Role of Reactive Metabolites in Drug-Induced Hepatotoxicity

ARTICLE *in* HANDBOOK OF EXPERIMENTAL PHARMACOLOGY · JANUARY 2010

DOI: 10.1007/978-3-642-00663-0_7 · Source: PubMed

6 AUTHORS, INCLUDING:

Abhishek Srivastava
AstraZeneca
17 PUBLICATIONS **301** CITATIONS

SEE PROFILE

Daniel J Antoine
University of Liverpool
98 PUBLICATIONS **1,733** CITATIONS

SEE PROFILE

Role of Reactive Metabolites in Drug-Induced Hepatotoxicity

A. Srivastava, J.L. Maggs, D.J. Antoine, D.P. Williams, D.A. Smith, and B.K. Park

Contents

Abstract Drugs are generally converted to biologically inactive forms and eliminated from the body, principally by hepatic metabolism. However, certain drugs undergo biotransformation to metabolites that can interfere with cellular functions through their intrinsic chemical reactivity towards glutathione, leading to thiol depletion, and functionally critical macromolecules, resulting in reversible modification, irreversible adduct formation, and irreversible loss of activity. There is now a great deal of evidence which shows that reactive metabolites are formed from drugs known to cause hepatotoxicity, such as acetaminophen, tamoxifen, isoniazid,

B.K. Park (✉)
Department of Pharmacology and Therapeutics, Centre for Drug Safety Science, School of Biomedical Sciences, University of Liverpool, Sherrington Buildings, Ashton Street, Liverpool, L69 3GE, UK
e-mail: b.k.park@liv.ac.uk

J. Uetrecht (ed.), *Adverse Drug Reactions*, Handbook of Experimental pharmacology 196,
DOI 10.1007/978-3-642-00663-0_7,

and amodiaquine. The main theme of this article is to review the evidence for chemically reactive metabolites being initiating factors for the multiple downstream biological events culminating in toxicity. The major objectives are to understand those idiosyncratic hepatotoxicities thought to be caused by chemically reactive metabolites and to define the role of toxic metabolites.

Keywords Metabolic activation · Reactive metabolites · Hepatotoxicity

Abbreviations

ADRs	Adverse drug reactions
DILI	Drug-induced liver injury
APAP	Acetaminophen
NAPQI	N-acetyl-p-benzoquinoneimine
GSH	Glutathione
ARE	Antioxidant response element
CCl_4	Carbon tetrachloride
NVP	Nevirapine
INH	Isoniazid
AQ	Amodiaquine
AQQI	Amodiaquine quinoneimine
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ULN	Upper limit of normal

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). There are many types of ADRs, affecting every organ in the body (Table 1). However, drug-induced liver injury (DILI) is the most frequent reason for the withdrawal of an approved drug from the market (Temple and Himmel 2002), and it accounts for approximately 50% of all acute liver failure cases (Kaplowitz 2001; Lee 2003; Ostapowicz 2002) and 15% of all liver transplantations in the United States (Russo et al. 2004).

Most drug-induced hepatotoxicities are unpredictable and poorly understood. The liver is the principal site of drug metabolism; therefore, it is often the target of toxicity. Typically they are idiosyncratic in nature. The manifestations range from mild, asymptomatic changes in serum transaminases, which are relatively common,

Table 1 Classification of adverse drug reactions on the basis of pathological effect (Liebler and Guengerich 2005)

Type	Definition	Examples
On-target or mechanism-related toxicity	Toxicity that occurs because of modulation of the primary pharmacological target (for example, receptor or enzyme), whether in the same cell/tissue or not	Statins
Off-target pharmacology	Adverse effects resulting from interaction of a drug with targets other than intended therapeutic targets	Terfenadine & hERG channel effects
Hypersensitivity & immunological reactions	Hypersensitivity refers to undesirable (damaging, discomfort-producing and sometimes fatal) reactions produced by the normal immune system	Penicillins
Bioactivation to reactive intermediates	The biological transformation of a drug to toxic metabolites, which often results in organ-and tissue-specific toxicity	Acetaminophen
Idiosyncratic toxicities	Rare toxicities that are thought to represent unique susceptibility of affected individuals	Nevirapine Isoniazid

to fulminant hepatic failure, which although rare, is potentially life threatening and may necessitate a liver transplant (Park et al. 1998).

The complete mechanisms of DILI remain largely unknown but appear to involve two pathways: direct hepatotoxicity and adverse immune reactions. In many instances, liver injury is thought to be initiated by the bioactivation of the drug to chemically reactive metabolites, which have the potential to modify the function of various critical cellular macromolecules and are therefore able to cause direct damage.

1.1 Drug Metabolism and Reactive Metabolites

The biotransformation of lipophilic compounds into water-soluble derivatives that are more readily excreted is the physiological role of drug metabolism. The principal site of drug metabolism is the liver. The liver is exposed to xenobiotics immediately after their absorption from the gastro-intestinal tract and has a high capacity for both phase I and phase II biotransformations. Usually, this conversion of the xenobiotic from a lipid to water soluble form results in loss of pharmacological/biological activity. Such metabolic reactions are, therefore, regarded as true "detoxication reactions", but certain xenobiotics additionally undergo biotransformation to toxic/reactive metabolites that can interfere with cellular functions and may have intrinsic chemical reactivity towards certain types of cellular macromolecules. Such biotransformations are therefore termed "intoxication" reactions. These toxic/reactive metabolites have the ability to interact with cellular proteins, lipids and nucleic acids, leading to protein dysfunction, lipid peroxidation, DNA

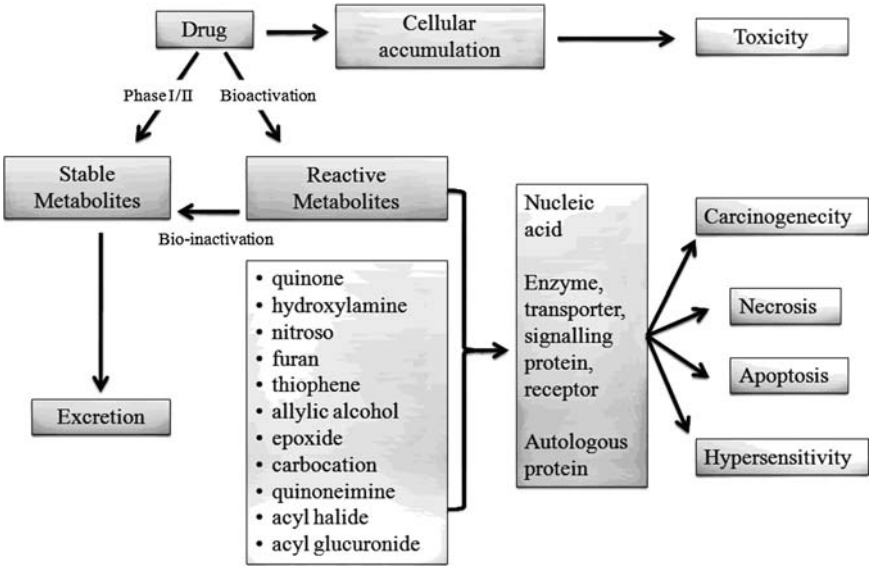


Fig. 1 Relationship between drug metabolism and toxicity. Toxicity may accrue through accumulation of parent drug or, via metabolic activation, through formation of a chemically reactive metabolite, which, if not detoxified, can effect covalent modification of biological macromolecules. The identity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

damage, and oxidative stress. Additionally, the metabolites may induce disruption of ionic gradients and intracellular calcium stores, resulting in mitochondrial dysfunction and loss of energy production. This impairment of cellular function can result in cell death and possible liver failure (Fig. 1).

The propensity of a molecule to form either toxic and/or chemically reactive metabolites is simply a function of its chemistry. Such metabolites are typically short-lived, with half-lives of generally less than 1 s, and are not usually detectable in plasma. Toxicophores are functional groups present in drugs which can be transformed into reactive species by normal biotransformations. The concept that small organic molecules can undergo bioactivation to electrophiles and free radicals, and elicit toxicity by covalent modification of cellular macromolecules, has its basis in chemical carcinogenicity and the pioneering work of the Millers (Miller and Miller 1947, 1952). They studied the hepatotoxic effects of p-dimethylaminoazobenzene in the rat and found that aminoazo dyes become tightly bound to the protein constituents of liver tissue. The application of such concepts to human drug-induced hepatotoxicity was established through the studies of Brodie, Gillette, and Mitchell (Brodie et al. 1971; Gillette et al. 1974) on the covalent binding to hepatic proteins of toxic (over) doses of the widely used analgesic acetaminophen.

Reactive metabolites may be broadly classified as either electrophiles or free radicals (Williams et al. 2002). In the vast majority of cases, the ultimate reactive

species is electrophilic in nature (Parkinson 1996), for example, epoxides and quinoids. Electrophiles are reactive because they are electron deficient and have either a high positive charge density (hard electrophiles) or a lower positive charge density (soft electrophiles) at the electrophilic center. Reactive metabolites that possess unpaired electrons are free radicals. Free radicals usually abstract a hydrogen atom from other molecules rather than becoming covalently bound; however, they can also add to double bonds. Free radical reactions can be self propagating: abstraction of a hydrogen atom from a lipid can initiate a chain reaction leading to lipid peroxidation, oxidative stress, or modification of other types of biological molecules by free radicals (Uetrecht 1995).

Formation of chemically reactive metabolites is mainly catalyzed by cytochromes P450 (Fig. 1). Quantitatively, the P450 isoforms in the endoplasmic reticulum are the most important group of enzymes involved in this process. Products of phase-II metabolism can also lead to toxicity. Additionally, non-cytochrome P450 oxidative enzymes, such as myeloperoxidase and prostaglandin H synthetase, have been implicated in the bioactivation of drugs and other chemicals, the metabolites of which are thought to be responsible for observed toxicity, e.g., clozapine and agranulocytosis, benzene and aplastic anemia (Fischer et al. 1991; Mason and Fischer 1992; Ross et al. 1996; Smith et al. 1989). Cytochrome P450 isoforms are present in different proportions in many organs, though most abundantly in the liver, and thus bioactivate the chemicals to cause organ-specific toxicity (Kao and Carver 1990; Pelkonen and Raunio 1997; Uetrecht 1992).

1.2 Relationship Between Metabolic Activation and Hepatotoxicity

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 absence 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 chemically reactive toxins. Typical examples of bioinactivation pathways include glutathione conjugation of quinones by glutathione *S*-transferases and hydration of arene oxides to dihydrodiols by epoxide hydrolases. The efficiency of a bioinactivation process is dependent on several factors including the inherent chemical reactivity of the substrate, substrate-selectivity of the enzymes, which is usually very broad, tissue expression of the enzymes, and the rapid upregulation of enzyme(s) and cofactors mediated by cellular sensors of chemical stress. It is only when a reactive metabolite can bypass or saturate these defense systems of bioinactivation and thereby damage proteins and nucleic acids through covalent binding that it exerts significant toxic effects.

