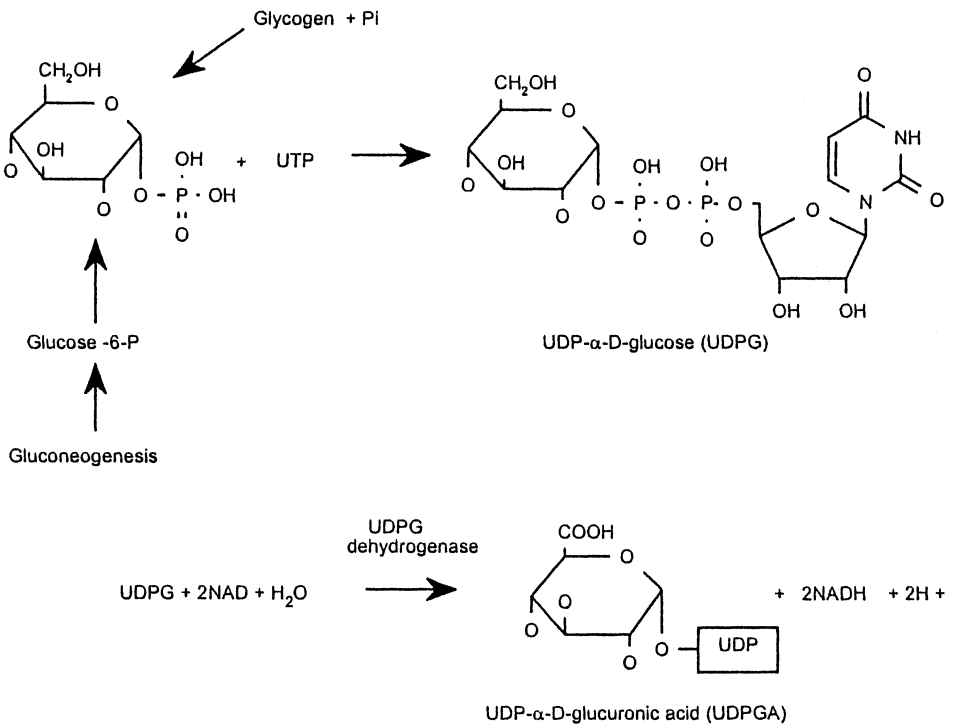


FIGURE 14.1 *Synthesis of UDPGA.*

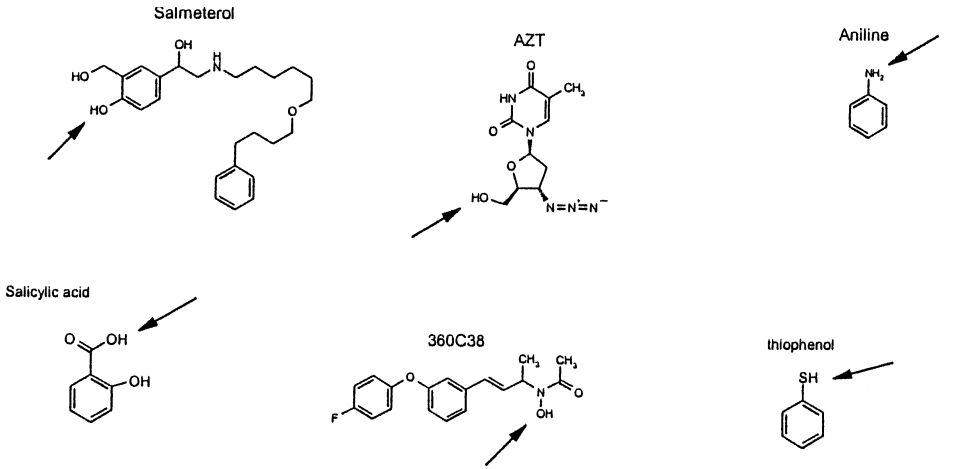


FIGURE 14.2 *Glucuronidation of some xenobiotic compounds.*

There is a wide variation in data obtained from microsomal UGT assays due to latency of the membrane-bound enzyme (Burchell and Coughtrie, 1989). Detergents such as Lubrol PX cause the release of this latent UDPGT activity by disrupting the membrane structure (Vanstapel and Blanckaert, 1988). Following on from this, a model has been proposed in which transport of UDPGA is rate limiting for glucuronidation in intact microsomes, but perturbation of the ER membrane by detergents allows free access of UDPGA and releases the latent UGT enzyme activity. However, substrate access to the enzyme may actually be more rate limiting than UDPGA access (Burchell and Coughtrie, 1989). Studies of the topology of UGTs have revealed a small C-terminal region on the cytoplasmic side of the membrane, linked by a transmembrane-spanning domain to the majority of the protein which is located in the lumen of the ER (Tephly and Burchell, 1990).

14.2.2 SULPHATION

Sulphation is a major pathway of Phase II drug metabolism and is carried out by a family of enzymes called the sulfotransferases (STs). These enzymes play a fundamental role in the metabolism and excretion of both endogenous compounds and xenobiotics. They exhibit a broad range of substrate specificities encompassing endogenous compounds, such as steroid hormones, bile acids, iodothyronines, monoamine neurotransmitters, sugar residues of glycoproteins and glycosaminoglycans, and tyrosine residues in proteins and peptides, as well as detoxifying xenobiotics including drugs, environmental pollutants and food additives (Falany *et al.*, 1993).

Two types of ST enzymes exist in man, which are distinguished by their subcellular localisation and functions. The first type is located in a membrane-bound state in the Golgi apparatus (Falany, 1991). These STs are responsible for the sulphation of endogenous proteins, peptides and glycans, often on tyrosine residues to produce post-translational modifications. The second type of STs is cytosolic enzymes involved in the detoxification and metabolism of endogenous and exogenous compounds (Weinshilboum and Otterness, 1994). Sulphation, like glucuronidation, also occurs in many mammalian tissues with generally greatest activity in the liver and small intestine (Wong and Yeo, 1982; Pacifici *et al.*, 1988). Sulphation of steroids occurs in most tissues, although the highest activity is found in adrenal, kidney, brain, etc. (Falany *et al.*, 1993). The cytosolic ST enzymes will be discussed in detail in this chapter. It is important to note that STs often form a low-capacity–high-affinity partner to the UGTs, which are typically high-capacity–low-affinity enzymes for xenobiotics.

