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Detoxification Pathways in the Liver

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Summary: The liver plays an important rôle in protecting the organism from potentially toxic chemical insults through its capacity to convert lipophiles into more water-soluble metabolites which can be efficiently eliminated from the body via the urine. This protective ability of the liver stems from the expression of a wide variety of xenobiotic biotransforming enzymes whose common underlying feature is their ability to catalyse the oxidation, reduction and hydrolysis (Phase I) and/or conjugation (Phase II) of functional groups on drug and chemical molecules. The broad substrate specificity, isoenzyme multiplicity and inducibility of many of these enzyme systems make them particularly well adapted to handling the vast array of different chemical structures in the environment to which we are exposed daily. However, some chemicals may also be converted to more toxic metabolites by certain of these enzymes, implying that variations in the latter may be important predisposing factors for toxicity. Pharmacogenetic defects of xenobiotic biotransformation enzymes, a subclass of inborn errors of metabolism which are manifested only upon drug challenge, introduce marked variation into human populations for the pharmacokinetics and pharmacodynamics of therapeutic and toxic agents, and thus may have important clinical consequences for drug efficacy and toxicity.

One of the most vital roles of the liver is in defence. Every day the human organism may be exposed, either intentionally or otherwise, to hundreds of exogenous chemical substances in food and water, in the air, or in purified formulations intended to elicit a specific therapeutic response. Many of these compounds, however, tend to be highly lipophilic and therefore to remain in the body for long periods of time. This would have drastic consequences for drug therapy and xenobiotic toxicity. Fortunately, a multitude of enzyme systems exist, many present in highest amounts in the liver, which are uniquely equipped for converting lipophilic substances into more water-soluble derivatives which can then be efficiently excreted by the kidney. This article presents an introductory discussion of the concepts and pathways of xenobiotic biotransformation by liver enzymes, an overview of some inherited pharmacogenetic defects affecting the activity of these enzymes and their potential clinical consequences, and finally an example of one such defect, the acetylation polymorphism.

DRUG BIOTRANSFORMATION REACTIONS

Depending on its structure, a drug may be subjected to two types of enzymatic manipulation which have been classified under the general headings of Phase I and Phase II reactions (Figure 1 and Table 1; Jakoby, 1988; Ziegler, 1988). Phase I reactions, which include oxidation, reduction and hydrolysis by a number of enzyme classes, tend to introduce or expose functional groups (often hydroxyl groups) on the drug molecule which may either directly improve its hydrophilicity for subsequent excretion, or allow it to be further acted upon by enzymes of the Phase II class. The latter reactions are conjugative or synthetic, that is, water-soluble side groups are added to enhance excretability. From Table 1 it can be seen that a number of the enzymes capable of biotransforming certain xenobiotics (e.g. xanthine dehydrogenase, catechol O-methyltransferase) also play an important rôle in the intermediary metabolism of endogenous substrates. However, some of the enzymes (e.g. arylamine N-acetyltransferase) possess no presently known endogenous substrates, implying that they may have evolved specifically for the purpose of protecting the organism from environmental chemical insults.

Although the title of this review suggests that the endpoint of drug biotransformation is detoxication, this is certainly not always the case. Indeed, it is important to emphasize that the only common underlying feature of biotransformation processes is that they attempt to convert chemicals from lipophiles to hydrophiles. As shown in Table 2, this process may have a number of biological consequences. A drug may lose its pharmacological activity as a result of biotransformation or be more rapidly eliminated so as to significantly decrease its duration of action. On the other hand, inactive prodrugs may be converted to active metabolites, or a drug may attain an altered pharmacological specificity due to the changes introduced into its structure.

