Metabolic Basis of Adverse Drug Reactions

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The rate of elimination of a lipophilic drug is governed largely by its rate of metabolism. Therefore, the ability of an individual to metabolise a particular drug will be an important determinant of the efficacy (intensity of effect), the duration of effect and the toxicity of that drug. Metabolism is usually associated with an increase in water-solubility of the drug, which in turn leads to an increase in the rate of either biliary or urinary excretion. The chemical changes involved usually result in a loss or diminution of pharmacological activity. Metabolism may therefore be considered a detoxification process. However, in certain circumstances, normal metabolic processes (biotransformations) may produce a toxic metabolite. Of particular importance in this context is the formation of chemically reactive metabolites which are responsible for various forms of drug toxicity.

Figure 1 shows that both the rate and route of drug metabolism are important determinants of drug toxicity,

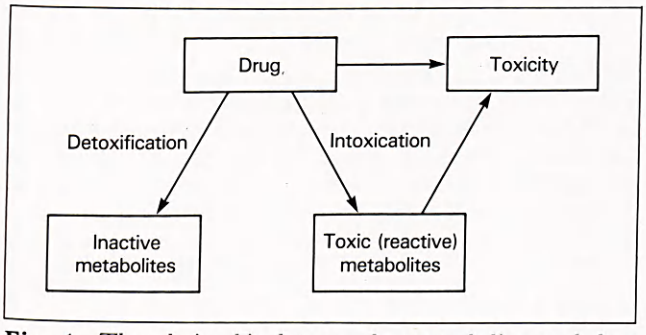

Fig. 1. *The relationship between drug metabolism and drug toxicity.*

so it is necessary to identify factors that may contribute to inter-individual variation in drug metabolism and to characterise metabolites which have toxicological activity.

Inter-individual Variation in Drug Metabolism

Drug metabolism reactions observed in man consist of phase I reactions (oxidation, reduction and hydrolysis) and phase II conjugation (Table 1). The enzyme systems responsible for these biotransformations appear to have the ability to metabolise an unlimited number of diverse organic compounds, including drugs. It is thought that one of the reasons for the versatility of the drug-metabolising enzyme system is that the enzymes exist in multiple forms which represent different gene products[1].

Table 1. Drug metabolism reactions observed in man.

Phase I	Phase II
Oxidation	Acetylation
Reduction	Glucuronidation
	Sulphation
Hydrolysis	Glutathione conjugation
	Amino acid conjugation
	N,S,O, — methylation

Population studies have shown that there are large differences between individuals in their capacity to metabolise drugs and other lipophilic xenobiotics. This inter-individual variability is due to a number of factors, centred on the genetic constitution of the individual and including an array of host factors, such as age, environmental considerations, disease and drugs, which interact dynamically with each other[2]. All of these factors may, in theory, partly determine the susceptibility of an individual to drug toxicity.

Genetic Variation

Given that the main purpose of drug metabolism is to convert lipophilic substances into more water-soluble metabolites and thereby prevent toxicity through accumulation, it is important to recognise individuals who have a genetically determined inability to perform a particular biotransformation. Genetic polymorphisms arise because of the occurrence of mutant alleles in the population which can influence either the structure or the amount of enzyme synthesised.

The two classical examples of polymorphic drug metabolism reactions are acetylation of various drugs and hydrolysis of succinylcholine. More recently, it has become apparent that certain drug oxidation reactions exhibit polymorphism.

Succinylcholine produces skeletal muscle relaxation of short duration because of its very rapid degradation, by plasma cholinesterase, to succinylmonocholine which is inactive. About one in 3,000 individuals is extremely sensitive to succinylcholine, responding to it by prolonged paralysis, because of an atypical plasma cholinesterase which hydrolyses the drug at a considerably reduced rate[3].

