143P MECHANISMS OF DRUG TOXICITY: AN OVERVIEW

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Drug toxicity is a major complication of drug therapy and drug development. Adverse drug reactions may be classified as either type A which are predictable (pharmacology or pharmacokinetics) or type B which are unpredictable. Type B adverse drug reactions include anaphylaxis, blood dyscrasias, severe cutaneous reactions, as well as hepatotoxicity. They are usually serious and can be fatal.

At present, prediction of idiosyncratic ADRs is not possible because of a lack of suitable animal models and a lack of understanding of the basic mechanisms involved in the toxicity when it does occur in man. An understanding of the role of drug metabolism, has provided insight into the mechanisms of many different types of drug toxicity.

The primary physiological role of drug metabolism is that of detoxication. Drug toxicity may involve 1) excessive drug accumulation (e.g. perhexilene), 2) drug bioactivation (e.g. paracetamol, tamoxifen), 3) immunoallergic hepatitis or novel mechanisms. Many idiosyncratic reactions appear to have an immunological aetiology. Nevertheless, the sequence of events by which a simple chemical can elicit severe damage to the liver remains poorly understood and alternative novel mechanisms of toxicity must also be explored.

There is increasing direct evidence for the role of T lymphocytes in severe skin reactions and an assumption that such lymphocytes play a central role in immunoallergic hepatitis. The clinically well characterised hypersensitivity reactions associated with drugs such as carbamazepine will be used as examples to attempt to define the pathophysiological pathway from the initial bioactivation of the parent drug right through to the clinical manifestation of tissue damage. In particular, the roles of cytokines and T cells in determining the nature and severity of the toxicity will be discussed, as will recent advances in the role of both inter- and intra-cellular signalling in the regulation of the immune response to drugs and their metabolites.

The possible role of secondary factors, such as concomitant disease processes, in determining inter-individual variation in susceptibility to idiosyncratic reactions will also be explored.

144P THE ROLE OF CYTOPROTECTIVE MECHANISMS IN MODULATING TOXICITY.

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Studies conducted over several decades have established the concept that the toxicity of many drugs and chemical is associated with their biotransformation to toxic metabolites (*q.v.*, Anders, M. W., Ed. (1985) *Bioactivation of Foreign Compounds*, Academic Press, Orlando). Several types of toxic metabolites may be formed: hard or soft electrophiles; stable, but toxic, metabolites; organic free radicals; and reduced oxygen species.

Although a clear association between the formation of toxic metabolites and chemically induced cytotoxicity is well established, the mechanism by which toxic metabolites produce cell injury and death is still poorly understood. Both chemical and enzymatic mechanisms contribute to cytoprotection, although the latter is generally more important.

Water, which is present at intracellular concentrations of about 55 M, affords some protection against hard electrophilic species, e.g., carbocationic species. Water also serves as a second substrate for epoxide hydrolase, which converts electrophilic epoxides to dihydrodiols, which may, however, be further biotransformed to toxic metabolites.

Glutathione, a soft nucleophile, plays a central role in cytoprotection, largely by serving as a second substrate for the glutathione transferases, which catalyze the reaction of glutathione with a range of soft electrophiles. This reaction is the first step in the mercapturic acid pathway and leads to the formation of readily excreted *S*-substituted *N*-acetyl-L-cysteine metabolites.

Although most mercapturic acids show little toxicity, glutathione *S*-conjugate formation is also the first step in the b-lyase-dependent bioactivation of nephrotoxic haloalkenes. Some of the glutathione transferases catalyze the reduction of organic hydroperoxides, which are formed during lipid peroxidation.

The formation of glucuronide and sulfate conjugates of phenolic metabolites is usually a detoxication mechanism, particularly with phenolic metabolites that may undergo redox cycling. Reduced oxygen species are formed in a range of cellular processes, and enzymatic mechanisms for their detoxication are available. The sequential one-electron reduction of oxygen affords superoxide anion radical, which is a substrate for superoxide dismutase, hydrogen peroxide, which is a substrate for selenium-dependent glutathione peroxidase, and the hydroxyl radical for which no enzymatic detoxication mechanism is available. Carbon- or oxygen-centered free radicals formed during lipid peroxidation may react chemically with chain-breaking antioxidants, *e.g.*, a-tocopherol.

145P PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL REGULATION OF CYTOCHROME P450.

