[REVIEW ARTICLE]

INHERITED VARIATIONS IN DRUGS EFFECT INDEPENDENT IN PHARMACOKINETIC: POLYMORPHISM IN PHASE II BIOTRANSFORMATION ENZYMES

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Abstract

Phase II 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 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. Polymorphism in Phase II drug metabolism enzymes may have role in diseases, cancer, and others. This review discuss brief about polymorphism in Phase II drug metabolism enzyme. ***[JuKe Unila 2014; 4(8):254-268]***

Keywords: *phase II biotransformation enzyme, drug effect, pharmacokinetic*

Introduction

There are four discrete processes in the pharmacokinetic phase during the biological disposition of a drug (or other xenobiotic), namely its absorption, distribution, metabolism and excretion – the ADME concept.[1]

Drugs are introduced into the body by several routes. They may be taken by mouth (orally); given by injection into a vein (intravenously), into a muscle (intramuscularly), into the space around the spinal cord (intrathecally), or beneath the skin (subcutaneously); placed under the tongue (sublingually); inserted in the rectum (rectally) or vagina (vaginally); instilled in the eye (by the ocular route); sprayed into the nose and absorbed through the nasal membranes (nasally); breathed into the lungs, usually through the mouth (by inhalation); applied to the skin (cutaneously) for a local (topical) or bodywide (systemic) effect; or delivered through the skin by a patch (transdermally) for a systemic effect. Each route has specific purposes, advantages, and disadvantages.[1]

After the drug is absorbed, it is then distributed to various organs of the body. Distribution is influenced by how well each organ is supplied by blood, organ size, binding of the drug to various components of blood and tissues, and permeability of tissue membranes. The more fat-soluble a drug is, the higher its ability to cross the cell membrane. The blood-brain-barrier restricts passage of drugs from the blood into the central nervous system and cerebrospinal fluid. Protein binding (attachment of the drug to blood proteins) is an important factor influencing drug distribution. Many drugs are bound to blood proteins such as serum albumin (the main blood protein) and are not available as active drugs.[1]

By the early part of the 20th centurythe major drug metabolizing reactions had been identified. A unifying

theory on the role of metabolism was developed by John Paxson Sherwin.[2]

Xenobiotic metabolizing enzymes have historically been grouped into the phase 1 reactions, in which enzymes carry out oxidation, reduction, or hydrolytic reactions, and the phase 2 reactions, in which enzymes form a conjugate of the substrate (the phase 1 product).[3]

Phase II biotransformation reactions that a drug or its metabolite typically undergo. In these so-called 'conjugation reactions', mediated by the appropriate enzymes, the drug becomes linked to an endogenous moiety through one or more functional groups, that may either be present on the parent drug, or which may have resulted from a phase I reaction of oxidation, reduction or hydrolysis. A characteristic of most conjugation reactions is the replacement of a hydrogen atom present in a hydroxyl, amino or carboxyl group, by the conjugating agent. In general, the resulting conjugated metabolites have no pharmacological activity, are highly water-soluble and therefore subsequently readily excreted in the urine. These reactions are usually considered as detoxication reactions, but in certain cases, toxication has been recorded, and examples of both are treated below. Major phase II reactions include glucuronidation, sulphation, acetylation, and conjugation with glutathione or amino acids.[1]

Parent drugs or their phase I metabolites that contain suitable chemical groups often undergo coupling or conjugation reactions with an endogenous substance to yield drug conjugates (Table 1). In general, conjugates are polar molecules that are readily excreted and often inactive. Conjugate formation involves high-energy intermediates and specific transfer enzymes. Such enzymes (transferases) may be located in microsomes or in the cytosol. Of these, uridine 5'-dphosphate[UDP]-glucuronosyl transferases [UGTs] are the most dominant enzymes. These microsomal enzymes catalyze the coupling of an activated endogenous substance (such as the UDP derivative

