Annu. Rev. Pharmacol. Toxicol. 2005. 45:177–202
doi: 10.1146/annurev.pharmtox.45.120403.100058

First published online as a Review in Advance on September 7, 2004

THE ROLE OF METABOLIC ACTIVATION IN DRUG-INDUCED HEPATOTOXICITY

B. Kevin Park, Neil R. Kitteringham, James L. Maggs, Munir Pirmohamed, and Dominic P. Williams

Department of Pharmacology and Therapeutics, University of Liverpool, Sherrington Buildings, Liverpool, Merseyside L69 3GE, United Kingdom; email: bkpark@liv.ac.uk, neilk@liv.ac.uk, j.l.maggs@liv.ac.uk, munirp@liv.ac.uk, dom@liv.ac.uk

Key Words bioactivation, chemically reactive metabolite, critical protein, acetaminophen, diclofenac, tamoxifen, troglitazone

■ **Abstract** The importance of reactive metabolites in the pathogenesis of drug-induced toxicity has been a focus of research interest since pioneering investigations in the 1950s revealed the link between toxic metabolites and chemical carcinogenesis. There is now a great deal of evidence that shows that reactive metabolites are formed from drugs known to cause hepatotoxicity, but how these toxic species initiate and propagate tissue damage is still poorly understood. This review summarizes the evidence for reactive metabolite formation from hepatotoxic drugs, such as acetaminophen, tamoxifen, diclofenac, and troglitazone, and the current hypotheses of how this leads to liver injury. Several hepatic proteins can be modified by reactive metabolites, but this in general equates poorly with the extent of toxicity. Much more important may be the identification of the critical proteins modified by these toxic species and how this alters their function. It is also important to note that the toxicity of reactive metabolites may be mediated by noncovalent binding mechanisms, which may also have profound effects on normal liver physiology. Technological developments in the wake of the genomic revolution now provide unprecedented power to characterize and quantify covalent modification of individual target proteins and their functional consequences; such information should dramatically improve our understanding of drug-induced hepatotoxic reactions.

INTRODUCTION

Adverse drug reactions (ADRs) are significant health problems that contribute to patient morbidity and mortality. There are many different types of ADRs, affecting every organ system in the body. However, drug-induced liver injury is the most frequent reason for the withdrawal of an approved drug from the market, and it also accounts for more than 50% of cases of acute liver failure in the United States today (1). More than 600 drugs have been associated with hepatotoxicity. The clinical picture is diverse, even for the same drug when given to different

patients. The manifestations range 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.

Most drug-induced hepatic injuries that occur in humans are unpredictable and poorly understood. Although the asymptomatic rises in transaminases are common, the more severe forms of liver damage are fortunately rare, generally occurring with a frequency between 1 in 1000 and 1 in 10,000. The patients present with a pattern of liver injury that is consistent for each drug and may therefore be termed idiosyncratic, a term that does not imply any particular mechanism. Drug-induced liver toxicity mimics natural disease, and therefore lessons learned from the study of drug-induced hepatotoxicity should not only enhance drug safety but also provide new pharmacological strategies for the treatment of liver disease.

The 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. In particular, we now have a better understanding of the events that link drug metabolism and the formation of toxic metabolites to changes in liver function and the evolution of liver pathology. In this review, we relate recent advances in molecular toxicology to the clinical problem of drug-induced hepatotoxicity.

HEPATOTOXICITY AND DRUG METABOLISM

The biotransformation of lipophilic compounds into water-soluble derivatives that are more readily excreted is a physiological role of the liver. The liver receives more than 80% of its blood flow from the gastrointestinal tract and has a high capacity for both phase I and phase II biotransformations. Cytochrome P450 enzymes play a primary role in the metabolism of an incredibly diverse range of foreign compounds, including therapeutic agents. Such compounds may undergo concentration in the liver by various processes, including active transport systems.

Although the major role of drug metabolism is detoxication, it can also act as an "intoxication" process. Thus, foreign compounds can undergo biotransformation to metabolites that have intrinsic chemical reactivity toward cellular macromolecules (Figure 1). The propensity of a molecule to form such chemically reactive metabolites—usually electrophiles—is simply a function of its chemistry, and structural alerts are now well defined. A number of enzymes, and in particular the cytochromes P450, can generate, and in many instances release, reactive metabolites. The versatility of P450 together with the reactivity of their oxygen intermediates enables them to functionalize even relatively inert substrates, leading to the direct formation of diverse chemically reactive species. Such metabolites are short-lived, with half-lives of generally less than one minute, and are not usually

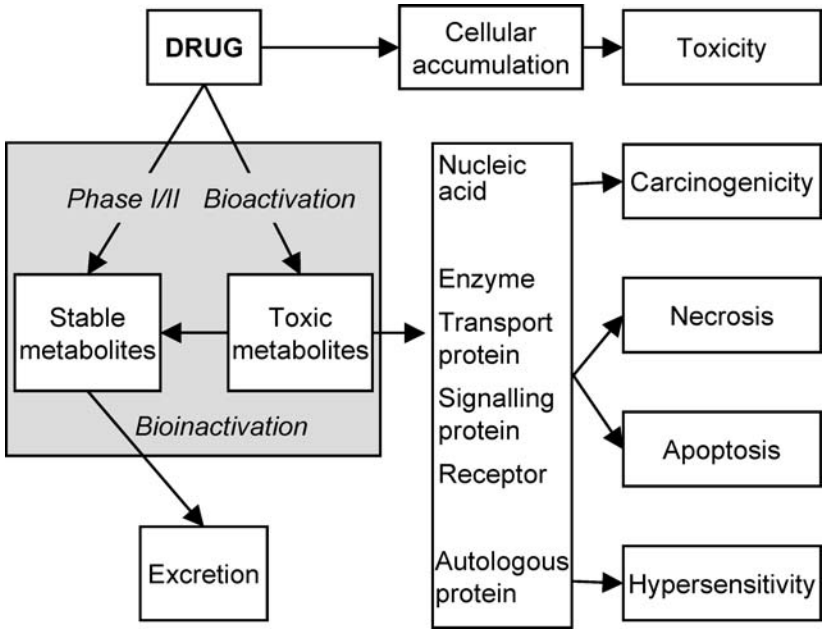

Figure 1 Relationship between drug metabolism and toxicity. Toxicity may accrue through accumulation of parent drug or, via metabolic activation, through formation of a chemically reactive metabolite, which, if not detoxified, can effect covalent modification of biological macromolecules. The identity of the target macromolecule and the functional consequence of its modification will dictate the resulting toxicological response.

detectable in plasma. Their intracellular formation can be inferred from endogenous trapping reactions or physico-chemical techniques. Their formation may be modulated by enzyme induction, enzyme inhibition, and gene deletion in animals. However, none of these experimental procedures is directly applicable to man. Hence, human exposure to chemically reactive metabolites in the liver is almost impossible to quantify.

The concept that small organic molecules can undergo bioactivation to electrophiles and free radicals and elicit toxicity by chemical modification of cellular macromolecules has its basis in chemical carcinogenicity and the pioneering work of the Millers (2, 3). The application of such concepts to human drug-induced hepatotoxicity was established through the studies of Brodie, Gillette, and Mitchell (4, 5) on the covalent binding to hepatic proteins of toxic (over) doses of the widely used analgesic acetaminophen.

However, the relationship between bioactivation and the occurrence of hepatic injury is not simple. For example, many chemicals undergo bioactivation in the liver but are not hepatotoxic. The best example is the lack of hepatotoxicity with

therapeutic doses of acetaminophen. Tight coupling of bioactivation with bioinactivation may be one reason for this. Many enzymic and nonenzymic pathways of bioinactivation are present in the liver, which is perhaps the best equipped of all the organs in the body to deal with toxins. Typical examples of bioinactivation pathways include glutathione conjugation of quinones by glutathione *S*-transferases (GSTs) and hydration of arene oxides to dihydrodiols by epoxide hydrolases. It is only when a reactive metabolite is a poor substrate for such enzymes that it can escape bioinactivation and thereby damage proteins and nucleic acids.

Moreover, covalent binding per se does not necessarily lead to drug hepatotoxicity. The regioisomer of acetaminophen, 3-hydroxyacetanilide, becomes covalently bound to hepatic proteins in rodents without inducing hepatotoxicity (6). It is therefore necessary to identify the subset of targets, i.e., covalently modified macromolecules, that is critical to the toxicological process. Hard electrophiles generally react with hard nucleophiles, such as functional groups in DNA and lysine residues in proteins. Soft electrophiles react with soft nucleophiles, which include cysteine residues in proteins and in glutathione, which has a concentration of approximately 10 mM in the liver. Free radicals can also react with lipids and initiate lipid peroxidative chain reactions. Unfortunately, there are no simple rules to predict the target macromolecule(s) for a particular chemically reactive metabolite or the biological consequences of a particular modification. Furthermore, noncovalent interactions also play a role because covalent binding of hepatotoxins is not indiscriminate with respect to proteins. Even within a single protein there can be selective modification of an amino acid side-chain found repeatedly in the primary structure. Thus, the microenvironment (pK_a, hydrophobicity, etc.) of the amino acid in the tertiary structure appears to be the crucial determinant of selective binding, and therefore the impact of covalent binding on protein 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 therefore not surprising that irreversible chemical modification of a protein, which has a profound effect on function, is a mechanism of drug-induced hepatotoxicity. However, it is also important to note that a number of drugs (e.g., penicillins, aspirin, omeprazole) rely on covalent binding to proteins for their efficacy, and thus prevention of covalent binding through chemical modification of the compound may also inadvertently lead to loss of efficacy. Similarly, endogenous compounds, such as cyclopentenone prostaglandins, are Michael acceptors, which react with specific cysteine residues in transcription factors to elicit their physiological effects in cell signaling (7).

The considerable task therefore facing the molecular toxicologist and drug metabolist is to differentiate between those protein modifications that are critical for a particular type of drug toxicity (and drug efficacy) and the "white noise" of noncritical, background covalent binding.