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Hepatic cytochrome P450 enzymes dictate the clearance of many drugs and toxins from the blood, while some drugs and toxins require P450-catalyzed metabolism to have their effects. The activities of these enzymes is modulated by genetic and epigenetic factors, that together determine the individual's drug metabolism phenotype. Epigenetic factors include physiological stimuli such as hormones or disease states. For example, important changes in P450 expression occur during development. Expression of hepatic P450 enzymes is sexually differentiated in the livers of rodents, due to sex differences in the plasma pattern of growth hormone secretion. Diabetes and starvation induce the expression of CYP2E1 and CYP4A family enzymes, by different mechanisms.

Our laboratory has focussed on the regulation of cytochrome P450s in models of infectious and inflammatory disease. Infections in humans cause a decrease in drug clearance that can lead to clinical drug toxicity. In animals, inflammatory stimuli cause decreases in hepatic P450 content and associated activities, as well as decreased expression of many cytochrome P450 mRNAs. The in vivo effects of inflammation can be mimicked by incubating hepatocytes with various inflammatory cytokines. While these cytokines must undoubtedly contribute to the down-regulation of P450 expression seen in vivo, other factors associated with the disease state, such as stress and undernutrition, may contribute. Not all P450s are suppressed by inflammatory stimuli; some are unaffected or induced.

The major form of P450 in the livers of untreated male rats is CYP2C11, whereas CYP2C12 is a female-specific enzyme. Transcription of these P450 genes is inhibited after treatment of rats with bacterial endotoxin (LPS). However, kinetic and other evidence suggests that inflammation causes changes in P450 mRNA and protein degradation that can in some cases complement, and in some cases oppose, the transcriptional effects.

Our laboratory has been studying the mechanism of suppression of *CYP2C11* gene transcription by inflammatory cytokines. We found a sequence that spans the transcription initiation site of the *CYP2C11* gene, and that binds the cytokine-regulated transcription factor NF-kB. Disruption of this binding site results in the inability of interleukin-1 and LPS to suppress reporter gene expression driven by the *CYP2C11* promoter, whereas the response to interleukin-6 is unaffected. This represents a first step in understanding the transcriptional regulation of P450 genes by cytokines.

Supported by grants GM46897 and GM53093 from the NIH.

146P WHAT IS NEW IN PARACETAMOL (ACETAMINOPHEN) POISONING ?

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Paracetamol poisoning accounts for 48% of hospital admissions for poisoning and 200-300 deaths every year in the UK. Recent evidence supports oral activated charcoal use if it is given within 1-2 hours of ingestion (Buckley *et al.*, 1999a).

There is good evidence to support the use of N-acetylcysteine in early paracetamol poisoning and its action by hepatic glutathione repletion is well understood. Whether oral or intravenous is the route of choice for N-acetylcysteine remains controversial (Buckley *et al.*, 199b)

A few cases suggest that patients with enzyme induction or glutathione depletion are at increased risk of paracetamol poisoning (McClements *et al.*, 1990). Alcohol is protective if co-ingested with paracetamol, but whether chronic excessive use increases risk when a paracetamol overdose is taken remains controversial (Prescott, 2000). Recent case reports have also raised the possibility of toxicity from paracetamol given in therapeutic doses but they include patients with acute overdose and other confounding factors (Bridger *et al.*, 1998; Miles *et al.*, 1998).

Comparatively little is known about the mechanisms of late paracetamol poisoning (Jones, 1998). N-acetylcysteine is given to patients who present late, until either their PTR improves or transplantation occurs. It may act by free radical scavenging, by haemodynamic actions or by oxygen kinetics (ones, 1998; Walsh *et al.*, 1998). Intensive care support for acute liver failure has progressed and transplantation offers a future for some patients (Larsen *et al.*,2000; Mutimer *et al.*,1994). The use of biological markers to identify those at risk of developing liver failure and identifying their pathophysiological role is still in its infancy (Simpson *et al.*,2000).

Various methods of preventing poisoning have been considered, including reduction of pack size, adding methionine (Jones *et al.*,1997 McAuley *et al.*,1999) and reduced publicity leading to less "copy cat"activity (Veysey *et al.*, 1999).

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147P THE TOXICOLOGY OF TAMOXIFEN

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The antioestrogenic drug tamoxifen is used successfully as an adjuvant therapy in the treatment of breast cancer. This drug has been approved by the FDA for prophylactic use in healthy women at increased risk of this disease. However, the use of tamoxifen is not without risk. Tamoxifen is a liver carcinogen in rats and increases the risk of endometrial cancer in women. Tamoxifen induces liver DNA damage in rodents as detected by the ^{32}P-postlabelling assay, demonstrating that tamoxifen is a genotoxic carcinogen *in vivo*.