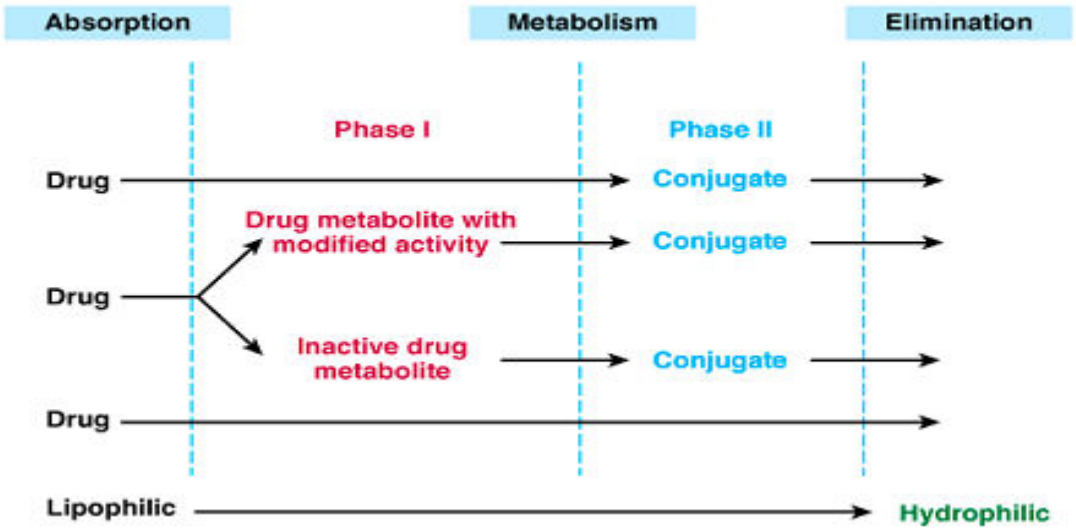

Figure 1. Phase I and II reaction
(Source: Katzung BG, Masters SB, and Trevor AJ; Basic and Clinical Pharmacology 11th ed., 2005)

of glucuronic acid) with a drug (or endogenous compound such as bilirubin, the end product of heme metabolism). Nineteen UGT genes (UGTA1and UGT2) encode UGT proteins involved in the metabolism of drugs and xenobiotics. Similarly, 11 human sulfo transferases [SULTs] catalyze the sulfation of substrates using 3'-phosphoadenosine 5'-phosphosulfate [PAPS] as the endogenous sulfate donor. Cytosolic and microsomal glutathione [GSH] transferases [GSTs] are also engaged in the metabolism of drugs and xenobiotics, and in that of leukotrienes and prostaglandins, respectively. Chemicals containing an aromatic amine or a hydrazine moiety (eg, isoniazid) are substrates of cytosolic N-acetyl transferases [NATs], encoded by NAT1 and NAT2 genes, which utilize acetyl CoA as the endogenous cofactor.[4]

S-adenosyl-L-methionine[SAMe; AdoMet]-mediated O-, N-, and S-methylation of drugs and xenobiotics bymethyltransferases [MTs] also occurs. Finally, endobiotic, drug, and xenobiotic epoxides generated via P450-catalyzed oxidations can also be hydrolyzed by microsomal or cytosolic epoxide hydrolases [EHs].

Conjugation of an activated drug such as the S-CoA derivative of benzoic acid, with an endogenous substrate, such as glycine, also occurs. Because the endogenous substrates originate in the diet, nutrition plays a critical role in the regulation of drug conjugations process.[4] Phase II reactions are relatively faster than P450-catalyzed reactions, thus effectively accelerating drug biotransformation process.

Drug conjugations were once believed to represent terminal inactivation events and as such have been viewed as "true detoxification" reactions. However, this concept must be modified, because it is now known that certain conjugation reactions (acyl glucuronidation of nonsteroidal anti-inflammatory drugs, O-sulfation of N-hydroxyacetylaminofluorene, and N-acetylation of isoniazid) may lead to the formation of reactive species responsible for the toxicity of the drugs. Furthermore, sulfation is known to activate the orally active pro drug minoxidil into a very efficacious vasodilator, and morphine-6-glucuronide is more potent than morphine itself.[4]

The term genetic polymorphism defines monogenic traits that exist in the normal population in at least two phenotypes, neither of which is rare. Genetic polymorphisms in drug metabolism are common occurrences. Mutations in the genes for numerous drug-metabolizing enzymes cause enzyme variants with higher or lower activity or lead to the partial or total absence of an enzyme protein. Genetic polymorphisms have been described for a wide variety of drug and xenobiotic metabolizing enzymes. Many of these variations were first identified by the occurrence of adverse reactions after normal doses of drugs inpatients or volunteers. The association between decreased drug clearance and decreased activity of a drug-metabolizing enzyme, the inherited nature of the deficiency, and its frequency and clinical importance was then evaluated. Phenotyping methods involving administration of probe drugs and measurement of metabolites in body fluids or determination of enzyme activity in families and populations were used for this purpose.