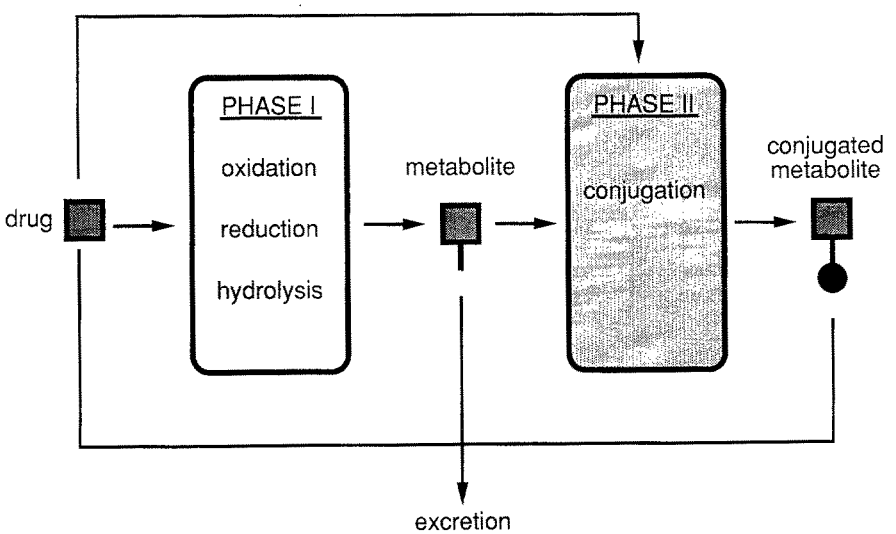

Figure 1 Potential routes of drug and xenobiotic biotransformation. Phase I and Phase II enzymes are generally present in the highest concentrations in the liver, but may also be found in significant amounts in many other tissues

Table 1 Enzymes of xenobiotic biotransformation

Phase I	*Phase II*
Dehydrogenases	Conjugation reactions
Alcohol dehydrogenase	UDP-glucuronyltransferase
Aldehyde dehydrogenase	Alcohol sulphotransferase
Dihydrodiol dehydrogenase	Amine O-sulphotransferase
Xanthine dehydrogenase	Phenol sulphotransferase
Reductases	Glutathione transferase
Ketoreductase	Phenol O-methyltransferase
Nitroreductase	Catechol O-methyltransferase
Azoreductase	Amine N-methyltransferase
N-oxide reductase	Histamine N-methyltransferase
Sulphoxide reductase	Thiol S-methyltransferase
Oxidases	Glycine acyltransferase
Aldehyde oxidase	Glutamate acyltransferase
Monoamine oxidase	Arylamine N-acetyltransferase
Mono-oxygenases	Cysteine N-acetyltransferase
Cytochromes P450	Cysteine conjugate β-lyase
Flavin-containing mono-oxygenase	Thioltransferase
Hydrolases	Rhodanese
Esterases and amidases	
Epoxide hydrolase	

Table 2 Biological consequences of drug biotransformation

Production of stable metabolites with:
Decreased pharmacological activity – inactivation
Increased pharmacological activity – prodrug activation
Altered pharmacological specificity
Production of chemically reactive metabolites:
Covalent binding with cellular macromolecules
Toxic consequences – hepatotoxicity, carcinogenicity, teratogenicity

Moreover, biotransformation may even produce an increase in chemical toxicity by formation of chemically reactive electrophiles with the potential to bind covalently to intracellular macromolecules and result in cell death, immune responses and mutational events.

A number of important features of xenobiotic metabolizing enzymes makes them particularly suited for the conversion of thousands of potential substrates in the environment to metabolites which will hopefully be eliminated from the body. Firstly, in contrast to many enzymes of intermediary metabolism which have a restricted substrate specificity for a particular endogenous compound, most drug-metabolizing enzymes have evolved to have an extremely broad substrate specificity, allowing them in some cases to biotransform hundreds of potential chemical compounds, albeit with varying degrees of success. Secondly, isozyme multiplicity is a common feature of these enzyme systems. Again, evolution has ensured, by the production of

many closely related enzyme isoforms with distinct but often overlapping substrate specificities, that chemicals with widely divergent structures are unlikely to escape biotransformation. Thirdly, the activity of many drug biotransforming enzymes may be induced, at the level of transcriptional activation, by exposure to certain drugs and environmental substances which are themselves often substrates for metabolism. These points are well illustrated by the microsomal cytochrome P450 monooxygenase enzyme superfamily, probably the most important and widely studied drug biotransformation enzyme system (Gonzalez, 1989; Okey, 1990). Not only are individual cytochrome P450 isozymes usually capable of adding hydroxyl groups to a wide variety of chemical structures, but the number of related isozymes with the potential to catalyse such reactions may number as many as 50–100 proteins produced from at least 14 different gene families (Nebert *et al.*, 1989).