The disposition and toxicity of many aromatic amine and hydrazine compounds are partly determined by the rate and extent of their N-acetylation. Thus, the capacity

of individuals to N-acetylate such drugs as isoniazid, hydralazine, procainamide, dapsone, sulphadimidine, phenelzine and sulphapyridine is genetically determined. Quantitative assessments of the rate and extent of acetylation yield bimodal frequency distributions which separate 'fast' and 'slow' acetylators; 52 per cent of the population are slow acetylators[4]. Drug acetylation is controlled by two autosomal alleles at a single gene locus, the trait for fast acetylation being dominant and that for slow, recessive[5].

The clinical implications of acetylator status illustrate the influence of phenotype on adverse drug reactions. Differences in acetylator status have marked effects on the pharmacological and toxicological profiles of a number of important drugs (Table 2). The neurotoxicities which result from isoniazid, hydralazine- and procainamide-induced systemic lupus erythematosus and sulphasalazine-induced toxicity, illustrate the significance of acetylator status in clinical medicine[6]. These toxicities are dose-dependent and therefore more common among slow acetylators, who usually have higher serum concentrations of the drug at any time after ingestion, than do rapid acetylators.

Table 2. Adverse reactions associated with slow acetylator status.

Drug	Toxic effect	Susceptible phenotype
Isoniazid	Peripheral neuritis	Slow
	SLE syndrome	Slow
	Hepatitis	Rapid/Slow
Hydralazine	SLE syndrome	Slow
Procainamide	SLE syndrome	Slow
Salicylazo-sulphapyridine	Cyanosis and haemolysis	Slow
Arylamines	Bladder cancer	Slow

Other disorders for which acetylator status has been claimed to be a predisposing factor include isoniazid-induced hepatitis[7,8], arylamine-associated bladder cancer[9] and haemolysis induced by sulphones and sulphonamides in glucose-6-phosphate deficiency[10,11]. Analysis of the relationship between the metabolism and toxicity of some of these compounds is complicated by the fact that a metabolite is thought to be responsible for the toxicity.

Acetylator phenotype may partly determine whether or not an individual is susceptible to drug interactions. For example, Kutt *et al.*[12] observed phenytoin intoxication in approximately 10 per cent of epileptics who took the drug together with isoniazid. All the patients were slow acetylators and intoxication could be avoided by simply reducing the dose of phenytoin.

Although there seems to be common agreement that acetylator status is an important determinant of an individual's susceptibility to the toxicity of certain drugs, the value of acetylator phenotype as a predictor of drug toxicity remains an open question[13,14]. However, the distinction made between the two phenotypes is only semi-quantitative and thus may not be a sufficiently powerful method to identify those individuals most at risk.

Until recently pharmacogenetic polymorphisms in drug oxidation were considered rare, despite the fact that the majority of lipophilic drugs are metabolised by the hepatic cytochrome P-450 enzymes, a family of enzymes with distinct but overlapping substrate specificity. A number of monogenically controlled polymorphic drug oxidation reactions have now been discovered. The principle biotransformations that have been investigated are debrisoquine 4-hydroxylation and sparteine oxidation; independent polymorphisms have been reported for tolbutamide hydroxylation[15], mephenytoin hydroxylation[16] and nifedipine oxidation[17].

The formation of the major metabolite of debrisoquine, 4-hydroxydebrisoquine, displays polymorphism in the British population. Two distinct phenotypes, 'extensive metabolisers' and 'poor metabolisers', are recognisable; the 'poor metaboliser' phenotype frequency is an autosomal Mendelian recessive character and has a frequency of 8.9 per cent[18]. Poor metabolisers have grossly impaired metabolism and excrete little or no metabolite.

Since the original report that debrisoquine hydroxylation in man exhibits genetic polymorphism, there has been much interest in other drug biotransformations which co-segregate with the defect in debrisoquine 4-hydroxylase activity and, perhaps more importantly, whether poor metabolisers are more susceptible to adverse drug reactions (Table 3).

Table 3. Adverse reactions associated with impaired debrisoquine oxidation.

