PHASE II DRUG METABOLIZING ENZYMES

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Background. Phase II biotransformation reactions (also 'conjugation reactions') generally serve as a detoxifying step in drug metabolism. Phase II drug metabolising enzymes are mainly transferases. This review covers the major phase II enzymes: UDP-glucuronosyltransferases, sulfotransferases, N-acetyltransferases, glutathione S-transferases and methyltransferases (mainly thiopurine S-methyl transferase and catechol O-methyl transferase). The focus is on the presence of various forms, on tissue and cellular distribution, on the respective substrates, on genetic polymorphism and finally on the interspecies differences in these enzymes.

Methods and Results. A literature search using the following databases PubMed, Science Direct and EBSCO for the years, 1969–2010.

Conclusions. Phase II drug metabolizing enzymes play an important role in biotransformation of endogenous compounds and xenobiotics to more easily excretable forms as well as in the metabolic inactivation of pharmacologically active compounds. Reduced metabolising capacity of Phase II enzymes can lead to toxic effects of clinically used drugs. Gene polymorphism/ lack of these enzymes may often play a role in several forms of cancer.

INTRODUCTION

It is generally accepted that the biotransformation of substances foreign to the body (xenobiotics) including drugs is divided into phases I and II. Phase I reactions include transformation of a parent compound to more polar metabolite(s) by unmasking or de novo formation of functional groups (e.g. -OH, -NH$_2$, -SH). Reactions include e.g. *N*- and *O*-dealkylation, aliphatic and aromatic hydroxylation, *N*- and *S*-oxidation, and deamination. The main enzymes in this phase are cytochromes P450 (CYPs) performing mainly hydroxylations and hence acting as monooxygenases, dioxygenases and hydrolases. The cytochromes P450 constitute a superfamily of heme enzymes responsible for the metabolism of xenobiotics and endobiotics. They are also involved in a variety of biosynthetic processes[1].

Phase II enzymes play also an important role in the biotransformation of endogenous compounds and xenobiotics to more easily excretable forms as well as in the metabolic inactivation of pharmacologically active substances. The purpose of phase II biotransformation is to perform conjugating reactions. These include glucuronidation, sulfation, methylation, acetylation, glutathione and amino acid conjugation. In general, the respective conjugates are more hydrophilic than the parent compounds.

Phase II drug metabolizing enzymes are mostly transferases and include: UDP-glucuronosyltransferases (UGTs), sulfotransferases (SULTs), N-acetyltransferases (NATs), glutathione S-transferases (GSTs) and various methyltransferases (mainly thiopurine S-methyl transferase (TPMT) and catechol O-methyl transferase (COMT)). The participation of phase II drug metabolizing enzymes in the metabolism of clinically used drugs is shown in Fig. 1.

Phase II enzymes have attracted much less attention in clinical pharmacology than cytochromes P450 because drug interactions involving these enzymes are relatively
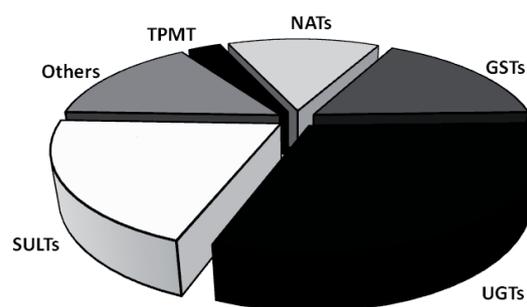

Fig. 1. Participation of major phase II enzymes in the metabolism of clinically used drugs.
UGTs, UDP-glucuronosyltransferases; SULTs, sulfotransferases; NATs, N-acetyltransferases; GSTs, glutathione S-transferases; TPMT, thiopurine S-methyltransferase. (According to Goodman and Gilman's manual of pharmacology and therapeutics (eleventh edition, 2007)).

rare. Nevertheless, the reduced metabolising capacity of the phase II enzymes can lead to the manifestation of the toxic effects of clinical drugs. Although phase II reactions are generally detoxifying, the conjugates formed may also mediate adverse effects (e.g. conjugates acting as carriers for potentially carcinogenic compounds in the activation of benzylic alkohols, polycyclic aromatic hydrocarbons, aromatic hydroxylamines, hydroxamic acid and nitroalkanes by sulphotransferases) (ref.[2]).

There are also individual differences in metabolic response for both Phase I and Phase II enzymes. Further, both external (smoking, medication, nutrition and effects of the environment) and internal (age, sex, diseases and genetics) factors are known to influence phase II enzymes.

UDP-GLUCURONOSYLTRANSFERASES
(UGTs; EC 2.4.1.17)

UDP-glucuronosyltransferases are the key enzymes of the process known as glucuronidation. The formation of glucuronide conjugates is the most important detoxication pathway of the Phase II of drug metabolism in all vertebrates. In humans, approximately 40–70% of all clinical drugs are subjected to glucuronidation reactions metabolized by UGTs[3]. UGT enzymes are responsible for the metabolism of many xenobiotics (e.g. drugs, chemical carcinogens, environmental pollutants and dietary substances) and endobiotics (e.g. bilirubin, steroid hormones, thyroid hormones, bile acids and fat-soluble vitamins) (ref.[4, 5]).

The UGTs are a superfamily of membrane-bound enzymes catalyzing the formation of a chemical bond between a nucleophilic O-, N-, S-, or C-atom with uridine-5´-diphospho-α-D-glucuronic acid (UDPGA). The glucuronic acid is in the α-configuration at the C1 atom when bound to the coenzyme and the transfer occurs with an inversion of configuration. This reaction leads to formation of the respective β-D-glucuronides (Fig. 2) with easy elimination via bile or urine.

All UGT enzymes are capable of forming O-linked glucuronides. These can be formed through conjugation of UDPGA with aliphatic alcohols, phenols, carboxylic acids, thiols and amines (primary, secondary, tertiary) (ref.[6]).

Forms of UGT, tissue and cellular distribution

Currently, the mammalian UGT gene superfamily is known to consist of 117 members. In humans, four UGT families have been identified: UGT1, UGT2 (divided into subfamilies, 2A and 2B), UGT3 and UGT8. First two families, UGT1 and UGT2, use UDPGA to glucuronidate endo- and xenobiotics. This is not valid for the UGT8 and UGT3 family. The UGT8 enzymes has a biosynthetic role in the nervous system and use the UDP-galactose to galactosidate ceramides (which is an important step in the synthesis of glycosfingolipids and cerebrosides). The function of the UGT3 family was unclear for a long time. Recently UGT3A1 was identified as a UDP N-acetylglucosaminyltransferase[7].