During the past 10 years, several of these polymorphisms have been studied at the protein and gene level, and simple DNA tests have been developed to predict the phenotype.[5]

Table 1. Phase II reaction*

Type of Conjugation	Endogenous Reactant	Transferase (Location)	Types of Substrates	Examples
Glucuronidation	UDP glucuronic acid	UDP glucuronosyl transferase (microsomes)	Phenols, alcohols, carboxylic acids, hydroxlamines, sulfonamides	Nitrophenol, morphine, acetamoniphen, diazepam, N-hydroxy dapsone, sulfathiazole, meprobamate, digitoxin, digoxin
Acetylation	Acetyl-CoA	N-Acetyl transferase (cytosol)	Amines	Sulfonamides, isoniazid, clonazepam, dapsone, mescaline
Glutathione conjugation	Glutathione (GSH)	GSH-S-transferase (cytosol, microsomes)	Epoxides, arene oxides, nitro groups, hydroxylamines	Acetaminophen, ethacrynicacid, bromobenzene
Glycine conjugation	Glycine	Acyl-CoA glycine transferase (mitochondria)	Acyl-CoA derivatives of carboxylic acids	Salycylic acid, benzoic acid, nicotinic acid, cinnamic acid, cholic acid, deoxycholic acid
Sulfation	Phosphoadenosylphosphosulfate	Sulfo transferase (cytosol)	Phenols, alcohols, aromatic amines	Estrone, aniline, phenol, 3-hydroxy-coumarin, acetaminophen, methyldopa
Methylation	S-adenosylmethionine	Transmethylases (cytosol)	Cathecolamine, phenols, amines	Dopamine, ephinephrine, pyridine, histamine, thiouracil
Water conjugation	Water	Epoxide hydrolase (microsomes)	Arene oxides, cis-disubstituted and mono-substituted oxiranes	Benzopyrene, 7,8-epoxide, styrene 1,2-oxyde, carbamazepine epoxide
		(cytosol)	Alkene oxides, fatty acid epoxides	Leukotriene A_4

Data source from Katzung BG *et al.*[4]

Discussion

All enzymes involved in the metabolism of drugs are regulated by genes and gene products. Because of evolutionary and environmental factors, there is a remarkable degree of genetic variability built into the population. Thus, the genetic factor represents an important source of inter individual variation in drug metabolism. Mutations in the gene for a drug-metabolizing enzyme result in enzyme variants with higher, lower, or no activity or may lead to a total absence of the enzyme. Therefore, it is not unusual to find a ten-fold or as much as a 50-fold difference in the rate of drug metabolism among patients. With the technological breakthroughs in molecular biology, significant progress has been made in understanding the role of genetic polymorphisms in drug metabolism process.[6]

A. Glucuronidation

Glucuronidation represents one of the most important phase II biotransformation reactions converting thousands of lipophilic endobiotics and xenobiotics (drugs, dietary plant constituents, etc.) and their phase I metabolites into hydrophilic and excretable conjugates. For many compounds such as plant constituents which already contain functional groups (-OH, -COOH, -SH, -NH_2) glucuronidation represents the primary biotransformation reaction. Glucuronidation is catalysed by a supergene family of UDP-glucuronosyl transferases (UGTs) which are integral proteins of the membranes of the endoplasmic reticulum and the nuclear envelope. UGTs are present in many tissues of vertebrates (mammals, fish and- although no sequences have been published-in amphibian, reptiles and birs).[7]

Conjugation reactions catalyzed by the super family of these enzymes serve as the most important detoxification pathway for broad spectrum of drugs, dietary chemicals, carcinogens and their oxidized metabolites, and other various environmental chemicals in all vertebrates. Furthermore, UGTs are involved in the regulation of several active endogenous compounds such as bile acids or hydroxysteroids due to their inactivation via glucuronidation.[8,9]

In humans, almost 40–70% of clinically used drugs are subjected toglucuronidation.[10] The mammalian UGT gene super family comprises of 117 members. Four UGT families have been identified in humans: UGT1, UGT2 involving UGT2A and UGT2B subfamily, UGT3 and UGT8. Enzymes included in the UGT1 and UGT2 subfamily are responsible for the glucuronidation of exo– and endogenous compounds, whereas members of the UGT3 and UGT8 subfamilies have their distinct functions.[11]

