New insights into drug metabolism and toxicology

Report of a symposium on drug metabolism and toxicity held at the British Pharmacological Society Meeting in Cardiff, UK, in July 2000

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Introduction

Adverse drug reactions present a major clinical problem: for example, recent data show that poisoning accounts for about 4000 deaths each year in England and Wales alone. Furthermore, in addition to being a major cause of morbidity and mortality, adverse drug reactions can prevent effective drug therapy and, in extreme cases, can lead to the withdrawal of potentially valuable medicines. Pharmacological and clinical aspects of drug toxicity were reviewed at a symposium, *Drug metabolism and toxicity*, which formed part of the British Pharmacological Society meeting in Cardiff, UK, in July 2000. The meeting was organized by P. J. Nicholls and P. A. Routledge (Welsh School of Pharmacy and University of Wales College of Medicine, Cardiff, UK) and chaired by P. J. Nicholls.

Mechanisms of drug toxicity

The mechanisms underlying drug toxicity were reviewed by B. K. Park (University of Liverpool, Liverpool, UK). Adverse drug reactions can be classified into four principal groups (Table 1). Type A reactions, which account for 70–80% of all reactions, represent an augmentation of the normal effect of the drug. Such reactions are dose-dependent and hence can usually be reversed by lowering the dose. By contrast, type B reactions are idiosyncratic and do not show a clear dose–response, and there is frequently a high degree of individual susceptibility to such reactions.

Type B reactions can result from accumulation of drug in individuals in whom drug metabolism is impaired.

For example, terfenadine is a prodrug that undergoes first pass carboxylation to form the active agent; terfenadine itself, however, can prolong the QT interval, leading to potentially fatal dysrhythmias (torsades des pointes) in some patients. This risk is increased in patients with liver disease, and in patients treated with drugs such as ketoconazole or erythromycin, in whom the conversion of terfenadine to its active metabolite is impaired. Most such reactions are now readily predictable since the role of specific cytochrome P450 isoforms in drug metabolism can be determined from *in vitro* studies, allowing potential drug interactions to be identified. The risk of such interactions in clinical practice can then be assessed in pharmacokinetic and pharmacodynamic studies.

There is considerable genetic variation in drug metabolism. Approximately 10% of individuals show a deficiency in the CYP2C6 isoform (the principal P450 isoform involved in drug metabolism in humans), and polymorphism of CYP2C9 is also attracting attention due to the involvement of this isoform in warfarin metabolism. The best documented example of toxicity arising as a result of such polymorphism is perhexilene, the half-life of which is markedly increased, from 2–6 days to 9–22 days, in individuals with CYP2D6 (debrisoquine hydroxylase) deficiency. This drug accumulation is associated with hepatotoxicity and peripheral neuropathy. A variety of *in vitro* and *in vivo* techniques are now available to predict such genetic variations in drug metabolism.

In addition to being responsible for drug elimination, drug metabolizing enzymes such as cytochromes P450 can also produce reactive metabolites (bioactivation). Such reactions are less predictable, and can cause potentially serious adverse effects. For example, paracetamol is primarily metabolized by phase II glucuronidation and sulphation, but approximately 5–10% is converted to a toxic quinoneimine metabolite, which is detoxified by glutathione. When paracetamol is taken in overdose,

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Table 1 Principal classes of adverse drug reactions [1]

Type A	Reactions that are predictable from the known primary or secondary pharmacology of the drug
Type B	Reactions that are not predictable from the known pharmacology of the drug
Type C	Reactions whose characteristics can be predicted or rationalized from the chemical structure of the drug
Type D	Carcinogenic or teratogenic reactions

the phase II pathways become saturated, leading to increased production of the quinoneimine. This in turn leads to glutathione depletion and hepatocellular damage. Recent studies have shown that exposure to paracetamol leads to induction of the *c-fos* and *c-jun* genes, resulting in increased expression of γ -glutamylcysteinyl synthetase. However, glutathione production is actually decreased, due to cellular damage resulting from a direct effect of paracetamol on the cell.