Table 2 Examples of hard and soft electrophiles and hard and soft nucleophiles

Type	Electrophiles	Nucleophiles
Hard	Alkyl carbonium ions	Oxygen atoms of purine/pyrimidine bases in DNA
	Benzylic carbonium ions	Endocyclic nitrogens of purine bases in DNA
	Iminium ions	Oxygen atoms of protein serine and threonine residues
	Aldehydes	
Soft	Epoxides	Protein thiol groups
	Enones	Sulfhydryl groups of glutatione
	Quinone imines	Primary/secondary amino group of protein lysine and histidine residues
	Quinone methides	
	Michael acceptors	

Moreover, covalent binding of a drug does not necessarily lead to hepatotoxicity. A regioisomer of acetaminophen, 3-hydroxyacetanilide, becomes covalently bound to hepatic proteins in rodents without inducing hepatotoxicity (Qiu et al. 2001; Tirmenstein and Nelson 1989). It is therefore necessary to identify the subset of targets, i.e., covalently modified macromolecules, that are critical to the toxicological process. Unfortunately, although some liver proteins have been identified which are modified covalently by several xenobiotics (Hanzlik et al. 2007), there are currently no techniques to predict the target macromolecule(s) for a particular chemically reactive metabolite let alone the biological consequences of a particular modification. However, from a simple chemical perspective, it is known that hard electrophiles generally react with hard nucleophiles, such as functional groups in DNA and lysine residues in proteins, whereas soft electrophiles react with soft nucleophiles, which include cysteine residues in proteins and in glutathione (Table 2), which has a concentration of approximately 10 mM in the liver.

Furthermore, noncovalent drug–protein interactions also play a role because covalent binding of hepatotoxins is not indiscriminate with respect to cellular proteins (Koen et al. 2007; Qiu et al. 1998). Even within a single liver protein there can be selective modification of an amino acid side-chain found repeatedly in the primary structure (Koen et al. 2006; Nerland et al. 2003). Thus, the microenvironment (pKa, 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, when it has a profound effect on function, is a potential mechanism of drug-induced hepatotoxicity. However, it is also important to note that a number of drugs (e.g., penicillins, clopidogrel, and omeprazole) rely on covalent binding to proteins for their efficacy, and thus prevention of adverse covalent binding through chemical modification of the compound may also inadvertently lead to loss of efficacy. Similarly, certain endogenous electrophiles, such as cyclopentenone prostaglandins, are Michael acceptors, which can react with specific cysteine residues in transcription factors to elicit their physiological effects in cell signaling (Kawamoto et al. 2000).

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 functionally inconsequential "background" covalent binding.

1.3 Relationship Between Dose of Drug and Incidence of Hepatotoxicity

Although liver toxicity does not show a classical dose response for any one drug, many of the drugs that are associated with high incidence of hepatotoxicity are given at high doses, a factor that drives the relatively high amounts of reactive metabolite formed (Smith and Obach 2005; Uetrecht 1999). Moreover, in a recent study of 233 most commonly prescribed oral medications that are associated with significantly higher incidences of DILI, it has been reported that the drugs that are prescribed at higher dose have a higher incidence of hepatotoxicity: out of 598 cases reported, 77% occurred in patients taking drugs at daily doses of at least 50 mg (higher doses), 14% at doses of 11-49 mg (intermediate doses), and only 9% for drugs at doses less than 10 mg (lower doses) (Lammert et al. 2008).

Table 3 lists the dose and incidence of the hepatotoxicity for various drugs, some of which have been withdrawn as a result of these findings. Some of the drugs show effects on liver function earlier than the actual onset of liver toxicity, with serum aminotransferase levels raised by threefold the upper limit of normal (ULN). The incidence of this is much higher and can show a classical dose response: for instance, tolcapone, a catechol-O-methyltransferase inhibitor used as an adjunct to levodopa in Parkinson's disease, produces threefold the ULN in 1–3% of patients receiving 100 mg TID and 3.7% of patients receiving 200 mg TID (Olanow and Watkins 2007). Tolcapone, an *o*-nitrocatechol, is metabolized to reactive intermediates – o-quinone or quinoneimine species – by human liver microsomes (Smith et al. 2003).

In the next section, we discuss a selection of hepatotoxic drugs and nondrug xenobiotics, and summarize the evidence for reactive metabolite formation and the current hypotheses of how this might lead to liver injury and defensive cellular responses.

2 Model Hepatotoxins: Role of Reactive Metabolite Formation

A number of simple chemical compounds such as acetaminophen, bromobenzene, furosemide, methapyrilene (Graham et al. 2008), and thiobenzamide (Ikehata et al. 2008) produce hepatotoxicity, with damage to extrahepatic tissues

Table 3 Incidance of hepatotoxicity associated with various drugs ([a]drugs discussed in this review, [b]cases with serum aminotransferase levels raised 3x ULN, but acute liver failure cases reported are 1:3)

Drugs	Dose (mg/day)	Incidence per treated patient	Metabolic activation
Acetaminophen[a]	>4,000	1:1[b] (Ostapowicz et al. 2002)	Yes (Mitchell et al. 1973; Raucy et al. 1989)
Acetaminophen[a]	<4,000	1:250 (Dart and Bailey 2007)	Yes (Mitchell et al. 1973; Raucy et al. 1989)
Nevirapine[a]	200–400	1:30 (de Maat et al. 2002)	Proposed (Uetrecht 2006)
Isoniazid[a]	300	1:250 (Derby et al. 1993)	Yes (Nelson et al. 1976; Sarich et al. 1995)
Troglitazone	400	1:3,000 (Graham et al. 2003)	Yes (He et al. 2004)
Bromfenac	100–200	1:15,000 (Goldkind and Laine 2006)	–
Amodiaquine[a]	200–400	1:15,650 (Phillips-Howard and West 1990)	Yes (Harrison et al. 1992; Jewell et al. 1995)
Clozapine	300	1:16,000 (Macfarlane et al. 1997)	Yes (Pirmohamed et al. 1995)
Trovafloxacin	200	1:18,000 (Ball et al. 1999)	Proposed (Sun et al. 2008)
Zileuton	2,400	1:30,000 (Watkins et al. 2007)	Yes (Joshi et al. 2004)
Tolcapone	300–600	1:30,000 (Olanow and Watkins 2007)	Yes (Smith et al. 2003)
Nimesulide	200	1:10,00000 (Boelsterli 2002)	Proposed (Boelsterli et al. 2006)

in certain cases (bromobenzene is also pneumotoxic and nephrotoxic; Dahl et al. 1990), in one or more rodent species after a single dose or a short-term regimen. The reproducibility of these injuries permits detailed mechanistic investigations that are impractical or unachievable in the case of idiosyncratic reactions; however, they may provide a source of crucial insights into the mechanisms of such reactions.

It is generally accepted that the toxicities of these model hepatotoxins are associated with the generation of chemically reactive metabolites, which can be detected and estimated indirectly through the irreversible binding of uncharacterized radiolabeled material to hepatic protein (Evans et al. 2004) and/or the formation of stable metabolites such as glutathione conjugates (Masubuchi et al. 2007). Until recently, most assays of reactive metabolite formation in vivo and in vitro depended on measuring irreversible binding of radioactivity using relatively laborious methods of exhaustive solvent extraction (Evans et al. 2004; Masubuchi et al. 2007; Takakusa et al. 2008). The possibility of an alternative, semi-quantitative assay of reactive metabolites in microsomal incubations based upon a novel glutathione analog and LC-MS/MS has been established by Soglia et al. (2006). Advanced mass spectrometry techniques now enable not only high-throughput screening of glutathione-trapped reactive metabolites but also structural characterization of adducts, which provides fundamental insights into the identities of the reactive metabolites (Ma et al. 2008; Wen et al. 2008). A complementary method exists for cyanide trapping of iminium ion intermediates

generated in microsomal incubations (Argoti et al. 2005). As an alternative technique, a peptide-based in vitro method for the detection of reactive metabolites, in which covalently bound adducts are detected by mass spectrometry, has been developed (Mitchell et al. 2008). A recent study on the mechanism-based inactivation of cytochrome P450s by 17-α-ethynylestradiol illustrated how the characterization of GSH adducts of reactive metabolites can complement parallel analyses of modified proteins (Kent et al. 2006). However, the structure of a GSH adduct of a reactive species is not invariably analogous to that of the protein adduct (Koenigs et al. 1999).

The metabolic activation of hepatotoxins and the covalent modification of cellular, sub-cellular, and blood plasma proteins by reactive metabolites, in general, has received considerable attention (Caldwell and Yan 2006; Kalgutkar and Soglia 2005; Park et al. 2005). Nevertheless, the structure of the metabolite-protein adduct has been determined in only a few cases (Baer et al. 2007; Bambal and Hanzlik 1995; Damsten et al. 2007; Sleno et al. 2007; Yukinaga et al. 2007; Zhang et al. 2003), and identification of the modified amino acid residue in vivo remains a major analytical challenge (Koen et al. 2006). Greater progress has been made in identification of the cellular proteins that are modified in vivo (Druckova et al. 2007; Ikehata et al. 2008; Koen et al. 2007; Qiu et al. 1998; Shipkova et al. 2004). Potential targets within individual organelles can now be identified by using model electrophiles in cell fractions (Shin et al. 2007; Wong and Liebler 2008). Adducted amino acid residues of hepatic proteins are likely to be more easily identified in vivo if an intrinsically reactive compound is administered (Nerland et al. 2003). However, the usefulness of this approach for identifying potential binding sites of reactive metabolites has not been established. Nevertheless, it has the potential to confirm the association of selective modification of liver proteins with loss of critical enzyme activities (Campian et al. 2002) suggested by various studies on APAP (Park et al. 1998). The extent to which a particular loss of activity in vivo is due to adduction by a reactive metabolite or drug-induced oxidative modifications of amino acid residues is a complex analytical problem (Andringa et al. 2008). Despite these difficulties, the expectation is that a comprehensive database of cellular proteins modified covalently by reactive metabolites in vivo should ultimately facilitate elucidation of the mechanisms of associated toxicities (Hanzlik et al. 2007). It is already apparent that, although each bioactivated xenobiotic may modify a unique set of hepatic proteins, there is a partial commonality with the proteins modified by other compounds (Koen et al. 2007). A similar selectivity is seen with model electrophiles in vitro (Shin et al. 2007). The proteins modified perform a great variety of biological functions, with corresponding potential for disruption of those functions. The relationship of the adduction of these proteins to the toxicity of reactive metabolites has been discussed in terms of inhibition of enzymes critical to maintenance of cellular energy and homeostasis, the unfolded protein response, and interference with kinase-based signaling pathways (Ikehata et al. 2008). The targeting of components of signaling pathways and metabolic networks has been proposed, alongside the need to understand mechanisms of damage at a systems level (Liebler 2008).

2.1 *Acetaminophen*

Acetaminophen (APAP) is a major cause of drug-related morbidity and mortality in humans, producing massive hepatic necrosis after a single toxic dose. APAP is thought to be safe at therapeutic doses, but some studies have claimed that it may be hepatotoxic at doses below 4 g per day (Dart and Bailey 2007; Watkins et al. 2006) (Fig. 2).