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. 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. Several UGT forms are expressed mainly in the gastrointestinal tract e.g. UGT1A7, UGT1A8 and UGT1A10. 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, brain, and placenta also exert glucuronidation activity.[8,11-17]

In steatosis mouse models, UGT induction was accompanied by increased aryl hydrocarbon receptor, constitutive androstane receptor (CAR), peroxisome proliferator-activated receptor (PPAR)-γ, pregnane X receptor, nuclear factor (erythroid-derived 2)-like2 (Nrf2), and peroxisome proliferator-activated receptor-γ coactivator-1αmRNA expression. In addition, fasting increased CAR, PPAR, and Nrf2 binding activity. The work points to hepatic triglyceride concentrations corresponding with nuclear receptor and UGT expression.[18]

Some polymorphisms in glucuronidation had been known. Sex steroids are metabolized to less active compounds by glucuronidation, catalyzed by UDP-glucuronosyl transferases (UGT) and sulfation, catalyzed by sulfotransferases (SULT). Women with the UGT1A1(TA7/TA7) genotype had 25% lower mean estradiol (E2) concentrations compared to the wild type (TA6/TA6).[19]

The mutations in UGT1A1 gene result in several syndromes connected with decreased bilirubin detoxification capacity of UGT1A1. More than 50 mutations of UGT1A1 causing Crigler–Najjar syndrome type I and type II.[20] Patients suffering from type I, also called congenital familial nonhemolytic jaundice with kernicterus, completely lack the UGT1A1 enzymatic activity resulting in toxic effects of bilirubin on the central nervous system. The most common deficiency of UGT1A1 enzyme is Gilbert's syndrome. 2–12% of the population suffer from this benign disorder characterized by intermittent unconjugated hyper-bilirubinemia. In most cases, this syndrome is caused by a mutation in the promotor region of UGT1A1 gene.[21] Increased toxicity of a pharmacologically active metabolite of irinotecan (SN–38) has been described in patients suffering from Gilbert's syndrome as UGT1A1 is the main enzyme responsible for the formation of the inactive SN–38 glucuronide.[22] The genetic variability in the UGT1 or UGT2 gene families was also suggested to alter risk of cancer either as a result of decreased inactivation of hormones such as estrogens or due to reduced detoxification of environmental carcinogens and their reactive metabolites.[23]

B. Sulfation

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. In general, O-sulfation represents the dominant cellular sulfonation reaction. N-sulfation is a crucial reaction in the modification of carbohydrate chains in macromolecules such as heparin and heparan sulfate, common components of proteoglycan.[24] N-Sulfoconjugation is also involved in the metabolism of xenobiotics such as quinolones and amino drugs.[25] The PAPS is a universal sulfate donor molecule required for all sulfonation reactions and it can be synthesized by all tissues in mammals.[26]

Table 2. Examples of Xenobiotics and Endogenous Compounds That Undergo Sulfate Conjugation

Functional Group	Example
Primary alcohol	Chloramphenicol, ethanol, hydroxymethyl polycyclic aromatic hydrocarbons, polyethylene glycols
Secondary alcohol	Bile acids, 2-butanol, cholesterol, dehydroepiandrosterone, doxaminol
Phenol	Acetaminophen, estrone, ethinylestradiol, naphthol, pentachlorophenol, phenol, picenadol, salicylamide, trimetrexate
Catechol	Dopamine, ellagic acid, -methyl-DOPA
N-oxide	Minoxidil
Aliphatic amine	2-Amino-3,8-dimethylimidazo[4,5,-f]-quinoxaline (MeIQx)* 2-Amino-3-methylinidazo-[4,5-f]-quinoline (IQ)* 2-Cyanoethyl-*N*-hydroxythioacetamide, despramine
Aromatic amine	2-Aminonaphthalene, aniline
Aromatic hydroxylamine	*N*-hydroxy-2-aminonaphthalene
Aromatic hydroxyamide	*N*-hydroxy-2acetylaminofluorene

*Amino acid pyrolysis products

To date, two large groups of SULTs have been identified. The first group includes membrane–bound enzymes with no demonstrated xenobiotic–metabolising activity. These enzymes are localized in the Golgi apparatus and they are involved in metabolism of endogenous peptides, proteins, glycosaminoglycans, and lipids.[24]