Hypersensitivity reactions such as anaphylaxis, blood dyscrasias, and skin reactions, account for approximately 3500 serious adverse events each year but remain poorly understood. Studies with drugs associated with well characterized hypersensitivity reactions, such as carbamazepine, have shown that these drugs are converted to reactive metabolites that provoke the formation of antibodies to the drug or, in some cases, to cytochrome P450. However, the development of such an immune response, depends on the prevailing cytokine profile. Studies with carbamazepine suggest that exposure of antigen-presenting cells to the drug or its reactive metabolites leads to recruitment of cytotoxic CD8 cells and reversal of the normal CD4:CD8 ratio. The CD8 cells then infiltrate the epidermis, resulting in tissue damage. Severe hypersensitivity reactions are extremely rare. Preliminary data suggest that severe skin reactions are associated with the HLA-DR3 phenotype, but further studies are needed to clarify the role of individual genes. It is clear that both changes in drug metabolism and immunological factors are involved in the development of hypersensitivity reactions.

Cytoprotective mechanisms and drug toxicity

The contribution of reactive metabolites to drug toxicity was discussed further by M. W. Anders (University of Rochester Medical Centre, Rochester, USA). Hard or soft electrophiles are probably the most common reactive metabolites but a variety of other types may also be formed, including stable toxic metabolites such as carbon monoxide and cyanide, organic free radicals, and reduced oxygen species. Cytoprotective mechanisms exist for most of these types of metabolite.

In general, soft acids or electrophiles react with soft bases or nucleophiles. Conversely, hard acids or electrophiles react with hard bases or nucleophiles. This makes

it possible to predict whether cytoprotective mechanisms will react with specific metabolites. For example, glutathione, which is a soft nucleophile, offers little protection against carcinogens, most of which are hard nucleophiles.

Glutathione is the most abundant sulphur-containing nucleophile, and is present in most tissues in concentrations of 2–10 mmol l⁻¹. It plays a key role in cytoprotection, primarily through its ability to serve as a second substrate for glutathione transferases which metabolize a variety of soft electrophiles. Glutathione transferase is the first step in the mercapturic acid pathway, which leads to the formation of readily excreted S-substituted N-acetyl-L-cysteine metabolites. Moreover, glutathione contributes to the detoxification of a variety of drugs via enzymes such as glutathione peroxidases, formaldehyde dehydrogenase, and glyoxylase I and II, in addition to nonenzymatic reactions with electrophiles and free radicals.

Organic free radicals can be produced by reduction of perhaloalkanes such as carbon tetrachloride, or during oxidative stress. These agents are primarily removed by chain-breaking antioxidants such as vitamin E, resulting in the formation of stable radicals. In addition, they may react with glutathione to produce glutathione thiol radicals. Organic free radicals may also dimerize to form two-electron species, but for kinetic reasons this mechanism is rare.

Reactive oxygen species, such as superoxide or hydroxyl radicals or peroxides, are produced in a variety of cellular processes, including oxidation of flavoproteins and redox cycling of quinones. Superoxide anions and hydrogen peroxide are detoxified by superoxide dismutase and selenium-dependent glutathione peroxidase, respectively. In addition, hydrogen peroxide can be broken down to water and oxygen by catalase. No specific cytoprotective mechanisms against hydroxyl radicals exist.

As described above, many enzymes involved in drug detoxification are also involved in bioactivation to potentially harmful compounds. Glutathione S-conjugation, for example, is both an important detoxification mechanism and the first step in the β -lyase-dependent activation of nephrotoxic haloalkanes. Thus cytotoxicity results from an imbalance between these detoxification and bioactivation functions, and this has important implications for drug design.

Regulation of cytochrome P450

In addition to drug metabolism, the cytochrome P450 enzyme family has a variety of physiological roles, including the activation of vitamin D₃, steroid synthesis, and eicosanoid signalling. As discussed by E. T. Morgan (Emory University School of Medicine, Atlanta, USA), cytochrome P450 activity is affected by a variety of physiological and pathophysiological factors that together determine the drug metabolism phenotype of an individual.