BIOACTIVATION AND HEPATOCARCINOGENESIS

The relationship between bioactivation, bioinactivation, and DNA adduct formation has been well established for a number of hepatocarcinogens. Aflatoxin, which is a hepatocarcinogen and a hepatotoxin, is converted into an epoxide, which is more readily detoxified by GST enzymes than by epoxide hydrolase. The balance of these reactions explains the greater DNA damage in humans compared with rodents because human forms of GST are less able to catalyze the conjugation of aflatoxin than their rodent counterparts (8, 9). Transgenic knockout mice have been used to establish the role of bioactivation by P450 (for review see 10) and bioinactivation by GSTs (11) for a number of carcinogenic polyaromatic hydrocarbons.

An important safety issue with respect to a therapeutic agent arose with the discovery that tamoxifen is a genotoxic hepatocarcinogen in the rat (12). Tamoxifen is a nonsteroidal antiestrogen used for the treatment of breast cancer (13). It has contributed to the reduction of deaths from breast cancer in the United States and the United Kingdom. There is now sufficient human experience to indicate that tamoxifen does not cause hepatic tumors in women after either prophylaxis or treatment. A consideration of the relative rates of bioactivation and bioinactivation provides a metabolic rationale for the safety of the drug in women.

The major route of bioactivation of tamoxifen to a genotoxic metabolite is known to be by sequential α-hydroxylation and sulphonation to a sulphate ester that collapses to a reactive carbocation and forms DNA adducts (14). Importantly, we observed that the corresponding glucuronide of α-hydroxytamoxifen is chemically very stable, and thus this biotransformation represents bioinactivation. There is no glutathione conjugate formed because the carbocation is a hard electrophile. A comparison of the relative rates of hydroxylation, sulphonation, and glucuronylation was performed in vitro between human and rodent enzymes. Rats had a greater propensity for sulphonation (bioactivation), whereas human liver had a much greater ability to effect glucuronylation (bioinactivation) (15, 16). An overall analysis of risk based on dose and the relative rates of metabolism suggested a 150,000-fold safety factor for the development of liver cancer from tamoxifen in humans when compared with rats (Figure 2).

BIOACTIVATION AND HEPATOTOXINS

A number of simple chemical compounds that produce selective hepatotoxicity after a single dose have been widely studied. These compounds are generally toxic in all species studied and include carbon tetrachloride, bromobenzene, furosemide, and acetaminophen. For each compound, there is compelling evidence that bioactivation is essential for hepatotoxicity. The use of transgenic null mice for certain P450 isoforms has been definitive in this regard. However, even for such simple compounds, the structure of the ultimate toxic metabolite is not known with

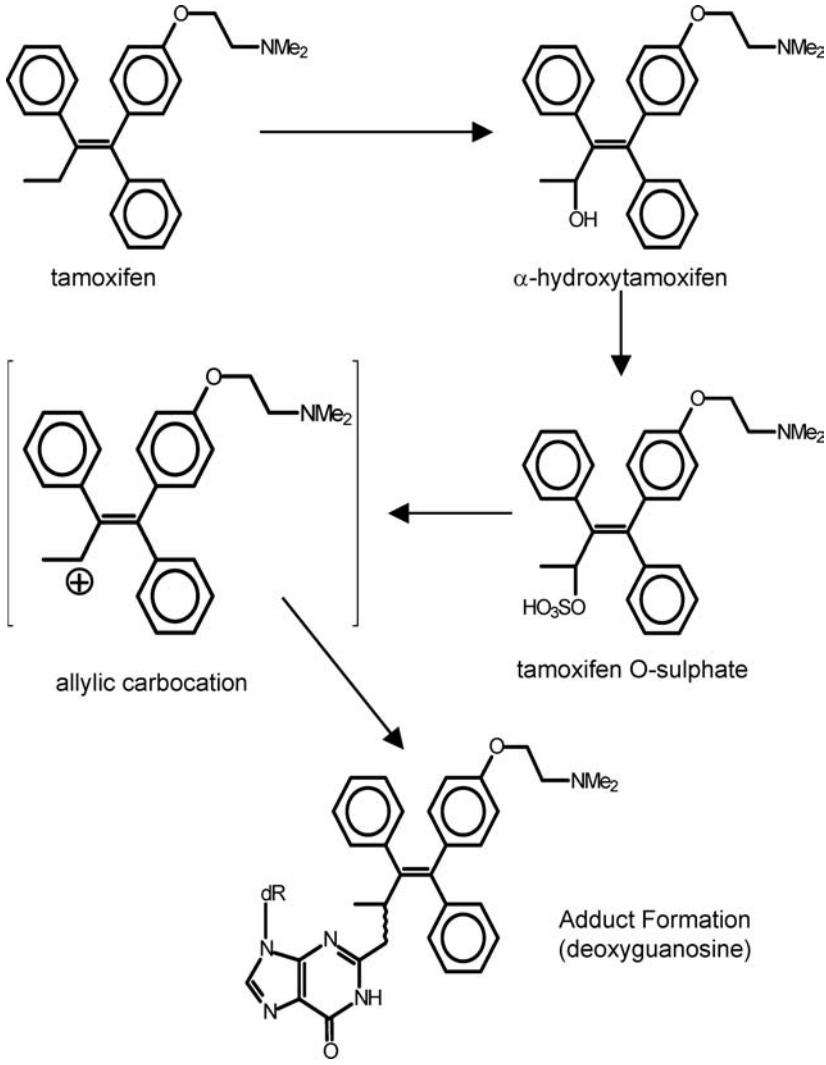

Figure 2 Metabolic bioactivation of tamoxifen. Tamoxifen undergoes sequential oxidation and sulphonation to form a carbocation that reacts covalently with DNA.

certainty. This information is essential if one is to relate global changes in gene expression, proteomics, and metabonomics in a way that can be used by the medicinal chemist in drug design.

Acetaminophen

Acetaminophen is a major cause of drug-related morbidity and mortality in humans, producing massive hepatic necrosis after a single toxic dose. A similar

pathological picture is observed in rodents. Toxicity is essentially dose-dependent, but there is interindividual variability in susceptibility, with alcoholics and patients on enzyme-inducing drugs perhaps being more susceptible. At therapeutic doses, acetaminophen is deactivated by glucuronylation and sulphation to metabolites, which are rapidly excreted in urine. However, a proportion of the drug undergoes bioactivation to N-acetyl-p-benzoquinoneimine (NAPQI) by CYP2E1, CYP1A2, and CYP3A4 (17, 18) (Figure 3).

NAPQI is rapidly quenched by a spontaneous reaction with hepatic glutathione after a therapeutic dose of acetaminophen. After a toxic (over) dose, glutathione

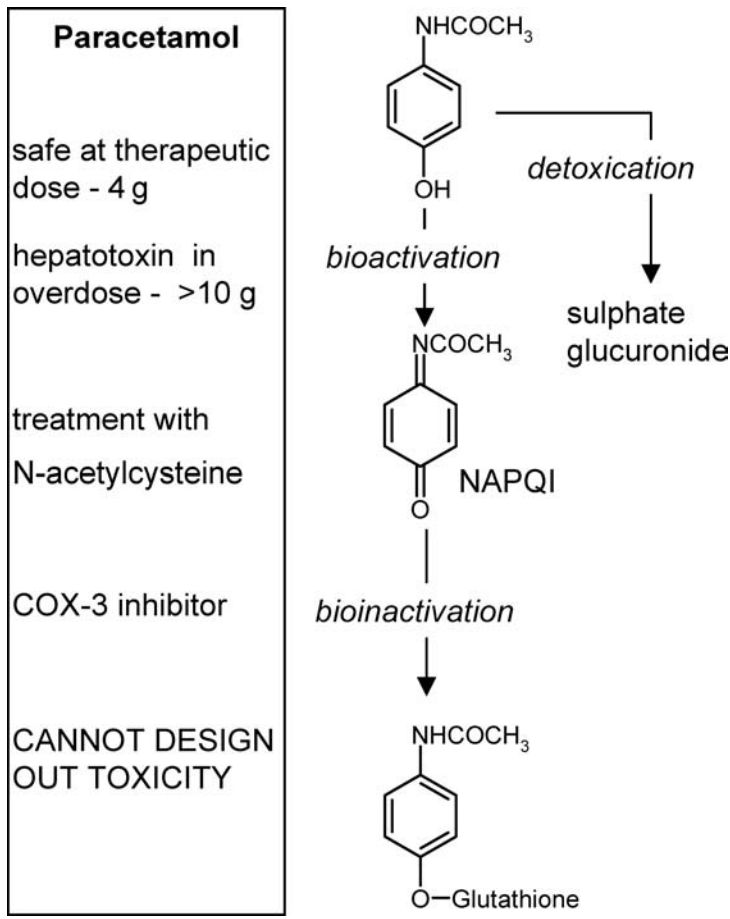

Figure 3 Bioactivation of acetaminophen. Acetaminophen can undergo conversion to the chemically reactive species N-acetyl-p-benzoquinoneimine, which can oxidize and covalently modify proteins. The toxicological and pharmacological properties of the molecule are a function of the redox potential of the molecule.

depletion occurs, which is an obligatory step for covalent binding and toxicity (19). The standard treatment for acetaminophen intoxication is N-acetylcysteine, which replaces hepatic glutathione and prevents toxicity. N-acetylcysteine is most beneficial if given within 16 h of the overdose. The early signs of cellular disruption in isolated hepatocytes can be reversed by a disulphide reductant, dithiothreitol (20, 21).