Toremifene, a structural analogue of tamoxifen, does not induce significant DNA damage or liver tumours in rats. In rats, neither drug results in uterine DNA damage detectable by ^{32}P-postlabelling. In women taking tamoxifen therapeutically, little or no DNA damage is detected in the liver or uterine tissues. Classical *in vitro* mutagenicity tests give negative results for tamoxifen. However, tamoxifen causes dose related increases in the mutation frequency of the *lacI* gene in liver of transgenic Big Blue rats. Uterine DNA shows no change in mutation frequencies. Toremifene is negative for both uterus and liver.

Phase 1 detoxication of tamoxifen produces N-desmethyltamoxifen, 4-hydroxytamoxifen and tamoxifen N-oxide as main metabolites. Tamoxifen itself is not genotoxic but can be metabolised by CYP to more reactive species. a-Hydroxytamoxifen, a minor phase 1 metabolite, is further activated to a sulphate-ester which reacts with DNA via a carbocation to form the major adducts in rat liver.

The therapeutic dose of tamoxifen is 20 mg daily ~ 0.3 mg/kg. To detect DNA damage and develop liver tumours, rats receive 30 mg/kg/day. Metabolism of tamoxifen to a-hydroxytamoxifen in rat liver microsomes is 3 fold higher than in human and subsequent bioactivation (sulphation) is ~5 faster in rats. Detoxication via glucuronidation of a-hydroxytamoxifen in human liver microsomes is ~100 fold faster than in rats. This gives a ~15000 fold safety factor for women.

Thus from our understanding of the mechanisms of toxicity and metabolism of tamoxifen, it is highly unlikely to be an hepatic carcinogen in humans. It is unclear whether the mechanism of uterine carcinogenicity is associated with genotoxicity. Even if tamoxifen undergoes uterine metabolic activation resulting in DNA damage, it is unlikely to be causally related to the mechanism of carcinogenesis.

Despite the risks, the prophylactic use of tamoxifen in women at risk of breast cancer provides considerable benefit in reducing mortality from this disease.

148P BIOCHEMICAL AND BIOPHYSICAL EVIDENCE OF G PROTEIN-COUPLED RECEPTOR DIMERIZATION

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A growing body of evidence suggests that G protein-coupled receptors (GPCRs) may form functionally relevant homo- and hetero-dimers. However, most biochemical approaches used to study such dimerization are based on co-immuno-precipitation techniques that require solubilization of these hydrophobic proteins that could lead to artefactual aggregation.

In an effort to directly assess the existence of GPCR dimers in whole cells, we used a newly developed assay known as Bioluminescence Resonance Energy Transfer (BRET). BRET is the non-radiative transfer of energy between a luminescent donor, e.g. luciferase (luc) and a fluorescent acceptor, e.g. the Green Fluorescent Protein (GFP). Upon di-merization of the donor and acceptor, the luminescence resulting from the catalytic degradation of coelanterazine by luc is transferred to the GFP that, in turn, emits fluorescence at its characteristic wavelength.

To apply this approach to the study of β-adrenergic receptor (βAR) dimerization, fusion βAR-luc and βAR-GFP constructs were co-expressed in HEK-293 cells and the occurrence of BRET assessed.

The detection of BRET under basal conditions unambiguously demonstrated that βARs form constitutive complexes that are minimally dimeric. The selectivity of these interactions was confirmed by showing that no BRET occurred between the βARs and other unrelated GPCRs.

Stimulation with the agonist isoproterenol led to an increase in BRET, indicating that receptor dimers play a role in signal transduction. This notion is further supported by the observation that a peptide that blocks dimerization also inhibits receptor function. Using the V2 vasopressin receptor (V2R) as another GPCR model, we found that the formation of dimers occurs very early following receptor biosynthesis suggesting that it could play a role in proper folding and maturation. Indeed, both immature (precursor) and mature forms of receptor were observed as dimers. Furthermore, mutant forms of the V2R that cannot reach the cell surface are also observed as dimeric complexes.

Taken together, our data demonstrate that GPCRs exist as dimers in whole cells, and suggest that dimerization could play important roles in both receptor ontogeny and function.