In rats, the expression of hepatic P450 is sexually differentiated. The CYP2C11 isoform predominates in males, whereas the CYP2C12 isoform is predominant in females. This differentiation is mediated by testosterone, which acts on the anterior pituitary to influence patterns of growth hormone secretion. In males, growth hormone is secreted episodically with peaks every 3–4 h, whereas in females secretion is virtually continuous.

Diabetes and starvation have marked effects on P450 expression. These stimuli tend to induce the expression of CYP2E1 and CYP4A, and inhibit the expression of CYP2C11 and CYP2C12. P450 expression also increases during growth and development, but different isozymes are expressed at different stages of ontogenesis.

Infections and inflammatory stimuli such as bacterial endotoxin (lipopolysaccharide) decrease the activity of many cytochrome P450 isozymes. In humans, such effects are manifested as alterations in drug pharmacokinetics in patients with infections. *In vitro*, incubation of cultured hepatocytes with inflammatory cytokines such as interleukin (IL)-1, IL-6, or tumour necrosis factor (TNF) reduce the expression of many cytochrome P450 messenger RNAs, as is seen in the livers of rats exposed to inflammatory stimuli. It has therefore been assumed that these cytokines are predominantly responsible for the decrease in P450 activity, but it seems likely that other physiological mediators, such as glucocorticosteroids, catecholamines, and growth hormone, also contribute.

Some cytochrome P450s can be induced by inflammatory stimuli in specific tissues. In rat kidney, for example, bacterial endotoxin leads to increased expression of CYP4A2, CYP4A3, and CYP2E1.

Recent studies have investigated the mechanisms by which inflammatory stimuli down regulate the transcription of cytochrome P450 mRNAs. A nuclear factor (NF)- κ B binding site has been identified, which spans the transcription initiation site of the CYP2C11 gene. Exposure to lipopolysaccharide leads to increased binding of NF- κ B to the CYP2C11 gene. Conversely, mutations at this binding site reduce the ability of IL-1 or lipopolysaccharide to suppress CYP2C11 gene expression.

In addition to these transcriptional effects on P450 gene expression, there is evidence that inflammatory

stimuli also exert post-transcriptional effects. For example, although exposure to lipopolysaccharide reduces CYP2C11 mRNA by approximately 80%, protein levels are not suppressed to the same extent. Moreover, CYP2C11 mRNA has a long half-life (approximately 9.5 h) in cultured hepatocytes, and hence down regulation of transcription alone is not sufficient to explain changes in mRNA levels. Such findings suggest that inflammatory stimuli may affect the stability of the expressed protein.

New insights into paracetamol toxicity

Despite the availability of an effective antidote, paracetamol poisoning remains a major public health problem, accounting for 48% of hospital admissions for poisoning and 200–300 deaths each year in the UK. Recent developments in the management of paracetamol poisoning were reviewed by A. L. Jones (National Poisons Information Service, London, UK).

The use of the paracetamol antidote *N*-acetylcysteine in early (<12 h) paracetamol poisoning is well established, but the choice of oral or intravenous administration has remained controversial. However, a recent meta-analysis suggests that both routes are equally effective. There is evidence that activated charcoal is useful in the treatment of early paracetamol poisoning, provided it is given within 1 h. For example, in a recent study the use of activated charcoal within 1–2 h significantly reduced the need for *N*-acetylcysteine.

In patients with late paracetamol poisoning, *N*-acetylcysteine is normally given until either the prothrombin time (a sensitive marker of paracetamol-induced liver damage) improves or liver transplantation takes place. The mechanisms by which *N*-acetylcysteine acts in patients with advanced paracetamol poisoning are unknown but free radical scavenging, haemodynamic effects, and changes in oxygen kinetics have all been proposed. However, there is no firm evidence that *N*-acetylcysteine improves cardiac output or oxygen uptake; it is possible that the compound has cytoprotective effects such as reduction of oxidized thiol groups or blockade of reactive electrophiles. Other developments in the management of late paracetamol poisoning include improvements in intensive care for patients with acute liver failure and the use of biological markers such as TNF α to identify patients at risk of liver failure.

A few case reports have suggested that patients with glutathione depletion or enzyme induction are at increased risk of paracetamol poisoning. In animals, enzyme induction due to chronic alcohol consumption is associated with paracetamol toxicity. However, in humans, alcohol has been shown to be protective if taken with paracetamol, but it is unclear whether chronic over-use increases the risk of paracetamol toxicity.