The massive chemical stress mediated by an acetaminophen overdose leads to an immediate adaptive defense response in the hepatocyte. This involves various mechanisms, including the nuclear translocation of redox-sensitive transcription factors such as Nrf-2, which "sense" chemical danger and orchestrate cell defense (Figure 4). Thus, with respect to acetaminophen, Nrf-2 genes of immediate significance are those involved in glutathione synthesis such as γ-glutamylcysteine

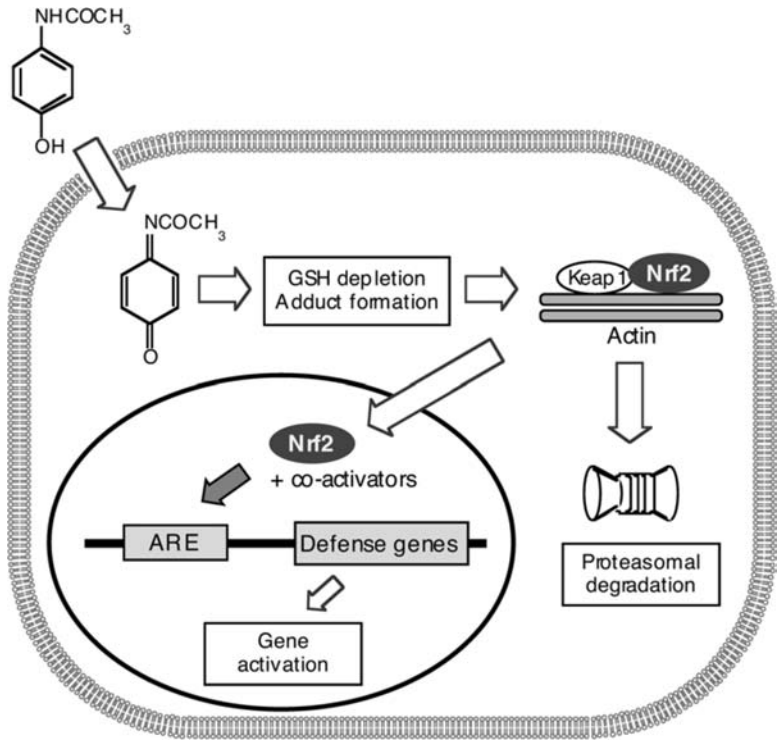

Figure 4 Activation of Nrf2 in hepatocytes in response to paracetamol exposure. Generation of NAPQI in the hepatocytes results in GSH depletion, protein adducts, and oxygen free radical formation. Each of these contributes to the release of Nrf2 from its cytoplasmic inhibitor, Keap1, and translocation to the nucleus. In the nucleus, Nrf2 heterodimerizes with small Maf or other proteins and activates the antioxidant response element (ARE), resulting in enhanced transcription of a battery of genes encoding antioxidant proteins and phase II drug metabolizing enzymes.

synthetase (γ-GCS), GSTs, glucuronyltransferases, and heme oxygenase (22). Importantly, it has been observed that nuclear translocation occurs at nontoxic doses of acetaminophen and at time-points before overt toxicity is observed. However, with increasing doses of acetaminophen, there is progressive dislocation of nuclear translocation, transcription, translation, and protein activity (23) as the rate of drug bioactivation overwhelms cell defense through the destruction of critical proteins.

THE CRITICAL PROTEIN HYPOTHESIS Since the initial discovery that covalent binding of acetaminophen to hepatic proteins was associated with hepatotoxicity, there has been a progression of techniques that have been used to identify the protein targets. Thus, radiolabeled drugs and Western blotting enable the detection and quantification of adduct formation, whereas more recently proteomics has allowed the simultaneous identification of several adducted proteins. The latter technique offers the possibility of determining the amino acids modified and the nature of that modification. This in turn allows a molecular rationale for the change in activity of that protein.

At least 17 liver enzymes that show a loss of activity ex vivo after administration of a toxic dose of acetaminophen to a rodent species have now been investigated; these are listed in Table 1. An additional 14 liver enzymes are known to be adducted by paracetamol in vivo and in vitro but have yet to be shown to be inhibited.

It is notable that modification of proteins can occur in most intracellular compartments of the hepatocyte, e.g., endoplasmic reticulum (ER), cytosol, mitochondria, and plasma membrane, which is an indication of the intracellular mobility of the reactive metabolite once glutathione is depleted (Figure 5). The loss of hepatocyte viability is likely to be a function of the summation and extent of inhibition of protein activity. Thus, inhibition of γ-GCS, glyceraldehydes-3-phosphate dehydrogenase (GAPDH), and Ca^{2+}/Mg^{2+} ATPase will severely impair hepatocyte function by uncoupling mitochondria, depleting glutathione and ATP, and disturbing Ca^{2+} homeostasis, which could lead to the expression of TNF and Fas receptors on cell membranes. γ-GCS catalyses the rate-limiting step in glutathione synthesis, the primary biochemical defense of the hepatocyte against NAPQI. GAPDH, which, as a component of the glycolytic pathway, contributes to ATP production, is more than 80% inhibited at 2 h after a toxic dose of acetaminophen. On the basis of reaction with NAPQI in vitro, inhibition is thought to be due to modification of a critical cysteine (cys-149) within the active site of the enzyme (24). The loss of calcium homeostasis is one of the first pathological features of acetaminophen toxicity. It is clear that as NAPQI diffuses from its site of formation, a number of enzymes are chemically modified—usually at cysteine or lysine residues—but there is a degree of protein selectivity and variation in amino acid modification: Acetaminophen appears to react with lysine residues of three intraluminal ER proteins (25). Presumably, noncovalent interactions and the microenvironment of amino acid residues determine the precise structure of modified proteins.

The rapid inactivation of several proteins suggests that cellular failure is a consequence of multiple parallel events rather than a simple cascade or signaling

TABLE 1 Hepatic enzymes inhibited by acetaminophen[a]

Fraction	Enzyme	Species	Dose/ concentration	Inhibition[b]	Modification[c]	Modified amino acid	Abundance[d]
Microsomes	Glutamine synthase (76, 77)	Mouse	400 mg/kg	65%, 6 h	Covalent	?	?
Cytosol	ADP-ribose pyrophosphatase-1 (78)	Rat	800 mg/kg	ca. 50%, ? h	Noncovalent	Cysteine?[e]	?
	γ-Glutamylcysteinyl synthetase (23)	Mouse	530 mg/kg	31%, 1 h	?	?	?
	GAPDH (24)	Mouse	500 mg/kg	83%, 2 h	Covalent	Cysteine?[f]	2.05%
	Glutathione *S*-transferase (79)	Mouse	500 mg/kg	46%, 6 h	Covalent	?	1.5–3.3
	Methionine adenosyltransferase (80)	Mouse	400 mg/kg	45%, 6 h	Covalent	?	0.2%
	MIF tautomerase (81)	Mouse	200 mg/kg	72%, 8 h	Covalent	Proline	?
	N-10-formyl-H4folate dehydrogenase (82)	Mouse	400 mg/kg	25%, 2 h	Covalent	?	0.55%
	Protein phosphatase (hepatocyte) (83)	Mouse	10 mM	18%, 4 h	Noncovalent?	?	?
	Proteasome (84)	Mouse	600 mg/kg	50%, 2 h	Covalent	?	?
	Tryptophan-2,3-dioxygenase (85)	Rat	3 × 100 mg/kg	44%, 3 h	?	?	?
Mitochondria	Aldehyde dehydrogenase (86)	Mouse	600 mg/kg	40%, 4 h	Covalent	?	0.71%
	Carbamyl phosphate synthase-1 (76)	Mouse	400 mg/kg	65%, 6 h	Covalent?	Cysteine?[g]	4.3%
	Glutamate dehydrogenase (87)	Mouse	600 mg/kg	35%, 1 h	Covalent	?	1.4%
	Mg^{2+} ATPase (88)	Rat	650 mg/kg	35%, 24 h	Covalent	?	2%
Cell membrane	Ca^{2+}/Mg^{2+}-ATPase (89)	Rat	2.5 g/kg	32%, 2.5 h	Covalent?	?	?
	Na^{+}/K^{+}-ATPase (90)	Rat	850 mg/kg	52%, 3 h	?	?	?

[a]These enzymes have been reported to be inhibited in animals or hepatocytes exposed to acetaminophen. A number of hepatic enzymes known to be covalently modified by acetaminophen in vitro or in vivo have yet to be reported to be inhibited by the drug: calreticulin and two protein disulfide isomerases (25); aryl sulfotransferase, carbonic anhydrase III, 2,4-dienoyl-CoA reductase, glutathione peroxidase, glycine *N*-methyltransferase, 3-hydroxyanthranilate 3,4-dioxygenase, inorganic pyrophosphatase, protein synthesis initiation factor 4A, sorbitol dehydrogenase, thioether *S*-methyltransferase, urate oxidase (not found in primates) (91).

[b]Measured ex vivo.

[c]Taken to be covalent modification (arylation) if radiolabel or immunologically reactive drug moiety coincided with protein on gel electrophoretogram except for the MIF tautomerase adduct, which was characterized by MALDI-TOF analysis of the adducted protein isolated from mouse liver.

[d]Relative expression levels in livers of male mice (92).

[e]Enzyme inhibition in vivo was reversible ex vivo with dithiothreitol.

[f]Cys-149 of GAPDH is modified by NAPQI in vitro.

[g]The inhibited enzyme's protein band was less extensively labeled by a thiol-modifying fluorescent reagent (monobromobimane).

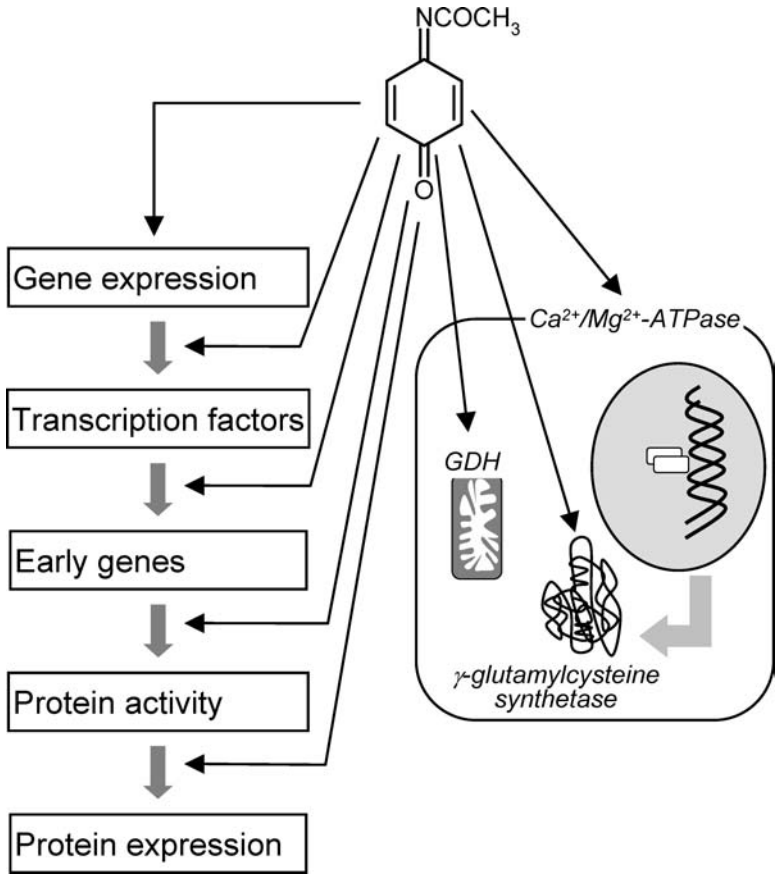

Figure 5 Levels of interaction of the chemically reactive metabolite of acetaminophen, NAPQI, with cellular function. The cellular locations of specific proteins involved in cell defense and cell damage, whose functions are known to be modified by NAPQI following exposure to acetaminophen, are indicated.

