Drug metabolism and hepatotoxicity

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Drug metabolism in the 1960s

At the time the Liver Unit was established in 1966, interest in the study of drug metabolism was growing, stimulated largely by the pharmaceutical industry, and the legislative requirements imposed upon it. It represented a remarkable change from the situation a few years previously when '... the study of the metabolism of foreign compounds was not a popular one; biochemists considered it rather a waste of time and effort, both of which could be better expended on looking at naturally occurring substances. Pharmacologists also had little interest in the subject and regarded it as unrelated to their immediate problems. Toxicology was in a primitive state of development and still largely concerned with cataloguing and describing the effects of various poisons'.¹ By the 1960s, however, centres in Britain, Europe, and the United States had begun to characterise the drug metabolising enzymes and their responses to repeated drug ingestion. In 1966, novel discoveries emphasised: (i) the existence of more than one form of cytochrome P-450,² the basic function³ and characterisation^{4,5} of which had been defined only in the previous three years; (ii) the clinical implications of enzyme induction to drug therapy, as illustrated by three case reports, one describing fatal haemorrhage after withdrawal of the enzyme inducing sedative chloral hydrate in a patient on anticoagulants,⁶ and two showing relief of paediatric jaundice with phenobarbitone;^{7,8} (iii) the hepatotoxicity of environmental toxins, after the outbreak of Epping jaundice resulting from contamination of flour with 4,4-diamino-diphenylmethane.⁹ The limited understanding of the mechanisms of hepatotoxic drug reactions at that time can be gauged from a 1969 review by Schaffner and Raisfeld.¹⁰ An abundance of important interactions between chemicals and endogenous biochemical pathways had been documented, however, as exemplified in the review of carbon tetrachloride hepatotoxicity by Recknagel¹¹ which continues to complement contemporary texts.¹²

In 1967 Conney published his almost timeless review of the agents responsible for enzyme induction in which he predicted that 'the genetic make-up of the individuals of a population within a given species may be important in determining the occurrence or magnitude of enzyme induction'.¹³ Support for these prophecies was some years away, but the monitoring of enzyme induction in man and its clinical features and benefits was the starting point for the King's Liver Unit Drug Metabolism Group in 1966. Studies on the clinical value of phenobarbitone in the treatment of unconjugated hyperbilirubinaemia dominated the late 1960s¹⁴⁻¹⁶ and prompted the search for alternative inducing therapies with fewer hypnotic side effects.¹⁷ In 1965 Aarts¹⁸ had suggested that urinary glucaric acid excretion might reflect

hepatic drug metabolising enzyme activity. Over the next five years interest at King's centred around screening for enzyme induction by measuring the urinary excretion of this compound.¹⁹ The test was validated by showing an association with hepatic cytochrome P-450 content,²⁰ and was used to show the enzyme inducing properties of drugs²¹ and environmental contaminants such as DDT.²² The observation that urinary D-glucaric acid excretion rose during the course of normal pregnancy indicated that changes in hepatic microsomal enzyme activity could be associated with endogenous compounds and physiological states.²³ Other studies showed a correlation between urinary D-glucaric acid excretion and low circulating levels of calcium and folic acid in epileptic patients.^{24,25} This prompted the suggestion that the enzyme inducing properties of anticonvulsant drugs could lead to increased consumption and eventual deficiency of vitamin D and folic acid.

The 1970s: the concepts of enzyme multiplicity and covalent binding

There was worldwide activity in cataloguing the metabolism and effects of many hundreds of drugs and chemicals throughout the 1970s, not least in relation to the molecular features of enzyme induction and inhibition. A growing awareness emerged of the multiplicity of both phase I and phase II metabolising enzymes, with different forms showing a specific yet overlapping capacity to catalyse particular drug transformations, as well as a selective responsiveness to both enzyme inducers and inhibitors.

Perhaps the most significant advances of the 1970s in relation to drug induced hepatotoxicity were the experimental observations by Gillette, Brodie and colleagues at the National Institutes of Health, USA, of the role played by reactive drug metabolites,²⁶ their covalent binding to cellular macromolecules²⁷ and the hepatoprotective role of glutathione.²⁸ In retrospect, it is perhaps remarkable that 20 years had elapsed since the Millers had first observed a similar phenomenon in aminoazo dye induced carcinogenesis.²⁹ Their comment in 1966 after 20 years work that '... we are still not in a position to define the molecular mechanism by which a chemical induces cancer'³⁰ had a prophetic parallel in arguments that raged for many years on the relevance of covalent binding of reactive metabolites to chemical and drug induced liver damage.