Second group, cytosolic SULTs are presumed to play a crucial role in the detoxification processes occurring in the developing human fetus since no UGTs transcripts have been detected in fetal liver at 20 weeks of gestation.[27] Sulfonation is generally described as a detoxification pathway for many xenobiotics. Furthermore, the role of sulfotransferases in the activation of various procarcinogens and promutagens was confirmed.[28]

We focus only in cytosolic sulfotransferases.The super family of cytosolic sulfotransferases is subsequently divided into families and subfamilies according to the amino acid sequence identity among individual SULTs. In detail, members of one SULTs family share at least 45% amino acid sequence identity, whereas SULTs subfamily involves individual members with at least 60% identity. There are four human SULT families, SULT1, SULT2, SULT4 and SULT6, have been identified. These SULT families include at least 13 different members. The SULT1 family comprises of 9 members divided into 4 subfamilies (1A1, 1A2, 1A3, 1A4, 1B1, 1C1, 1C2, 1C3 and 1E1). SULT2A (SULT2A1) and SULT2B (SULT2B1a and SULT2B1b) belong to SULT2 family. The SULT4A1 and SULT6B1 are the only members of the SULT4 and SULT6 family, respectively.[29] Cytosolic sulfo-transferases exert relatively broad tissue distribution.

Genetic polymorphism was detected in many SULT forms such as the SULT1A1, SULT1A3, SULT1C2, SULT2A1, SULT2A3 and SULT2B1 enzyme.[29] Single nucleotide polymorphism in the SULT1A1 gene leading to an Arg213 → His amino acid substitution is relatively frequent in the Caucasian population (25.4–36.5%).[30] This mutation results in a variation of SULT1A1 thermal stability and enzymatic activity. SULT1A1 polymorphism might play role in the pathophysiology of lung cancer, urothelial carcinoma, and meningiomal brain tumors.[31-33]

C. Glutathion

The glutathione S-transferasesare capable of catalyzing a seemingly protean spectrum of reactions; they are widely distributed and are present at high concentrations in the cytosol. The overall hypothesis for what follows is based on the ability of these proteins to bind hydrophobic compounds and, specifically, to increase the nucleophilicity of the sulfhydryl group of glutathione (GSH). These two attributes of the glutathione transferases are sufficient to account for the extensive list of titles that have been bestowed on them. In current usage are the following terms: glutathione S-alkyltransferase, glutathione S-aryl-transferase, glutathione S-aralkyltransferase, glutathione S-alkenetransferase, glutathione S-epoxide transferase, nitrate ester reductase, steroid isomerase, A'-3-ketosteroid isomerase, and ligandin. With the exception of the steroid-designated enzymes, all these activities should now be identified solely as glutathione S-transferase. (34)

Glutathione exists in the cell as oxidized (GSSG) or reduced (GSH),and the ratio of GSH: GSSG is critical in maintaining acellular environment in the reduced state. In addition to affecting xenobiotic conjugation with GSH, a severereduction in GSH content can predispose cells to oxidative damage, a state that has been linked to a number ofhuman health issues.[35]

Two distinct super families of GSTs have been described. One comprises soluble dimeric enzymes that areinvolved in biotransformation of toxic xenobiotics and endobiotics.The soluble GST super family 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 and peroxisomes.[36,37] 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.[38,39] Members of both GST families exhibit selenium-independent glutathione peroxidase activity.

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.[40,41] Little is known about polymorphism in membrane-associated proteins in eicosanoid and glutathione metabolism genes. Iida et al. described single-nucleotide polymorphism of MGST1 (a member of MAPEG) in healthy Japanese volunteers.[42]

D. Acetylation

By now, you will hopefully be familiar with the central idea that biotransformation increases water solubility at the expense of lipid solubility. Acetylation is generally classified as a Phase II process, although it appears to be rather contradictory as acetylated metabolites are less water-soluble than the parent drug; indeed, this often makes acetylated metabolites difficult to eliminate in urine. In extreme , acetylated metabolites of some old sulphonamides were so poorly