mechanism. It is well established that one of the main events in isolated hepatocytes is overall energy failure (26, 27), which is accompanied by the generation of megamitochondria that are apparently ATP-depleted and nonfunctional (28). The execution of hepatocytes involves interplay between hepatocyte damage mediated by chemical stress and the activation of nonparenchymal cells and the subsequent release of various mediators. The role of Kupffer cells has been demonstrated by the fact that mice treated with dichloromethylene diphosphonate (DMDP), which depletes 99% of macrophages from the liver, were protected against acetaminophen toxicity (29). Indeed, acetaminophen-treated rats have four- to sevenfold more infiltrating macrophages than resident Kupffer cells (30). Furthermore, neutralization of Fas ligand (31) and TNF (32) affords a degree of protection against the early

apoptotic processes and the final overwhelming necrosis, which is the overriding feature of acetaminophen's hepatotoxicity.

Nitric oxide has a dual role in the hepatic response to acetaminophen. Nitric oxide derived from iNOS contributes to acetaminophen-induced parenchymal cell injury and to microvascular disturbances, whereas nitric oxide derived from constitutive NOS exerts a protective role in liver microcirculation and thereby minimizes liver injury. In this context, it is of interest to note that glutathione depletion can lead to oxidative deactivation of nitric oxide and thus produce hypertension (33).

It has also been suggested that liver blood flow is an important determinant of toxicity. Consistent with this, it has been demonstrated that alpha-blockers, which mediate vasodilatation, protect against acetaminophen toxicity even when given after bioactivation and covalent binding of the drug has occurred (L. Randle, unpublished data).

THE ASSOCIATION BETWEEN DRUG BIOACTIVATION AND HEPATOTOXICITY IN MAN

Acetaminophen-induced hepatic necrosis is the best-described form of injury induced by reactive metabolites, but this type of toxicity is unusual in that it is caused by a single dose as well as being clearly dose-dependent. In most instances, drug-induced injury in man is an infrequent and variable event and a number of general mechanisms have been proposed (1). Chemically reactive metabolites have been proposed as being responsible for most types of drug-induced injury, but direct evidence for the role of such metabolites is difficult to obtain because of the lack of suitable in vitro and in vivo models.

Some drug reactions have all the clinical hallmarks of an immunological mechanism, which include time of presentation, general clinical features, greatly enhanced reaction on reexposure to the drug, and some laboratory evidence of drug-induced immunological perturbation. In such cases, the liver alone may be involved, or liver injury may be part of a more complex hypersensitivity syndrome, as has been observed for anticonvulsants. Thus, for a series of drugs, there is chemical evidence for bioactivation, based largely on in vitro or animal studies, and some evidence of drug-induced antibody formation or a drug-related T cell response (Table 2). The question is whether the association between bioactivation and an immune response is coincidental or consequential.

Halothane

Halothane is the best-studied drug with respect to immunoallergic hepatitis. A significant proportion of patients exposed to this inhalation anesthetic develop asymptomatic rises in transaminases. Fulminant irreversible hepatitis is a rare but life-threatening phenomenon. Most of the patients recorded in the literature with immunoallergic hepatitis had more than one exposure (34). Antibodies have been detected in such patients that recognize autoantigens and neoantigens created by

TABLE 2 Chemical and immunological basis of drug-induced immunoallergic hepatitis

Drug	Bioactivation	Immune response	Reference
Halothane	Oxidative dehalogenation	Drug metabolite IgG Anti-CYP2E1 IgG Autoantibodies	(39, 45)
Tienilic acid	Thiophene sulphoxidation	Anti-CYP2C9 IgG	(42)
Dihydralazine	?	Anti-CYP1A2 IgG	(44)
Sulphamethoxazole	N-hydroxylation	IgG antibodies Drug and metabolite T cells	(93) (94)
Carbamazepine	Arene oxidation	Drug T cell	(46, 95)
Nevirapine	Arene oxidation	Drug T cell	Unpublished data

trifluoroacetylation of hepatic proteins (Figure 6). Preincubation of halothane-pretreated, but not of control, rabbit hepatocytes with sera from patients with halothane-induced fulminant hepatic failure rendered the hepatocytes susceptible to the cytotoxic effects of normal lymphocytes in vitro (35). It is thus likely that drug-specific T cells may play a role in the pathogenesis of hepatocyte injury, but direct evidence for this is lacking.

It is likely that the common chemical trigger for both the mild and severe forms of hepatocyte injury is drug bioactivation to an acyl halide. Bioactivation of halothane is substantial and is a consequence of the presence of a vulnerable proton alpha to halide groups, which are effective leaving groups. In this sense, the only metabolic route available to the molecule is bioactivation. There is direct and indirect evidence for this concept. First, the detection of drug metabolite-specific antibodies in affected patients. Second, a global evaluation of the relationship between the metabolism and toxicity of inhalation anesthetics reveals that the newer, metabolically inert anesthetics such as enflurane and isoflurane are rarely associated with hepatotoxicity in man. Pohl and colleagues (36–39) have identified a number of target proteins modified by halothane; trifluoroacetylation of lysine residues is believed to be the principal chemical modification. Precisely how such chemical modifications trigger an immune response and what is the immunological mechanism of cell killing is still very much a matter of debate. Animal models of experimental autoimmune hepatitis indicate that T cells rather than immunoglobulins provide the immunological trigger for cell death (40, 41). A feature of such animal models is the minimal level of tissue injury, with protection partly afforded by the presence of T suppressor cells. In man, therefore, the balance between the different T cell subsets with different functions may be crucial in determining not only individual susceptibility but also the severity of the injury.

A further clue to the mechanisms involved in such reactions was the discovery of antibodies directed against the P450 enzymes responsible for the bioactivation of tienilic acid, dihydralazine, and halothane (42–44). In the case of halothane,

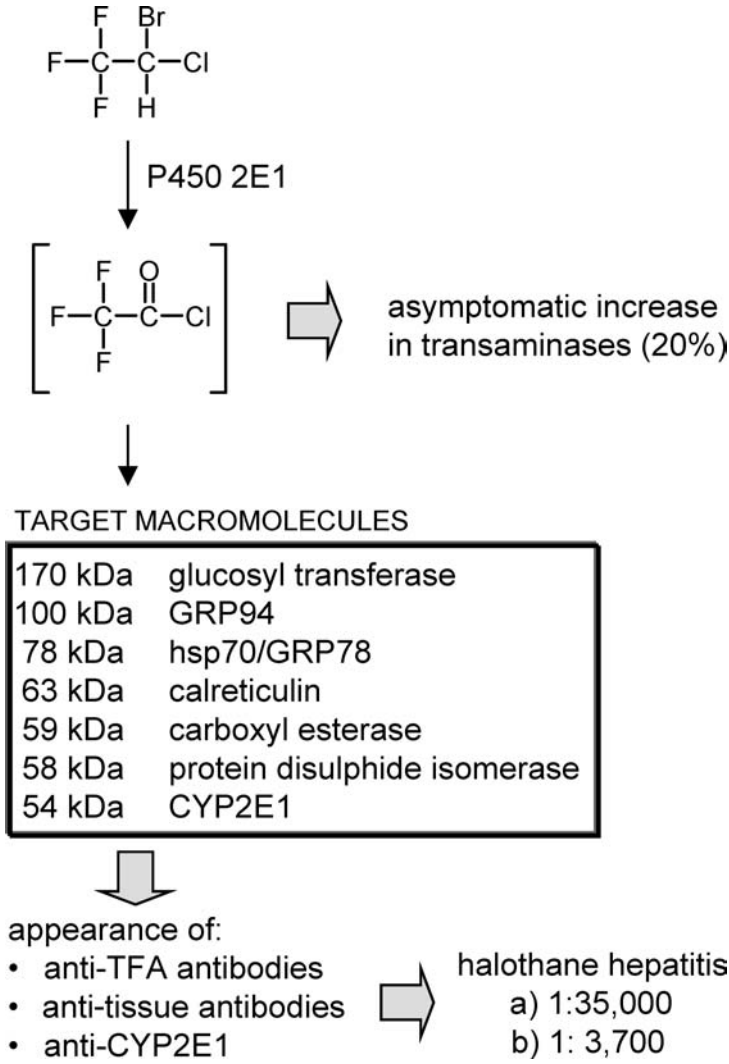

Figure 6 Bioactivation of halothane. Halothane is metabolized by cytochrome P450 2E1 to a chemically reactive trifluoroacetyl radical, which has been shown to covalently modify lysine residues in a range of target proteins, including CYP2E1 itself (39). Chemical modification of protein(s) leads to the immune response associated with halothane hepatitis.

autoantibody generation was extensive (45). Collectively, these data provide evidence for a loss of tolerance to autologous proteins chemically modified as a consequence of drug bioactivation. Further studies are required to examine patients with immunoallergic hepatitis for drug-specific T cells (46) and for genetic variants in drug metabolism and immune responsiveness, which might provide the key to understanding the idiosyncratic nature of such reactions.

Studies from our laboratories have already shown that patients with deranged liver function as a consequence of taking carbamazepine or nevirapine have circulating T cells that recognize the drug (D.J. Naisbitt, unpublished data). Thus most of the available information is compatible with the hapten mechanism of drug-induced immunoallergic toxicity outlined in Figure 7.