The enzymes catalyse the transfer of a charged sulphonate group from the cofactor PAPS by electrophilic attack at the oxygen and nitrogen atoms of –C–OH, –N–OH and –NH groups. The cofactor PAPS is synthesised from ATP and inorganic sulfate by the sequential action of two enzymes (Figure 14.3), ATP sulphurylase and adenosine 5′-phosphosulphate kinase. Some examples of the sulphation of xenobiotic compounds are shown in Figure 14.4.

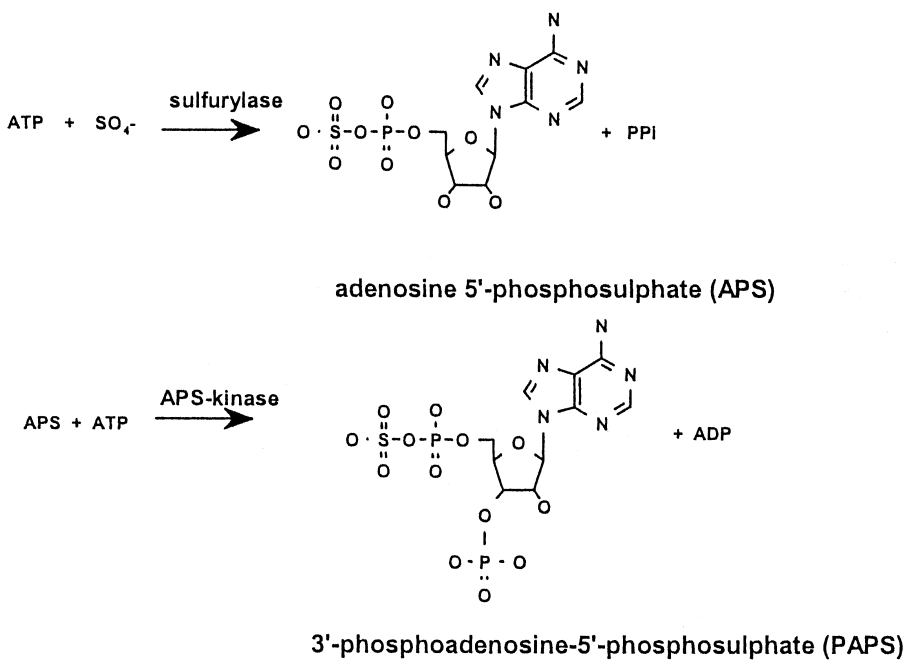


FIGURE 14.3 *Synthesis of PAPS.*

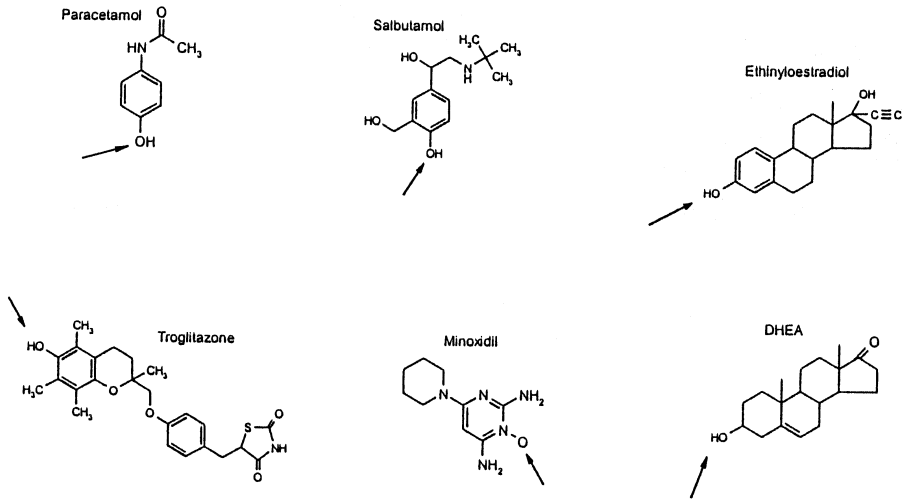


FIGURE 14.4 *Sulphation of some xenobiotic compounds.*

14.2.3 METHYLATION

Methylation is a minor pathway for xenobiotic metabolism although it is an essential step for some endogenous compounds such as catechols, catecholamines, histamine and *N*-acetylserotonin. Methylation differs from other Phase II reactions because it usually results in a decrease in the water solubility of xenobiotics. This is not always the case since N-methylation of pyridine groups, e.g. nicotine can produce quaternary ammonium ions that are very water soluble.

The cofactor for methylation is *S*-adenosylmethionine (SAM). The synthesis of SAM is shown in Figure 14.5. The methyl group bound to the sulphonium ion in SAM is transferred to acceptor substrates by nucleophilic attack from an electron-rich heteroatom (*O*, *N* or *S*).

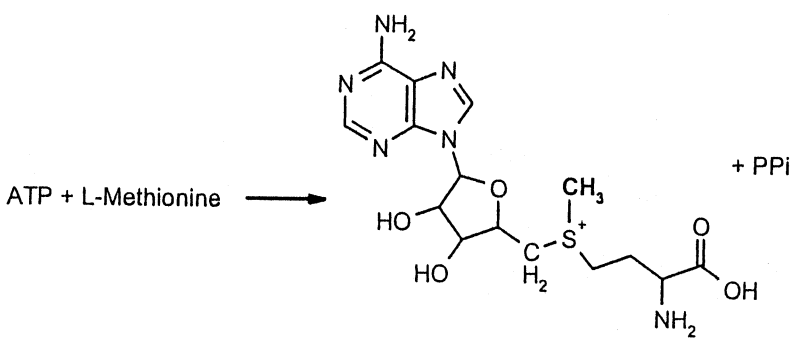


FIGURE 14.5 *Synthesis of SAM.*

Methylation of phenols and catechols is catalysed by two different enzymes known as phenol *O*-methyltransferase (POMT) and catechol *O*-methyltransferase (COMT) (Weinshilboum, 1989, 1992). POMT is a microsomal enzyme that methylates phenols but not catechols, and COMT is a cytosolic enzyme with the converse substrate specificity. COMT has a more important role in the metabolism of catechols than POMT has in the metabolism of phenols. COMT is present in most tissues with the highest concentrations in the liver and kidney. Substrates for COMT include neurotransmitters like adrenaline, noradrenaline and dopamine and catechol drugs like L-dopa, isoprenaline and isoetharine. In humans, COMT is encoded by a single gene with alleles for a low- and high-activity forms (Weinshilboum, 1989, 1992). In Caucasians, these allelic variants are expressed with equal frequency whereas in African Americans the higher activity form is more prevalent.