The enzymes of each family share at least 40% homology in their DNA sequences and the enzymes of each subfamily share at least 60% homology in their DNA sequences[8]. According to the nomenclature, Arabic numerals represent the family (e.g., UGT1). A letter designates the subfamily (e.g., UGT1A) and the second Arabic numeral denotes the individual gene (e.g., UGT1A1) (ref.[9]).

Recently, twenty-two human UGT proteins were identified: UGT1A1, 1A3, 1A4, 1A5, 1A6, 1A7, 1A8, 1A9, 1A10, 2A1, 2A2, 2A3, 2B4, 2B7, 2B10, 2B11, 2B15, 2B17, 2B28, 3A1, 3A2 and 8A1[7, 10-13]. Many of these forms, but not all, are shown to have broad tissue distribution with liver as a major location. The UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B7 and 2B15 enzymes are considered to be the most important human liver drug metabolising UGT forms. Extrahepatic glucuronidation has also been described. Several UGT forms are expressed mainly in the gastrointestinal tract e.g. UGT1A7, UGT1A8 and UGT1A10[14-16]. Intestinal UGTs are presumed to be of particular importance in the first-pass metabolism of dietary supplements and drugs. They can also influence their oral bioavailability. Kidney[17], brain and pancreas[18], placenta[19] and nasal epithelium[13] also exert glucuronidation activity.

In general, the UGTs are bound to the endoplasmic reticulum and the substrate binding sites are exposed to the lumen[20].

Substrates, inhibitors and inductors of UGTs

Most UGTs have been shown to exhibit overlap in substrate specificities. To date, only a few substrate-selective forms of UGT have been identified. UGT1A1 is
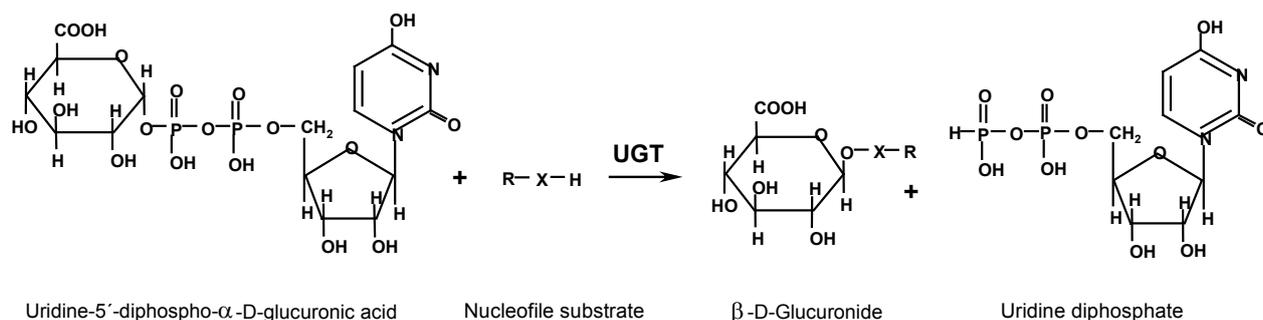

Uridine-5´-diphospho-α-D-glucuronic acid   Nucleofile substrate   β-D-Glucuronide   Uridine diphosphate

Fig. 2. Conjugation of a nucleophile substrate with uridine-5'-diphospho-α-D-glucuronic acid.

the only isoform responsible for bilirubin glucuronidation[21]. UGT1A1 exhibits moderate activity in the conjugation of simple phenols, anthraquinones/flavonones and C_{18} steroids and low activity in the conjugation of complex phenols and coumarins[20]. UGT2B7 is the major enzyme responsible for the glucuronidation of opioids[22]. UGT1A3, UGT1A9, and UGT2A1 are the major enzymes of the conjugation of carboxylic acids, UGT1A4 and UGT1A3 catalyze the N-glucuronidation of amines. UGT1A6 preferentially conjugates complex phenols and primary amines[10, 20, 23]. The selectivity of UGT1A3 toward carboxylic acid-containing compounds (aliphatic or aromatic) has also been described[24]. Chen et al.[25] confirmed the formation of glucuronides of flavonoids by UGT1A9 and 1A3. Lewinsky et al.[26], found that 34 of 42 tested bioflavonoids were glucuronidated by UGT1A10.

Two selective inhibitors of UGT forms have been discovered. Hecogenin (steroidal saponin) is responsible for inhibition of UGT1A4, and flucoconazole inhibits UGT2B7 activity[27, 28]. Bilirubin, the specific substrate of UGT1A1, has been shown to inhibit the enzymatic activity of UGT1A4[29].

Analgetics, nonsteroidal anti-inflammatory drugs (NSAD), antiviral drugs, anticonvulsants and anxiolytics/sedatives have been described as putative inhibitors of drug glucuronidation in humans.

Further, some drugs (analgetics, antivirals and anticonvulsants) may act as putative UDP-glucuronosyltransferase inducers in humans (e.g. rifampin increases codeine oral clearance and glucuronidation of debrisoquine) (ref.[23, 30]).

Genetic polymorphism in UGTs

Studies of UGTs in humans have shown that several diseases are directly related to the pharmacogenetics of these enzymes. Genetic polymorphisms have been identified for the following UGT enzymes: UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2A1, UGT2B4, UGT2B7, UGT2B10, UGT2B15, and UGT2B28[31-34].

Kadakol et al.[35] compiled information on more than 50 mutations of UGT1A1 that cause Crigler-Najjar syndrome type I (including 9 novel mutations) or type II (including 3 novel mutations). These authors also presented a correlation of structure to function for UGT1A1.

The Crigler-Najjar syndrome was first described under the title 'congenital familial nonhemolytic jaundice with kernicterus', in 1952. Crigler-Najjar types I and II are autosomal recessive disorders[36, 37]. Type I patients have complete absence of bilirubin UDP-glucuronosyltransferase (UGT1A1). Type II patients have a partial deficiency of this enzyme are less severely jaundiced and generally survive into adulthood without neurologic or intellectual impairment.

Gilbert syndrome is an autosomal dominant disorder caused by mutation in the *UGT1* gene causing mild hyperbilirubinemia compared to the Crigler-Najjar syndrome. Gilbert syndrome is a benign, mild, unconjugated hyperbilirubinemia that is found in approximately 10% of the population[38].