At therapeutic doses, APAP is safely cleared by glucuronylation and sulphation to metabolites that are rapidly excreted in urine. However, a proportion of the drug undergoes bioactivation to N-acetyl-p-benzoquinoneimine (NAPQI) by CYP2E1, CYP1A2, and CYP3A4 (Raucy et al. 1989; Thummel et al. 1993). NAPQI is rapidly quenched by a spontaneous and enzymatic reaction (Coles et al. 1988) with hepatic glutathione after a therapeutic dose of APAP (Fig. 3). However, after a toxic (over) dose, glutathione depletion occurs, which is an obligatory step for covalent binding and toxicity (Davis et al. 1974). The standard treatment for APAP 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 (Albano et al. 1985; Rafeiro et al. 1994).

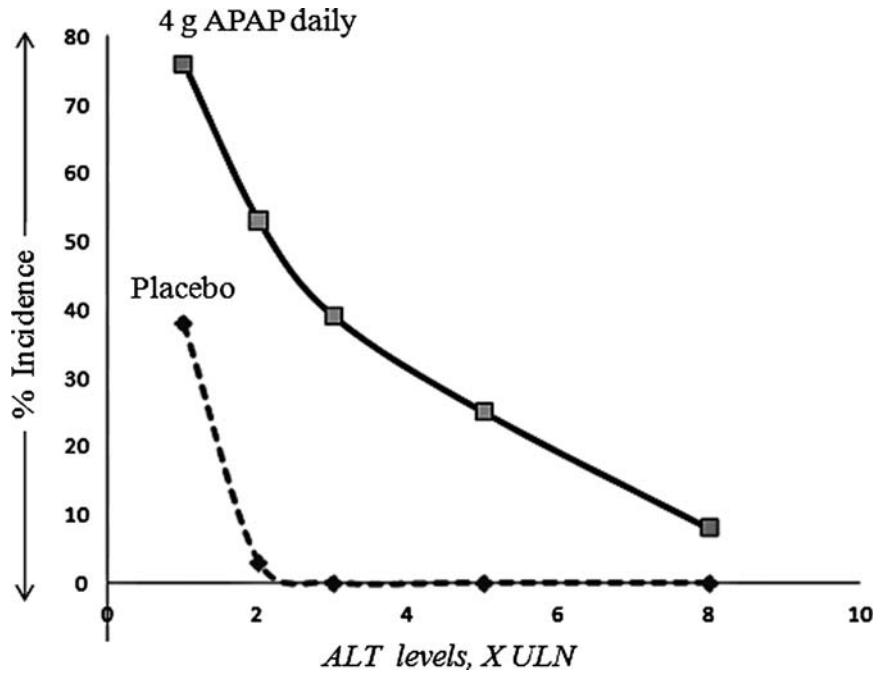


Fig. 2 Incidence of peak alanine aminotransferase elevations by multiples of upper limit of normal for therapeutic dose (4 g/day) of APAP and placebo, in 145 healthy adults (Watkins et al. 2006)

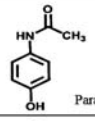
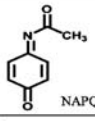
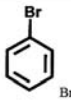
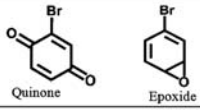
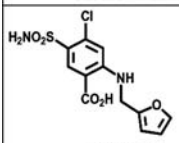
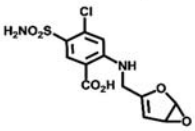
Parent Compound	Reactive Metabolite	GSH Depletion	Covalent Binding
 Paracetamol	 NAPQI	Required	Yes
 Bromobenzene	 Quinone Epoxide	Required	Yes
CCl_4 Carbon Tetrachloride	$CCl_3^{\bullet}$ Trichloromethyl radical	May occur	Yes
 Furosemide	 Furosemide epoxide	May occur	Yes

Fig. 3 Bioactivation of model hepatotoxins. The structures of the toxic metabolites that are formed from oxidative metabolism of acetaminophen, bromobenzene, carbon tetrachloride, and furosemide are given alongside the requirement for GSH depletion and covalent binding

The massive biochemical stress produced by an APAP 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. Thus, with respect to APAP, Nrf-2-activated genes of immediate significance are those coding for γ-glutamylcysteine synthetase (γ-GCS), GSTs, glucuronyltransferases, and heme oxygenase. Importantly, it has been observed that nuclear translocation occurs at nontoxic doses of APAP and at time-points before overt toxicity is observed (Goldring et al. 2004). However, with increasing doses of APAP, there is progressive dislocation of nuclear translocation, transcription, translation, and protein activity (Kitteringham et al. 2000), as the rate of drug bioactivation overwhelms cell defense through the destruction of critical proteins.

The cytoplasmic protein Keap1 directly binds to Nrf2 and represses transcription by promoting proteasome-dependent degradation of the protein. It has also been demonstrated that Keap1 is an adaptor molecule for the ubiquitin ligase complex and directs the rapid degradation of Nrf2. When cells are exposed to electrophiles, Nrf2 is liberated from the Keap1-mediated degradation process and accumulates in the nucleus to activate antioxidant response element (ARE)-mediated gene transcription (Okawa et al. 2006). Somatic disruption of the Keap1 gene does not interfere with the development of the morphological and physiological integrity of the liver. However, specific knockout of the Keap1 gene in mouse hepatocytes confers a strong resistance to drug-induced toxicity (Okawa et al. 2006). This indicates that the constitutive activation of Nrf2, and concomitantly activation of its target genes, is advantageous for mice to overcome xenobiotic toxicity.

2.2 Bromobenzene

Bromobenzene is an industrial solvent and environmental pollutant, which causes centrilobular hepatic necrosis in rats and mice (Casini et al. 1985; Jollow et al. 1974) and is toxic to isolated rodent and human hepatocytes (Grewal et al. 1996; Mennes et al. 1994). The hepatic damage is generally believed to be mediated through P450-generated reactive intermediates (Fisher et al. 1993), especially bromobenzene 3,4-epoxide, although alkylation of protein sulfur nucleophiles in vivo by quinone metabolites is 10–15 times more extensive than alkylation by epoxides (Slaughter and Hanzlik 1991) (Fig. 3). The 3, 4-epoxide can be hydrolysed by microsomal epoxide hydrolase to form the dihydrodiol (den Besten et al. 1994) or detoxified by conjugation to GSH (Jollow et al. 1974). The epoxide intermediate may also undergo nonenzymatic isomerisation to 4-bromophenol (Selander et al. 1975), which can be further metabolized to yield catechols and quinones, some of which have been associated with hepatotoxicity via redox cycling and oxidative stress (Mizutani and Miyamoto 1999). The postulated mechanisms of bromobenzene-induced hepatotoxicity include GSH depletion, lipid peroxidation, covalent modification of critical cellular proteins, and mitochondrial dysfunction (Casini et al. 1985; Fisher et al. 1993; Koen et al. 2007; Maellaro et al. 1990). Modification of rat hepatic proteins by bromobenzene's reactive metabolites has been analyzed in some detail and undoubtedly appears to be a highly selective process at both the molecular (Koen et al. 2006) and cellular levels (Koen et al. 2007), although there is partial commonality with the proteins modified by other bioactivated hepatotoxins such as APAP (Koen et al. 2007). Nevertheless, no specific mechanistic linkage with hepatotoxicity has been elucidated to date. Some early studies obtained a dissociation of bromobenzene-induced liver necrosis from covalent binding in mice administered an antioxidant that largely blocked the necrosis (Casini et al. 1984, 1985). Major interindividual differences between the susceptibility of isolated primate hepatocytes to bromobenzene were observed by Mennes et al. (1994), which could not be attributed to rates of cytochrome P450-mediated bioactivation. This suggested that the combined activities of metabolic deactivating reactions might be the major determinant of cell damage.

2.3 Carbon Tetrachloride

Carbon tetrachloride (CCl_4) was a widely used industrial solvent and cleaner. It produces liver injury in rats and mice that has served as a useful experimental model for certain hepatotoxic effects (Renner 1985; Slater 1981). A single dose of CCl_4 when administered to a rat produces centrilobular necrosis and fatty degeneration of the liver, and chronic low dose exposure is associated with liver cirrhosis (Slater et al. 1985). These hepatotoxic effects of CCl_4 are mediated through its metabolic activation in the liver by CYP2E1 to reactive intermediates, including the

trichloromethyl free radical (Fig. 3). The radicals formed are capable of covalently binding to lipids and proteins, with lipids being the favored option. The availability of oxygen determines the nature of the pathological outcome, with low oxygen levels favoring covalent binding to cellular protein as well as to CYP450s (Recknagel et al. 1989). Hepatotoxic mechanisms are thought to be through lipid peroxidation and disruption of cellular homeostasis, which can lead to disruption of the cytoskeleton, cell signaling, and gene expression pathways (Clawson 1989; Recknagel et al. 1989).

2.4 Furosemide

Furosemide is a highly potent loop diuretic, which causes massive hepatic necrosis in mice (Mitchell et al. 1974; Williams et al. 2007; Wong et al. 2000). It has been postulated that the hepatocellular damage caused by furosemide is a result of cytochrome P450-mediated bioactivation of the furan ring to an epoxide intermediate (Mitchell et al. 1974, 1976a), which binds covalently with hepatic macromolecules both in vivo and in vitro (Mitchell et al. 1976a) (Fig. 3). The proteins modified by the reactive metabolite have not been identified. Recently, we have found that furosemide is metabolized in mice to a γ-ketocarboxylic acid, a product of furan-ring opening, which further indicates the formation of a reactive epoxide intermediate (Williams et al. 2007). Detoxification of furosemide's epoxide is thought to occur via hydration to a dihydrodiol and conjugation with GSH (Williams et al. 2007). Depletion of GSH has been observed in mouse (Grewal et al. 1996) and rat (Williams et al. 2007) hepatocytes. However, furosemide has no effect on hepatic GSH levels in vivo (Mitchell et al. 1974; Wong et al. 2000).

3 Hepatocarcinogenesis: Role of Reactive Metabolite Formation

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 (Guengerich et al. 1996; Wilson et al. 1997). Transgenic knockout mice have been used to establish the role of bioactivation by P450 (Gonzalez and Kimura 1999) and bioinactivation by GSTs (Henderson et al. 1998) for a number of carcinogenic polyaromatic hydrocarbons.

3.1 *Tamoxifen*

Tamoxifen is a nonsteroidal antiestrogen used for the treatment of breast cancer (Benson and Pitsinis 2003; Clemons et al. 2002; Fisher et al. 1998; Furr and Jordan 1984; U.S. Food and Drug Administration 2005b). It has a good tolerability profile and moreover, unlike many other endocrine therapies, it is efficacious in both pre- and postmenopausal women (Clemons et al. 2002). However, an increased risk of developing uterine sarcomas and endometrial carcinomas has been observed in patients treated with tamoxifen (Lavie et al. 2008). Although this drug has been reported to be a potent genotoxic hepatocarcinogen in rats (Williams et al. 1993), it 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.

Tamoxifen is mainly metabolized to *N*-oxide, *N*-desmethyl, α-hydroxy, and 4-hydroxy metabolites (Carter et al. 2001; De Vos et al. 2001; Li et al. 2001; MacCallum et al. 2000). Multiple CYPs including CYP 1A1, 2D6, 3A4, 2B6, and 3A5 are involved in the metabolism of tamoxifen to reactive catechol products that have the potential to form protein and DNA adducts (Davis et al. 1998; Dehal and Kupfer 1999; Fan and Bolton 2001; Notley et al. 2002). The major route of bioactivation of tamoxifen to a genotoxic metabolite is thought to be by sequential α-hydroxylation and sulphonation to a sulfate ester that collapses to a reactive carbocation and forms DNA adducts (Martin et al. 1995) (Fig. 4). Importantly, it was 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) (Boocock et al. 1999, 2000). 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.