Two *N*-methyltransferases have been described in humans, one is known as histamine *N*-methyltransferase which methylates the imidazole ring of histamine and related compounds. The other enzyme is known as nicotinamide *N*-methyltransferase which methylates compounds containing a pyridine ring, like nicotine or an indole ring like serotonin. Classification of human *N*-methyltransferases may not be applicable to other species. S-methylation is also an important pathway in the metabolism of some xenobiotics such as captopril, D-penicillamine and 6-mercaptopurine. In humans, S-methylation is catalysed by two enzymes, thiopurine methyltransferase (TPMT) and thiol methyltransferase (TMT). TPMT is a cytoplasmic enzyme with preference for aromatic and heterocyclic compounds like 6-mercaptopurine and azathioprine. TMT is a microsomal enzyme with preference for aliphatic sulph-hydryl compounds like captopril. TPMT is encoded by a single gene with alleles for low- and high-activity forms. The gene frequency of TPMT, low (6 per cent) and high (94 per cent), activity forms produces a trimodal distribution of TPMT activity expressed in 0.3, 11.1 and 88.6 per cent of the population, respectively. Low TPMT activity in cancer patients can lead to toxicity, whereas high TPMT activity may result in poor exposure to the drug. Some examples of the methylation of xenobiotic compounds are shown in Figure 14.6.

14.2.4 Acetylation

Acetylation is a major route of metabolism for xenobiotics containing an aromatic amine ($R–NH_2$) or a hydrazine group ($R–NH–NH_2$), which are converted to

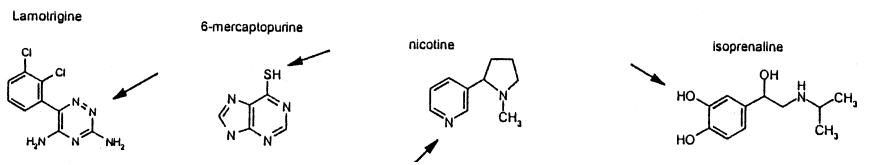


FIGURE 14.6 *Methylation of some xenobiotic compounds.*

aromatic amides (R–NH–$COCH_3$) and hydrazides (R–NH–NH–$COCH_3$), respectively. The acetylation of xenobiotics requires the cofactor acetyl-coenzyme A. The acetyl group of acetyl-CoA is transferred to the enzyme with the release of CoA. The acetyl group is then transferred to the amino group of the substrate.

Xenobiotics containing primary aliphatic amines are not a common substrate for N-acetylation, although cysteine conjugates, which are formed from glutathione conjugates, are converted to mercapturic acids by N-acetylation in the kidney. N-acetylation often causes a reduction in water solubility of the parent molecule via addition of a non-ionisable group.

N-acetylation of xenobiotics is catalysed by the cytoplasmic enzyme *N*-acetyltransferases (NAT) which is present in the liver (Kupffer cells) and other tissues of mammalian species, with the notable exception being the dog. There are only 2–3 enzymes present in any one species and in humans they are called NAT1 and NAT2. NAT1 is expressed in most tissues, whereas NAT2 is expressed in the liver and gut. There are no specific substrates although *p*-aminosalicylic acid, sulphamethoxazole and sulphanilamide are preferentially acetylated by NAT1 while isoniazid, procainamide dapsone and hydralazine are preferentially acetylated by NAT2. The carcinogenic amine, 2-aminofluorine is acetylated equally well by NAT1 and NAT2. Some examples of the acetylation of xenobiotic compounds are shown in Figure 14.7.

14.2.5 Amino acid conjugation

There are two mechanisms for the conjugation of xenobiotics with amino acids. Xenobiotics with a carboxylic acid group can react via the amino group of amino acids such as glycine and taurine. This biotransformation step first requires the activation of the xenobiotic with CoA by acyl-CoA synthase, which produces an acyl-CoA thioester. The second step, catalysed by acyl-CoA: amino acid *N*-acyltransferase, involves the transfer of the acyl group of the xenobiotic to the amino group of the amino acid (Figure 14.8). Substrates for amino acid conjugation are limited to certain aliphatic, aromatic, heteroaromatic, cinnamic and arylacetic acids. Xenobiotics with aromatic hydroxylamine groups can react with a carboxylic acid of amino acids