A further extension from these points and from the existence of a variety of biotransforming enzyme systems (Table 1) is the fact that a given drug may undergo a wide variety of competing biotransformation reactions to produce multiple metabolites, each with its own potential for pharmacological activity or for toxicity. There are many examples of xenobiotics with relatively simple chemical structures which undergo a remarkably complex series of metabolic conversions. Examples of such compounds include the most widely used drug in the world, caffeine (Arnaud, 1984), and the potent experimental carcinogen 2-aminofluorene (Miller, 1978). The latter compound in fact requires metabolic activation for its carcinogenic potential to be manifested. Thus the balance of competing biotransformation pathways, each with the potential for variations in activity due to environmental (i.e. enzyme induction) and genetic factors, may ultimately determine the efficacy or toxicity of drugs and environmental chemicals.

INHERITED DEFECTS OF DRUG BIOTRANSFORMATION

It is well recognized that the response to drugs and toxic substances varies widely among individuals in a given population. The discipline termed pharmacogenetics aims to assess the rôle of inheritance in producing such variation, most prominently in recent years with respect to genetic defects in the enzymes of drug biotransformation (Meyer *et al.*, 1990). Numerous pharmacogenetic variants affecting drug disposition have been detected (Table 3), encompassing both polymorphisms and rare defects. The most important feature which distinguishes these pharmacogenetic defects from other inborn errors of metabolism is that they are manifested only upon drug challenge. Consequently they are not in themselves life threatening, eliminating any discussion of prenatal diagnosis and genetic counselling. However, because of their potential clinical consequences for affecting drug action and chemical toxicity (Table 4), it is often important to identify affected individuals before or during exposure to substances whose biotransformation leads to alterations in their potential for efficacy or toxicity.

The detection and study of pharmacogenetic defects can be approached from two different directions. One is the classical, event-based approach where affected probands are identified and family pedigrees are then investigated in detail for the presence of

Table 3 Genetic variations affecting the pharmacokinetics of drugs and xenobiotics

Polymorphisms	*McKusick no.*	*Rare defects*	*McKusick no.*
Arylamine N-acetyltransferase	24340	Pseudocholinesterase	17740
Alcohol dehydrogenase	10370	Catalase	11150
Aldehyde dehydrogenase	10064	Xanthine dehydrogenase	27830
Paraoxonase	16882	Glucuronyltransferase	21880
Glutathione transferase	13834	Trimethylamine N-oxidase	27570
Glucosyltransferase	20480		
Thiopurine methyltransferase	18768		
Catechol O-methyltransferase	11679		
Carboxymethylcysteine S-oxidase			
Cytochromes P450:		Cytochromes P450:	
Debrisoquine 4-hydroxylase	12403	Phenacetin O-de-ethylase	20030
S-Mephenytoin oxidase	12402	Phenytoin oxidase	22275
Antipyrine oxidase	10729	Coumarin hyroxylase	12272
Theophylline oxidase (?)	18765		
Nifedipine oxidase (?)	12401		
Tolbutamide oxidase (?)			

Table 4 Clinical consequences of genetically defective drug metabolizing enzymes

Functional overdose due to inefficient elimination of active drug
Lack of efficacy due to inefficient prodrug activation
Idiosyncratic toxicity unrelated to the intended drug effect
Associations with apparently spontaneous diseases

defective gene products. The second approach, which is unique to pharmacogenetics and is likely to become increasingly popular in the pharmaceutical industry for new drug development, is drug-based. Starting with a specific chemical structure, *in vitro* studies with human tissues or cell culture systems expressing specific xenobiotic-metabolizing enzymes can predict the occurrence of pharmacogenetic variation *in vivo*. At this stage, experimental methodologies for the two approaches converge. Since these defects are silent in the absence of a drug, patient testing methods often involve the administration of a specified dose of a so-called 'probe' drug whose disposition (1) is affected by the genetic defect, and (2) may be conveniently monitored by measurement of plasma elimination kinetics or urinary metabolite profiles. However, such screening approaches may present a risk of producing toxicity from the probe drug itself if genetically predisposed individuals exist in the population studied. For this reason, testing methods which minimize or avoid toxic drug exposures continue to be developed. These include the development of safer probe drugs without toxic potential, such as caffeine for the determination of acetylator phenotype (see below), the use of peripheral blood lymphocytes expressing xenobiotic-metabolizing enzymes for *in vitro* toxicology testing (Spielberg, 1984), and the use of molecular genetic tests for the direct diagnosis of mutations in the genes encoding drug-metabolizing enzymes (Meyer, 1990).