The Liver Unit's interests in this area focused initially on paracetamol hepatotoxicity.³¹ Suicidal overdose with paracetamol was becoming increasingly common in Great Britain,³² and the growing number of referrals of such patients to the Liver Unit allowed the metabolic basis for hepatotoxicity from the drug to be studied in

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Figure 1: Current concepts in the mechanisms of paracetamol induced hepatotoxicity.

man. Early investigations showed that liver damage arises after ingestion of high doses of the drug when the balance in metabolism switches from the detoxication pathways of glucuronide and sulphate conjugation to cytochrome P-450-dependent activation steps.³³ This leads to an increased production of an unstable metabolite with the ability to bind irreversibly to liver cell proteins. Normally this metabolite is detoxified by conjugation with glutathione, but stores of this nucleophile become depleted leaving the reactive metabolite free to exert its toxic effects. By showing that the same mechanisms applied in man³⁴ (Fig 1), the value of studies in animal models for developing treatment regimens was emphasised and stimulated the study of potential new antidotes. This led to the clinical use of methionine,³⁵ cysteamine and dimercaprol,³⁶ initiating an interest in the treatment of paracetamol induced fulminant hepatic failure discussed elsewhere in this volume.

Idiosyncratic drug hepatotoxicity

In the mid-1970s, no role had yet emerged for reactive metabolites in idiosyncratic drug toxicity (hepatotoxicity produced unpredictably by therapeutic doses of drugs in only a minority of susceptible individuals). Zimmerman, in his authoritative and still invaluable text of 1978 commented that 'relatively few experimental approaches have thrown any light on the hepatic injury caused by host idiosyncrasy'.³⁷ Within two years, however, important observations on individual variability in the rate of metabolism of

drugs and in the responses to their potentially toxic metabolites was to transform this area.

It was well known that the capacity of an individual to acetylate drugs is genetically determined,³⁸ with the population divided into rapid and slow acetylators. Research from St Mary's Hospital Medical School, London showed that a similar polymorphism applied to a pathway of oxidative metabolism, the hydroxylation of debrisoquine.³⁹ This provided a potential for determining susceptibility to liver damage from some therapeutic agents as a result of differences in the rates at which individuals metabolised a drug through 'safe' or potentially hepatotoxic pathways. It was some years, however, before well characterised examples emerged. Thus, the half life of the antianginal drug, perhexiline maleate, is much prolonged in poor hydroxylators of debrisoquine⁴⁰ and this seems to be associated with drug accumulation and microvesicular phospholipidosis.⁴¹ Different considerations apply to liver damage from the antituberculous drug isoniazid. Slow acetylators of the drug metabolise more through oxidation which can produce a reactive hydrazine derivative, and there is a higher incidence of liver damage amongst individuals with the slow acetylator phenotype.⁴² Genetic variations in oxidative metabolism are also likely to be important, as are responses to environmental influences. For example, concomitant exposure to rifampicin induces the metabolism of hydrazine and increases the risk of hepatotoxicity.⁴³

Another factor determining susceptibility to some types of hepatotoxic drug reaction is the capacity to mount an immune response against drug altered liver components. A series of investigations carried out in the Liver Unit identified an antibody directed against a hepatocyte surface antigen altered by a halothane metabolite.⁴⁴ The altered antigenic determinant probably results from oxidative halothane metabolism⁴⁵ which generates trifluoroacetylated proteins⁴⁶⁻⁴⁸ (Fig 2). It is likely that all individuals exposed to the drug generate altered hepatocyte membrane determinants,⁴⁹ but only a small minority mount an immunological reaction against them.⁵⁰ The fact that many patients with severe halothane hepatitis have circulating antibodies directed against other organs in the body⁵¹ strongly suggests an underlying, genetically determined defect in immune regulation.⁵² In contrast, some patients with hepatitis from the drug have no evidence of immune involvement, and liver damage in these cases is probably the result of overproduction of a hepatotoxic derivative produced by reductive halothane metabolism. Preferential stimulation of this pathway in experimental animals produces dose related hepatotoxicity⁵³ (Fig 2).

Subsequently, the methods validated with halothane have been used to show a possible immune basis for drug hepatotoxicity from α -methyl dopa⁵⁴ tienilic acid⁵⁵ and ethanol.⁵⁶ In addition to expressing an antibody reacting with tienilic acid derived neoantigens, more than 50% of patients with tienilic acid hepatitis produce an antibody (α -LKM2) directed against a cytochrome P-450 II isoenzyme (P-450 8; P-450 MP). Both this and the analogous LKM1 antibody (α -P-450 IID) have been associated with

Figure 2: Mechanisms underlying predictable and immune mediated hepatotoxicity from halothane.

autoimmune chronic active hepatitis⁵⁷ and are discussed in greater detail elsewhere in this volume.