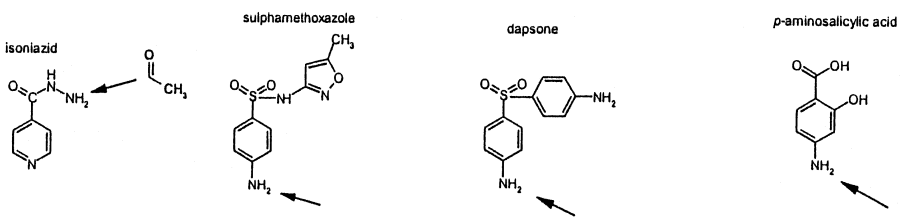


Figure 14.7 *Acetylation of some xenobiotic compounds.*

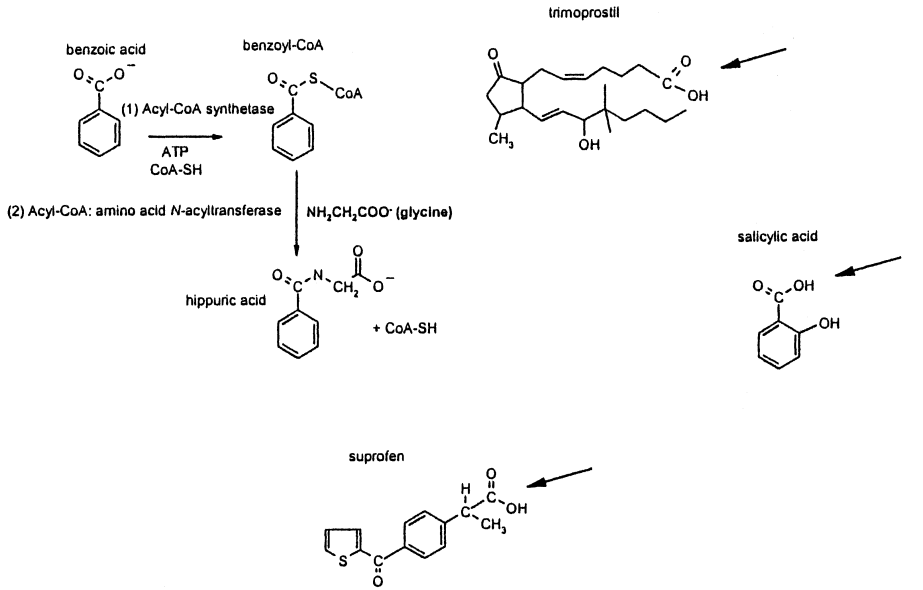


FIGURE 14.8 *Amino acid conjugation of some xenobiotic compounds.*

like proline or serine to produce *N*-esters that can degrade to form reactive electrophilic nitrenium and carbonium ions.

Amino acid conjugation of xenobiotics depends on steric hindrance and substitution on aromatic rings or aliphatic side-chains. In rats, ferrets and monkeys, phenylacetic acid undergoes amino acid conjugation whereas diphenylacetic acid undergoes glucuronidation in these species. Bile acids are endogenous substrates for taurine and glycine conjugation with activation to an acyl-CoA thioester via the microsomal enzyme cholyl-CoA synthetase. Conjugation with glycine and taurine is catalysed by a single cytosolic enzyme, bile acid-CoA:amino acid *N*-acyltransferase (Falany, 1991). In contrast, xenobiotic activation occurs mostly in mitochondria which contain a number of acyl-CoA synthetases. Subsequent xenobiotic conjugation occurs via cytosolic and/or mitochondrial *N*-acyltransferases. Two types of *N*-acyltransferases have been isolated from hepatic mitochondria, one preferring benzoyl-CoA, the other arylacetyl-CoA. The acceptor amino acid is both species and xenobiotic-dependent. For benzoic, heterocyclic and cinnamic acids the acceptor amino acid is usually glycine. Aryl acetic acids are also conjugated with glycine except in primates which use glutamine. In mammals, taurine is also used.

14.2.6 GLUTATHIONE CONJUGATION

Glutathione *S*-transferases (GSTs) are a family of mainly cytosolic enzymes which are present in most tissues, with high concentrations in liver, intestine, kidney

adrenal and lung. Glutathione is a tripeptide which is comprised of glycine, cysteine and glutamic acid – the latter is linked to the cysteine via the γ-carboxyl group rather than the more usual α-carboxyl function (Figure 14.9). Glutathione conjugation occurs with a wide variety of electrophilic compounds or compounds that can be transformed into electrophiles. The mechanism of the reaction is one of nucleophilic attack of glutathione thiolate anion (GS^-) with an electrophilic carbon, oxygen, nitrogen or sulphur atoms in the molecule concerned. Substrates for GSTs have three common features, they are hydrophobic, contain an electrophilic atom and

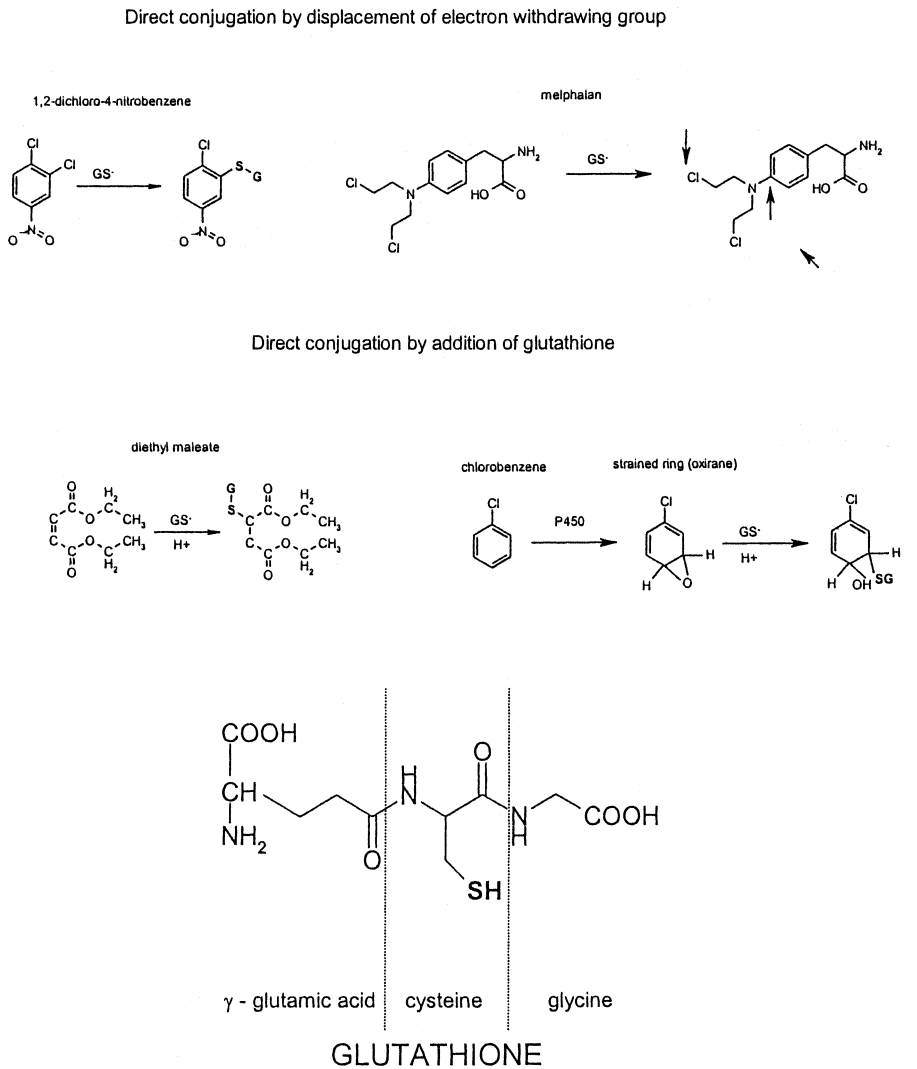


FIGURE 14.9 *Glutathione conjugation with an electrophilic carbon atom.*

react chemically with glutathione to some degree (e.g. paracetamol, nitrosurea). Substrates for GSTs can be classified into two groups, those that are sufficiently electrophilic for direct conjugation and those which undergo biotransformation to an electrophilic metabolite prior to conjugation. The conjugation reactions can be divided into two types, addition and displacement reactions (Figure 14.9).