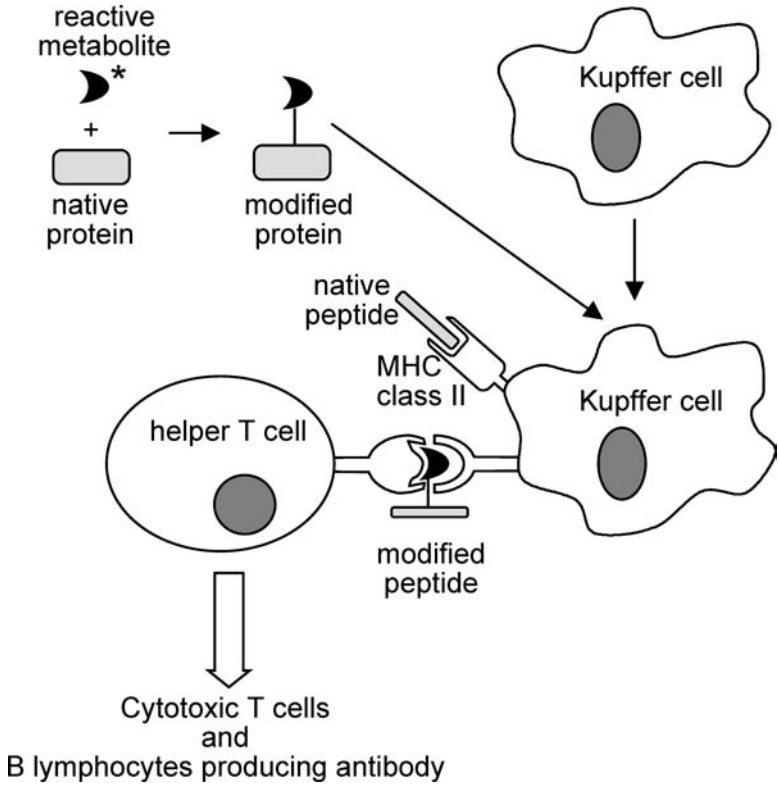

Figure 7 Proposed mechanism for the role of reactive metabolites in immunoallergic hepatitis. The drug undergoes bioactivation in the hepatocytes leading to drug-protein conjugate formation in the liver. The resulting modified protein is internalized by Kupffer cells and presented to cognate T cells that recognize modified peptide and native peptide. This in turn can lead to the generation of cytotoxic T cells and B lymphocytes producing antibody. In theory, such an unregulated response could explain the severe idiosyncratic hepatotoxicity associated with halothane.

Isoniazid

Isoniazid (INH) is still the most widely used drug in the treatment of tuberculosis (TB) and has high activity against *Mycobacterium tuberculosis*, although resistant strains have emerged. INH is used in combination with drugs such as rifampicin and pyrazinamide to reduce the chance of inducing resistant strains of the mycobacterium.

INH causes two major adverse reactions: hepatitis and peripheral neuropathy. The incidence and severity of the adverse drug reactions are related to dose and duration of therapy. Toxicity may be delayed by several weeks. A minor asymptomatic increase in liver aminotransferases (less than threefold) is seen in 10%–20% of patients within the first two months of therapy, whereas fatal hepatitis is seen in less than 1% of patients. Mortality is greater than 10% in patients with jaundice (47, 48). INH typically produces diffuse massive necrosis or chronic hepatitis. Clinical features resemble acute viral-induced hepatitis. Anorexia, fatigue, nausea, and vomiting are the usual prodromal features, but jaundice and dark urine may be the first evidence of injury (49). Combination therapy is a risk factor for hepatitis, although formal studies evaluating the mechanisms of this have not been undertaken. Interestingly, of the three anti-TB compounds, it has been suggested that pyrazinamide is the most hepatotoxic, with a rate of hepatitis three and five times higher than that of rifampicin and INH, respectively (50–52).

Studies in the rat (53) and rabbit (54), along with in vitro studies, indicate that INH undergoes acetylation to give N-acetylisoniazid. This is then hydrolyzed to acetylhydrazine, which undergoes bioactivation by P450 enzymes to give an acetyl radical, a reactive species identified by trapping as a glutathione conjugate (53). Precisely how such a reactive intermediate induces hepatocyte damage remains to be elucidated, as do the reasons for the increased incidence of hepatotoxicity when combination therapy is used. Target proteins have not been identified for the reactive metabolite formed from INH. To date, there is no convincing clinical or laboratory evidence to suggest an immunological mechanism.

Interestingly, bioactivation plays a role in the pharmacology of INH, with elimination of nitrogen being the driving force for the formation of an isonicotinoyl radical intermediate (Figure 8). INH can thus be considered a prodrug, which is activated by the mycobacterial catalase-peroxidase enzyme KatG. The product of bioactivation forms a covalent adduct with NADH, which is an inhibitor of InhA, an enoyl-acyl carrier protein reductase that is involved in the biosynthesis of mycolic acids present in the mycobacterium cell wall (55, 56).

Diclofenac

The nonsteroidal antiinflammatory drugs (NSAIDs) as a class have a strong association with hepatotoxicity. Several NSAIDs have been withdrawn after obtaining approval for a license, the most recent being bromfenac (57). The mechanism of hepatotoxicity appears to be complex and multifactorial, involving both pharmacological and metabolic mechanisms. For example, inhibition of the COX

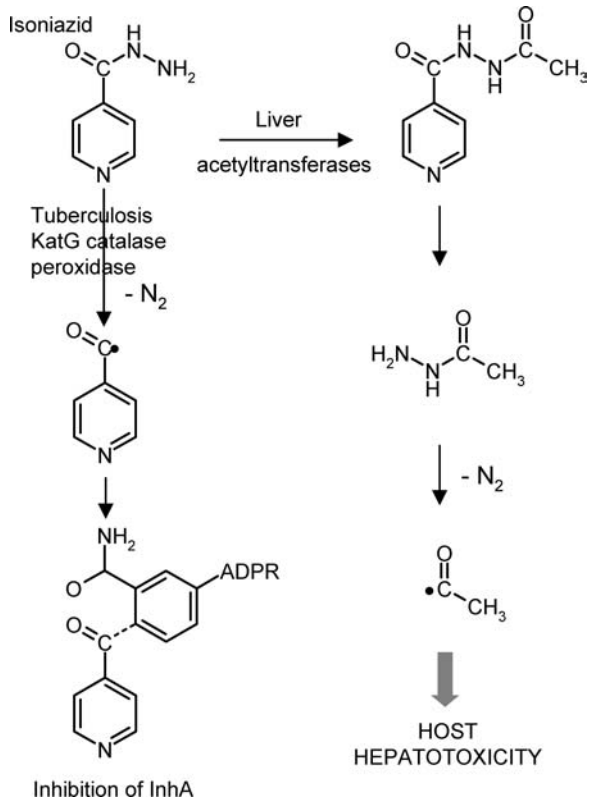

Figure 8 Metabolic bioactivation of isoniazid. Reactive metabolites are responsible for the pharmacology and toxicology of isoniazid. In *Mycobacterium tuberculosis*, generation of the isonicotinoyl radical leads to the formation of an adduct with NADH, which in turn inhibits an enoyl-acyl carrier protein reductase (InhA) (53, 56).

enzymes may lead to a reduction in cytoprotective prostaglandins, whereas bioactivation may occur by both oxidation and conjugation. This metabolic complexity is illustrated with reference to diclofenac, which undergoes acyl glucuronylation (58), acyl thiolation (59), and multiple P450-catalyzed oxidations producing two *p*-benzoquinoneimines via phenols and an as yet uncharacterized intermediate—possibly an epoxide—of mechanism-based inhibition (60–62). The relative contributions of these metabolites to protein adduction, cytotoxicity, and hepatotoxicity in vivo remains to be determined. In isolated rat hepatocytes, although the binding of diclofenac to protein appears to derive principally from reactions of the acyl glucuronide, the cytotoxicity has been attributed to products of oxidative pathways (63). However, diclofenac-protein adduct formation—and especially on the cell surface—might be causally relevant to the expression of immune-mediated hepatitis.

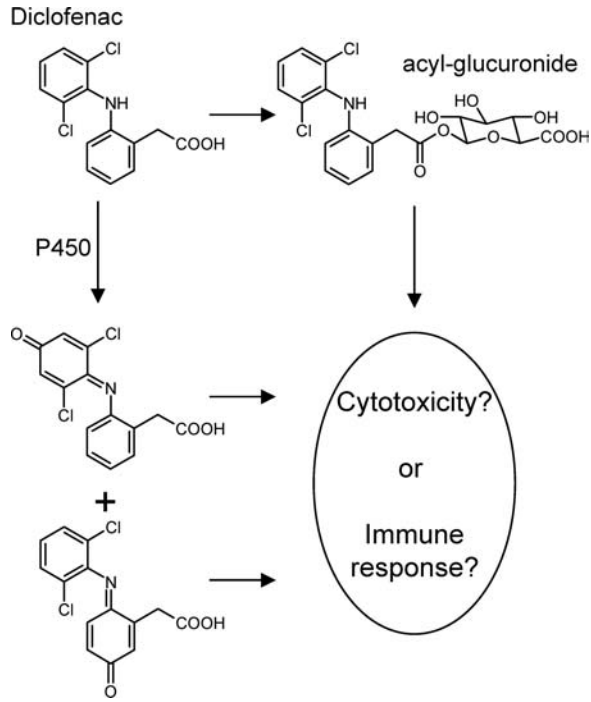

Figure 9 Metabolic bioactivation of diclofenac. Diclofenac can form electrophilic metabolites by either oxidation or glucuronylation. The precise role of such metabolites in the rare hepatotoxicities associated with diclofenac remains to be elucidated (96).

Immunological and nonimmunological mechanisms have been proposed for diclofenac toxicity. The acyl glucuronide can achieve concentrations in bile up to 5000-fold higher than those in peripheral blood because of a potent export pump located in the canalicular membrane of hepatocytes (64). The acyl glucuronide is protein reactive and forms covalent adducts with circulating proteins and hepatic proteins (Figure 9). A particular target is the canalicular ectoenzyme dipeptidyl peptidase (DPP) IV (CD26), which shows a decrease in activity following administration of diclofenac to rats (65).