THE ACETYLATION POLYMORPHISM

This example, reviewed in depth recently (Evans, 1989), illustrates some of the features unique to pharmacogenetic defects as a subset of inborn errors of metabolism. It was in the mid-1950s, before the term pharmacogenetics was even devised, that a significant incidence of unwanted side effects associated with the use of the antitubercular drug isoniazid led to a closer examination of its disposition in healthy human subjects. Population frequency histograms of plasma ioniazid concentrations after a single oral dose were distinctly bimodal, allowing for segregation of roughly equal numbers of 'slow' and 'rapid' eliminators of the drug in unrelated Caucasian subjects. The inherited nature of this phenomenon was first suggested by twin studies and by the observation of a marked interethnic difference in the proportions of the two isoniazid elimination subgroups, with a number of Oriental populations displaying a much lower frequency of the slow eliminator phenotype. Pedigree analysis verfied the genetic hypothesis and suggested that the ability to eliminate isoniazid was controlled by the action of two alleles at a single autosomal gene locus, with rapid elimination as the apparently dominant trait.

The biochemical basis of these population variations was soon found to be related to differences in the rate of isoniazid N-acetylation taking place predominantly in the liver. The reaction is catalysed by the soluble enzyme arylamine N-acetyltransferase (EC 2.3.1.5), which mediates the transfer of an acetyl group from the essential cofactor acetyl coenzyme A to aromatic amines or hydrazines, producing an acetamide. Many investigations have established that the acetylation of a large number of arylamine and hydrazine xenobiotics is under the same genetic control.

Evans (1989) has critically assessed the large body of literature documenting the possible clinical and toxicological consequences of the acetylation polymorphism (Tables 5 and 6). The first studies of isoniazid showed that neurological side effects were indeed more frequent among genetically slow acetylators due to functional overdose on standardized drug administration schedules. Dose-related phenomena have also been reported with numerous other drug therapies (Table 5). It could be argued that such toxicities or therapeutic failures could be avoided by individualizing drug dosing, by therapeutic drug monitoring in patients taking these medications, or by using related drugs whose disposition is unaffected by the genetic defect. These alternatives, however, are often not possible or practical. In addition, there exist several associations of acetylator phenotype with apparently spontaneous disorders (Table 6) where exposure to causative agents is either undocumented or uncontrolled.

Table 5 Adverse reactions related to the acetylation polymorphism

Drug	*Phenotype*	*Effect observed*
Isoniazid	Slow	Peripheral neuropathy
		Phenytoin adverse effects
	Rapid	Therapeutic failure with 1x weekly doses
Hydralazine	Slow	Development of antinuclear antibodies and SLE-like syndrome
	Rapid	Higher dose required to treat hypertension
Salicylazosulphapyridine	Slow	More adverse reactions to sulphapyridine
	Rapid	Methaemoglobinaemia
Procainamide	Slow	More prone to SLE-like syndrome
	Rapid	More ventricular premature beats in cardiac patients

Table 6 Associations of acetylator phenotype with certain disorders

Disorder	*Phenotype*
Bladder cancer in workers exposed to aromatic amines	Slow
Colorectal cancer	Rapid
Laryngeal cancer	Slow
Advanced cancer of the breast	Rapid
Diabetes (Caucasians)	Rapid
Gilbert's disease	Slow
Leprosy (Chinese patients)	Slow