Glutathione can also conjugate xenobiotics with electrophilic heteroatoms (*O*, *N*, and *S*). An example of this is trinitroglycerine (Figure 14.10). In many cases, the initial conjugate formed between glutathione and the heteroatom is cleaved by a second molecule of glutathione to form oxidised glutathione. The second step is usually non-enzymatic in nature.

Glutathione conjugates can be excreted intact in bile or they can be transformed to mercapturic acids in the kidney and excreted in urine. The process for the generation of mercapturic acids involves the sequential cleavage of the glutamic acid and glycine residues, followed by N-acetylation of the remaining cysteine conjugate. The glutathione conjugate, leukotriene C_4, undergoes metabolism to leukotriene D_4 and then leukotriene E_4 via sequential loss of amino acids. Similarly, the glutathione conjugate of naphthalene undergoes sequential degradation to 1-naphthylmercapturic acid. Also, cysteine conjugates can be degraded by kidney β-lyase which leads to the generation of thiol metabolites which can then undergo further metabolism via methylation or oxidation at the sulphur atom.

14.3 *Nomenclature of phase II enzymes*

The nomenclature systems used for each of the four major Phase II enzyme families are discussed below, accompanied by a table of the major enzymes in humans for each family.

14.3.1 UDP-GLUCURONOSYLTRANSFERASE (UGT) NOMENCLATURE

A nomenclature system, based on evolutionary divergence, has been adopted for *UGT* genes (Burchell *et al.*, 1991; Mackenzie *et al.*, 1997). Each UGT encoding

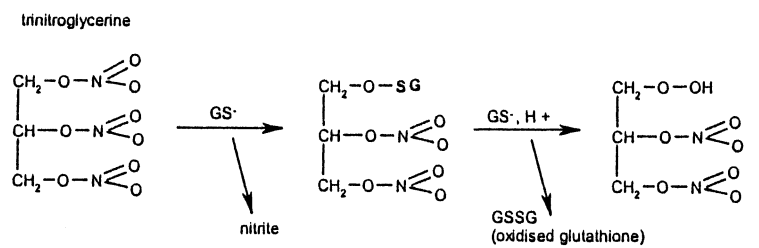


FIGURE 14.10 *Glutathione conjugation of trinitroglycerine.*

gene, *UGT*, is followed by an arabic numeral for the family it belongs to, a letter to denote the subfamily, and finally another arabic numeral for each individual gene (Burchell *et al*., 1991). A UGT protein sequence from one gene family must have at least 45 per cent sequence identity with another member of that family, whilst subfamilies have greater than 60 per cent sequence identity.

In mammals at least 47 cDNAs and genes have been described which belong to three families, namely *UGT1*, *UGT2* and *UGT8*. The *UGT1* family comprises two subfamilies named *UGT1A* and *UGT1B*, whilst the *UGT2* family contains three subfamilies designated *A*, *B* and *C*, respectively. To date no subfamilies have been identified for the *UGT8* family. This is summarised in Table 14.2.

At least 15 human UGTs have been identified. The *UGT1* gene is unusual in that it is a single gene locus which can generate a number of different UGT isozymes. The *UGT1* gene consists of four common exons (exons 2–5) and a variable exon 1. The presence of a unique exon 1 for each UGT1 isozyme (produced by alternative splicing of exon 1) means that UGT1A1, UGT1A3, UGT1A4, etc. are all transcripts of the *UGT1* gene. In contrast, the *UGT2* genes each generate an individual UGT2 isoform (UGT2B7, UGT2B8, etc.).

14.3.2 Sulphotransferase (SULT) nomenclature

As a result of advances in the cDNA cloning of SULT enzymes in recent years, the nomenclature in this field has become increasingly difficult to understand. Indeed at recent meetings, prizes should have been awarded to those actually able to

Table 14.2 *Human UDP-glucuronosyltransferase enzyme nomenclature*

UGT1 family	UGT2 family	UGT8 family
UGT1A1	UGT2B4	UGT8
UGT1A2P	UGT2B7	
UGT1A3	UGT2B10	
UGT1A4	UGT2B15	
UGT1A5	UGT2B17	
UGT1A6		
UGT1A7		
UGT1A8		
UGT1A9		
UGT1A10		
UGT1A11P		
UGT1A12P		

This table includes all UDPGT enzymes for which a human cDNA sequence has been elucidated. Adapted from Mackenzie *et al*. (1997) *Pharmacogenetics* 7, 225–269.

identify the SULTs being discussed in posters! In an attempt to clarify the situation and devise a universal nomenclature system, the SULT nomenclature committee has proposed some guidelines.

The abbreviation SULT will be used to refer to all cytosolic sulfotransferase enzymes and will be in italics when referring to a gene rather than the enzyme protein or mRNA. For enzymes isolated from mice only the first letter will be in capitals, e.g. Sult.

A family of sulfotransferase enzymes will include proteins with 45 per cent or greater amino acid sequence identity, whilst subfamilies will have 60 per cent or greater identity. Families will be assigned arabic numerals, subfamilies capital letters, and individual gene products another arabic numeral e.g. SULT1A1. Orthologues across different species will have the same designation but will be followed by a three-letter species suffix in lower case (Table 14.3).

At least 11 human SULTs have been identified. In addition to the individual isoforms such as SULT1A1, SULT1A2, etc. major variant alleles have also been described which differ by single amino acids e.g. SULT1A1*1 (Arg213) and SULT1A1*2 (His213).