Thiazolidinedione Antidiabetics

Chemically reactive metabolites have also been described for a number of drugs that cause idiosyncratic hepatotoxicity, but for which no mechanistic studies are available. An important example is troglitazone, a 2,4-thiazolidinedione, which was the first of a new class of drugs for type 2 diabetes. Troglitazone was associated with a significant frequency of reversible increases in serum transaminases. Reports of severe and fatal liver injury finally led to the withdrawal of this

important new drug (66). Fortunately, it could be replaced by newer and safer 2,4-thiazolidinediones (glitazones): pioglitazone and rosiglitazone (Figure 10). The mechanism of troglitazone-induced hepatic injury is not known. The drug undergoes oxidative bioactivation at both the chroman ring—which is unique to troglitazone—and the thiazolidinedione ring in rats, forming several reactive metabolites that are eliminated as thioether and thioester conjugates of glutathione (67, 68). It is also bioactivated in human hepatocytes (69, 70) and is cytotoxic (71). An association of troglitazone hepatotoxicity in diabetic patients with a glutathione S-transferase double null genotype provides indirect evidence for the importance of bioactivation and bioinactivation in its pathogenesis (72). However, the less hepatotoxic and cytotoxic glitazones—pioglitazone and rosiglitazone—as well as troglitazone undergo NADPH-dependent covalent binding to human microsomal protein (73). At present, the toxicological significance of troglitazone's metabolic activation remains an open question; even the relative extents of the glitazones' bioactivation in vitro is unquantified. Finally, it is important to note that the heterogeneous clinical picture of troglitazone hepatotoxicity has prompted the suggestion that this may be a reflection of interindividual variation in the balance of different mechanisms of drug toxicity as well as varying patient characteristics (74).

CONCLUSIONS AND SOLUTIONS

There is overwhelming evidence that chemically reactive metabolites derived from simple organic molecules, including therapeutic agents, can cause a wide range of hepatic injuries. There are short- and long-term solutions to the problem.

In the short term, the drug metabolist can determine the propensity of a novel chemical entity to undergo bioactivation in model systems ranging from expressed enzymes, through genetically engineered cells, to whole animals. Bioactivation can be assessed by trapping experiments with model nucleophiles in vitro or by measurement of uncharacterized covalent binding to endogenous proteins in vitro and in vivo. The chemistry of the process needs to be defined, and the medicinal chemist can then address the issue by seeking a metabolically stable pharmacophore to replace the potential toxicophore. Such an approach will minimize chemical hazard, but cannot give any insight into biological risk in man, or in any other species for that matter. Evans et al. (75) have provided an industrial perspective on this topic and adopted a pragmatic approach to minimize reactive metabolite formation at an early stage in drug development. A decision tree has been designed based on a target covalent binding value of 50 pmol drug equivalent /mg protein in vitro and in vivo. The standard method measures covalent binding of radiolabeled drug to hepatic microsomes. Such an approach seems to suggest that there may be a threshold level of covalent binding, above which critical proteins necessarily become damaged. Appropriate dose-ranging studies are, however, required to validate this concept.

In the long term, we require a more fundamental understanding of the role of chemically reactive metabolites in human hepatotoxicity. We need to know how

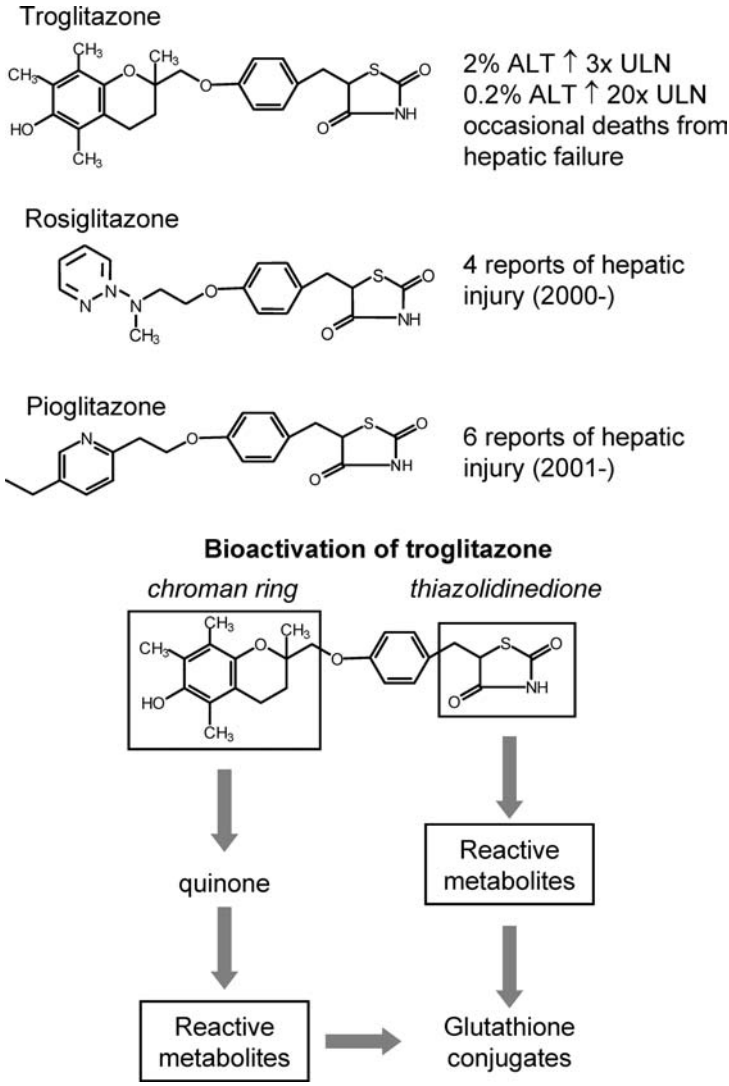

Figure 10 Metabolism and toxicity of glitazones. Troglitazone, a novel antidiabetic agent, was withdrawn because of rare but serious hepatotoxicity. Rosiglitazone and pioglitazone are now firmly established in the treatment of diabetes. It has been established that troglitazone undergoes bioactivation to several chemically reactive metabolites. Novel test systems are required to define the possible role of such metabolites in hepatotoxicity (97, 98).

the ultimate hepatotoxin interferes with signaling, and the sequence of molecular events that impair cell defense, which ultimately lead to hepatocyte destruction. It is only when such a mechanistic framework is established that we will be in position to understand the time-course of the toxicity, the nature of the toxicity, and the direction that the toxicity takes in a particular patient. It is therefore imperative that such studies begin at the clinical level, but are then translated into molecular studies in the laboratory with the design of appropriate in vitro and in vivo model systems to fully exploit the molecular technology now available in the post genomic era.

ACKNOWLEDGMENT

The authors wish to acknowledge the generous and continuing support of the Wellcome Trust.

The *Annual Review of Pharmacology and Toxicology* is online at http://pharmtox.annualreviews.org

LITERATURE CITED

1. Lee WM. 2003. Drug-induced hepatotoxicity. *New Engl. J. Med.* 349:474–85
2. Miller E, Miller J. 1947. The presence and significance of bound aminoazo dyes in the livers of rats fed p-dimethylaminobenzene. *Cancer Res.* 7:468–80
3. Miller E, Miller J. 1952. In vivo combinations between carcinogens and tissue constituents and their possible role in carcinogenesis. *Cancer Res.* 12:547–56
4. Brodie BB, Reid WD, Cho AK, Sipes G, Krishna G, Gillette JR. 1971. Possible mechanism of liver necrosis caused by aromatic organic compounds. *Proc. Natl. Acad. Sci. USA* 68:160–64
5. Gillette JR, Mitchell JR, Brodie BB. 1974. Biochemical mechanisms of drug toxicity. *Annu. Rev. Pharmacol.* 14:271–88
6. Tirmenstein MA, Nelson SD. 1989. Subcellular binding and effects on calcium homeostasis produced by acetaminophen and a nonhepatotoxic regioisomer, 3′-hydroxyacetanilide, in mouse liver. *J. Biol. Chem.* 264:9814–19
7. Kawamoto Y, Nakamura Y, Naito Y, Torii Y, Kumagai T, et al. 2000. Cyclopentenone prostaglandins as potential inducers of phase II detoxification enzymes. 15-deoxy-delta(12,14)-prostaglandin j2-induced expression of glutathione S-transferases. *J. Biol. Chem.* 275:11291–99
8. Guengerich FP, Johnson WW, Ueng YF, Yamazaki H, Shimada T. 1996. Involvement of cytochrome P450, glutathione S-transferase, and epoxide hydrolase in the metabolism of aflatoxin B1 and relevance to risk of human liver cancer. *Environ. Health Perspect.* 104(Suppl. 3): 557–62
9. Wilson AS, Williams DP, Davis CD, Tingle MD, Park BK. 1997. Bioactivation and inactivation of aflatoxin B1 by human, mouse and rat liver preparations: effect on SCE in human mononuclear leucocytes. *Mutat. Res.* 373:257–64
10. Gonzalez FJ, Kimura S. 1999. Role of gene knockout mice in understanding the mechanisms of chemical toxicity and carcinogenesis. *Cancer Lett.* 143:199–204
11. Henderson CJ, Smith AG, Ure J, Brown K, Bacon EJ, Wolf CR. 1998. Increased skin tumorigenesis in mice lacking pi class