4 Idiosyncratic Hepatotoxicity: Role of Reactive Metabolite Formation

Idiosyncratic reactions are extremely host-dependent and uncommon reactions that cannot be either predicted or rationalized from the known pharmacology of the drug. The exact mechanism behind these idiosyncratic reactions is still unclear; however, it is believed that many idiosyncratic reactions are initiated by reactive drug metabolites, e.g., nevirapine, isoniazid, and amodiaquine, which bind covalently to macromolecules and either cause direct cell damage or trigger an immune response.

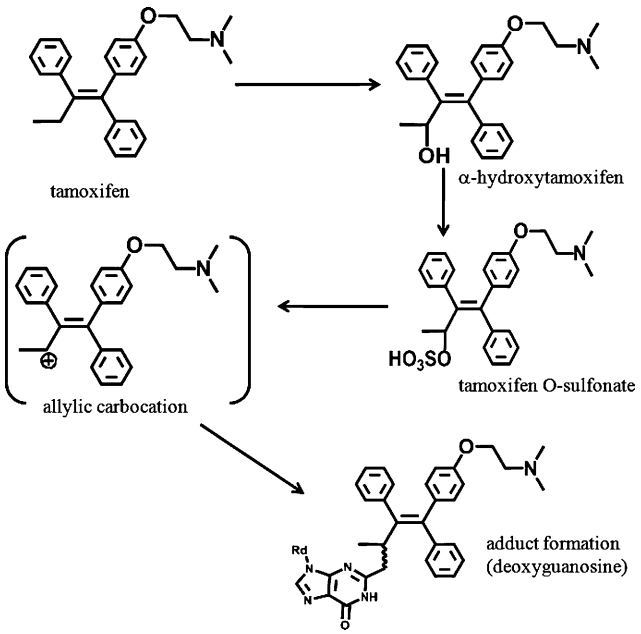


Fig. 4 Metabolic activation of tamoxifen

4.1 Nevirapine

Nevirapine (NVP), a nonnucleoside reverse transcriptase inhibitor, is widely used for the treatment of human immunodeficiency virus (HIV) infections. It is the main option for the first-line treatment of HIV-1, together with two nucleoside reverse transcriptase inhibitors, in countries with limited resources. NVP is associated with two serious clinically restrictive side effects: skin reactions and hepatotoxicity. Severe, life threatening, and in some cases fatal hepatotoxicity, including fulminant and cholestatic hepatitis, hepatic necrosis, and hepatic failure, has been reported in HIV-infected patients taking NVP (DHHS Panel on Antiretroviral Guidelines for Adults and Adolescents 2008). For this reason, NVP is given a black box warning for hepatotoxicity, and concern has been raised over NVP-based treatment.

NVP-induced hepatotoxicity is idiosyncratic in nature and no clear mechanistic understanding of human toxicity exists. Thus, it is impossible to predict which patients will suffer from hepatic damage. For instance, among persons taking NVP, the incidence of an asymptomatic increase of liver enzyme levels in plasma is approximately 5–15% (de Maat et al. 2003; Martin-Carbonero et al. 2003; Martinez et al. 2001; Prakash et al. 2001), and the occurrence of clinically symptomatic hepatotoxicity of NVP is approximately 4% (Boehringer-Ingelheim International 2005; DHHS Panel on Antiretroviral Guidelines for Adults and Adolescents 2008; Martinez et al. 2001; U.S. Food and Drug Administration 2005a). However, patients having concomitant infection with hepatitis viruses and a higher CD4

cell count prior to starting NVP ($\geq$250 cells/mm^3 in females and 400 cells/mm^3 in males) are at a higher risk of developing hepatotoxicity (Boehringer-Ingelheim International 2005; de Maat et al. 2003, 2005; Piliero and Purdy 2001). Multivariate analysis in a recent study showed that the independent risk factors for severe hepatotoxicity were a body mass index (BMI) lower than 18.5, female sex, serum albumin level of less than 35 gl^{-1}, mean corpuscular volume above 85 fl, plasma HIV-1 RNA load of lower than 20,000 copies/ml, aspartate aminotransferase level of less than 75 IU l^{-1}, and lactate dehydrogenase level of less than 164 IU l^{-1}. It is unclear why low baseline enzyme levels should act as predictive factors for hepatotoxicity (Sanne et al. 2005).

The pattern of NVP-induced liver injury varies, not only in terms of severity (Buyse et al. 2006; Centers for Disease Control and Prevention. 2001; Maniar et al. 2006) but also in time to onset (Clarke et al. 2000; de Maat et al. 2003). For instance, some reactions occur early while others occur late after prolonged exposure and, indeed, are not always accompanied by hypersensitivity manifestations, such as fever and eosinophilia. It is possible that the late-occurring reactions may be nonimmune in nature, while those occurring early (e.g., within the first 3 months) may have an immune pathogenesis. This is consistent with our recent observation of NVP-reactive T cells in a patient with early-onset hepatitis in the absence of any cutaneous manifestations (Drummond et al. 2006). The patient's T cells proliferated in vitro on exposure to NVP but not its stable metabolites.

Studies with human liver microsomes suggest that oxidative metabolism of NVP is mediated primarily by cytochrome P450 (CYP) isozymes CYP3A4 and CYP2B6. Among the phase-1 metabolites, 2-hydroxy NVP, 3-hydroxy NVP, 8-hydroxy NVP, and 12-hydroxy NVP are the major metabolites (Riska et al. 1999), mainly formed by CYP3A4, CYP2B6, CYP3A4, and CYP2D6, respectively (Erickson et al. 1999).

The enzymes involved in the metabolism of NVP show considerable variation in expression and activity. CYP3A4 is the most variable enzymatic complex and displays a wide range of inter-individual polymorphic expression (Dai et al. 2001; Wojnowski 2004). In one study, association between the frequent CYP2B6 variant (516G$\rightarrow$T) and NVP pharmacokinetics has been observed. Patients homozygous for the variant allele (516G$\rightarrow$T/T) presented 1.7-fold higher NVP plasma levels than those homozygous for the common allele (516G$\rightarrow$G/G) (Rotger et al. 2005). The clinical implication of this observation remains unclear; however, high NVP plasma levels have been associated with an increased risk of liver toxicity (De Requena et al. 2005; Nunez et al. 2003). Although this variable expression may result in hepatotoxicity in patients homozygous for the variant allele, the relationship between NVP plasma concentrations and the risk of skin rash and hepatic injury appears to be unclear because contradictory reports also exist (Almond et al. 2004). The association of these adverse effects with a higher starting dose, low BMI, and increased age (all of which are associated with higher plasma concentrations) would suggest that there is a relationship with NVP pharmacokinetics (Pirmohamed et al. 2007).

In another study, MDR1 3435C$\rightarrow$T was significantly associated with decreased risk of hepatotoxicity (Haas et al. 2006). This gene is thought to be associated with

altered expression of the P-glycoprotein. It is possible that altered P-glycoprotein activity in the intestine associated with MDR1 variants alters disposition of NVP and/or its metabolites, which in turn affects intracellular concentrations of NVP and/or its metabolites and ultimately toxicity in the liver (Saitoh and Spector 2008).

Although the role of an immune-mediated mechanism in NVP-induced skin rash and hepatotoxicity has been strongly advocated (Pirmohamed et al. 2007; Popovic et al. 2006; Shenton et al. 2007), it is not yet clear whether immune induction in patients is due to a (reactive) metabolite or NVP itself. Highly circumstantial evidence for reactive metabolite involvement comes from a case reported by Claes et al. (2004), where a patient suffering from NVP-induced toxic epidermal necrolysis and toxic hepatitis was successfully treated with intravenous human immunoglobulins and high doses of N-acetylcysteine (300 mg kg^{-1} per day in a continuous infusion until recovery). As a precursor of glutathione, N-acetylcysteine may have helped to restore levels of glutathione, thus enhancing detoxification of toxic metabolites (De Rosa et al. 2000), and resulted in exceptionally fast clinical recovery in this case.

Hypothetically, there may be several pathways for NVP bioactivation. For example, the cyclopropylamine group has the potential to become bioactivated to an aminium cation radical (Shaffer et al. 2001). 12-Hydroxy NVP, which is the major phase-1 metabolite in human liver microsomes, is a substrate for sulphotransferase, and it has been suggested that the sulfate ester dissociates to form a reactive quinonemethide intermediate (Uetrecht 2006; Chen et al. 2008). However, in the case of the hepatotoxicity associated with NVP in humans, Chen et al. (2008) have proposed that tissue injury is due to quinonemethide produced by P450. NVP also has the potential to form an epoxide intermediate in either of the pyridine rings (Fig. 5). Srivastava et al. (2009) have found a pyridino-substituted mercapturate of NVP in human urine that could be derived from a glutathione conjugate of an epoxide. A reactive intermediate (aminium cation/epoxide/quinonemethide) might deplete hepatic glutathione (GSH) in certain patients because the synthesis of GSH is often reduced in HIV infection (Otis et al. 2008; Stehbens 2004). Depletion of GSH, if it is uncompensated, may lead to oxidative stress and covalent binding of NVP to critical hepatic proteins; as a consequence, triggering apoptosis and necrosis of liver cells. Adduct formation with proteins under these circumstances might also lead to the initiation of the immune response, and possibly explain the hypersensitivity observed with NVP.

A fully robust animal model of NVP-induced hepatotoxicity has yet to be developed (Walubo et al. 2006), but Uetrecht and colleagues have characterized extensively a dose-dependent, NVP-induced, skin rash in female Brown Norway rats that resembles the idiosyncratic cutaneous reaction seen in humans and appears to be immune-mediated (Popovic et al. 2006; Shenton et al. 2007). An association between 12-hydroxy NVP metabolism and skin toxicity has been shown in a recent study (Chen et al. 2008), when it was found that 12-OH-NVP caused a rash at a lower dose than required for NVP. The authors proposed that the rash produced in rats may be due to quinonemethide formed in the skin via sulfation of 12-hydroxy NVP metabolite followed by loss of inorganic sulfate.

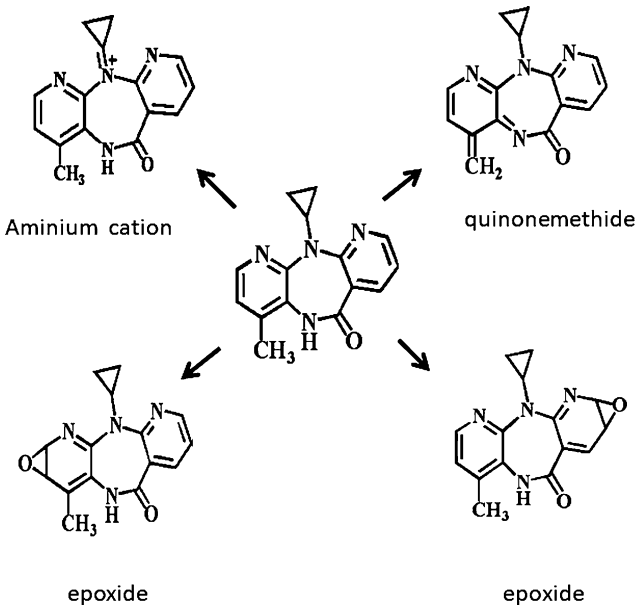


Fig. 5 Possible bioactivation pathways for nevirapine

4.2 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 2 months of therapy, whereas fatal hepatitis is seen in less than 1% of patients. Mortality is greater than 10% in patients with jaundice (Mitchell et al. 1976b; Zimmerman 1990). 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 (Ishak and Zimmerman 1995). 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 (Durand et al. 1995; Ormerod et al. 1996; Schaberg et al. 1996).