14.3.3 *N*-ACETYLTRANSFERASE (NAT) NOMENCLATURE

N-acetyltransferase enzyme activity was one of the first drug-metabolising activities shown to exhibit polymorphic variation (Meyer, 1993), which results in inter-individual variation such that individuals can be separated into slow or rapid acetylators.

Three *NAT** loci (*NAT1**, *NAT2** and *NAT3**) have been found in vertebrates. Advances in DNA technology have been responsible for a vast expansion in the acetylation field, allowing the identification and characterisation of numerous allelic variants at these *NAT** loci.

A nomenclature system has been adopted (Vatsis *et al*., 1995) based upon nucleotide changes observed in cDNA and genomic clones, or within PCR-generated fragments of *NAT* genes. A nomenclature system based on evolutionary divergence

TABLE 14.3 *Human sulphotransferase enzyme nomenclature*

Phenol STs		Estrogen STs		Hydroxysteroid STs	
New name	**Current name**	**New name**	**Current name**	**New name**	**Current name**
SULT1A1	HTSPST1 or PPST1	SULT1E1	hEST	SULT2A1	hDHEAST
SULT1A2	HTSPST2 or PPST2				
SULT1A3	HTLPST MPST				

This table includes the proposed new nomenclature and the current (old) name for all SULT enzymes for which a human cDNA sequence has been elucidated.

is not possible due to insufficient structural information on *NAT** loci. The *N*-acetyltransferases are assigned the root symbol *NAT*, which is followed by an arabic numeral denoting the gene family and an asterisk which indicates that this is a gene. Individual alleles are then assigned a combination of up to three arabic numerals and latin letters (e.g. NAT2*2, NAT2*5A, NAT1*10, etc.) in chronological order irrespective of the species they were derived from (Table 14.4).

14.3.4 Glutathione-*S*-transferase (GST) nomenclature

Molecular cloning of GSTs in mouse, rat and human has allowed the identification of four classes of enzyme. Within each class, GSTs possess greater than 40 per cent identity whilst those with less than 30 per cent are assigned to a different class (Hayes and Pulford, 1995). The elucidation of gene structures and chromosomal localisation for a wide range of rat and human *GST* genes supports the hypothesis that each class does indeed represent a separate GST family (Rushmore and Pickett, 1993).

The classes of GST enzymes have been designated alpha, mu, pi and theta. The diversity of GST enzymes found in mammals is achieved by the dimeric nature in which subunits come together to form GST enzymes. A novel nomenclature system designates the alpha, mu and pi enzymes as GSTA, GSTM and GSTP, respectively.

Table 14.4 *Human* N-*acetyltransferase alleles and their corresponding proteins*

NAT1* locus		**NAT2* locus**		**NATP locus**	
Allele	**Protein**	**Allele**	**Protein**	**Allele**	**Protein**
*NAT1*3*	NAT1 3	NAT2*4	NAT2 4	*NATP1*	None
*NAT1*4*	NAT1 4	*NAT2*5A*	NAT2 5A		
*NAT1*5*	NAT1 5	*NAT2*5B*	NAT2 5B		
*NAT1*10*	NAT1 10	*NAT2*5C*	NAT2 5C		
*NAT1*11*	NAT1 11	*NAT2*6A*	NAT2 6A		
		*NAT2*6B*	NAT2 6B		
		*NAT2*7A*	NAT2 7A		
		*NAT2*7B*	NAT2 7B		
		*NAT2*12A*	NAT2 12A		
		*NAT2*12B*	NAT2 12B		
		*NAT2*13*	NAT2 13		
		*NAT2*14A*	NAT2 14A		
		*NAT2*14B*	NAT2 14B		
		*NAT2*17*	NAT2 17		
		*NAT2*18*	NAT2 18		

In the case of NATP there is no corresponding protein as this is a psuedogene in humans. Adapted from Vatsis *et al*. (1995).

This is followed by arabic numerals which denote the subunits present in the dimeric enzyme e.g. GSTA1-1. Allelic variants can also be indicated by lower case letters e.g. GSTM1b-1b which is a homodimeric Mu class enzyme composed of the 1b allelic variant (Table 14.5).

14.4 *Phase II enzymes and drug development*

14.4.1 Toxicity of conjugated metabolites

As eluded to in the introduction, the conjugated metabolites produced by Phase II conjugation reactions do not always result in a reduction in activity. Situations in which conjugates, particularly glucuronides and sulphates give rise to reactive, potentially toxic metabolites are described below.

Glucuronides

A number of compounds which contain a carboxylic acid grouping are susceptible to conjugation catalysed by UGTs, leading to the formation of acyl glucuronides (reviewed in Spahn-Langguth and Benet, 1992). A number of these acyl glucuronides have been shown to bind irreversibly to proteins and exert toxic effects. In addition, acyl glucuronides undergo a reaction known as acyl migration where the aglycone can move from the 1′-hydroxyl group to other hydroxyl groups on the glucuronic acid sugar residue. Such changes in the molecule render the conjugate resistant to β-glucuronidase, and the protein-bound adducts formed may pre-dispose individuals to immunologic problems. Typical substrates which are metabolised to acyl glucuronide conjugates include the non-steroidal anti-inflammatory drugs (NSAIDs, such as the profens), and these compounds have been associated with toxic responses.

Table 14.5 *Human glutathione-S-transferase enzyme nomenclature for gene locus and corresponding protein*

Alpha class		**Mu class**		**Pi class**	
Gene locus	**Protein**	**Gene locus**	**Protein**	**Gene locus**	**Protein**
GSTA1	GSTA1-1	*GSTM1*	GSTM1a-1a	*GSTP1*	GSTP1-1
GSTA2	GSTA2-2	*GSTM1*	GSTM1b-1b		
		GSTM2	GSTM2-2		
		GSTM3	GSTM3-3		

Adapted from Mannervik *et al*. (1992) *Biochem. J*. **282**, 305–308.