glutathione S-transferases. *Proc. Natl. Acad. Sci. USA* 95:5275–80
12. Carthew P, Rich KJ, Martin EA, De Matteis F, Lim CK, et al. 1995. DNA damage as assessed by 32P-postlabelling in three rat strains exposed to dietary tamoxifen: the relationship between cell proliferation and liver tumour formation. *Carcinogenesis* 16:1299–304
13. Group EBCTC. 1998. Tamoxifen for early breast cancer: an overview of the randomised trials. *Lancet* 351:1451–67
14. Martin EA, Rich KJ, White IN, Woods KL, Powles TJ, Smith LL. 1995. 32P-postlabelled DNA adducts in liver obtained from women treated with tamoxifen. *Carcinogenesis* 16:1651–54
15. Boocock DJ, Maggs JL, White IN, Park BK. 1999. Alpha-hydroxytamoxifen, a genotoxic metabolite of tamoxifen in the rat: identification and quantification in vivo and in vitro. *Carcinogenesis* 20: 153–60
16. Boocock DJ, Maggs JL, Brown K, White INH, Park BK. 2000. Major inter-species differences in the rates of O-sulphonation and O-glucuronylation of alpha-hydroxytamoxifen in vitro: a metabolic disparity protecting human liver from the formation of tamoxifen-DNA adducts. *Carcinogenesis* 21:1851–58
17. Raucy JL, Lasker JM, Lieber CS, Black M. 1989. Acetaminophen activation by human liver cytochromes P450IIE1 and P450IA2. *Arch. Biochem. Biophys.* 271:270–83
18. Thummel KE, Lee CA, Kunze KL, Nelson SD, Slattery JT. 1993. Oxidation of acetaminophen to N-acetyl-p-aminobenzoquinone imine by human CYP3A4. *Biochem. Pharmacol.* 45:1563–69
19. Davis DC, Potter WZ, Jollow DJ, Mitchell JR. 1974. Species differences in hepatic glutathione depletion, covalent binding and hepatic necrosis after acetaminophen. *Life Sci.* 14:2099–109
20. Albano E, Rundgren M, Harvison PJ, Nelson SD, Moldeus P. 1985. Mechanisms of N-acetyl-p-benzoquinone imine cytotoxicity. *Mol. Pharmacol.* 28:306–11
21. Rafeiro E, Barr SG, Harrison JJ, Racz WJ. 1994. Effects of N-acetylcysteine and dithiothreitol on glutathione and protein thiol replenishment during acetaminophen-induced toxicity in isolated mouse hepatocytes. *Toxicology* 93:209–24
22. Goldring C, Kitteringham N, Elsby R, Randle L, Clement Y, et al. 2004. Activation of hepatic Nrf2 in vivo by acetaminophen in CD-1 mice. *Hepatology* 39:1267–76
23. Kitteringham NR, Powell H, Clement YN, Dodd CC, Tettey JN, et al. 2000. Hepatocellular response to chemical stress in CD-1 mice: induction of early genes and gamma-glutamylcysteine synthetase. *Hepatology* 32:321–33
24. Dietze EC, Schafer A, Omichinski JG, Nelson SD. 1997. Inactivation of glyceraldehyde-3-phosphate dehydrogenase by a reactive metabolite of acetaminophen and mass spectral characterization of an arylated active site peptide. *Chem. Res. Toxicol.* 10:1097–103
25. Zhou L, McKenzie BA, Eccleston ED Jr, Srivastava SP, Chen N, et al. 1996. The covalent binding of [14C]acetaminophen to mouse hepatic microsomal proteins: the specific binding to calreticulin and the two forms of the thiol:protein disulfide oxidoreductases. *Chem. Res. Toxicol.* 9:1176–82
26. Andersson BS, Rundgren M, Nelson SD, Harder S. 1990. N-acetyl-p-benzoquinone imine-induced changes in the energy metabolism in hepatocytes. *Chem. Biol. Interact.* 75:201–11
27. Burcham PC, Harman AW. 1991. Acetaminophen toxicity results in site-specific mitochondrial damage in isolated mouse hepatocytes. *J. Biol. Chem.* 266: 5049–54
28. Karbowski M, Kurono C, Wozniak M,

Ostrowski M, Teranishi M, et al. 1999. Free radical-induced megamitochondria formation and apoptosis. *Free Radic. Biol. Med.* 26:396–409

29. Goldin RD, Ratnayaka ID, Breach CS, Brown IN, Wickramasinghe SN. 1996. Role of macrophages in acetaminophen (paracetamol)-induced hepatotoxicity. *J. Pathol.* 179:432–35
30. Laskin DL, Pilaro AM. 1986. Potential role of activated macrophages in acetaminophen hepatotoxicity. I. Isolation and characterization of activated macrophages from rat liver. *Toxicol. Appl. Pharmacol.* 86:204–15
31. Zhang H, Cook J, Nickel J, Yu R, Stecker K, et al. 2000. Reduction of liver Fas expression by an antisense oligonucleotide protects mice from fulminant hepatitis. *Nat. Biotechnol.* 18:862–67
32. Blazka ME, Elwell MR, Holladay SD, Wilson RE, Luster MI. 1996. Histopathology of acetaminophen-induced liver changes—role of interleukin-1-alpha and tumor-necrosis-factor-alpha. *Toxicol. Pathol.* 24:181–89
33. Ito Y, Abril ER, Bethea NW, McCuskey RS. 2004. Role of nitric oxide in hepatic microvascular injury elicited by acetaminophen in mice. *Am. J. Physiol. Gastrointest. Liver Physiol.* 286:G60–67
34. Kenna JG. 1997. Immunoallergic drug-induced hepatitis: lessons from halothane. *J. Hepatol.* 26(Suppl. 1):5–12
35. Vergani D, Mieli-Vergani G, Alberti A, Neuberger J, Eddleston AL, et al. 1980. Antibodies to the surface of halothane-altered rabbit hepatocytes in patients with severe halothane-associated hepatitis. *New Engl. J. Med.* 303:66–71
36. Martin JL, Kenna JG, Martin BM, Thomassen D, Reed GF, Pohl LR. 1993. Halothane hepatitis patients have serum antibodies that react with protein disulfide isomerase. *Hepatology* 18:858–63
37. Martin JL, Pumford NR, LaRosa AC, Martin BM, Gonzaga HM, et al. 1991. A metabolite of halothane covalently binds to an endoplasmic reticulum protein that is highly homologous to phosphatidylinositol-specific phospholipase C-alpha but has no activity. *Biochem. Biophys. Res. Commun.* 178:679–85
38. Hayden PJ, Ichimura T, McCann DJ, Pohl LR, Stevens JL. 1991. Detection of cysteine conjugate metabolite adduct formation with specific mitochondrial proteins using antibodies raised against halothane metabolite adducts. *J. Biol. Chem.* 266:18415–18
39. Bourdi M, Chen W, Peter RM, Martin JL, Buters JT, et al. 1996. Human cytochrome P450 2E1 is a major autoantigen associated with halothane hepatitis. *Chem. Res. Toxicol.* 9:1159–66
40. Lohse AW, Manns M, Dienes HP, Meyer zum Buschenfelde KH, Cohen IR. 1990. Experimental autoimmune hepatitis: disease induction, time course and T-cell reactivity. *Hepatology* 11:24–30
41. Lohse AW, Brunner S, Kyriatsoulis A, Manns M, Meyer zum Buschenfelde KH. 1992. Autoantibodies in experimental autoimmune hepatitis. *J. Hepatol.* 14:48–53
42. Lecoeur S, Andre C, Beaune PH. 1996. Tienilic acid-induced autoimmune hepatitis: anti-liver and -kidney microsomal type 2 autoantibodies recognize a three-site conformational epitope on cytochrome P4502C9. *Mol. Pharmacol.* 50:326–33
43. Eliasson E, Kenna JG. 1996. Cytochrome P450 2E1 is a cell surface autoantigen in halothane hepatitis. *Mol. Pharmacol.* 50:573–82
44. Belloc C, Gauffre A, Andre C, Beaune PH. 1997. Epitope mapping of human CYP1A2 in dihydralazine-induced autoimmune hepatitis. *Pharmacogenetics* 7: 181–86
45. Kitteringham NR, Kenna JG, Park BK. 1995. Detection of autoantibodies directed against human hepatic endoplasmic reticulum in sera from patients with halothane-associated hepatitis. *Br. J. Clin. Pharmacol.* 40:379–86

46. Naisbitt DJ, Britschgi M, Wong G, Farrell J, Depta JP, et al. 2003. Hypersensitivity reactions to carbamazepine: characterization of the specificity, phenotype, and cytokine profile of drug-specific T cell clones. *Mol. Pharmacol.* 63:732–41
47. Mitchell JR, Zimmerman HJ, Ishak KG, Thorgeirsson UP, Timbrell JA, et al. 1976. Isoniazid liver injury: clinical spectrum, pathology, and probable pathogenesis. *Ann. Intern. Med.* 84:181–92
48. Zimmerman HJ. 1990. Update of hepatotoxicity due to classes of drugs in common clinical use: non-steroidal drugs, anti-inflammatory drugs, antibiotics, antihypertensives, and cardiac and psychotropic agents. *Semin. Liver Dis.* 10:322–38
49. Zimmerman HJ, Ishak KG. 1995. Morphologic spectrum of drug-induced hepatic disease. *Gastroenterol. Clin. North Am.* 24:759–86
50. Durand F, Bernuau J, Pessayre D, Samuel D, Belaiche J, et al. 1995. Deleterious influence of pyrazinamide on the outcome of patients with fulminant or subfulminant liver failure during antituberculous treatment including isoniazid. *Hepatology* 21:929–32
51. Ormerod LP, Skinner C, Wales J. 1996. Hepatotoxicity of antituberculosis drugs. *Thorax* 51:111–13
52. Schaberg T, Rebhan K, Lode H. 1996. Risk factors for side-effects of isoniazid, rifampin and pyrazinamide in patients hospitalized for pulmonary tuberculosis. *Eur. Respir. J.* 9:2026–30
53. Nelson SD, Mitchell JR, Timbrell JA, Snodgrass WR, Corcoran GB 3rd. 1976. Isoniazid and iproniazid: activation of metabolites to toxic intermediates in man and rat. *Science* 193:901–3
54. Sarich TC, Zhou T, Adams SP, Bain AI, Wall RA, Wright JM. 1995. A model of isoniazid-induced hepatotoxicity in rabbits. *J. Pharmacol. Toxicol. Methods* 34:109–16
55. Rozwarski DA, Grant GA, Barton DH, Jacobs WR Jr, Sacchettini JC. 1998. Modification of the NADH of the isoniazid target (InhA) from Mycobacterium tuberculosis. *Science* 279:98–102
56. Rawat R, Whitty A, Tonge PJ. 2003. The isoniazid-NAD adduct is a slow, tight-binding inhibitor of InhA, the Mycobacterium tuberculosis enoyl reductase: adduct affinity and drug resistance. *Proc. Natl. Acad. Sci. USA* 100:13881–86
57. Fontana RJ, McCashland TM, Benner KG, Appelman HD, Gunartanam NT, et al. 1999. Acute liver failure associated with prolonged use of bromfenac leading to liver transplantation. The Acute Liver Failure Study Group. *Liver Transpl. Surg.* 5:480–84
58. Kenny JR, Maggs JL, Meng X, Sinnott D, Clarke SE, et al. 2004. Syntheses and characterisation of the acyl glucuronide and hydroxy metabolites of diclofenac. *J. Med. Chem.* 47:2816–25
59. Grillo MP, Hua F, Knutson CG, Ware JA, Li C. 2003. Mechanistic studies on the bioactivation of diclofenac: identification of diclofenac-S-acyl-glutathione in vitro in incubations with rat and human hepatocytes. *Chem. Res. Toxicol.* 16:1410–17
60. Shen S, Marchick MR, Davis MR, Doss GA, Pohl LR. 1999. Metabolic activation of diclofenac by human cytochrome P450 3A4: role of 5-hydroxydiclofenac. *Chem. Res. Toxicol.* 12:214–22
61. Tang W, Stearns RA, Bandiera SM, Zhang Y, Raab C, et al. 1999. Studies on cytochrome P-450-mediated bioactivation of diclofenac in rats and in human hepatocytes: identification of glutathione conjugated metabolites. *Drug Metab. Dispos.* 27:365–72
62. Masubuchi Y, Ose A, Horie T. 2002. Diclofenac-induced inactivation of CYP3A4 and its stimulation by quinidine. *Drug Metab. Dispos.* 30:1143–48
63. Kretz-Rommel A, Boelsterli UA. 1993. Diclofenac covalent protein binding is dependent on acyl glucuronide formation and is inversely related to P450-mediated acute cell injury in cultured rat