These and many other studies from groups throughout the world have given support to Hans Popper's thesis that improved understanding of underlying mechanisms could turn idiosyncratic drug reactions into predictable ones.⁵⁸

The 1980s: linking *in vitro* events with *in vivo* consequences

The profile of multiple cytochrome P-450 isoenzymes present in different individuals is determined primarily by genetic factors, but can be modified by environmental manipulations, particularly exposure to substances which induce or inhibit these enzymes.⁵⁹ The implications of this heterogeneity to drug metabolism and hepatotoxicity were to dominate most of the 1980s. The identification of multiple enzymic forms was achieved initially with chemicals (specific inducers and inhibitors), then by purification and isolation, and subsequently with specific antibodies which competitively inhibited substrate transformations. At the start of the decade, a role for gene probing of drug metabolising enzymes was still some way off, with no mention of it as an investigative tool made by Lu and West in their 1979 review of the multiplicity of cytochromes P-450.⁶⁰ Accumulating knowledge, however, showed that association could be made between the metabolism of a specific drug and defined P-450 isoenzymes. This made it increasingly possible to predict which enzyme inducing

drugs might influence that compound's metabolism and which agents could competitively inhibit its clearance. So arose the concept that P-450 doctors might emerge as a future subspeciality.⁶¹ Experimental studies in the Liver Unit were directed towards characterising the enzyme inducing properties of rifampicin.⁶² Clinically significant effects were demonstrated on corticosteroid requirements in asthmatics⁶³ and on theophylline pharmacokinetics.⁶⁴ Rifampicin also induced the metabolism of testosterone and its hydroxylation at multiple sites on the molecule provided a powerful method for investigating enzyme induction.^{65,66} The potential for urinalysis of endogenous steroid metabolites as a means of fingerprinting cytochrome P-450 isoenzyme profiles in individuals, however, was overtaken by the upsurge in molecular biology techniques. These not only added a further dimension to detection and characterisation, but also provided a long awaited basis for logical classification and nomenclature of a host of P-450s previously existing under numerous aliases.

In the late 1970s and early 1980s enormous information accrued on the potential mechanisms by which drugs caused and antidotes prevented hepatic damage. A large number of studies were carried out *in vitro* and used single end points such as covalent binding, depletion of intracellular glutathione or the impairment of cellular and biochemical events. The dangers of interpreting such *in vitro* observations in isolation soon became clear, however, stressing the value to toxicology of *in vivo* investigations for several reasons. First, the interplay between different enzymes acting on the same substrate was difficult to assess except *in vivo*, because drugs are not metabolised by a single isoenzyme. Secondly, competing detoxication pathways could determine a drug's toxic potential. In addition, non-parenchymal cells were increasingly being shown to be important in mediating and promulgating hepatotoxic events,⁶⁷ and the emerging complexity and polymorphism of cytoprotective mechanisms could also influence susceptibility to tissue damage. Experimental studies in the Liver Unit confirmed that chronic ingestion of ethanol potentiated paracetamol hepatotoxicity, whereas acute administration provided a protective effect.⁶⁸ These findings could not be explained solely by induction or inhibition of the enzymes producing the toxic metabolite, however, as had previously been considered to be the case,⁶⁹ and complementary roles for effects on glutathione depletion and intermediary metabolism were proposed.⁷⁰ More recent evidence from convincingly large series of patients suggests that chronic alcohol consumption and anticonvulsant therapy adversely affect outcome after paracetamol overdose (Bray *et al*, in preparation).

From 1986 . . . to the future

Advances over the past five years have been dominated by the characterisation of genetic factors regulating the control of drug metabolising enzymes, together with an increased understanding of the roles of glutathione and calcium

Figure 3: Pharmacological influences on the response to cyclosporine immunosuppression.