Sulphates

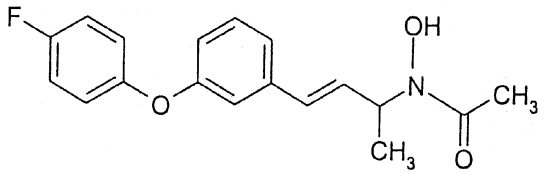
It has been known for a number of years that sulphation is traditionally associated with inactivation and detoxication of xenobiotics (reviewed in Glatt, 1997). However, the sulphate group is electron withdrawing and can act as a good leaving group, thus generating reactive, potentially toxic metabolites. Typical substrates which generate toxic metabolites via this mechanism include heterocyclic aromatic hydroxylamines, formed as a result of N-hydroxylation of the primary amine group. The hydroxyl group introduced into the molecule (typically by CYP1A2) is then available for conjugation with sulphate catalysed by SULT enzymes. Chemical degradation of the sulphate conjugate occurs, generating a reactive, electrophilic species which can bind covalently to nucleophiles such as DNA and protein. Metabolites generated in this way via CYP and SULT pathways have been shown to be potent mutagens *in vitro* and thus provide a mechanism for the genotoxic and carcinogenic properties of heterocyclic amines and polycyclic aromatic hydrocarbons containing benzylic groups.

14.4.2 Stereoselectivity in metabolism by Phase II enzymes

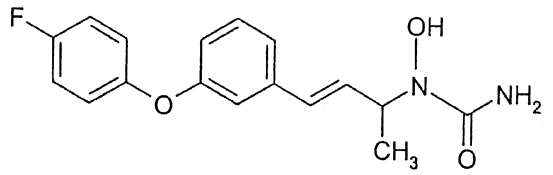
A number of UGT substrates have shown various degrees of selectivity in relation to the glucuronidation of chiral compounds. Glucuronidation of NSAIDs with chiral centres has shown this route of metabolism for several compounds (e.g. naproxen, ketoprofen) to be stereoselective. The enantiomers of a series of reverse hydroxamic acids (e.g. BW360C) (Figure 14.11) which possessed a single chiral centre showed marked differences in their metabolism by human liver microsomal UGTs. In such cases, the *R*-enantiomers were preferentially and extensively metabolised by human UGTs whereas the corresponding *S*-enantiomers were almost resistant to glucuronidation. In contrast, a series of close structural analogues of the reverse hydroxamates (*N*-hydroxyureas with a chiral centre e.g. BW70C) (Figure 14.11) were much less rapidly metabolised by glucuronidation and showed the opposite stereoselectivity with respect to metabolism by UGTs, i.e. the *S*-enantiomers were the preferred substrates.

A number of chiral substituted *N*-hydroxyureas which are 5-lipoxygenase (5-LO) inhibitors have been screened by Abbott as UGT substrates (Bouska *et al*., 1997). For these compounds, glucuronidation was shown to be the major route of metabolism and this markedly limited the duration of action of the compounds *in vivo*. In addition, the first generation 5-LO inhibitor (zileuton) (Figure 14.11) was marketed as the racemic mixture. The *S*-enantiomer was metabolised at a greater rate (3.5x) by human liver UGT than the *R*-enantiomer. *In vivo*, the *S*-isomer was cleared more rapidly in humans indicating that metabolism by UGT was the major factor involved in clearance of zileuton (Sweeny and Nellans, 1995).

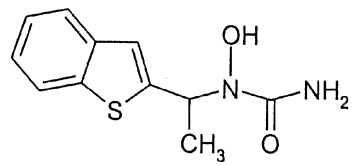
Salbutamol and many other β-2 agonists contain one or more asymmetric carbon atoms. Salbutamol is metabolised in man pre-dominantly by sulphation catalysed



BW360C



BW70C



ZILEUTON

FIGURE 14.11 *The enantiomers of a series of reverse hydroxamic acids.*

by SULT isozymes. The formation of salbutamol sulphate has been shown to be stereoselective in human liver and intestine *in vitro* with a marked preference for the active (−) enantiomer (Walle *et al.*, 1993).

14.4.3 Polymorphisms in Phase II enzymes

A number of Phase II enzymes have been shown to exist in multiple forms which elicit clinically relevant genetic polymorphisms. These include the UGT-glucuronosyltransferase (UGT1A1), *N*-acetyltransferase (NAT2), thiopurine methyltransferase (TPMT) and catechol *O*-methyltransferase (COMT) (reviewed in Parkinson, 1996; Evans and Relling, 1999).

UGT1A1, the enzyme responsible for the conjugation of bilirubin and certain xenobiotics, is subject to inter-individual variation. Thirty-one allelic variants have been identified by cDNA cloning which are implicated in the bilirubin conjugation disorders of Criggler-Najar and Gilberts syndrome. Patients with Criggler-Najar syndrome are deficient in UGT1A1 and hence lack the ability to conjugate bilirubin resulting in unconjugated hyperbilirubinaemia, which causes severe jaundice and often leads to death in infancy. In Gilberts syndrome, the milder form

of the disease, patients have reduced levels of active UGT1A1 and hence reduced metabolic capacity so they experience a milder form of unconjugated hyperbilirubinaemia that often goes undetected for many years.

Slow acetylator phenotypes, poor metabolisers or PMs for the soluble enzyme NAT2, were originally suspected to be under genetic influence because of differences in the numbers of adverse effects in patients receiving the NAT2 substrate isoniazid in Japan and US. Orientals typically have $\leq$10 per cent of PMs whereas about 50 per cent of Caucasians have the PM genotype. In all cases, metabolism (N-acetylation) of amine-containing drugs by NAT2 results in deactivation of the compound and is thus a detoxifying pathway. Thus, PMs for NAT2 are at risk from a number of drug classes which are NAT2 substrates (e.g. dapsone, sulphonamides) which generate potentially toxic hydroxylamines via CYP-mediated metabolism.

TPMT-deficient patients are adversely affected by standard doses of anticancer drugs which contain a thiol group such as 6-mercaptopurine (6-MP) and 6-thioguanine (6-TG). These compounds are widely used in leukaemia therapy and a closely related compound azathioprine (which is metabolised to 6-MP) is an immunosuppressant. Patients with the wild-type TPMT enzyme are able to effectively methylate thiopurines such as 6-MP and 6-TG at the thiol group.