Interspecies differences in UGT enzymes

In man and some other species, conjugation with glucuronic acid represents by far the most important metabolic pathway. A couple of studies have shown interspecies differences in the glucuronidation process. For example, differences in the formation and stereoselectivity of silybin glucuronides by liver microsomes of man, monkey, pig, dog and rat were described by Matal et al.[39]. Species differences in the glucuronidation of Beviramat, an anti-HIV drug candidate, have been demonstrated in human, rat, mouse, dog and marmoset liver microsomes[24]. Species differences between human, rats, dogs and monkeys in the N-glucuronidation of the muscle relaxant Afloqualone were described by Kaji and Kume[40]. Some studies have demonstrated that cats have remarkably low hepatic levels of UGT1A6. This means that this species exhibits deficient paracetamol[41], acetaminophen[42] and serotonin glucuronidation[43].

SULFOTRANSFERASES (SULTs; EC 2.8.2.1)

Sulfotransferases are a supergene family of enzymes that catalyse the conjugation of 3'-phosphoadenosine 5'-phosphosulphate (PAPS) with an O-, N- or S- acceptor group of an appropriate molecule (Fig. 3). In general, *O*-sulfation represents the dominant cellular sulfonation reaction. Nevertheless, *N*-sulfation is a crucial reaction in the modification of carbohydrate chains in macromolecules such as heparin and heparan sulfate, common components of proteoglycan[44]. *N*-Sulfoconjugation is also involved in the metabolism of xenobiotics such as quinolones and amino drugs[45]. The PAPS is a universal sulfate (or, correctly sulfonate) donor molecule required for all sulfonation reactions and shown that it can be synthesized by all tissues in mammals[46].

Sulfonate conjugation was first described by Baumann in 1876. SULTs are probably the major detoxification enzyme system in the developing human fetus: no UGT transcripts were detected in fetal liver at 20 weeks of gestation[47]. Human fetal liver cytosolic fractions have demonstrated significant sulfotransferase activity towards a large number of substrates (e.g. cortisol, dopamine, paracetamol, testosterone, pregnenolone, estrogen) (ref.[48]).

Sulfonation has a significant role in the biotransformation of a number of endogenous low-molecular compounds (e.g. steroids, catecholamines, serotonin, iodothyronines, eicosanoids, some tyrosine-containing peptides, retinol, 6-hydroxymelatonin, ascorbate and vitamin D) (ref.[49]). Moreover, it is an important pathway in the biotransformation of numerous xenobiotics such as drugs and chemicals[50]. On the other hand, a number of compounds (procarcinogens) are converted by sulfonation into highly reactive intermediates which can act as chemical carcinogens and mutagens by covalently binding to DNA[51].
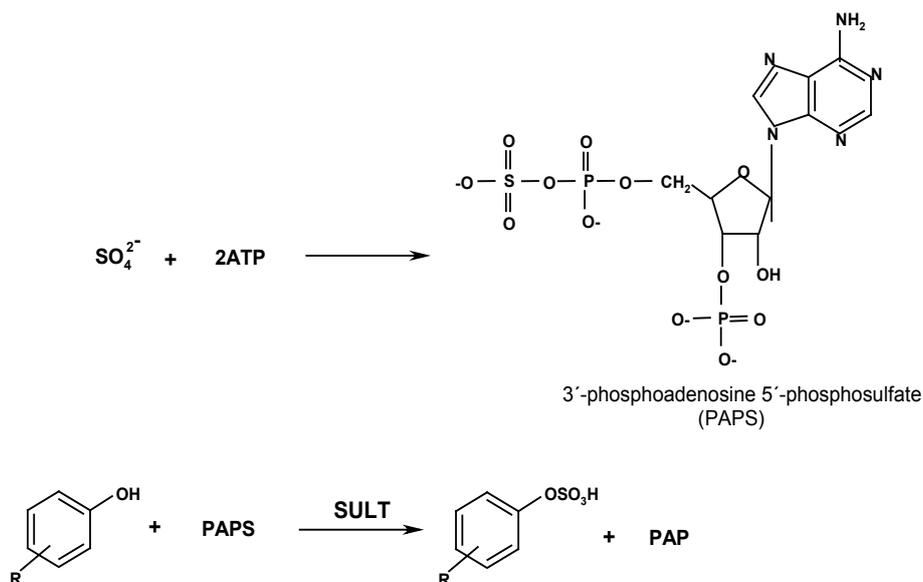
3′-phosphoadenosine 5′-phosphosulfate
(PAPS)

Fig. 3. General reaction catalyzed by SULT.

SULTs: Forms, tissue and cellular distribution

To date, four humans SULT families, SULT1, SULT2, SULT4 and SULT6, have been identified with at least 13 distinct members. The SULT1 family involves 9 members divided into 4 subfamilies (1A1, 1A2, 1A3 and 1A4; 1B1; 1C1, 1C2 and 1C3; 1E1). The SULT2 family can be divided into two subfamilies, SULT2A (SULT2A1) and SULT2B. The SULT2B subfamily is comprised of two isoforms SULT2B1a and SULT2B1b. The SULT4A1 and SULT6B1 are the only members of the SULT4 and SULT6 family respectively[52]. The members of the same SULT gene family share at least 45% amino-acid sequence identity while members of subfamilies share at least >60% identity in amino acid sequence[53].

Two broad classes of sulfotransferases have been identified: namely the cytosolic and membrane-bound ones. Membrane-bound SULTs are localized in the Golgi apparatus and are responsible for the sulfonation of peptides, proteins, lipids and glycosamoniglycans. Cytosolic SULTs catalyze sulfonation of xenobiotics and small endobiotic molecules such as steroids, bile acids and neurotransmitters[50].

SULTs exhibit wide tissue distribution. The members of SULT1A subfamily have been identified in liver, brain, breast, intestine, jejunum, lung, adrenal gland, endometrium, placenta, kidney and blood platelets. SULT1A1 exhibits the highest level of expression of all SULT1 enzymes in the liver. In contrast, SULT1A3 is expressed in most tissues with the exception of adult liver and SULT1B1 in liver, small intestine, colon and leukocytes. Expression of the SULT1C subfamily is found predominantly in the human fetus (fetal kidney, lung, heart and gastrointestinal tract). SULT1E1 is expressed in the human liver and jejunum. Fetal liver, lung and kidney also showed high level of SULT1E1. SULT2A1 shows the highest level of expression in liver, adrenal, duodenum and fetal adrenal

gland. Subfamily SULT2B is localized in human prostate, placenta, adrenal gland, ovary, lung, kidney and colon[49, 50, 52]. Human SULT4A1 has been identified in brain[54, 55] and SULT6B1 in testis and kidney[56].