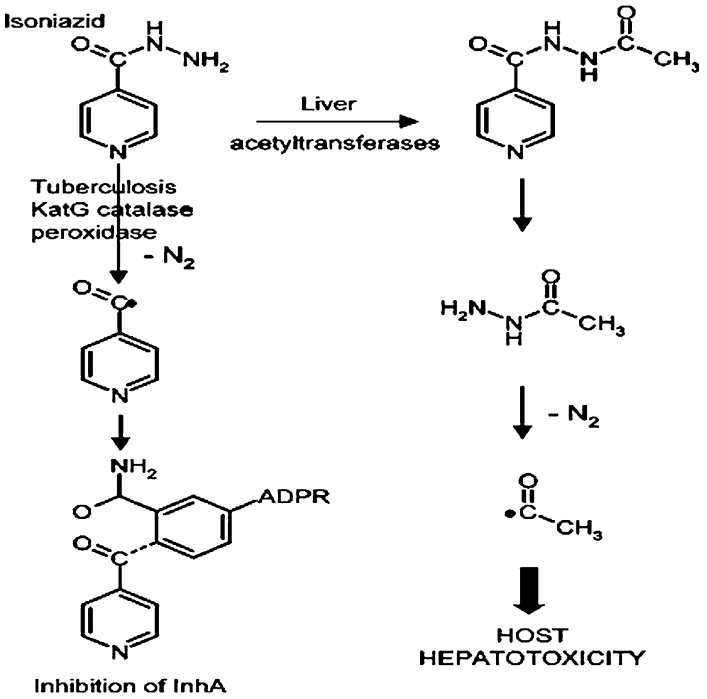


Fig. 6 Metabolic activation of isoniazid. Reactive metabolites are responsible for the pharmacology and toxicology of isoniazid. In *Mycobacterium tuberculosis*, generation of the isonicotinoyl radical leads to the formation of an adduct with NADH, which in turn inhibits an enoyl-acyl carrier protein reductase (InhA) (Rawat et al. 2003; Rozwarski et al. 1998)

Studies in the rat (Nelson et al. 1976) and rabbit (Sarich et al. 1995, 1996), along with in vitro studies, indicate that INH undergoes acetylation to give N-acetylisoniazid. This is hydrolyzed to acetylhydrazine (Lauterburg et al. 1985a, b; Preziosi 2007; Ryan et al. 1985; Sarich et al. 1996), which undergoes bioactivation by P450 to give the acetyl radical (Fig. 6); a reactive species identified by trapping as a glutathione conjugate (Nelson et al. 1976). 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. However, it has been found that rifampicin exacerbates INH-induced toxicity in human though not in rat hepatocytes (Shen et al. 2008). In another study, GST-T1 homozygous null polymorphism was found to be a risk factor for antituberculosis drug-induced hepatotoxicity in humans (Leiro et al. 2008). 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 (Fig. 6). 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 (Rawat et al. 2003; Rozwarski et al. 1998).

4.3 Amodiaquine

Amodiaquine (AQ), a 4-aminoquinoline antimalarial which is effective against many chloroquine-resistant strains of *Plasmodium falciparum*, produces idiosyncratic toxicity in man. Clinical use of AQ has been severely restricted because of life-threatening agranulocytosis and hepatotoxicity seen in about 1 in 2,000 patients during prophylactic administration (Hatton et al. 1986; Larrey et al. 1986; Neftel et al. 1986). The metabolism of AQ has been clearly implicated and there is potential for designing out the toxicity.

AQ, like APAP, undergoes extensive bioactivation to an electrophilic quinoneimine metabolite (AQQI) (Fig. 7), which has been detected in vivo in rats (Harrison et al. 1992) and in vitro (Jewell et al. 1995). However, unlike APAP, AQ can undergo autoxidation in solution. Subsequent oxidative stress or conjugation to

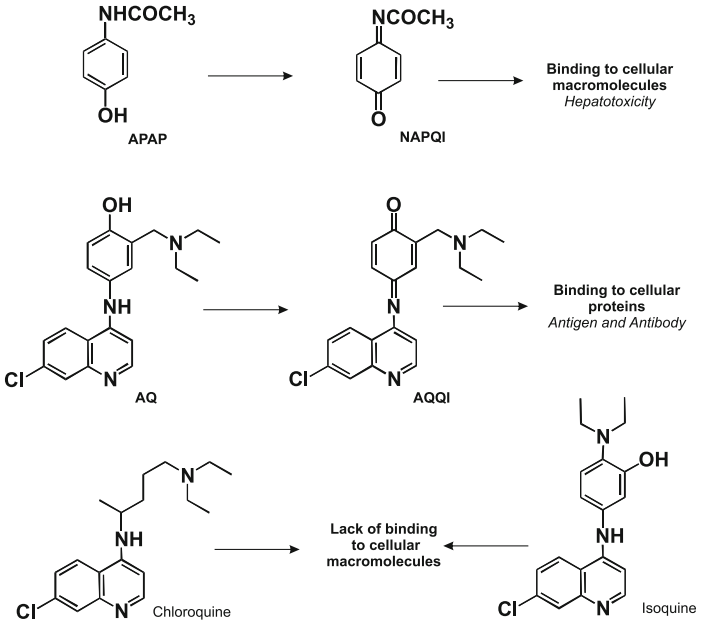


Fig. 7 Metabolic activation of acetaminophen (APAP) and amodiaquine (AQ) to their respective quinoneimines. The structures of chloroquine and isoquine demonstrate their relative lack of chemical reactivity

cysteinyl sulfhydryl groups on proteins is likely to be involved in the induction of toxicity by either cytotoxic or immunological mechanisms (Clarke et al. 1990; Maggs et al. 1988). IgG antibodies, which recognize the 5′-cysteinyl group, have been detected in patients with adverse reactions to AQ (Clarke et al. 1990). However, the factors determining individual susceptibility are unknown.

Modification of AQ has been carried out in an attempt to design a safe replacement for AQ. Interchange of the 3′ hydroxyl and the 4′ Mannich side-chains provided a new series of analogs that cannot form toxic quinoneimine metabolites via cytochrome P450-mediated metabolism (Fig. 7). The prototype, isoquine, emerged as an AQ regioisomer that cannot form toxic metabolites by simple oxidation, and which is potent against chloroquine-resistant parasites in vitro. In sharp contrast to AQ, isoquine (and phase I metabolites) undergoes clearance by glucuronidation instead of GSH conjugation (O'Neill et al. 2003). Isoquine has the potential to be a cost-effective and safer alternative to AQ. Knowledge of AQ's mechanism of toxicity was essential for this development.

5 Conclusion

Clearly, drug metabolism can play an important initiating step in the development of hepatotoxicity, and chemically reactive metabolites have been correlated with the hepatotoxicity of most of the drugs. For this reason, it appears that, if a pharmacophore replaces the potential toxicophore in a new chemical entity, toxicity can be prevented. Additionally, the propensity of a new chemical entity to undergo bioactivation needs to be determined at an early stage of the drug developmental process. High throughput screens have been developed to identify reactive metabolites early in lead optimization based inter alia on covalent binding potential and glutathione adduct formation (Caldwell and Yan 2006; Kalgutkar and Soglia 2005). A retrospective analysis of covalent binding of drugs associated with idiosyncratic drug toxicity (Takakusa et al. 2008) concluded that such studies could be informative in terms of risk assessment. However, it is clear that such studies, when conducted in the preclinical phase of drug development, cannot predict the risk of drug toxicity. Moreover, the use of transgenic animals has provided enhanced definition of the role of P450 enzymes in chemical-induced toxicity. However, these studies, at best, predict the potential of a compound to undergo bioactivation, with the ultimate effect on the liver dependent upon a range of factors such as dose, fractional clearance, rate of metabolic turnover, systemic exposure, activation of cellular signaling pathways, and host-specific factors. In particular, the administration of higher mass doses of drug has been associated with a greater incidence of ADRs (Lammert et al. 2008). Therefore, the development of improved physiological test systems based on information gained from studies with model hepatotoxins such as APAP are required that encompass both chemical and biological factors associated with hepatotoxicity to try and screen for rare but often fatal idiosyncratic hepatotoxicities earlier on in drug development. Finally, the methodology used to

identify protein modification through the irreversible association of radiolabeled compound to general microsomal protein has not changed since the 1960s. Further utilization of shotgun proteomic mass spectrometric techniques combined with LC-MS-MS now allow the identification of adducted amino acids within peptides (Koen et al. 2006). Identification of critical residues within proteins that become adducted, leading to loss of protein function, enhances the biological information that can be obtained from these outdated irreversible binding assays.

References

Albano E, Rundgren M, Harvison PJ et al (1985) Mechanisms of N-acetyl-p-benzoquinone imine cytotoxicity. Mol Pharmacol 28:306–311

Almond LM, Boffito M, Hoggard PG et al (2004) The relationship between nevirapine plasma concentrations and abnormal liver function tests. AIDS Res Hum Retroviruses 20:716–722

Andringa KK, Bajt ML, Jaeschke H et al (2008) Mitochondrial protein thiol modifications in acetaminophen hepatotoxicity: effect on HMG-CoA synthase. Toxicol Lett 177:188–197

Argoti D, Liang L, Conteh A et al (2005) Cyanide trapping of iminium ion reactive intermediates followed by detection and structure identification using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Chem Res Toxicol 18:1537–1544

Baer BR, Wienkers LC, Rock DA (2007) Time-dependent inactivation of P450 3A4 by raloxifene: identification of Cys239 as the site of apoprotein alkylation. Chem Res Toxicol 20:954–964

Ball P, Mandell L, Niki Y et al (1999) Comparative tolerability of the newer fluoroquinolone antibacterials. Drug Saf 21:407–421

Bambal RB, Hanzlik RP (1995) Bromobenzene 3, 4-oxide alkylates histidine and lysine side chains of rat liver proteins in vivo. Chem Res Toxicol 8:729–735

Benson JR, Pitsinis V (2003) Update on clinical role of tamoxifen. Curr Opin Obstet Gynecol 15:13–23

Boehringer-Ingelheim International (2005) Viramune product monograph. Boehringer-Ingelheim Pharmaceuticals Inc, Ridgefield, CT, USA

Boelsterli UA (2002) Nimesulide and hepatic adverse effects: roles of reactive metabolites and host factors. Int J Clin Pract 56(Suppl 128):30–36

Boelsterli UA, Ho HK, Zhou S, and Leow KY (2006) Bioactivation and hepatotoxicity of nitroaromatic drugs. Curr Drug Metab 7:715–727