If the TPMT enzyme is missing or deficient, there is a major risk of thiopurine toxicity because of an accumulation of these toxic compounds. In addition the compounds are themselves carcinogenic which could lead to the development of further cancers if the thiopurines are not cleared because of this metabolic defect.

It should be noted that other major Phase II enzymes (SULTs, GSTs) also exhibit polymorphisms but an association with changes in the effects of drugs (as a result of changed metabolic capability of the mutant forms) has yet to be established. GST isoforms are known to be important in the detoxication of reactive species from environmental sources as well as reactive drug metabolites such as epoxides. However, GST polymorphism has yet to be unequivocally associated with changes in drug metabolism. There is evidence for an association of GST isoforms with several disease states (e.g. cancer susceptibility). SULT polymorphisms have been readily demonstrated in human platelets (Weinshilboum *et al.*, 1997). More importantly, large differences in the expression of SULT2A1 (DHEA ST) in human liver and intestine (major sites of sulphation activity *in vivo*) and of SULT1E1 (EST) in human small intestine suggest that polymorphic forms of human SULTs exist.

14.4.4 Drug–drug interactions

UGTs

A number of drugs are known to be cleared by UGT-mediated metabolism as a primary route of clearance (reviewed in Burchell *et al.*, 1995). Of these compounds one of the most extensively studied is the anti-HIV compound AZT (zidovudine).

Since AZT is routinely administered as part of a drug cocktail in AIDS therapy, the potential for drug interaction is large. A number of compounds have been shown to inhibit AZT glucuronidation *in vitro* in human liver microsomes but relatively few appear to have major clinical significance (Rajaonarison *et al.*, 1992). The UGT isoform involved in the metabolism of AZT is UGT2B7 (Barbier *et al.*, 2000).

Lamotrigine (LTG), the antiepileptic drug undergoes a number of drug–drug interactions with other anticonvulsant agents. LTG is metabolised primarily in man by N-glucuronidation, and is thought to be a substrate for UGT1A4. Both carbamazepine and phenytoin, both CYP inducing agents, have been shown to increase the clearance of LTG resulting in a corresponding reduced elimination half-life for LTG. This was considered to be due to induction of the UGT enzyme(s) catalysing the metabolism of LTG. In contrast, valproate, which is also metabolised in part by UGT, causes reduced clearance of LTG *in vivo*, by inhibiting the UGT responsible for LTG metabolism. In both cases, dosage adjustments for LTG are required to adjust the circulating levels of LTG in the presence of the inducing or inhibiting coadministered antiepileptic drugs (reviewed in Anderson, 1998).

A recent study to investigate the metabolism of an anticancer prodrug irinotecan was reported (Iyer *et al.*, 1998). The experimental approach was similar to that used extensively to implicate CYP isoforms in Phase I metabolism. Metabolism of the active (de-esterified) metabolite of irinotecan (SN-38) by a panel of human liver microsomes correlated with bilirubin (a UGT1A1 substrate) conjugation for those liver preparations and expressed human UGT1A1 (but not UGT1A4 or 2B7) catalysed the glucuronidation reaction. In addition, patients with Crigler-Najjar syndrome and Gunn rats (both of which are markedly deficient in UGT1A1) lacked glucuronidating activity towards SN-38. Thus various experimental approaches indicated that SN-38 was conjugated by UGT1A1, the major bilirubin UGT isoform. Patients with Gilbert's syndrome (which have partially impaired bilirubin UGT activity) would hence expect to have reduced levels of UGT1A1 and hence associated deficiency of SN-38 metabolism. Since SN-38 has been implicated with GI toxicity, it is apparent that impairment of its major route of metabolism would have undesirable side effects. Likewise, coadministration of other drugs which are substrates/inhibitors of UGT1A1 may lead to an unwanted drug–drug interaction.

SULTs

Sulphation plays an important role in the modulation of activity of many key endogenous compounds e.g. steroids and neurotransmitters such as dopamine. Thus interference with these functions could markedly affect homeostasis in the body. A number of commonly used drugs, such as clomiphene, ibuprofen chlorpromazine and tamoxifen have been shown to inhibit the sulphation of DHEA and oestrone by their respective SULT enzymes. Dietary chemicals such as vanillin and tartrazine are potent inhibitors of SULT isozymes. Various flavanoids such as quercetin are SULT

inhibitors, and polyphenolic components of red wine are potent and selective inhibitors of human SULT1A1. Such compounds may act as chemical protectants against procarcinogens such as heterocyclic amines which require sulphation for activation (reviewed in Burchell and Coughtrie, 1997).

14.4.5 Induction

UGTs

A number of agents (particularly rifampicin and antiepileptic drugs) are known to induce UGT isozymes as well as CYP isozymes (especially CYP3A4) (Anderson, 1998; Tanaka, 1999). Oral contraceptive agents are also known to induce UGT isozymes. The ability of a compound to induce UGT has been used in a therapeutic situation. In Crigler-Najjar patients (which lack or are severely deficient in UGT1A1 which conjugates bilirubin), phenobarbital has been used to induce UGT1A1 *in vivo* and has been shown to induce this isozyme in human hepatocyte cultures. However, there is also a genetic influence in the degree of induction of UGT1A1 which can be achieved and recent work has shown that weak induction may in part be associated with variation in the UGT1A1 promoter sequence (Ritter *et al*., 1999).

SULTs

SULT isozymes are generally considered to be refractory to induction by xenobiotics which induce CYPs and UGTs. However, Li *et al*. (1999) have recently reported induction of ethinyloestradiol sulphation in human hepatocyte cultures by rifampicin.

14.5 *Summary*

In summary, there is a rapidly growing knowledge base around the role of Phase II enzymes in the metabolism of xenobiotic and endogenous compounds. When new drugs are shown to undergo Phase II metabolism, we should be aware of the potential not only for drug–drug interactions via direct competition or effects on enzyme expression, but also the potential for interaction with endogenous compound metabolism which may affect physiological processes.

Differences in the gene expression of Phase II enzymes in the human population must also be taken into account when we assess human drug response where these enzymes are important for metabolic deactivation. New tools and technologies currently being developed, will allow us to probe further the understanding of the activity and function of these enzymes in the future.

14.6 *References*

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