Substrates, inhibitors and inductors of SULTs

SULT enzymes have different substrate preferences although there is evidence of substrate overlap at the levels of subfamilies and families.

SULT1A1 is a xenobiotic-conjugating enzyme with a broad substrate range. It has also been termed phenol sulfotransferase (P-PST) and thermostable phenol sulfotransferase (TS PST1). This form is responsible for the sulfoconjugation of phenolic compounds such as monocyclic phenols, naphtols, benzylic alkohols, aromatic amines, hydroxylamines, dopamine and iodothyronines[49]. 4-nitrophenol has been widely used to selectively detect SULT1A1 activity[57].

SULT1A2 (TS PST2) appears to be an efficient enzyme for sulfoconjugation of several aromatic hydroxylamines[58], and this reaction may be taken as an example of a toxification reaction, contrary to detoxication reactions occuring in the majority of cases. Charged species (the sulfoconjugates of hydroxylamines) formed in this reaction are chemically reactive and mutagenic. The physiological role of SULT1A2 has not been identified yet. SULT1A2 can sulfoconjugate substrates such as 2-naphtol or 4-nitrophenol[49]. Although, SULT1A2 shares >93% aminoacid identity with SULT1A1 and SULT1A3, this enzyme exhibits no activity toward dopamine as a substrate[50].

SULT1A3 was previously known as thermolabile phenol SULT (TL PST) and monoamine sulfotransferase (M-PST). It displays high affinity for monocyclic phenols. SULT1A3 has a specific role in the sulfonation of catecholamines and as such is responsible for the regula-

tion of the rapidly fluctuating levels of neurotransmitters. Dopamine is often used as a selective substrate for the detection of SULT1A3 activity[59]. Other substrates include norepinephrine, catechols, monocyclic phenols and aromatic molecules[50].

The substrate specifity of SULT1B1 is restricted to thyroid hormones[60] and small phenolic compounds such as 1-naphtol and 4-nitrophenol[61].

SULT1C1 conjugates some iodothyronines[62] but a good substrate for this enzyme has not been identified. SULT1C2 showed activity for substrates as 4-nitrophenol and *N*-hydroxy-2-acetylaminofluoren[63].

SULT1E1 was also called estrogen sulfotransferase (EST). This enzyme has a greater affinity for estrogen sulfation[64] than any other SULTs which conjugate estrogen. SULT1E1 may be important in both the metabolism of estrogens and in the regulation of their activities. This enzyme also shows activity towards iodothyronines, pregnenolon, 1-naphtol, naringenin, genistein and 4-hydroxytamoxifen[50].

SULT2A1 was termed dehydroepiandrosterone-sulfotransferase (DHEA ST). This form is responsible for the sulfoconjugation of hydroxysteroids such as DHEA, androgens, bile acids and oestrone[65].

SULT2A and SULT2B subfamilies metabolize similar substrates but members of the SULT2B subfamily are predominantly cholesterol sulfotransferases[66].

To date no substrates have been identified for SULT4A1 or SULT6B1.

SULT activity may be inhibited in humans exposed to certain therapeutic drugs, dietary or environmental chemicals[67]. The inhibitory effects of various compounds have been examined mainly for the SULT1A subfamily. Vietri et al.[68] described curcumin as a potent inhibitor of SULT1A1 in human liver. De Santi et al.[69] showed inhibition of SULT1A1 by quercetin in human adult and fetal liver. The inhibitory effects of various beverages and catechins in tea were investigated by Nishimuta et al.[70]. Their results showed inhibition of recombinant SULT1A1 and 1A3 by grapefruit juice, orange juice, green tea, black tea and oolong tea. An inhibitory effect of some non-steroidal anti-inflammatory agents on SULT1A1 and SULT1E1 activity was demonstrated by King et al.[71]. Nimesulide, meclofenamate, piroxicam were selective inhibitors of SULT1A1 while sulindac and ibuprofen were more selective for SULT1E1 inhibition.

Maiti et al.[72] found that retinoic acid can increase sulfotransferase expression and activity in cultured human cells. They reported retinoic acid induction of human SULT1A1, 2A1 and 1E1 in hepatic carcinoma cells (HepG2) and in intestinal carcinoma cells (Caco-2).

Methotrexate induced human SULTs in HepG2 and Caco-2 cells[73]. Chen el al.[73] showed that protein and mRNA expression of human SULT1A1, 1A3, 2A1, 1E1 were induced in HepG2 cells; SULT1A3 and 2A1 were induced in Caco-2 cells. Sulfotransferase expression in HepG2 and Caco-2 cell lines was also investigated by Chen et al.[74]. Their data suggested that genistein, a natural isoflavone found in soybean products induced SULT1A1 and SULT2A1 gene and protein expression in these cells.

Genetic polymorphism in SULTs

Genetic polymorphism is known for the major form in adult human liver SULT1A1. Common single nucleotide polymorphism results in an $Arg_{213} \rightarrow His$ amino acid substitution resulting in variation of activity and thermal stability. This mutation was found with a frequency of 25.4 – 36.5% in Caucasians[49]. Individuals who are homozygous for His_{213}/His_{213} have significantly reduced platelet sulfotransferase activity.

Genetic polymorphism is also known for SULT1A2, 1A3, 1C2, 2A1, 2A3, 2B1[52]. Several studies have demonstrated that SULT1A1 polymorphism may play a role in the development of cancers such as lung cancer[75], urothelial carcinoma[76] and meningiomal brain tumors[77].

Interspecies differences in SULTs

There is a dearth of information about interspecies differences in SULT enzymes. In some species, SULT forms were isolated that have no equivalent human form. Tsoi et al.[78] identified a canine SULT1D1. No equivalent human form of this enzyme has been identified. The SULT3 family was found in rabbit[79] and *SULT5A1* was confirmed in mice[80]. On the other hand, SULT1A2 has not been identified in any other species than human[50].

Moreover, Wang et al.[81] described a different dopamine metabolism in man and rat showing that dopamine was entirely sulfoconjugated in human but glucuronidated in rat.

In rodents, SULTs exhibit dramatic sexual dimorphism in SULT expression. The SULT enzyme RNAs from both male and female rats were found in highest concentration in liver. SULT1A1, 1C1 and 1E2 are designated as male-dominant sulfotransferases. On the other hand, members of the SULT2 family are predominant for females[82].