hepatocytes. *Toxicol. Appl. Pharmacol.* 120:155–61

64. Boelsterli UA. 2003. *Mechanistic Toxicology*. Chapter 9:202. London: Taylor & Francis
65. Hargus SJ, Martin BM, George JW, Pohl LR. 1995. Covalent modification of rat liver dipeptidyl peptidase IV (CD26) by the nonsteroidal anti-inflammatory drug diclofenac. *Chem. Res. Toxicol.* 8:993–96
66. Tolman KG, Chandramouli J. 2003. Hepatotoxicity of the thiazolidinediones. *Clin. Liver Dis.* 7:369–79, vi
67. Kassahun K, Pearson PG, Tang W, McIntosh I, Leung K, et al. 2001. Studies on the metabolism of troglitazone to reactive intermediates in vitro and in vivo. Evidence for novel biotransformation pathways involving quinone methide formation and thiazolidinedione ring scission. *Chem. Res. Toxicol.* 14:62–70
68. Tettey JN, Maggs JL, Rapeport WG, Pirmohamed M, Park BK. 2001. Enzyme-induction dependent bioactivation of troglitazone and troglitazone quinone in vivo. *Chem. Res. Toxicol.* 14:965–74
69. Satoh H, Dilone E, Nangia A, Zhao S, Frank K. 2000. Covalent adducts in human primary hepatocytes cultured with [14C]-troglitazone. *Drug Metab. Rev.* 32(Suppl. 2):204
70. Prabhu S, Fackett A, Lloyd S, McClellan HA, Terrell CM, et al. 2002. Identification of glutathione conjugates of troglitazone in human hepatocytes. *Chem. Biol. Interact.* 142:83–97
71. Haskins JR, Rowse P, Rahbari R, de la Iglesia FA. 2001. Thiazolidinedione toxicity to isolated hepatocytes revealed by coherent multiprobe fluorescence microscopy and correlated with multiparameter flow cytometry of peripheral leukocytes. *Arch. Toxicol.* 75:425–38
72. Watanabe I, Tomita A, Shimizu M, Sugawara M, Yasumo H, et al. 2003. A study to survey susceptible genetic factors responsible for troglitazone-associated hepatotoxicity in Japanese patients with type 2 diabetes mellitus. *Clin. Pharmacol. Ther.* 73:435–55
73. Jayyosi Z, Muc M, Gallagher M, Toutain H, Stevens J, Kelley M. 1999. Covalent binding of troglitazone and other members of the thiazolidinedione family to human liver microsomes, implications for cases of idiosyncratic hepatotoxicity. *Proc. N. Am. ISSX Meet., 9th, Nashville,* Oct. 24–28, p. 200
74. Smith MT. 2003. Mechanisms of troglitazone hepatotoxicity. *Chem. Res. Toxicol.* 16:679–87
75. Evans DC, Watt AP, Nicoll-Griffith DA, Baillie TA. 2004. Drug-protein adducts: an industry perspective on minimizing the potential for drug bioactivation in drug discovery and development. *Chem. Res. Toxicol.* 17:3–16
76. Gupta S, Rogers LK, Taylor SK, Smith CV. 1997. Inhibition of carbamyl phosphate synthetase-I and glutamine synthetase by hepatotoxic doses of acetaminophen in mice. *Toxicol. Appl. Pharmacol.* 146:317–27
77. Bulera SJ, Birge RB, Cohen SD, Khairallah EA. 1995. Identification of the mouse liver 44-kDa acetaminophen-binding protein as a subunit of glutamine synthetase. *Toxicol. Appl. Pharmacol.* 134: 313–20
78. Ribeiro JM, Costas MJ, Cameselle JC. 1999. ADP-ribose pyrophosphatase-I partially purified from livers of rats overdosed with acetaminophen reveals enzyme inhibition in vivo reverted in vitro by dithiothreitol. *J. Biochem. Mol. Toxicol.* 13:171–77
79. Ozdemirler G, Aykac G, Uysal M, Oz H. 1994. Liver lipid peroxidation and glutathione-related defence enzyme systems in mice treated with paracetamol. *J. Appl. Toxicol.* 14:297–99
80. Shirota FN, DeMaster EG, Shoeman DW, Nagasawa HT. 2002. Acetaminophen-induced suppression of hepatic AdoMet synthetase activity is attenuated by

prodrugs of L-cysteine. *Toxicol. Lett.* 132: 1–8

81. Senter PD, Al-Abed Y, Metz CN, Benigni F, Mitchell RA, et al. 2002. Inhibition of macrophage migration inhibitory factor (MIF) tautomerase and biological activities by acetaminophen metabolites. *Proc. Natl. Acad. Sci. USA* 99:144–49
82. Pumford NR, Halmes NC, Martin BM, Cook RJ, Wagner C, Hinson JA. 1997. Covalent binding of acetaminophen to N-10-formyltetrahydrofolate dehydrogenase in mice. *J. Pharmacol. Exp. Ther.* 280:501–5
83. Bruno MK, Khairallah EA, Cohen SD. 1998. Inhibition of protein phosphatase activity and changes in protein phosphorylation following acetaminophen exposure in cultured mouse hepatocytes. *Toxicol. Appl. Pharmacol.* 153:119–32
84. Pierce RH, Franklin CC, Campbell JS, Tonge RP, Chen W, et al. 2002. Cell culture model for acetaminophen-induced hepatocyte death in vivo. *Biochem. Pharmacol.* 64:413–24
85. Daya S, Anoopkumar-Dukie S. 2000. Acetaminophen inhibits liver trytophan-2,3-dioxygenase activity with a concomitant rise in brain serotonin levels and a reduction in urinary 5-hydroxyindole acetic acid. *Life Sci.* 67:235–40
86. Landin JS, Cohen SD, Khairallah EA. 1996. Identification of a 54-kDa mitochondrial acetaminophen-binding protein as aldehyde dehydrogenase. *Toxicol. Appl. Pharmacol.* 141:299–307
87. Halmes NC, Hinson JA, Martin BM, Pumford NR. 1996. Glutamate dehydrogenase covalently binds to a reactive metabolite of acetaminophen. *Chem. Res. Toxicol.* 9:541–46
88. Parmar DV, Ahmed G, Khandkar MA, Katyare SS. 1995. Mitochondrial ATPase: a target for paracetamol-induced hepatotoxicity. *Eur. J. Pharmacol.* 293:225–29
89. Tsokos-Kuhn JO, Hughes H, Smith CV, Mitchell JR. 1988. Alkylation of the liver plasma membrane and inhibition of the Ca^{2+} ATPase by acetaminophen. *Biochem. Pharmacol.* 37:2125–31
90. Corcoran GB, Chung SJ, Salazar DE. 1987. Early inhibition of the Na^{+}/K^{+}-ATPase ion pump during acetaminophen-induced hepatotoxicity in rat. *Biochem. Biophys. Res. Commun.* 149:203–7
91. Qiu Y, Benet LZ, Burlingame AL. 1998. Identification of the hepatic protein targets of reactive metabolites of acetaminophen in vivo in mice using two-dimensional gel electrophoresis and mass spectrometry. *J. Biol. Chem.* 273:17940–53
92. Fountoulakis M, Berndt P, Boelsterli UA, Crameri F, Winter M, et al. 2000. Two-dimensional database of mouse liver proteins: changes in hepatic protein levels following treatment with acetaminophen or its nontoxic regioisomer 3-acetamidophenol. *Electrophoresis* 21:2148–61
93. Gruchalla RS, Sullivan TJ. 1991. Detection of human IgE to sulfamethoxazole by skin testing with sulfamethoxazoyl-poly-L-tyrosine. *J. Allergy Clin. Immunol.* 88:784–92
94. Farrell J, Naisbitt DJ, Drummond NS, Depta JP, Vilar FJ, et al. 2003. Characterization of sulfamethoxazole and sulfamethoxazole metabolite-specific T-cell responses in animals and humans. *J. Pharmacol. Exp. Ther.* 306:229–37
95. Madden S, Maggs JL, Park BK. 1996. Bioactivation of carbamazepine in the rat in vivo. Evidence for the formation of reactive arene oxide(s). *Drug Metab. Dispos.* 24:469–79
96. Boelsterli UA. 2003. Diclofenac-induced liver injury: a paradigm of idiosyncratic drug toxicity. *Toxicol. Appl. Pharmacol.* 192:307–22
97. Scheen AJ. 2001. Hepatotoxicity with thiazolidinediones: is it a class effect? *Drug Saf.* 24:873–88
98. Graham DJ, Green L, Senior JR, Nourjah P. 2003. Troglitazone-induced liver failure: a case study. *Am. J. Med.* 114:299–306

Annual Review of Pharmacology and Toxicology
Volume 45, 2005

CONTENTS