Boocock DJ, Maggs JL, White IN et al (1999) α-Hydroxytamoxifen, a genotoxic metabolite of tamoxifen in the rat: identification and quantification in vivo and in vitro. Carcinogenesis 20:153–160

Boocock DJ, Maggs JL, Brown K et al (2000) Major inter-species differences in the rates of O-sulphonation and O-glucuronylation of α-hydroxytamoxifen in vitro: a metabolic disparity protecting human liver from the formation of tamoxifen-DNA adducts. Carcinogenesis 21:1851–1858

Brodie BB, Reid WD, Cho AK et al (1971) Possible mechanism of liver necrosis caused by aromatic organic compounds. Proc Natl Acad Sci USA 68:160–164

Buyse S, Vibert E, Sebagh M et al (2006) Liver transplantation for fulminant hepatitis related to nevirapine therapy. Liver Transplant 12:1880–1882

Caldwell GW, Yan Z (2006) Screening for reactive intermediates and toxicity assessment in drug discovery. Curr Opin Drug Discov Devel 9:47–60

Campian EC, Cai J, Benz FW (2002) Acrylonitrile irreversibly inactivates glyceraldehyde-3-phosphate dehydrogenase by alkylating the catalytically active cysteine 149. Chem Biol Interact 140:279–291

Carter SJ, Li XF, Mackey JR et al (2001) Biomonitoring of urinary tamoxifen and its metabolites from breast cancer patients using nonaqueous capillary electrophoresis with electrospray mass spectrometry. Electrophoresis 22:2730–2736

Casini AF, Pompella A, Comporti M (1984) Glutathione depletion, lipid peroxidation, and liver necrosis following bromobenzene and iodobenzene intoxication. Toxicol Pathol 12: 295–299

Casini AF, Pompella A, Comporti M (1985) Liver glutathione depletion induced by bromobenzene, iodobenzene, and diethylmaleate poisoning and its relation to lipid peroxidation and necrosis. Am J Pathol 118:225–237

Centers for Disease Control and Prevention (2001) Serious adverse events attributed to nevirapine regimens for postexposure prophylaxis after HIV exposures-worldwide, 1997–2000. MMWR Morb Mortal Wkly Rep 49:1153–1156

Chen J, Mannargudi BM, Xu L, Uetrecht J (2008) Demonstration of the metabolic pathway responsible for nevirapine-induced skin rash. Chem Res Toxicol 21:1862–1870

Claes P, Wintzen M, Allard S et al (2004) Nevirapine-induced toxic epidermal necrolysis and toxic hepatitis treated successfully with a combination of intravenous immunoglobulins and N-acetylcysteine. Eur J Intern Med 15:255–258

Clarke JB, Maggs JL, Kitteringham NR et al (1990) Immunogenicity of amodiaquine in the rat. Int Arch Allergy Appl Immunol 91:335–342

Clarke S, Harrington P, Condon C et al (2000) Late onset hepatitis and prolonged deterioration in hepatic function associated with nevirapine therapy. Int J STD AIDS 11:336–337

Clawson GA (1989) Mechanisms of carbon tetrachloride hepatotoxicity. Pathol Immunopathol Res 8:104–112

Clemons M, Danson S, Howell A (2002) Tamoxifen ("Nolvadex"): a review. Cancer Treat Rev 28:165–180

Coles B, Wilson I, Wardman P et al (1988) The spontaneous and enzymatic reaction of N-acetyl-p-benzoquinonimine with glutathione: a stopped-flow kinetic study. Arch Biochem Biophys 264:253–260

Dahl JE, Becher R, Aarstad K et al (1990) Species differences in short term toxicity from inhalation exposure to bromobenzene. Arch Toxicol 64:370–376

Dai D, Tang J, Rose R et al (2001) Identification of variants of CYP3A4 and characterization of their abilities to metabolize testosterone and chlorpyrifos. J Pharmacol Exp Ther 299:825–831

Damsten MC, Commandeur JN, Fidder A et al (2007) Liquid chromatography/tandem mass spectrometry detection of covalent binding of acetaminophen to human serum albumin. Drug Metab Dispos 35:1408–1417

Dart RC, Bailey E (2007) Does therapeutic use of acetaminophen cause acute liver failure? Pharmacotherapy 27:1219–1230

Davis DC, Potter WZ, Jollow DJ et al (1974) Species differences in hepatic glutathione depletion, covalent binding and hepatic necrosis after acetaminophen. Life Sci 14:2099–2109

Davis W, Venitt S, Phillips DH (1998) The metabolic activation of tamoxifen and α-hydroxytamoxifen to DNA-binding species in rat hepatocytes proceeds via sulphation. Carcinogenesis 19:861–866

de Maat MM, Mathot RA, Veldkamp AI et al (2002) Hepatotoxicity following nevirapine-containing regimens in HIV-1-infected individuals. Pharmacol Res 46:295–300

de Maat MM, ter Heine R, van Gorp EC et al (2003) Case series of acute hepatitis in a non-selected group of HIV-infected patients on nevirapine-containing antiretroviral treatment. AIDS 17:2209–2214

de Maat MM, Beijnen JH, Schellens JH et al (2005) Chronic hepatotoxicity after long-term antiretroviral treatment including nevirapine. J Infect 50:262–264

De Requena DG, Jimenez-Nacher I, Soriano V (2005) Changes in nevirapine plasma concentrations over time and its relationship with liver enzyme elevations. AIDS Res Hum Retroviruses 21:555–559

De Rosa SC, Zaretsky MD, Dubs JG et al (2000) N-acetylcysteine replenishes glutathione in HIV infection. Eur J Clin Invest 30:915–929

De Vos F, Van de Wiele C, Vandecapelle M et al (2001) High performance liquid chromatographic determination of 123-I labeled tamoxifen metabolites in human plasma. Nucl Med Biol 28:335–338

Dehal SS, Kupfer D (1999) Cytochrome P-450 3A and 2D6 catalyze ortho hydroxylation of 4-hydroxytamoxifen and 3-hydroxytamoxifen (droloxifene) yielding tamoxifen catechol: involvement of catechols in covalent binding to hepatic proteins. Drug Metab Dispos 27:681–688

den Besten C, Brouwer A, Rietjens IM et al (1994) Biotransformation and toxicity of halogenated benzenes. Hum Exp Toxicol 13:866–875

Derby LE, Gutthann SP, Jick H et al (1993) Liver disorders in patients receiving chlorpromazine or isoniazid. Pharmacotherapy 13:353–358

DHHS Panel on Antiretroviral Guidelines for Adults and Adolescents (2008) Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Office of AIDS Research Advisory Council (OARAC), pp 1–134

Druckova A, Mernaugh RL, Ham AJ et al (2007) Identification of the protein targets of the reactive metabolite of teucrin A in vivo in the rat. Chem Res Toxicol 20:1393–1408

Drummond NS, Vilar FJ, Naisbitt DJ et al (2006) Drug-specific T cells in an HIV-positive patient with nevirapine-induced hepatitis. Antivir Ther 11:393–395

Durand F, Bernuau J, Pessayre D et al (1995) Deleterious influence of pyrazinamide on the outcome of patients with fulminant or subfulminant liver failure during antituberculous treatment including isoniazid. Hepatology 21:929–932

Erickson DA, Mather G, Trager WF et al (1999) Characterization of the in vitro biotransformation of the HIV-1 reverse transcriptase inhibitor nevirapine by human hepatic cytochromes P-450. Drug Metab Dispos 27:1488–1495

Evans DC, Watt AP, Nicoll-Griffith DA et al (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

Fan PW, Bolton JL (2001) Bioactivation of tamoxifen to metabolite E quinone methide: reaction with glutathione and DNA. Drug Metab Dispos 29:891–896

Fischer V, Haar JA, Greiner L et al (1991) Possible role of free radical formation in clozapine (clozaril)-induced agranulocytosis. Mol Pharmacol 40:846–853

Fisher R, Brendel K, Hanzlik RP (1993) Correlation of metabolism, covalent binding and toxicity for a series of bromobenzene derivatives using rat liver slices in vitro. Chem Biol Interact 88:191–198

Fisher B, Costantino JP, Wickerham DL et al (1998) Tamoxifen for prevention of breast cancer: report of the national surgical adjuvant breast and bowel project P-1 study. J Natl Cancer Inst 90:1371–1388

Furr BJ, Jordan VC (1984) The pharmacology and clinical uses of tamoxifen. Pharmacol Ther 25:127–205

Gillette JR, Mitchell JR, Brodie BB (1974) Biochemical mechanisms of drug toxicity. Annu Rev Pharmacol 14:271–288

Goldkind L, Laine L (2006) A systematic review of NSAIDs withdrawn from the market due to hepatotoxicity: lessons learned from the bromfenac experience. Pharmacoepidemiol Drug Saf 15:213–220

Goldring CE, Kitteringham NR, Elsby R et al (2004) Activation of hepatic Nrf2 in vivo by acetaminophen in CD-1 mice. Hepatology 39:1267–1276

Gonzalez FJ, Kimura S (1999) Role of gene knockout mice in understanding the mechanisms of chemical toxicity and carcinogenesis. Cancer Lett 143:199–204

Graham DJ, Drinkard CR, and Shatin D (2003) Incidence of idiopathic acute liver failure and hospitalized liver injury in patients treated with troglitazone. Am J Gastroenterol 98:175–179