Drug	Adverse reaction
Metoprolol	Excessive β-blockade[21,22]
Nortriptyline	Confusional state[35,36]
Phenacetin	Methaemoglobinaemia[32]
Phenformin	Lactic acidosis[28,29]
Perhexiline	Neuropathy and hepatotoxicity[25]

A number of reports have linked adverse reactions to lipophilic β-adrenoceptor blockers with impaired ability to hydroxylate debrisoquine[19,20]. Lennard *et al.*[21,22] found that plasma metoprolol concentrations and areas under the plasma concentration-time curve were greater in poor metabolisers than in normal metabolisers. A significant correlation was found between debrisoquine metabolic ratio, metoprolol elimination half-life and percentage reduction in exercise-induced tachycardia (24 hours) which was taken as a measure of β-blockade. However, Clark *et al.*[23] found in hypersensitive subjects no relationship between adverse reactions necessitating metoprolol withdrawal, and oxidation status.

It has been tentatively suggested that patients due to receive lipophilic β-blockers should first have their drug oxidation status determined. Jack and Kendall[24] have questioned the need for this and suggested that subjects at risk can be detected by measuring pulse rate. Neverthe-

less, measurement of oxidation status may be of value in (a) investigating the mechanism of the adverse reaction, (b) detecting individuals potentially at risk from the conventional doses of β-adrenoceptor blockers and (c) recognising drugs subject to wide inter-individual variation in metabolism within the population.

The anti-anginal drug perhexiline is associated with a number of adverse reactions, the most important of which are peripheral neuropathy and hepatotoxicity. The drug is amphiphilic and thus forms stable, non-degradable complexes with phospholipids which accumulate in certain cells and thereby produce cytotoxicity.

Shah *et al.*[25] have suggested that determination of debrisoquine oxidation status may be of predictive value in determining perhexiline dosage and in controlling the neurotoxicity of this drug, the incidence of which appears to be related to individual half-life[26]. The metabolism of perhexiline is associated with that of debrisoquine and poor oxidisers of perhexiline are also poor metabolisers of debrisoquine[27]. Shah *et al.*[25] compared a group of patients who had perhexiline-induced neuropathy with a control group who had no serious adverse effects on long-term treatment. The percentage of poor metabolisers of debrisoquine in the group who had suffered peripheral neuropathy was about 50 per cent, whereas the proportion of poor metabolisers in the control group was similar to that expected in a normal, healthy population.

The oral hypoglycaemic agent phenformin has been withdrawn from use in many countries because of its association with lactic acidosis. The oxidation of phenformin is thought to be linked with debrisoquine 4-hydroxylation and it has been suggested that phenformin toxicity might have arisen because of poor metabolism[28]. There is no direct clinical evidence to support this hypothesis, although it has been shown that volunteers, phenotyped as debrisoquine poor metabolisers, given phenformin had significantly higher blood lactate concentrations than corresponding extensive metaboliser phenotypes[29].

The analgesic phenacetin is associated with a risk of renal toxicity and was therefore virtually prohibited in the UK in 1980. It is converted into paracetamol in the liver by oxidative de-ethylation. In poor metabolisers of debrisoquine, the rate of formation of paracetamol is slower than in extensive metabolisers[30] and phenacetin produces methaemoglobin in poor metabolisers but not in extensive metabolisers[31]. It has been suggested that, in the poor metaboliser, more of the drug is converted into a toxic metabolite, 2-hydroxyphenetidine, via an alternative metabolic pathway (de-acetylation and aromatic 2-hydroxylation) not controlled by the same gene locus responsible for de-ethylation[32].

The metabolism of tricyclic antidepressants is related to that of sparteine and dibrisoquine[33,34]. Indeed, it has been suggested that it is possible to predict steady-state plasma concentrations from an individual's debrisoquine metabolic ratio[35]. The poor metaboliser appears to be at greater risk to nortryptyline-induced vertigo, dizziness and confusional state[36] but the clinical significance of this observation has not been defined.

Thus it can be seen that where metabolism is strongly influenced by a major gene effect, there may be pronounced differences in drug response, and toxicity may ensue through drug accumulation. Adverse effects are generally more frequent in the poor metaboliser, but encainide provides an example of the extensive metaboliser possibly being more predisposed, as the metabolites of the drug are pharmacologically active[37].