N-ACETYLTRANSFERASES (NATs; EC 2.3.1.5)

Liver arylamine *N*-acetyltransferases (acetyl CoA-dependent N-acetyltransferases, NATs) of adults are involved in the biotransformation of aromatic amines and hydrazines by transfer of the acetyl group from acetylcoenzyme A to the free amino group of parent compound (Fig. 4). NATs catalyze the activation of aromatic and heterocyclic amines (4-aminobiphenyls) via *O*-acetylation while *N*-acetylation of the parent amines is considered a detoxification step[83]. Arylamine *N*-acetyltransferases are present in eukaryotic organisms, including humans, and their existence has been also confirmed in the prokaryote *Salmonella typhimurium*[84, 85].

The role of NAT in endogenous metabolism is unclear.

NAT forms; tissue and cellular distribution

NATs are cytosolic enzymes found in many tissues of various species. In humans, two forms are known, NAT1 and NAT2. Two functional human gene loci, *NAT1* and *NAT2* were identified and characterized for humans and mapped to the short arm of human chromosome 8. The nucleotide sequences of these two genes show 85% homology and code two enzymes of different substrate spe-
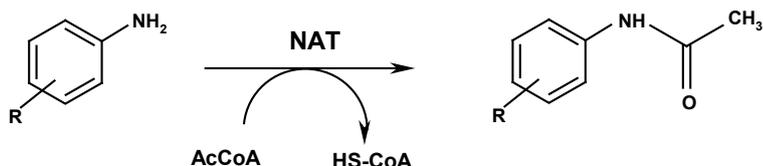
Fig. 4. Acetylation of arylamines by NAT.

cificity. In 2000, 25 human *NAT1* and 27 human *NAT2* alleles were identified[83]. Despite this high level of homology, NATs have distinct tissue distribution and substrate specificity. NAT1 has a ubiquitous tissue distribution and its expression has been demonstrated to be realated to cancers. NAT2 activity has been described in liver, colon and intestinal epithelium.

Substrates, inhibitors and inductors of NATs

Human NAT1 and human NAT2 have different substrate specificities. Typical specific substrates for human NAT1 are: *p*-aminobenzoic acid (PABA), *p*-aminosalicylic acid and *p*-aminobenzylglutamate[86]. Sulfamethazine is used as a NAT2-selective substrate[87]. Human NAT2 provides a major route for detoxification of drugs such as isoniazid (antituberculotic drug), hydralazine (antihypertensive drug) and sulphonamides (antibacterial drugs) (ref.[86]).

The inhibitory effect of polyphenolic compounds on human NATs has been described. Caffeic acid, esculetin, quercetin, kaemferol and genistein inhibited NAT1 whereas scopuletin and coumarin inhibited NAT2[88]. Chen et al.[89] and Lin et al.[90] described the effect of diallyl sulfide (DAS) and diallyl disulfide (DADS), major components of garlic, on NAT activity in human colon tumor cells and human promyelocytic leukemia cells. These studies demonstrated that DAS and DADS markedly inhibited NAT activity in these cells and would thus assist the organism in defense against cancer.

To date, little is known about the induction of NATs. In 2007, Butcher et al.[91] investigated the effects of androgens on the expression of NAT1 in human prostate cancer cells. The results showed that human NAT1 is induced by androgens.

Genetic polymorphism in NATs

Arylamine N-acetyltransferases, NAT1 and NAT2, are the polymorphic enzymes responsible for the ´acetylator phenothype´. Individual differences in the NATs metabolic capacity are caused by allelic variations of the NATs gene which are determined by pattern single nucleotide polymorphisms resulting in slow, intermediate or rapid acetylator phenotypes. Rapid and slow acetylations have been demonstrated to be a predisposing factor for the sensitivity of individuals to toxicity through exposure to a large number of arylamines. The frequency of the specific mutations within the NAT loci depends on racial and ethnic origin. Phenotyping analyses have revealed an association between NAT2 slow acetylation genotype and the risk of developing of several forms of cancer such as lung, colon, liver or bladder cancer[92]. Lammer et al.[93]

found evidence suggesting an interaction between NAT1 polymorphism, lack of maternal multivitamin use and association with birth defects (cleft lip).

Interspecies differences in NATs

NATs are cytosolic enzymes found in many tissues of a number of species. Animal models, such as mice, rats, hamsters or rabbits, have been used to study the relationship between the human NATs polymorphism and toxicity. It has been shown that acetylation of heterocyclic amines is species- and substrate-dependent. The substrate specificity in some species differs from the human one. Substrate *p*-aminobenzoic acid (PABA) is a selective substrate for human NAT1. In rodents it is a substrate for NAT2. There is an 82% identity at the amino acid level between mouse NAT2 and human NAT1[94]. In 2006, Walraven et al.[95] identified and characterised a third rat NAT gene (*NAT3*).

NATs have been found to have high activity in rats. Humans exhibit an intermediate activity of NATs and dogs totally lack this enzyme family[96].

In the rabbit, more than 80% of all acetylating capacity is localized in the liver and gut. Polymorphic genes leading to slow and rapid acetylating phenotypes have also been found in rabbits. Hence, rabbit NAT represents a good animal model for the human acetylation polymorphism[97].

GLUTATHIONE S-TRANSFERASES
(GSTs; 2.5.1.18)

Glutathione S-transferases, one of the major phase II detoxification enzymes are involved in the metabolism of xenobiotics and play an important role in cellular protection against oxidative stress.

The GSTs are a family of enzymes that catalyze the formation of thioether conjugates between the endogenous tripeptide glutathione and xenobiotic compounds (Fig. 5). GSTs can catalyze a large number of reactions including nucleophilic aromatic substitutions, Michael additions, isomerations and reduction of hydroperoxides, conjugation of many hydrophobic and electrophilic compounds with reduced glutathione. GSTs play a major role in the detoxication of epoxides derived from polycyclic aromatic hydrocarbons (PAHs) and alpha-beta unsaturated ketones. Moreover, a number of endogenous compounds such as prostaglandins and steroids are metabolized via glutathione conjugation[98].