Graham EE, Walsh RJ, Hirst CM et al (2008) Identification of the thiophene ring of methapyrilene as a novel bioactivation-dependent hepatic toxicophore. J Pharmacol Exp Ther 326:657–671
Grewal KK, Rafeiro E, Racz WJ (1996) Bromobenzene and furosemide hepatotoxicity: alterations in glutathione, protein thiols, and calcium. Can J Physiol Pharmacol 74:257–264
Guengerich FP, Johnson WW, Ueng YF et al (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):557–562
Haas DW, Bartlett JA, Andersen JW et al (2006) Pharmacogenetics of nevirapine-associated hepatotoxicity: an adult AIDS Clinical Trials Group collaboration. Clin Infect Dis 43:783–786
Hanzlik RP, Koen YM, Theertham B et al (2007) The reactive metabolite target protein database (TPDB) – a web-accessible resource. BMC Bioinformat 8:95
Harrison AC, Kitteringham NR, Clarke JB et al (1992) The mechanism of bioactivation and antigen formation of amodiaquine in the rat. Biochem Pharmacol 43:1421–1430
Hatton CSR, Bunch C, Peto TEA et al (1986) Frequency of severe neutropenia associated with amodiaquine prophylaxis against malaria. Lancet 327:411–414
Henderson CJ, Smith AG, Ure J et al (1998) Increased skin tumorigenesis in mice lacking pi class glutathione S-transferases. Proc Natl Acad Sci USA 95:5275–5280
He K, Talaat RE, Pool WF et al (2004) Metabolic activation of troglitazone: identification of a reactive metabolite and mechanisms involved. Drug Metab Dispos 32:639–646
Ikehata K, Duzhak TG, Galeva NA et al (2008) Protein targets of reactive metabolites of thiobenzamide in rat liver in vivo. Chem Res Toxicol 21:1432–1442
Ishak KG, Zimmerman HJ (1995) Morphologic spectrum of drug-induced hepatic disease. Gastroenterol Clin North Am 24:759–786
Jewell H, Maggs JL, Harrison AC et al (1995) Role of hepatic metabolism in the bioactivation and detoxication of amodiaquine. Xenobiotica 25:199–217
Jollow DJ, Mitchell JR, Zampaglione N et al (1974) Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3, 4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacology 11:151–169
Joshi EM, Heasley BH, Chordia MD et al (2004) In vitro metabolism of 2-acetylbenzothiophene: relevance to zileuton hepatotoxicity. Chem Res Toxicol 17:137–143
Kalgutkar AS, Soglia JR (2005) Minimising the potential for metabolic activation in drug discovery. Expert Opin Drug Metab Toxicol 1:91–142
Kao J, Carver MP (1990) Cutaneous metabolism of xenobiotics. Drug Metab Rev 22:363–410
Kaplowitz N (2001) Drug-induced liver disorders: implications for drug development and regulation. Drug Saf 24:483–490
Kawamoto Y, Nakamura Y, Naito Y et al (2000) Cyclopentenone prostaglandins as potential inducers of phase II detoxification enzymes. 15-deoxy-Δ12, 14-prostaglandin J2-induced expression of glutathione S-transferases. J Biol Chem 275:11291–11299
Kent UM, Lin HL, Mills DE et al (2006) Identification of 17-α-ethynylestradiol-modified active site peptides and glutathione conjugates formed during metabolism and inactivation of P450s 2B1 and 2B6. Chem Res Toxicol 19:279–287
Kitteringham NR, Powell H, Clement YN et al (2000) Hepatocellular response to chemical stress in CD-1 mice: induction of early genes and γ-glutamylcysteine synthetase. Hepatology 32:321–333
Koen YM, Yue W, Galeva NA et al (2006) Site-specific arylation of rat glutathione S-transferase A1 and A2 by bromobenzene metabolites in vivo. Chem Res Toxicol 19:1426–1434
Koen YM, Gogichaeva NV, Alterman MA et al (2007) A proteomic analysis of bromobenzene reactive metabolite targets in rat liver cytosol in vivo. Chem Res Toxicol 20:511–519
Koenigs LL, Peter RM, Hunter AP et al (1999) Electrospray ionization mass spectrometric analysis of intact cytochrome P450: identification of tienilic acid adducts to P450 2C9. Biochemistry 38:2312–2319
Lammert C, Einarsson S, Saha C et al (2008) Relationship between daily dose of oral medications and idiosyncratic drug-induced liver injury: Search for signals. Hepatology 47:2003–2009

Larrey D, Castot A, Pessayre D et al (1986) Amodiaquine-induced hepatitis. A report of seven cases. Ann Intern Med 104:801–803

Lauterburg BH, Smith CV, Todd EL et al (1985a) Oxidation of hydrazine metabolites formed from isoniazid. Clin Pharmacol Ther 38:566–571

Lauterburg BH, Smith CV, Todd EL et al (1985b) Pharmacokinetics of the toxic hydrazino metabolites formed from isoniazid in humans. J Pharmacol Exp Ther 235:566–570

Lavie O, Barnett-Griness O, Narod SA et al (2008) The risk of developing uterine sarcoma after tamoxifen use. Int J Gynecol Cancer 18:352–356

Lazarou J, Pomeranz BH, Corey PN (1998) Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies. JAMA 279:1200–1205

Lee WM (2003) Acute liver failure in the United States. Semin Liver Dis 23:217–226

Leiro V, Fernandez-Villar A, Valverde D et al (2008) Influence of glutathione S-transferase M1 and T1 homozygous null mutations on the risk of antituberculosis drug-induced hepatotoxicity in a Caucasian population. Liver Int 28:835–839

Li XF, Carter S, Dovichi NJ et al (2001) Analysis of tamoxifen and its metabolites in synthetic gastric fluid digests and urine samples using high-performance liquid chromatography with electrospray mass spectrometry. J Chromatogr A 914:5–12

Liebler DC (2008) Protein damage by reactive electrophiles: targets and consequences. Chem Res Toxicol 21:117–128

Liebler DC, Guengerich FP (2005) Elucidating mechanisms of drug-induced toxicity. Nat Rev Drug Discov 4:410–420

Ma L, Wen B, Ruan Q et al (2008) Rapid screening of glutathione-trapped reactive metabolites by linear ion trap mass spectrometry with isotope pattern-dependent scanning and postacquisition data mining. Chem Res Toxicol 21:1477–1483

MacCallum J, Cummings J, Dixon JM et al (2000) Concentrations of tamoxifen and its major metabolites in hormone responsive and resistant breast tumours. Br J Cancer 82:1629–1635

Macfarlane B, Davies S, Mannan K et al (1997) Fatal acute fulminant liver failure due to clozapine: a case report and review of clozapine-induced hepatotoxicity. Gastroenterology 112:1707–1709

Maellaro E, Del BB, Casini AF et al (1990) Early mitochondrial disfunction in bromobenzene treated mice: a possible factor of liver injury. Biochem Pharmacol 40:1491–1497

Maggs JL, Tingle MD, Kitteringham NR et al (1988) Drug-protein conjugates-XIV : Mechanisms of formation of protein-arylating intermediates from amodiaquine, a myelotoxin and hepatotoxin in man. Biochem Pharmacol 37:303–311

Maniar JK, Shah SR, Verma R et al (2006) Nevirapine-induced fulminant hepatitis. J Assoc Physicians India 54:957–958

Martin EA, Rich KJ, White IN et al (1995) ^{32}P-postlabelled DNA adducts in liver obtained from women treated with tamoxifen. Carcinogenesis 16:1651–1654

Martin-Carbonero L, Nunez M, Gonzalez-Lahoz J et al (2003) Incidence of liver injury after beginning antiretroviral therapy with efavirenz or nevirapine. HIV Clin Trials 4:115–120

Martinez E, Blanco JL, Arnaiz JA et al (2001) Hepatotoxicity in HIV-1-infected patients receiving nevirapine-containing antiretroviral therapy. AIDS 15:1261–1268

Mason RP, Fischer V (1992) Possible role of free radical formation in drug-induced agranulocytosis. Drug Saf 7(Suppl 1):45–50

Masubuchi N, Makino C, Murayama N (2007) Prediction of in vivo potential for metabolic activation of drugs into chemically reactive intermediate: correlation of in vitro and in vivo generation of reactive intermediates and in vitro glutathione conjugate formation in rats and human. Chem Res Toxicol 20:455–464

Mennes WC, van Holsteijn CW, van Iersel AA et al (1994) Interindividual variation in biotransformation and cytotoxicity of bromobenzene as determined in primary hepatocyte cultures derived from monkey and human liver. Hum Exp Toxicol 13:415–421

Miller EC, Miller JA (1947) The presence and significance of bound aminoazo dyes in the livers of rats fed p-dimethylaminobenzene. Cancer Res 7:468–480

Miller EC, Miller JA (1952) In vivo combinations between carcinogens and tissue constituents and their possible role in carcinogenesis. Cancer Res 12:547–556
Mitchell JR, Jollow DJ, Potter WZ et al (1973) Acetaminophen-induced hepatic necrosis. I. Role of drug metabolism. J Pharmacol Exp Ther 187:185–194
Mitchell JR, Potter WZ, Hinson JA et al (1974) Hepatic necrosis caused by furosemide. Nature 251:508–511
Mitchell JR, Snodgrass WR, Gillette JR (1976a) The role of biotransformation in chemical-induced liver injury. Environ Health Perspect 15:27–38
Mitchell JR, Zimmerman HJ, Ishak KG et al (1976b) Isoniazid liver injury: clinical spectrum, pathology, and probable pathogenesis. Ann Intern Med 84:181–192
Mitchell MD, Elrick MM, Walgren JL et al (2008) Peptide-based in vitro assay for the detection of reactive metabolites. Chem Res Toxicol 21:859–868
Mizutani T, Miyamoto Y (1999) Modulation of halobenzene-induced hepatotoxicity by DT-diaphorase modulators, butylated hydroxyanisole and dicoumarol: evidence for possible involvement of quinone metabolites in the toxicity of halobenzenes. Toxicol Lett 105:25–30
Neftel KA, Woodtly W, Schmid M et al (1986) Amodiaquine induced agranulocytosis and liver damage. BMJ 292:721–723
Nelson SD, Mitchell JR, Timbrell JA et al (1976) Isoniazid and iproniazid: activation of metabolites to toxic intermediates in man and rat. Science 193:901–903
Nerland DE, Cai J, Benz FW (2003) Selective covalent binding of acrylonitrile to Cys 186 in rat liver carbonic anhydrase III in vivo. Chem Res Toxicol 16:583–589
Notley LM, De Wolf CJ, Wunsch RM et al (2002) Bioactivation of tamoxifen by recombinant human cytochrome P450 enzymes. Chem Res Toxicol 15:614–622
Nunez M, Gonzalez-Requena D, Gonzalez-Lahoz J et al (2003) Interactions between nevirapine plasma levels, chronic hepatitis C, and the development of liver toxicity in HIV-infected patients. AIDS Res Hum Retroviruses 19:187–188
Okawa H, Motohashi H, Kobayashi A et al (2006) Hepatocyte-specific deletion of the keap1 gene activates Nrf2 and confers potent resistance against acute drug toxicity. Biochem Biophys Res Commun 339:79–88
Olanow CW, Watkins PB (2007) Tolcapone: an efficacy and safety review (2007). Clin Neuropharmacol 30:287–294
O'Neill PM, Mukhtar A, Stocks PA et al (2003) Isoquine and related amodiaquine analogues: a new generation of improved 4-aminoquinoline antimalarials. J Med Chem 46:4933–4945
Ormerod LP, Skinner C, Wales J (1996) Hepatotoxicity of antituberculosis drugs. Thorax 51:111–113
Ostapowicz G (2002) Summaries for patients. Acute liver failure in the United States. Ann Intern Med 137:I24
Ostapowicz G, Fontana RJ, Schiodt FV et al (2002) Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. Ann Intern Med 137:947–954
Otis JS, Ashikhmin YI, Brown LA et al (2008) Effect of HIV-1-related protein expression on cardiac and skeletal muscles from transgenic rats. AIDS Res Ther 5:8
Park BK, Pirmohamed M, Kitteringham NR (1998) Role of drug disposition in drug hypersensitivity: a chemical, molecular, and clinical perspective. Chem Res Toxicol 11:969–988
Park BK, Kitteringham NR, Maggs JL et al (2005) The role of metabolic activation in drug-induced hepatotoxicity. Annu Rev Pharmacol Toxicol 45:177–202
Parkinson A (1996) Biotransformation of xenobiotics. In: Klaassen CD (ed) Toxicology – the basic science of poisons, 5th edn. McGraw-Hill, New York, pp 113–186
Pelkonen O, Raunio H (1997) Metabolic activation of toxins: tissue-specific expression and metabolism in target organs. Environ Health Perspect 105(Suppl 4):767–774
Phillips-Howard PA, West LJ (1990) Serious adverse drug reactions to pyrimethamine-sulphadoxine, pyrimethamine-dapsone and to amodiaquine in Britain. J R Soc Med 83:82–85
Piliero PJ, Purdy B (2001) Nevirapine-induced hepatitis: A case series and review of the literature. AIDS Read 11:379–382