Recent case reports have queried whether a tiny minority of patients may develop liver failure with therapeutic doses of paracetamol. However, the combined data from all these reports suggest that this is very unlikely and that the case reports represent overdose rather than therapeutic dose. Interpretation of such reports is also complicated by other confounding factors such as errors in recalling the dose and products taken, delayed gastric emptying, enzyme induction, and glutathione depletion.

What can be done to prevent paracetamol poisoning? Recent studies suggest that the introduction in 1998 of statutory restrictions on paracetamol sales in the UK may be associated with decreases in paracetamol overdoses and severe paracetamol poisoning but the data are equivocal. The addition of methionine to paracetamol preparations was potentially dangerous. Limiting publicity given to overdoses in order to prevent 'copycat' activity, has also been suggested as a possible approach.

The toxicology of tamoxifen

The nonsteroidal antioestrogen tamoxifen has been used for many years as a well tolerated adjuvant therapy in breast cancer, and more recently has been approved for use as a chemopreventive agent in healthy women at risk of the disease. However, this use raises potential safety concerns since tamoxifen has been shown to induce liver tumours in rats and to increase the risk of endometrial cancer in women. The implications of these findings were discussed by E. A. Martin (Medical Research Council Toxicology Unit, Leicester, UK).

In rats, treatment with tamoxifen, at doses comparable with those used therapeutically, leads to the dose-dependent formation of hepatic DNA adducts. By contrast the tamoxifen analogue toremifene does not induce DNA damage in rats at doses 100-fold higher than those used therapeutically. A threshold value of 128 adducts/ 10^8 nucleotides appears to be required for tumour formation in rats.

There are marked species differences in the metabolism of tamoxifen, which are related to the genotoxic potential of the compound. In both rats and humans, the major metabolic pathways are 4-hydroxylation, *N*-oxidation and *N*-demethylation, although the relative contributions of these pathways differ between species. However, in rats the minor metabolite α -hydroxytamoxifen forms a sulphate ester which reacts with DNA to form the

major adducts in the liver. By contrast, in humans α -hydroxytamoxifen undergoes glucuronidation to form a stable compound which does not interact with DNA. Furthermore α -hydroxytamoxifen undergoes metabolism in rat liver to produce *N*-desmethyl, *N*-oxide and quinone derivatives, all of which form adducts with DNA.

In contrast to those in the rat liver, studies in humans have shown no significant increase in hepatic DNA damage during tamoxifen treatment. Furthermore, the relative risk of DNA damage is substantially lower in humans than in rats. The normal therapeutic dose of tamoxifen is 20 mg day^{-1} , which corresponds to approximately 0.3 mg kg^{-1} , whereas in rats doses of $30 \text{ mg kg}^{-1} \text{ day}^{-1}$ are required to cause tumour formation. Furthermore, the formation of α -hydroxytamoxifen is three times faster, and subsequent sulphation five times faster, in rat liver than in human liver. Conversely, the rate of glucuronidation of α -hydroxytamoxifen is approximately 100 times faster in humans than in rats. Together, these figures give a safety factor of about 150 000 for humans, compared with rats.

It is known that the risk of endometrial cancer is increased in tamoxifen-treated women, but it is unclear whether this is associated with genotoxicity. It has not been established whether α -hydroxytamoxifen is formed in human uterine tissue, and accelerator mass spectrometry studies suggest that, following a single dose of tamoxifen, the frequency of DNA adducts is approximately $0.052/10^8$ nucleotides in the myometrium and $0.013/10^8$ nucleotides in the endometrium. These values are considerably lower than those shown to be required for tumour formation in rat liver, which suggests that, even if tamoxifen does undergo metabolic activation leading to DNA damage in the uterus, this is unlikely to be related to the increased risk of endometrial cancer.

In conclusion, tamoxifen continues to provide considerable benefit in terms of reducing mortality from breast cancer, and the available data suggest that the prophylactic use of tamoxifen is unlikely to produce a significant risk of genotoxicity.

Reference

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