The major biological function of glutathione transferases appears to be defense against reactive and toxic
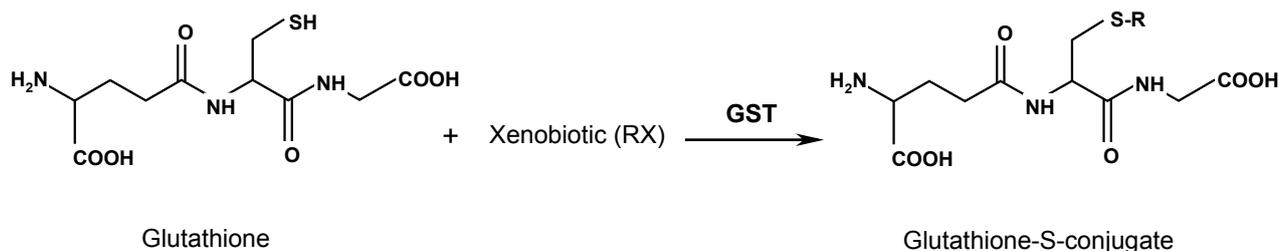
Fig. 5. Formation of glutathione conjugate.

electrophiles such as reactive oxygen species (superoxide radical and hydrogen peroxide) that arise through normal metabolic processes. Many of these are formed by cellular oxidative reactions catalyzed by cytochrome P450 and other oxidases[99].

GST forms; tissue and cellular distribution

Two distinct superfamilies of GSTs have been described. One comprises soluble dimeric enzymes that are involved in biotransformation of toxic xenobiotics and endobiotics. The soluble GST superfamily is subdivided into eight separate classes designated Alpha, Kappa, Mu, Pi, Sigma, Theta, Zeta and Omega. Soluble GSTs have been described mainly in cytoplasm but they are also presented in nucleus, mitochondria[100] and peroxisomes[101]. A number of GST classes were identified first in non-mammalian species and later recognized in mammals too. Human GST enzymes belong to classes Alpha (A1-A4), Mu (M1-M5), Pi (P1), Kappa (K1) and Theta (T1, T2) with their subunit composition or isoenzyme type designated by Arabic numerals. GSTs share more than 60% identity within a class but less than 30% identity with separate classes.

The other superamily of GSTs designated as MAPEG (membrane-associated proteins in eicosanoid and glutathione metabolism), probably with trimeric structure, is involved in arachidonic acid metabolism[99, 102]. Members of both GST families exhibit selenium-independent glutathione peroxidase activity.

Soluble GSTs and MAPEG are widely distributed throughout the body and found in liver, kidney, brain, pancreas, testis, heart, lung, small intestine, sceletal musles, prostate and spleen[103].

GSTs substrates, inhibitors and inductors

Substrates for GSTs are all compounds able to react with the thiol moiety of glutathione. These are electrophilic compounds such as epoxides, α,β-unsaturated ketones, quinones, sulfoxides, esters, peroxides and ozonides. The Alpha (A), Mu (M) and Pi (P) GST detoxify commonly incident harmful α,β-unsaturated carbonyls (e.g. acrolein, 4-hydroxynonenal, adenine, thymine propenals) (ref.[103]). A number of specific substrates of GSTs have been described. Ethacrynic acid has been shown to be a very specific substrate for GST-P1 and *trans*-stilbene oxide is a diagnostic substrate for GST-M1. Relatively small molecules e.g. methylene chloride, ethylene dibromide or isoprene derivates have been shown to be conjugated by GST-T1[98]. The 1-chloro-2,4-dinitrobenzene

(CDNB) has been described as a 'universal' GST substrate. However, theta class enzymes lack activity with this substrate[99].

A large number of inhibitors of GST are known, e.g. synthetic and naturally-occurring phenols, quinones or derivatives of vitamin C. Kulkarni et al.[104] described all-*trans* retinoic acid as an inhibitor of human placental and liver glutathione transferases in the micromolar range. GSTs have been found to be inhibited by glutathione derivatives or substrate analogs. Ploemen et al.[105] describe inhibition of human GSTs by dopamine, α-methyldopa and 5-*S*-glutathionyldopamine.

Extracts of *Ginkgo biloba* have been found to induce GST-P1 and elevated cellular GST activity in human cell lines[106]. Moreover, Williamson et al.[107] demonstrated which foods were inducers of the GST activity in humans. Their results showed that extracts from cruciferous vegetables (e.g. broccoli, Brussels sprouts, cabbage) as well as grapefruit extract act as inducers of human GSTs.

Genetic polymorphism in GSTs

Several types of allelic variations have been identified in the class Alpha, Mu, Pi, Theta GST gene families. Individuals lacking GST-M1, GST-T1 and GST-P1 genes have a higher incidence of bladder, breast, colorectal, head/neck and lung cancer. Loss of these genes have also been found to increase susceptibility to asthma and allergies, atherosclerosis and rheumatoid arthritis[98, 108]. Little is known about polymorphism in MAPEG genes. Iida et al.[109] described single-nucleotide polymorphism of MGST1 (a member of MAPEG) in healthy Japanese volunteers.

Interspecies differences in GSTs

Examination of hepatic cytosolic fractions prepared from mice, rats, Syrian Golden hamsters and humans show that murine liver possesses a significantly greater capacity to conjugate dichlormethane with GSH than livers from other species[110]. Sherratt et al.[111] confimed that mouse GST-T1 had a higher specific activity than the human transferase toward dichlormethane and 1,2-epoxy-3-(4′-nitrophenoxy) propane (1.8- and 16-fold higher, respectively). On the other hand, human GST-T1 had a 4.8-fold higher capacity than mouse isoenzyme to catalyze the reduction of cumene hydroperoxide.

Glutathione S-transferase activity toward 1-chloro-2,4-dinitrobenzene, the 'universal' GST substrate, was investigated by Igarashi et al.[112]. These authors used GSTs of the hepatic cytosol of rats, mice, guinea pigs, rabbits and

hamster. They showed that activity towards 1,2-dichloro-4-nitrobenzene was the highest in hamster, followed by rabbits, guinea pigs, mice and rats.

THIOPURINE S-METHYLTRANSFERASE (TPMT; EC 2.1.1.67)

Thiopurine *S*-methyltransferase is an *S*-adenosyl-L-methionine dependent enzyme that catalyzes *S*-methylation of aromatic heterocyclic sulfhydryl compounds including anticancer and immunosuppressive thiopurines such as 6-mercaptopurine (6-MP), 6-thioguanine (6-TG) and azathioprine (Fig. 6). These drugs are used to treat acute lymphoblastic leukemia, autoimmune disorders, inflammatory bowel disease and organ transplant recipients[113]. Thiopurines 6-MP, 6-TG and azathioprine are prodrugs which need to be activated by the hypoxanthine phosphoribosyltransferase (Fig. 6); metabolic conversion by TPMT leads to the formation of inactive methylated metabolites[114]. Impaired activity of TPMT causes an accumulation of thiopurine nucleotides and manifestation of cytotoxicity leading to the failure of haemopoiesis in most cases.