In no instance has the determination of phenotype provided an absolute test for drug toxicity. Indeed, it would have been naive to suppose that it would. Nevertheless, such information should be useful for detecting individuals potentially at risk and in the evaluation of the relationship between the metabolism and toxicity of new drugs.

Modulation of Drug Metabolism

Numerous factors may alter the capacity of an individual to metabolise drugs[1,2] and thereby increase the risk of toxicity because of either drug accumulation or enhanced rate of formation of a toxic metabolite. In practice, the most important considerations are age, enzyme induction and enzyme inhibition.

Age

The incidence of adverse drug reactions in elderly patients is approximately twice that found in younger patients. Numerous factors such as multiple disease states and multi-drug therapy contribute to this difference. There is, however, an expanding literature of clinical studies in man which clearly indicate that metabolism of some drugs is impaired with older age[38]. Therefore the elderly may be more susceptible to adverse drug reactions, especially from drugs with long half-lives.

This point is illustrated by the experience with the anti-inflammatory drug benoxaprofen (Opren), which was withdrawn because of adverse effects which were more severe and frequent in elderly patients[39-41].

The agent was designed with a longer half-life than other non-steroidal anti-inflammatory drugs, so that it could be administered once daily, while maintaining a therapeutic response. However, the rate of metabolism showed a profound dependence on age, the half-life in elderly patients being approximately four times longer than in younger patients[42]. The fatal hepatic and renal cytotoxicity of benoxaprofen appears to have been related to the excessive accumulation of the drug.

After paracetamol overdose, children experience relatively mild liver involvement, despite paracetamol serum concentrations that would be associated with life-threatening hepatotoxicity in adults. Paracetamol toxicity is due to formation of a toxic reactive metabolite. The lower incidence of severe toxicity in children may be related to a greater ability to metabolise paracetamol via non-toxic pathways[43].

Enzyme Inhibition and Enzyme Induction

The clinical implications of induction and inhibition of drug metabolism have been reviewed elsewhere[44]. Drug interactions which occur as a result of enzyme

induction or inhibition are usually produced by drugs prescribed in doses of 100 mg or more daily. Interactions involving such changes in enzyme activity are usually of importance only for drugs with a narrow therapeutic index such as anticonvulsants, anticoagulants, anti-arrhythmics and oral contraceptives (Table 4). With these drugs, a relatively small alteration in elimination rate may be associated with a change from a therapeutic to a toxic response.

Table 4. Clinical importance of enzyme induction and enzyme inhibition.

Substrates	Enzyme Inducers	Enzyme Inhibitors
Anti-arrhythmics	Carbamazepine	Cimetidine
Anticoagulants	Phenobarbitone	Erythromycin
Anticonvulsants	Phenytoin	Isoniazid
Oral contraceptives	Rifampicin	Sulphaphenazole
Tolbutamide		

For example, co-administration of the H_2-antagonist cimetidine increased warfarin plasma steady-state concentration and prolonged prothrombin time to a dangerous level[45]. More recent work[46] has shown that cimetidine stereoselectively inhibits the metabolism of R-warfarin. Phenobarbitone, on the other hand, stimulates the metabolism of warfarin. Concurrent administration of phenobarbitone and warfarin produces a change in steady-state plasma warfarin and anticoagulant effect within six days[47]. However, after withdrawal of phenobarbitone, drug metabolism returns to normal and this may lead to fatal haemorrhage during continued anticoagulant therapy[48].

New drugs thought to be either enzyme inhibitors or enzyme inducing agents may be screened using model drug substrates[1]. The time-course of enzyme induction may be monitored by simply measuring changes in the disposition of an endogenous compound, 6 β-hydroxycortisol.

Theoretically, enzyme induction could lead to a selective increase in toxic metabolite formation. Although such a mechanism has been demonstrated for carcinogens and hepatotoxins in sensitive animal test systems[49,50], there is no direct evidence for it in man. Furthermore, White *et al.*[51] did not find an increased rate of cancer in patients on long-term anticonvulsant therapy, as might have been expected if there was enhanced activation of aromatic hydrocarbons. Animal studies have shown that toxicity may be reduced by inhibition of paracetamol and isoniazid reactive metabolite formation with cimetidine, but this has not been achieved in man[52,53].