homeostasis in mediating and moderating tissue damage. Gene probing has provided a molecular basis for phenotypic expression of extensive and poor debrisoquine hydroxylation, where functionally defective gene products rather than complete gene deletions appear responsible.⁷¹ Parallel studies are strengthening links between the functional activities of structurally related isoenzymes across species; the extent of structural homology is providing clues as to the most appropriate animal models for drug metabolism in some cases. Experimental work is also beginning to clarify the role of endogenous compounds in regulating drug metabolism. For example, glucocorticoids have been shown to enhance the transcriptional activation of both cytochrome P-450 1A and glutathione S-transferase genes after enzyme induction by polycyclic aromatic hydrocarbons.⁷² The glutathione transferase isoenzymes are important in a transport and metabolic capacity and their polymorphism may contribute towards variability in the intracellular transport of drugs and the activity of detoxication pathways with glutathione.⁷³ Also emerging has been the importance of subcellular glutathione pools and their relevance to the regulation and maintenance of organelle function – for example, the mitochondria.⁷⁴ There is increasing interest in the role of interorgan glutathione homeostasis in determining toxic events outside the liver. For example, the kidney and lung extract glutathione from the plasma pool, which is apparently replenished only by the liver. Glutathione also maintains thiol/disulphide status, which in turn is crucial to the maintenance of subcellular integrity by calcium, so controlling the activity of plasma membrane ATPases, mitochondrial dehydrogenases and the activation of phospholipases and endonucleases. Disturbance of glutathione and calcium homeostasis is seen increasingly as a unifying mechanism in toxin related cell injury of different types.⁷⁵

Recent studies in the Liver Unit have shown that S-adenosylmethionine is beneficial and possibly complementary to N-acetylcysteine,

another glutathione precursor, in preventing experimentally induced paracetamol hepatotoxicity (Bray, Tredger, and Williams, in preparation). The benefit of late treatment with N-acetylcysteine (after the 16 h limit previously suggested) for reducing mortality and preventing worsening encephalopathy in paracetamol induced fulminant hepatic failure has also been convincingly shown.⁷⁶ Another late treatment studied experimentally was calcium channel blockade with diltiazem. This reduced paracetamol hepatotoxicity and stimulated regenerative activity when given to mice late after dosing with paracetamol when conventional antidotes had ceased to be maximally effective.⁷⁷

LIVER TRANSPLANTATION

The increasing use of liver transplantation at King's has dominated research activity in the drug metabolism group over the last five years. Selection of patients for transplantation demanded improved methods for evaluating prognosis and rekindled an interest in evaluating liver function. Stimulated by Rudi Preisig's sabbatical visit in 1987, studies were made of the value of the cytochrome P-450 I-based caffeine clearance test but it was found susceptible to interference by intensive drug therapy in fulminant hepatic failure and after liver transplantation.⁷⁸ Measurements of coagulation factors V and VIII showed no such interference and their value in predicting prognosis in paracetamol-induced fulminant hepatic failure⁷⁹ and acute alcoholic hepatitis (Pereira, Langley, Bird *et al*, in preparation) has been recently demonstrated.

The widespread use of cyclosporine after liver transplantation has stimulated Liver Unit research on drug monitoring and pharmacokinetics. Immunosuppression with cyclosporin is complicated in liver transplant recipients by the role played by the graft in influencing absorption (through bile production) as well as metabolism and the biliary excretion of metabolites.⁸⁰ The majority of immunosuppressive activity is associated with the parent drug and management has been improved by specific monitoring of cyclosporin itself. The value of radioimmunoassay with a specific monoclonal antibody was validated in liver graft recipients, showing a close agreement of cyclosporine results with those measured by liquid chromatography.⁸¹ In later collaborative studies, the influence of biliary T-tube status and graft function on cyclosporine bioavailability and clearance was clearly observed.⁸² Recent technical developments in cyclosporine assay procedures have also been appraised, but there is a growing need for alternative methods to routine drug monitoring in evaluating the efficacy of immunosuppressive therapy, as Figure 3 illustrates. One option presently under consideration involves the screening of activation markers on peripheral blood lymphocytes (Gonde, Cohen, Tredger *et al*, in preparation).

It is clear that the future for research by the drug metabolism group in the Institute of Liver Studies will extend beyond the perspective of metabolism and hepatotoxicity. Continued research in particular areas of clinical relevance,

however, such as paracetamol hepatotoxicity, seems assured. Here, and for many hepatotoxic drugs, characterisation of qualitative aspects of the covalent binding process is of obvious relevance. Obtaining evidence for the intracellular sites involved, the enzymes and cellular functions affected and the possibility of reversing such interactions is of high priority. This approach may be relevant to those drugs which are toxic directly through reactive metabolites and those invoking immune responses. For the latter, one emphasis in the immediate future must be to identify how drug related antigens are translocated to the cell surface, complementing investigations into the role of endogenous molecules such as cytochrome P-450 in autoimmune disease. Drug metabolists and immunologists together must then unravel the mechanisms underlying idiosyncrasy in immune mediated drug reactions. This may not only refine preclinical screening for drug toxicity, but will also lead to new treatment regimens to improve the safety of existing drugs.

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