Tissue and cellular distribution of TPMT

TPMT is a cytosolic enzyme with the highest levels in liver and kidney and relatively low levels in brain and lungs. Levels of TPMT activity in the red blood cells (RBC) correlate highly with levels of the enzyme activity in other human tissues (liver, kidney) and cells (lymphocyte) (ref.[115, 116]). Klemetsdal et al.[117] found that RBC TPMT activity was 8.3% higher in healthy males than healthy females. Erythrocyte activity in newborns is higher (by about 50% grater) than in healthy adults[118].

TPMT substrates and inhibitors

TPMT is an important enzyme in the metabolism of thiopurine substances. No endogenous substrate is known for this enzyme and its biological role remains unidentified. Recently, Oselin and Anier[114] have investigated the inhibitory potential of 15 non-steroidal anti-inflammatory drugs on human TPMT activity in vitro. Naproxen, mefenamic and tolfenamic acid inhibited TPMT activity in a noncompetitive manner. These autors described weak inhibition of TPMT by ketoprofen and ibuprofen. Olsalazine, 5-aminosalicylic acid and sulphasalazine have also been described as noncompetitive inhibitors of TPMT as well[119].

Genetic polymorphism in TPMT

The level of TPMT activity in human tissues is regulated by genetic polymorphism. Allele frequencies for genetic polymorphism are such that ~1 in 300 Caucasians is homozygous for a defective allele or alleles for the trait of very low activity, ~11% of people are heterozygous and have intermediate activity[120]. In 2008, 23 alleles of TPMT were identified and this may be associated with large interindividual variations in thiopurine drug toxicity and therapeutic efficacy[121]. Novel human thiopurine S-methyltransferase (TPMT) variant allele was identified in a Thai renal transplantation recipient with reduced erythrocyte TPMT activity[122]. TPMT activity is inherited as an autosomal co-dominant trait[115]. Humans with genetically determined low or intermediate TPMT activity have a higher risk for side-effects when treated with standard doses of thiopurines. On the other hand, wild-type individuals with high TPMT activity have a lower risk of toxicity but optimal concentration of drugs in blood cannot be achieved. In this instance, there is an increased risk of leukemia relapse[123].

Interspecies differences in TPMT

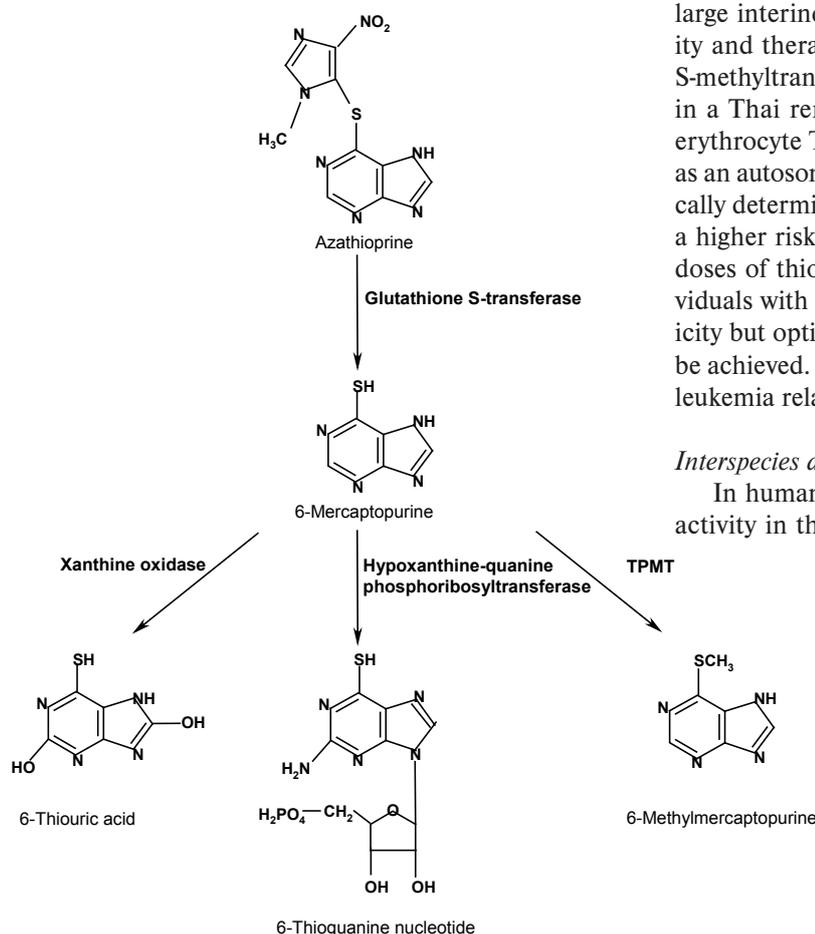In humans and the other species, the level of TPMT activity in the liver and other tissues correlates with its

Fig. 6. Thiopurine metabolism.

activity in peripheral red blood isolates. White et al.[124] demonstrated significant differences in red blood cell TPMT activity values of three species (dog, cat, horse). Values from canine samples were significantly higher than those from cats or horses. These relatively low levels of activity may help to explain the sensitivity of cats to thiopurine therapy.

A difference in TPMT activity between human and pig has also been described. The levels of TPMT activity in human have been found to be twise as high as the TPMT activity in pig samples[125].

CATECHOL O-METHYL TRANSFERASE (COMT; EC 2.1.1.6)

Catechol O-methyltransferase is responsible for transfer of a methyl group from S-adenosylmethionine to catecholamines. This O-methylation results in one of the major degradative pathways of the catecholamine transmitters (Fig. 7). COMT is an enzyme that plays a key role in the modulation of catechol-dependent functions such as cognition, cardiovascular function and pain processing.

COMT substrates include not only neurotransmitters such as norepinephrine, epinephrine and dopamine but also drugs having a catechol structure used in the treatment of hypertension, asthma and Parkinson's disease[126]. COMT was first discribed by Axelrod in 1957.

COMT forms; tissue and cellular distribution

COMT is an intracellular enzyme located in the postsynaptic neuron. COMT is presented in mammalian cells in two forms: in a cytoplasmic soluble form (S-COMT) and a membrane-bound form (MB-COMT) located in the cytosolic side of the rough endoplasmic reticulum[127].