For these reasons and for basic mechanistic studies it is often of value to know the acetylator phenotype of individuals in a population. Until very recently, phenotyping tests have made use of probe drugs which are themselves polymorphically acetylated. Among the drugs which have been used for this purpose are isoniazid itself, the antibacterial agents sulphamethazine and dapsone, and the antiarrhythmic procainamide. Analytical methods such as colorimetric, fluorimetric or high performance liquid chromatographic (HPLC) assays are then employed to quantify the amount of parent drug and/or its acetylated metabolite in plasma or urine samples at a specified time after drug intake. These tests suffer from two significant shortcomings: firstly, the drugs are potentially toxic, especially to genetically defective subjects; and secondly, the test parameters are generally not sensitive enough to distinguish between the three acetylation genotypes, even though detailed kinetic analyses have established that significant activity differences exist between heterozygous and homozygous rapid individuals, implying an additive gene dosage effect. It has been observed more recently, however, that the excretion of a caffeine metabolite is related to the acetylation polymorphism, leading to the development of a caffeine test for acetylator phenotype (Grant *et al.*, 1984). The test is safe, analytically simple and rapid, and sensitive enough to discriminate between the three genotypes of acetylation capacity, a feature that allows for the assessment of differential susceptibility of heterozygous and homozygous individuals to toxicity from certain drugs and xenobiotics.

Until only a few years ago, the low liver content and *in vitro* instability of human N-acetyltransferase enzyme protein(s) had hindered studies of the biochemical and molecular mechanisms leading to the occurrence of the slow acetylator phenotype in man. This situation has changed with recent technical improvements in analytical methods coupled with the use of recombinant DNA technologies to clone and express mutant alleles at the polymorphic gene locus. Using a specific polyclonal antiserum raised against a purified human arylamine N-acetyltransferase protein (Grant *et al.*, 1989) and with access to liver tissue from individuals whose *in vivo* acetylator phenotype could often be determined with the caffeine test, we were able to demonstrate that at the protein level, the slow acetylator phenotype is associated with a decrease in the quantity of functional enzyme present in human liver rather than a change in the substrate kinetics of a variant gene product (Grant *et al.*, 1990).

To study the molecular basis of the defect, a full-length cDNA encoding rabbit arylamine N-acetyltransferase (Blum *et al.*, 1989) was used to screen a human genomic DNA library, resulting in the isolation of two independently regulated N-acetyltransferase genes, designated *NAT*1 and *NAT*2, at separate loci on chromosome 8, pter-q11 (Blum *et al.*, 1990a). These genes both encode functional acetylating enzymes upon transient expression in mammalian (COS-1) cell culture. The recombinant *NAT*2 gene product shows protein immunoreactivity and enzyme kinetic characteristics identical to those of the human liver enzyme whose content is decreased in slow acetylators, suggesting that the *NAT*2 gene locus is the site of the acetylation polymorphism (Grant *et al.*, 1991). To date, four mutant alleles at the *NAT*2 gene locus have been identified; fortuitously, the three most common of these may be detected by RFLP analysis on Southern blots even though each contains only a

small number of nucleotide substitutions relative to the wild-type allele. Of the two most common mutant alleles, accounting for roughly 95% of those present in a Caucasian population, one contains point mutations which may impair mRNA translation efficiency, while the other contains a point mutation which appears to decrease enzyme stability (Blum *et al.*, 1991). The third mutant allele, identified by cloning and expression of its cDNA (Ohsako and Deguchi, 1990), is much less frequently observed both in Caucasian and Oriental populations, and a fourth mutant has been detected only once so far.

Interestingly, the *NAT*1 gene produces an acetylating enzyme with kinetic characteristics different from any that we had observed in human liver until quite recently. *NAT*1 is indeed expressed in human liver and probably also in many other tissues, but is unrelated to the acetylation polymorphism (Grant *et al.*, 1991; Ohsako and Deguchi, 1990). However, the existence of *NAT*1 has provided the solution to one of the puzzling clinical aspects related to the acetylation polymorphism, namely the existence of so-called 'monomorphic' arylamine substrates such as *p*-aminosalicylic acid, which are very efficiently acetylated *in vivo* but do not correlate with acetylator phenotype. It is now clear that such substrates are selectively metabolized by *NAT*1. This gene multiplicity, although certainly not as extensive as that displayed by many other drug biotransformation enzyme systems, nevertheless imparts upon the organism the capability to metabolize and thereby hopefully to detoxify a wider diversity of xenobiotics.

CONCLUSION

The purpose of this review has been to illustrate the importance of hepatic xenobiotic biotransforming enzymes in the conversion of drugs and environmental chemicals to metabolic products with altered potential for toxicity. It is clear that pharmacogenetic defects affecting many of these enzymes may be significant predisposing factors for the occurrence of toxic events associated with chemical exposures, and the mechanisms underlying these defects continue to be very active areas of research in pharmacology.

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