water-soluble that they crystallized (painfully) in the patient's kidneys. You may be wondering what acetyltransferases are actually for, since they do not at fi rst appear to contribute positively to the clearance of their substrates. There are a number of acetyltransferase gene families found in most cells and they have a large number of functions connected with cell homeostasis; for example, histone acetyl transferases (HATs) regulate DNA transcription through activating histone proteins by acetylating them, whilst some hormones such as melatonin are regulated through acetyltransferases. Although most metabolic reactions are to some extent reversible, acetylation is much more reversible than most. Indeed, there are many of these enzymes that are predominantly deacetylases, as well as acetylases. Many acetylated molecules can act as 'on' and'off ' switches in the regulation of the functions of nuclear receptor systems. The acetyl transferases relevant to human drug metabolism include two families of genes which are expressed, N-acetyltransferase 1 (NAT-1) and N-acetyltransferase 2 (NAT-2). These enzymes use acetyl Co enzyme A as a cofactor to acetylate their substrates by using whatis known as a double displacement (ping-pong) mechanism. There are two sequential steps to the reaction: firstly, the acetyl group is moved from acetyl CoA to form an acetylated enzyme intermediate, then the substrate is acetylated and CoA is released. Iodacetate andN-ethylmaleide are irreversible inhibitors of the process, whilst reversible inhibitors (salicylamide) are similar in structure to the substrates. NAT-1 is found in many tissues, particularly in the colon, but also in erythrocytes. NAT-1 expression was thought to be fairly constant through human populations, but recent studies have shown that there is at least a two-fold difference in some populations and it is believed that NAT-1 may be inducible in response to certain xenobiotic and endogenous substrates.[43]

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. Sulfame-thazine is used as a NAT2-selective substrate.Human NAT2 provides a major route for detoxification of drugs such as isoniazid (antituberculoticdrug), hydralazine (antihypertensive drug) and sulphonamides (antibacterial drugs).[44,45]

Variation among the human population in the ability to acetylate drugs has been known sincethe observations on individual variations in isoniazid toxicity in the 1950s. The genetic basis forthis was soon appreciated and has come to be known as the N-acetylation polymorphism. Acetylationwas variable in the human population, that it is inherited, and the molecular basis for this phenomenon. Since accurate determination of acetylator phenotype is critical to testing a number ofimportant hypotheses, including the link between disease risk and acetylator phenotype, limitations and potential problems with various methods of phenotype determination are discussed.[46]

N–acetylation polymorphism represents one of the oldest and most intensively studied pharmacogenetic traits and refers to hereditary differences concerning the acetylation

of drugs and toxicants. The genetic polymorphism in NAT activity was first recognised in tuberculosis patients treated with isoniazid, which is metabolised principally by N–acetylation. The polymorphism causes individual differences in the rate of metabolism of this drug. Individuals with a faster rate are called rapid acetylators and individuals with a slower rate are called slow acetylators. Rapid acetylators were competent in isoniazid acetylation but the drug was cleared less efficiently in the slow acetylator group, which resulted in elevated serum concentration and led to adverse neurologic side effects due to an accumulation of unmetabolized drug.[47] Consistent with the toxicity of isoniazid in slow acetylators, there is an increased incidence of other drug toxicities in subjects carrying defective NAT2 alleles, such as lupus erythematosus in patients treated with hydralazine or procainamide, and haemolytic anemia and inflammatory bowel disease after treatment with sulfasalazine[48,49]. The high frequency of the NAT2 and also NAT1 acetylation polymorphism in human population together with ubiquitous exposure to aromatic and heterocyclic amines suggest that NAT1 and NAT2 acetylator genotypes are important modifiers of human cancer susceptibility. Many studies suggested a relationship between acetylation phenotypes (in particular, arising from NAT2 genotypes) and the risk of various cancer including colorectal, liver, breast, prostate, head and neck, and other disease conditions such as birth defects or neurodegenerative and autoimmune diseases.[50-52]

E. Methylation

Methylation reactions are mainly involved with endogenous compound metabolism but some drugs may be methylated by non-specific methyltransferases foundin lung, and by the physiological methyltransferases. A list of the methyltransferases and their substrates is given in Table 3.

The cofactor, S-adenosyl methionine (SAM), is required to form methylconjugates and is produced from L-methionine and ATP under the influence ofthe enzyme, L-methionine adenosyltransferase. SAM can also be considered as a high-energy intermediate. The non-specific N-methyltransferase found in the lung can reverse the N-demethylation reactions of phase 1 metabolism but most ofthe other methyltransferases are specific for endogenous compounds except the S-methyltransferase that is foundin the microsomal fraction and which will methylate many thiols such as thiouracil.