Fig. 7. Degradative pathway of dopamine.

Primary structures of the two COMT forms are otherwise identical but differences between S-COMT and MB-COMT reside within the N-termini. The MB-COMT has an N-termini extension of about 50 amino acids. S-COMT is expressed at higher levels in most tissues than MB-COMT. The highest COMT activities have been found in liver, kidney, intestine, and brain[128]. S-COMT is predominatly expressed in peripheral tissues, while MB-COMT is mostly expressed in the brain. In the blood, COMT is found mainly in erythrocytes; in leukocytes its activity is low.

COMT substrates and inhibitors

Catechol-O-methyl transferase is involved in the inactivation of catecholamine transmitters such as norepinephrine, epinephrine and dopamine and also catecholestrogens and catechol drugs.

Several COMT inhibitors have been described such as entcapone, and tolcapone. COMT inhibitors have also been found in green tea e.g. flavonoid quercetin[129]. COMT inhibitors, entacapone and tolcapone, protect L-dopa from the action of COMT and thus prolong the action of this compound. Hence, they are a widely-used adjunct drugs in L-dopa therapy. When COMT inhibitors are given to patients together with an inhibitor of dopa decarboxylase (carbidopa or benserazide), L-dopa is optimally protected from degradation. This "triple therapy" is used in the treatment of Parkinson's disease[130].

Genetic polymorphism in COMT

A functional single nucleotide polymorphism of the gene for catechol-O-methyl transferase (VAL 108/158 MET) has been identified. The level of COMT enzyme activity (low, intermediate and high levels) is genetically polymorphic in human red blood cells and liver. This polymorphism is due to a G-to-A transition at codon 158 (for MB-COMT) or codon 108 (for S-COMT) of the *COMT* gene and results in the substitution of the amino acid valine for methionine causing a decrease in the activity level of the COMT enzyme 3 to 4 fold[131].

Functional polymorphism in the *COMT* gene (VAL 108/158 MET) has been examined in relationship to a number of neurological disorders involving the noradren-
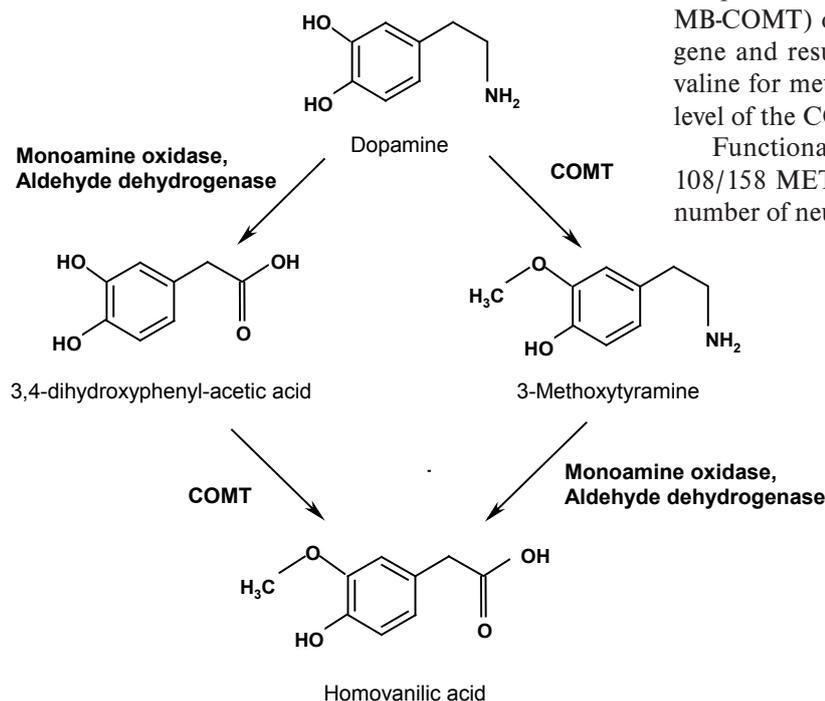
ergic or dopaminergic systems, such as schizophrenia[132-134] and Parkinson's disease[135, 136].

It has been suggested that a common functional genetic polymorphism in the *COMT* gene may contribute to the etiology of alcoholism. The results of Tiihonen et al.[137]; Kauhanen et al.[138] indicate that COMT polymorphism contributes significantly to the development of late-onset (≥25 years) alcoholism. An association of COMT low activity with early-onset (<25 years) alcoholism has been demonstrated as well[139].

Interspecies differences in COMT

COMT influences dopamine concentration in the prefrontal cortex (PFC). Dopaminergic neurotransmission in the PFC contributes to individual cognitive differences in animals and humans.

The human COMT amino acid sequence was compared with those of the chimpanzee, gorilla, orangutan, bonobo, dog, cat, rat, mouse and pig. Alanine at codon position 52/102 was found in all these species except the pig. Pig had the variant human amino acid (threonine) at this position. Methionine, the amino acid encoded by the variant human allele at codon 108/158 was not confirmed among tested species at that position[126].

The amino acid at the Val/Met locus is important for COMT activity. It has been hypothesised that COMT activity has decreased over the course of evolution[140].

CONCLUSION

Products of Phase II reactions are conjugates that are rapidly excreted in bile and urine. They are generated by the transfer of a specific moiety from coenzyme to endogenous or exogenous substrates. The enzymes are generally known as transferases. Phase II reactions involve glucuronidation, sulfation, methylation, acetylation, glutathione and amino acid conjugation. UDP-glucuronosyltransferases, sulfotransferases, N-acetyltransferases, glutathione S-transferases and various methyltransferases (thiopurine S-methyl transferase and catechol O-methyl transferase) are considered the major phase II enzymes. Phase II drug metabolizing enzymes play an important role in the biotransformation of endogenous compounds and xenobiotics to more easily excretable forms as well as in the metabolic inactivation of pharmacologically active compounds. Although phase II reactions are generally detoxifying, the conjugates formed may also mediate adverse effects. A number of compounds (procarcinogens) are converted by Phase II enzymes into highly reactive intermediates which can act as chemical carcinogens and mutagens by covalent binding to DNA. The reduced capacity of the Phase II enzymes to metabolise drugs may lead to the appearance of toxic effects of clinically used drugs. Moreover, several studies have provided evidence that gene polymorphism or lack of the genes for these enzymes may play a role in the development of several forms of cancer. Phase II biotransformation is hence an important aspect of human toxicology.

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