Toxic Metabolites

For most drugs metabolism represents a clearance mechanism. However, in certain circumstances, a normal biotransformation may lead to the formation of a toxic metabolite (Fig.2). Chemically reactive metabolites are particularly important in this respect because their covalent interaction with biopolymers *in vivo* might induce tumorogenicity, cytotoxicity and hypersensitivity[54].

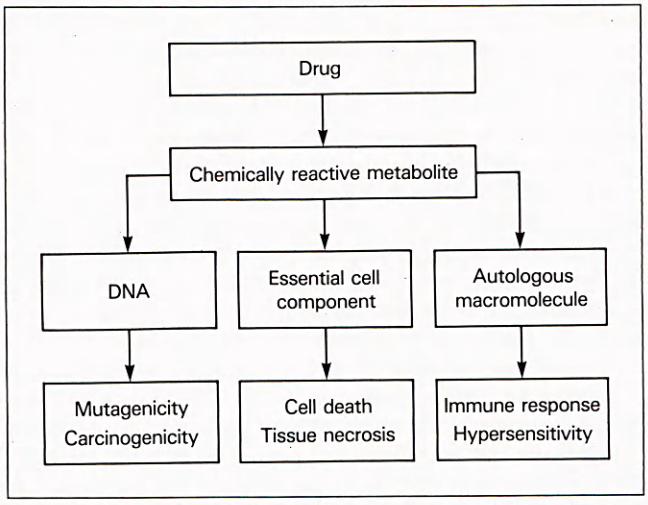

Fig. 2. *The role of chemically reactive metabolites in drug toxicity.*

In the process of the chemical induction of a tumour, binding of that chemical or one of its metabolites to a biological macromolecule seems to be the initial step. Good correlations between carcinogenicity and covalent binding to DNA as target macromolecule rather than RNA or protein have been observed[55,56]

The liver is the major site of drug metabolism and a number of drugs, including paracetamol (overdose), isoniazid and halothane, are thought to produce hepatotoxicity by generating chemically reactive metabolites which react indiscriminately with vital cellular macromolecules (reviewed by Timbrell[57]). The relationship between the toxicity and metabolism of paracetamol has been investigated extensively in both man and experimental animals[49,58,59].

Paracetamol is largely metabolised via glucuronidation and sulphation which account for approximately 50 per cent and 25 per cent of the drug, respectively. In addition, about 10 per cent of the drug is oxidised to a chemically reactive metabolite N-acetylbenzoquinone imine, which is normally detoxified immediately by conjugation with glutathione. However, after an overdose (10-20g) the sulphation and glutathione pathways become saturated, allowing the chemically reactive metabolite to arylate essential cell structures[60]. The severity of paracetamol-induced cellular necrosis varies in proportion to the amount of arylation. Administration of N-acetylcysteine may afford protection by providing more glutathione for detoxification of the reactive metabolite[61].

Work on ipomeanol-induced lung disease in animals provides further convincing evidence for the role of metabolic activation in chemical-induced tissue injury[50]. In particular, formation of chemically reactive metabolites in tissues other than liver, may produce organ-selective toxicity.

Our current understanding of drug hypersensitivity (allergy) is based on the assumption that drugs form haptens *in vivo*. This concept derives from classical