In general, unlike other conjugation reactions, methylation leads to a less polar product and thus hinders excretion of the drug.[53] The methyl transferases represent a relatively large number of enzymes that utilize the cofactor, S-adenosyl-L-methionine, in which the methyl group is bound to a positively charged sulfur, to transfer a methyl group to an oxygen, sulfur, or nitrogen atom in anappropriate substrates.[54]

E.1. S–methylation

Thiol methylation is important in the metabolism of many sulfhydryl drugs. Human tissues contain two separate genetically regulated enzymes that can catalyze thiol S–methylation. Thiolmethyltransferase (TMT) is a

membrane–bound enzyme that preferentially catalyzes the S–methylation of aliphatic sulfhydryl compounds such as captopril and D–penicillamine, whereas thiopurine S–methyltransferase (TPMT) is a cytoplasmic enzyme that preferentially catalyzes the S–methylation of aromatic and heterocyclic sulfhydryl compounds including anti cancer and immunosuppressive thiopurines such as 6–mercaptopurine, 6–thioguanine, and azathioprine. Thiopurine drugs have a relatively narrow therapeutic index and are capable of causing life–threatening toxicity, most often myelosuppression.[55] TPMT genetic polymorphism represents a striking example of the clinical importance of pharmacogenetics.

In 2010, 29 different variant TPMT alleles have been described and this may be associated with large interindividual variations in thiopurine drug toxicity and therapeutic efficacy.[56] 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.

Subjects homozygous for low TPMT activity have a high risk of myelosuppression after treatment with standard dose of azathioprine. Generally, TPMT–deficient patients (homozygous mutant) can be treated with 6–10% of the standard dose of thiopurines.[57] TPMT shows the highest level of expression in liver and kidney and the physiological role of this enzyme, despite extensive investigation, remains unclear.

E.2. CathecolO-Methyl Transferase (COMT)

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. 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

Tabel 3. The methyltransferases

Enzyme	Substrate	Site
Phenylethanolamine *N*-methyltransferase	Noradrenaline	Adrenals
Non-specific *N*-methyltransferase	Various (desmethylimipramine)	Lung
Imidazole *N*-methyltransferase	Histamine	Liver
Catechol *O*-methyltransferase	Catechols	Liver Kidney Skin Nerve tissue
Hydroxyindole *O*-methyltransferase	*N*-Acetylserotonin	Pineal gland
S-Methyltransferase	Thiols	Liver Kidney Lung

Source: Gibson GG *et al.*[53]

and dopamine but also drugs having a catechol structure used in the treatment of hypertension, asthma and Parkinson's disease.[58] COMT was first described by Axelrod in 1957.

COMT is an intracellular enzyme located in the post-synaptic 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.[59]

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. S-COMT is predominantly 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.

A functional single nucleotide polymorphism of the gene for catechol-O-methyl transferase (VAL 108/158 MET) has been identified. The level of COMT enzymeactivity (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.

Functional polymorphism in the COMT gene (VAL 108/158 MET) has been examined in relationship to a number of neurological disorders involving the noradrenergic or dopaminergic systems, such as schizophrenia, and Parkinson's disease. It has been suggested that a common functional genetic polymorphism in the COMT gene may contribute to the etiology of alcoholism. The 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.

Conclusion

Phase II enzymes play an important role in the detoxification of various xenobiotics. They significantly contribute to maintaining of homeostasis by binding, transport or inactivation of biologically active compounds such as hormones, bile acids, or other mediators. In contrast to their beneficial effects, these enzymes also participate in formation of reactive intermediates of various compounds. The most–discussed example of toxification reactions is the conjugation of N–hydroxy aromaticamines. These compounds undergo activation to toxic metabolites by numerous reactions, including N–glucuronidation by UGTs, O–acetylation by NATs, O–sulfonation by SULTs, and conjugation with amino acids by aminoacyl–tRNA–synthetase. The newly formed reactive electrophilic nitrenium and carbonium ions can act as carcinogens and mutagens due to covalent binding to DNA or to other biomolecules. Genetic polymorphisms of Phase II enzymes is another

noteworthy issue. Impaired metabolism of drugs due to genetically based dysfunction of competent enzymes may lead to manifestation of toxic effects of clinically used drugs. Moreover, it is evident that genetic polymorphisms in these enzymes are responsible for the developement of a number of neurological disorders or cancers. In conclusion, Phase II enzymes are an interesting research field since they play an essential role in the metabolism of hundreds of foreign compounds as well as in regulation of metabolism and disposition of various endogenous biologically active substances and thus maintaining homeostasis in the human body.

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