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Toxicophores: Investigations in drug safety

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Abstract

Adverse drug reactions, such as hepatotoxicity, blood dyscrasias and hypersensitivity are a major obstacle for the use and the development of new medicines. Many forms of organ-directed toxicity can arise from the bioactivation of drugs to the so-called *chemically reactive* metabolites, which can modify tissue macromolecules. It is well established that the toxicities of model hepatotoxins, such as acetaminophen, furosemide, bromobenzene and methapyrilene can be correlated with the generation of chemically reactive metabolites, which can be detected by measurement of the irreversible binding of radiolabelled material to hepatic protein and/or the detection of stable phase II metabolites such as glutathione conjugates. The basic chemistry of the reaction of such metabolites with model nucleophiles is relatively well understood. A major challenge is to define how certain reactive intermediates may chemically modify critical proteins and how modification of specific amino acids may alter protein function which in turn may affect cell signalling, regulation, defence, function and viability. This in turn will determine whether or not bioactivation will result in a particular form of drug-induced injury. It is now clear that even relatively simple reactive intermediates can react in a discriminative manner with particular cellular proteins and even with specific amino acids within those proteins. Therefore, both non-covalent, as well as covalent bonds will be important determinants of the target protein for a particular reactive metabolite. Mammalian cells have evolved numerous defence systems against reactive intermediates. Sensitive redox proteins such as Nrf-2 recognise oxidative stress and electrophilic agents, through oxidation or covalent modification of thiol groups. Defence genes, such as epoxide hydrolase and glutamate cysteine ligase then become up-regulated in an attempt to reduce the oxidising environment. However, whether the liver receives mild or severe injury depends upon extra-cellular signalling processes between the hepatocytes and non-parenchymal cells, particularly kupffer and natural killer cells (NK/NK T cells). Determination of the nature and downstream effect of these extra-cellular signalling processes is critical in order to design better predictive hepatotoxicity screens. More importantly, to understand and manipulate these signalling processes will aid in the design of safer therapeutic agents, but also contribute to the clinical management of liver disease.

Keywords: Toxicophore; Metabolism; Bioactivation; Drug design; Hepatotoxicity; Liver

1. Introduction

Adverse drug reactions (ADRs) are a major cause of patient morbidity and a significant cause of patient mortality (Lazarou et al., 1998; Pirmohamed et al., 1998). ADRs can be classified into three categories (Table 1) (Park et al., 1998). Type A reactions account for approximately 80% of ADRs and are predictable from the known primary or secondary pharmacology of the drug. They show simple dose–response relationships and therefore, can usually be avoided by dose reduction and are only rarely life-threatening. Examples of this type of reac-

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Table 1
Mechanistic classification of adverse drug reactions (Park et al., 1998)

TYPE A (augmented)	Reactions which are predictable from the known pharmacology often representing an exaggeration of the pharmacological effect of the drug
TYPE B (idiosyncratic)	Reactions are not predictable from a knowledge of the basic pharmacology of the drug and exhibit marked inter-individual susceptibility
TYPE C (chemical)	Reactions whose biological characteristics can either be predicted or rationalised in terms of chemical structure

tion are bleeding with anticoagulants and confusion with antidepressants. In contrast, type B reactions cannot be either predicted or rationalised from the pharmacological profile of the drug. Therefore, at present, type B reactions cannot be predicted either during the preclinical or early clinical phases of drug development. Although less common than type A reactions, type B reactions can be serious and may be life-threatening. Such reactions do not show any simple relationship to dose, in that some patients can tolerate very high doses, whereas others are sensitive to smaller doses (Park et al., 1998; Uetrecht, 1999, 2003). Type B reactions are extremely host-dependent, usually uncommon and therefore referred to as idiosyncratic. The definition of the term idiosyncratic suggests that there may be a genetic component in the susceptibility to such reactions. Thus, it is essential to understand the reason for the idiosyncratic nature of a drug reaction by investigation of the biology of the patient, as well as the pharmacology and the chemistry of the drug. Type C reactions are those that can be predicted from the chemical structure of either the drug itself or that of a metabolite. The drug metabolism literature indicates that there is an increasing awareness of chemical sub-structures that can lead to toxic metabolite formation, a concept that is now being incorporated into drug design (Park et al., 1998; Uetrecht, 1999, 2003; Williams et al., 2002; Williams and Naisbitt, 2002; Williams and Park, 2003).

There are many different types of ADRs, affecting every organ system within the body. However, drug-induced liver injury (DILI) is the most frequent reason for the withdrawal of an approved drug from the market and is also a major cause of attrition in drug development. More than 600 drugs have been associated with hepatotoxicity, with manifestations ranging from mild, asymptomatic changes in serum transaminases, which occur at a relatively high frequency with a number of drugs, to fulminant hepatic failure, which although rare, is potentially life threatening and may necessitate a liver transplant.

Major advances in molecular toxicology over the past decade have provided a conceptual framework for the mechanism of action of model hepatotoxins at the chemical, molecular, biochemical, and cellular levels. Drug metabolism provides a logical framework to define concentration–effect relationships for drug and metabolites linking *in vitro*, cellular and whole animal studies to man, which is essential for the development of safer drugs.

One of the liver's main physiological roles is the clearance and metabolism of xenobiotics into hydrophilic metabolites in order to facilitate their excretion. The liver is exposed to high concentrations of drugs after oral administration because of extraction, which leads to systemic exposure. The liver is often a primary target for chemical-induced toxicity due to its abundance of xenobiotic metabolising enzymes and its high capacity for both phase I and phase II biotransformations. Cytochrome P450 enzymes play a primary role in the phase I metabolism of an incredibly diverse range of foreign compounds, including therapeutic agents. Although the biotransformation sequence generally provides a detoxification pathway, there is the possibility that these reactions catalysed by CYP450 enzymes may generate metabolites that are not only more toxic, but also more reactive than the original xenobiotic. However, the relationship between bioactivation and the occurrence of hepatotoxicity is not simple. It is possible for chemicals to undergo bioactivation in the liver without causing hepatotoxicity. An example of this is the lack of hepatotoxicity seen with therapeutic doses of acetaminophen. This tightly coupled bioactivation and bioinactivation represents a mechanism for physiological clearance of relatively inert substrates. Ultimately, it is the balance between bioactivation, detoxification and defence mechanisms that determine whether a reactive metabolite may elicit a toxic effect (Castell et al., 1997; Park et al., 1998; Uetrecht, 1999, 2003; Williams et al., 2002; Williams and Naisbitt, 2002; Williams and Park, 2003) (Fig. 1).

One mechanism of drug-induced hepatotoxicity is irreversible chemical modification of a protein by a chemically reactive metabolite, which then has a profound effect on its function. The extent of binding and the biochemical role of the protein will in turn determine the toxicological insult of drug bioactivation. The resulting pathological consequences will be a balance between the rates of protein damage and the rates of protein replacement and cellular repair. It is important to note that the efficacy of a number of drugs (e.g., penicillins, aspirin, omeprazole) relies on their ability to covalently bind to