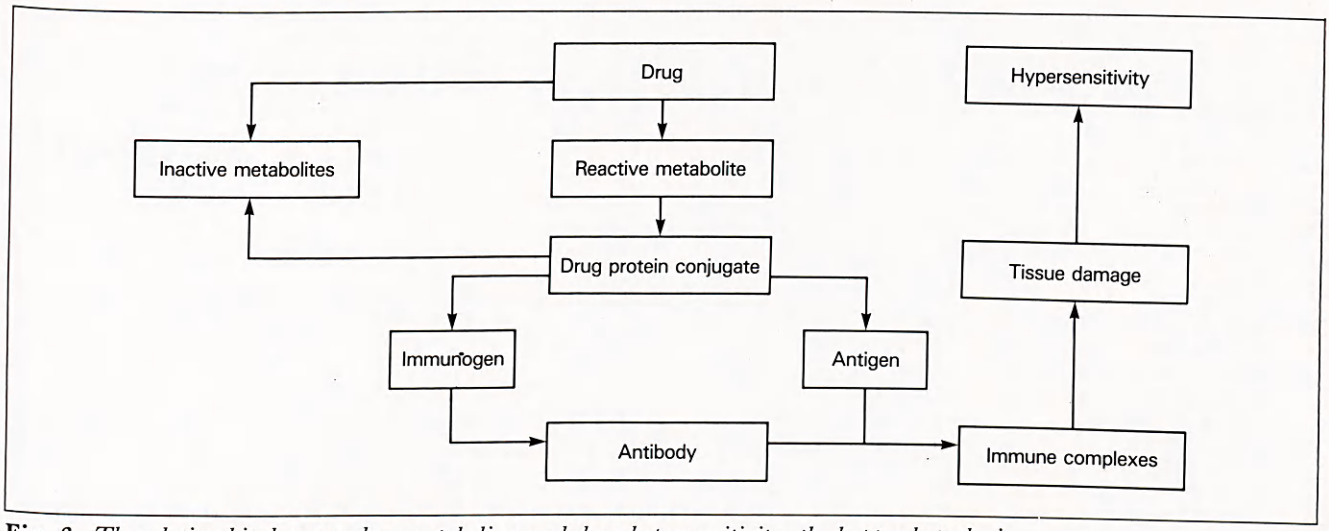

Fig. 3. *The relationship between drug metabolism and drug hypersensitivity: the hapten hypothesis.*

immunochemical studies[62,63] which showed that low molecular weight compounds (e.g. drugs) must be linked by a covalent bond to a macromolecular carrier in order to elicit an immune response (Fig. 3).

Evidence that drugs produce hypersensitivity in man, by acting as haptens, came from several groups working on penicillin allergy[64-66]. Antibodies directed against antigenic determinants derived from penicillin can be detected in the majority of patients treated with penicillin.

The sensitising capacity of penicillin can be explained by the inherent chemical reactivity of the β-lactam nucleus. However, the question of whether the ultimate immunogen is formed *in vivo* from autologous proteins or is in fact an impurity from the manufacturing process has not been resolved[67].

Most drugs do not possess direct protein reactivity, and it is assumed that haptens are formed from chemically reactive metabolites (Fig. 3). Although this is an attractive hypothesis, there is no direct experimental evidence to confirm it. A number of drugs with suspected immunological adverse effects, such as practolol, procainamide, chlorpromazine, sulphonamides, ethynyloestradiol, halothane and hydralazine readily form 'reactive metabolites' in *in vitro* drug-metabolising systems[68]. It is therefore possible that such metabolites might form effective (immunogenic) haptenated protein conjugates in certain individuals, especially those with deficient detoxification mechanisms (e.g. glutathione conjugation).

Conclusion

Individuals show remarkable variation in their ability and capacity to metabolise drugs. Drug toxicity may occur because of excessive accumulation of the parent drug or formation of a toxic (reactive) metabolite. To avoid adverse reactions, it is important to understand factors that affect dosage requirements and thus identify, within the population, individuals susceptible to dose-dependent drug toxicities.

Drug toxicity may be a function of the route rather than the rate of drug metabolism. In such circumstances, toxicity will be partly dependent on the balance between activation and deactivation pathways. At present it is not possible to assess an individual's capacity for generating such toxic metabolites. However, chemical studies of the *in vitro* and *in vivo* metabolism can provide some insight into the potential toxicity of a particular drug.

Acknowledgements

I should like to thank Professor A. M. Breckenridge for many helpful discussions.

This article is based on a paper read at the College Regional Conference in Liverpool in September 1985.

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