Pirmohamed M, Williams D, Madden S et al (1995) Metabolism and bioactivation of clozapine by human liver in vitro. J Pharmacol Exp Ther 272:984–990
Pirmohamed M, Breckenridge AM, Kitteringham NR et al (1998) Adverse drug reactions. BMJ 316:1295–1298
Pirmohamed M, Drummond NS, Naisbitt DJ et al (2007) Drug hypersensitivity reactions in patients with HIV disease. Expert Rev Clin Immunol 3:395–410
Popovic M, Caswell JL, Mannargudi B et al (2006) Study of the sequence of events involved in nevirapine-induced skin rash in Brown Norway rats. Chem Res Toxicol 19:1205–1214
Prakash M, Poreddy V, Tiyyagura L et al (2001) Jaundice and hepatocellular damage associated with nevirapine therapy. Am J Gastroenterol 96:1571–1574
Preziosi P (2007) Isoniazid: metabolic aspects and toxicological correlates. Curr Drug Metab 8:839–851
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 273:17940–17953
Qiu Y, Benet LZ, Burlingame AL (2001) Identification of hepatic protein targets of the reactive metabolites of the non-hepatotoxic regioisomer of acetaminophen, 3′-hydroxyacetanilide, in the mouse in vivo using two-dimensional gel electrophoresis and mass spectrometry. Adv Exp Med Biol 500:663–673
Rafeiro E, Barr SG, Harrison JJ et al (1994) Effects of N-acetylcysteine and dithiothreitol on glutathione and protein thiol replenishment during acetaminophen-induced toxicity in isolated mouse hepatocytes. Toxicology 93:209–224
Raucy JL, Lasker JM, Lieber CS et al (1989) Acetaminophen activation by human liver cytochromes P450IIE1 and P450IA2. Arch Biochem Biophys 271:270–283
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 100:13881–13886
Recknagel RO, Glende EA Jr, Dolak JA et al (1989) Mechanisms of carbon tetrachloride toxicity. Pharmacol Ther 43:139–154
Renner (1985) The limited relevance of models used for testing human hepatic diseases and their prevention. In: Keppler E, Popper H, Bianchi L, Reutter W (eds) Mechanisms of hepatocyte injury and death. MTP Press Ltd., Lancaster, pp 311–3133
Riska P, Lamson M, MacGregor T et al (1999) Disposition and biotransformation of the antiretroviral drug nevirapine in humans. Drug Metab Dispos 27:895–901
Ross D, Siegel D, Schattenberg DG et al (1996) Cell-specific activation and detoxification of benzene metabolites in mouse and human bone marrow: identification of target cells and a potential role for modulation of apoptosis in benzene toxicity. Environ Health Perspect 104 (Suppl 6):1177–1182
Rotger M, Colombo S, Furrer H et al (2005) Influence of CYP2B6 polymorphism on plasma and intracellular concentrations and toxicity of efavirenz and nevirapine in HIV-infected patients. Pharmacogenet Genomics 15:1–5
Rozwarski DA, Grant GA, Barton DH et al (1998) Modification of the NADH of the isoniazid target (InhA) from *Mycobacterium tuberculosis*. Science 279:98–102
Russo MW, Galanko JA, Shrestha R et al (2004) Liver transplantation for acute liver failure from drug induced liver injury in the United States. Liver Transpl 10:1018–1023
Ryan DE, Ramanathan L, Iida S et al (1985) Characterization of a major form of rat hepatic microsomal cytochrome P-450 induced by isoniazid. J Biol Chem 260:6385–6393
Saitoh A, Spector SA (2008) Effect of host genetic variation on the pharmacokinetics and clinical response of NNRTIs. Future HIV Ther 2:69–81
Sanne I, Mommeja-Marin H, Hinkle J et al (2005) Severe hepatotoxicity associated with nevirapine use in HIV-infected subjects. J Infect Dis 191:825–829
Sarich TC, Zhou T, Adams SP et al (1995) A model of isoniazid-induced hepatotoxicity in rabbits. J Pharmacol Toxicol Methods 34:109–116

Sarich TC, Youssefi M, Zhou T et al (1996) Role of hydrazine in the mechanism of isoniazid hepatotoxicity in rabbits. Arch Toxicol 70:835–840
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 9:2026–2030
Selander HG, Jerina DM, Daly JW (1975) Metobolism of chlorobenzene with hepatic microsomes and solubilized cytochrome P-450 systems. Arch Biochem Biophys 168:309–321
Shaffer CL, Morton MD, Hanzlik RP (2001) N-dealkylation of an N-cyclopropylamine by horseradish peroxidase. Fate of the cyclopropyl group. J Am Chem Soc 123:8502–8508
Shen C, Meng Q, Zhang G et al (2008) Rifampicin exacerbates isoniazid-induced toxicity in human but not in rat hepatocytes in tissue-like cultures. Br J Pharmacol 153:784–791
Shenton J, Popovic M and Uetrecht J (2007) Nevirapine Hypersensitivity. In: Pichler WJ (ed) Drug Hypersensitivity. Karger AG, Basel, pp 115–128
Shin NY, Liu Q, Stamer SL et al (2007) Protein targets of reactive electrophiles in human liver microsomes. Chem Res Toxicol 20:859–867
Shipkova M, Beck H, Voland A et al (2004) Identification of protein targets for mycophenolic acid acyl glucuronide in rat liver and colon tissue. Proteomics 4:2728–2738
Slater (1981) Activation of carbon tetrachloride: chemical principles and biological significance. In: McBrien DCH, Slater TF (eds) Free radicals, lipid peroxidation and cancer. Academic Press, London, pp 243–270
Slater TF, Cheeseman KH, Collins M, Maddix S, Milia A, Proudfoot K, Burton GW, Webb AC, Ingold KU (1985) Carbon tetrachloride toxicity as a model for studying free-radical mediated liver injury. Philos Trans R Soc Lond B 311:633–645
Slaughter DE, Hanzlik RP (1991) Identification of epoxide- and quinone-derived bromobenzene adducts to protein sulfur nucleophiles. Chem Res Toxicol 4:349–359
Sleno L, Varesio E, Hopfgartner G (2007) Determining protein adducts of fipexide: mass spectrometry based assay for confirming the involvement of its reactive metabolite in covalent binding. Rapid Commun Mass Spectrom 21:4149–4157
Smith DA, Obach RS (2005) Seeing through the mist: abundance versus percentage. Commentary on metabolites in safety testing. Drug Metab Dispos 33:1409–1417
Smith MT, Yager JW, Steinmetz KL et al (1989) Peroxidase-dependent metabolism of benzene's phenolic metabolites and its potential role in benzene toxicity and carcinogenicity. Environ Health Perspect 82:23–29
Smith KS, Smith PL, Heady TN et al (2003) In vitro metabolism of tolcapone to reactive intermediates: relevance to tolcapone liver toxicity. Chem Res Toxicol 16:123–128
Soglia JR, Contillo LG, Kalgutkar AS et al (2006) A semiquantitative method for the determination of reactive metabolite conjugate levels in vitro utilizing liquid chromatography-tandem mass spectrometry and novel quaternary ammonium glutathione analogues. Chem Res Toxicol 19:480–490
Srivastava A, Williams DP, Maggs JL, et al (2009) Characterization and Quantification of Mercapturate and Glutathione Conjugates of Nevirapine. Toxicology 262:6–7
Stehbens WE (2004) Oxidative stress in viral hepatitis and AIDS. Exp Mol Pathol 77:121–132
Sun Q, Zhu R, Foss FW Jr et al (2008) In vitro metabolism of a model cyclopropylamine to reactive intermediate: insights into trovafloxacin-induced hepatotoxicity. Chem Res Toxicol 21:711–719
Takakusa H, Masumoto H, Yukinaga H et al (2008) Covalent binding and tissue distribution/retention assessment of drugs associated with idiosyncratic drug toxicity. Drug Metab Dispos 36:1770–1779
Temple RJ, Himmel MH (2002) Safety of newley approved drugs: implications for prescribing. JAMA 287:2273–2275
Thummel KE, Lee CA, Kunze KL et al (1993) Oxidation of acetaminophen to N-acetyl-p-aminobenzoquinone imine by human CYP3A4. Biochem Pharmacol 45:1563–1569

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 264:9814–9819

U.S. Food and Drug Administration (2005a) FDA public health advisory for nevirapine (Viramune)

U.S. Food and Drug Administration (2005b) Tamoxifen Information

Uetrecht JP (1992) The role of leukocyte-generated reactive metabolites in the pathogenesis of idiosyncratic drug reactions. Drug Metab Rev 24:299–366

Uetrecht JP (1995) Myeloperoxidase as a generator of drug free radicals. Biochem Soc Symp 61:163–170

Uetrecht JP (1999) New concepts in immunology relevant to idiosyncratic drug reactions: the "danger hypothesis" and innate immune system. Chem Res Toxicol 12:387–395

Uetrecht J (2006) Evaluation of which reactive metabolite, if any, is responsible for a specific idiosyncratic reaction. Drug Metab Rev 38:745–753

Walubo A, Barr S, Abraham AM (2006) Rat CYP3A and CYP2B1/2 were not associated with nevirapine-induced hepatotoxicity. Methods Find Exp Clin Pharmacol 28:423–431

Watkins PB, Kaplowitz N, Slattery JT et al (2006) Aminotransferase elevations in healthy adults receiving 4 grams of acetaminophen daily: a randomized controlled trial. JAMA 296:87–93

Watkins PB, Dube LM, Walton-Bowen K et al (2007) Clinical pattern of zileuton-associated liver injury: results of a 12-month study in patients with chronic asthma. Drug Saf 30:805–815

Wen B, Ma L, Nelson SD et al (2008) High-throughput screening and characterization of reactive metabolites using polarity switching of hybrid triple quadrupole linear ion trap mass spectrometry. Anal Chem 80:1788–1799

Williams GM, Iatropoulos MJ, Djordjevic MV et al (1993) The triphenylethylene drug tamoxifen is a strong liver carcinogen in the rat. Carcinogenesis 14:315–317

Williams DP, Kitteringham NR, Naisbitt DJ et al (2002) Are chemically reactive metabolites responsible for adverse reactions to drugs? Curr Drug Metab 3:351–366

Williams DP, Antoine DJ, Butler PJ et al (2007) The metabolism and toxicity of furosemide in the Wistar rat and CD-1 mouse: a chemical and biochemical definition of the toxicophore. J Pharmacol Exp Ther 322:1208–1220

Wilson AS, Williams DP, Davis CD et al (1997) Bioactivation and inactivation of aflatoxin B1 by human, mouse and rat liver preparations: effect on SCE in human mononuclear leucocytes. Mutat Res 373:257–264

Wojnowski L (2004) Genetics of the variable expression of CYP3A in humans. Ther Drug Monit 26:192–199

Wong HL, Liebler DC (2008) Mitochondrial protein targets of thiol-reactive electrophiles. Chem Res Toxicol 21:796–804

Wong SG, Card JW, Racz WJ (2000) The role of mitochondrial injury in bromobenzene and furosemide induced hepatotoxicity. Toxicol Lett 116:171–181

Yukinaga H, Takami T, Shioyama SH et al (2007) Identification of cytochrome P450 3A4 modification site with reactive metabolite using linear ion trap-Fourier transform mass spectrometry. Chem Res Toxicol 20:1373–1378

Zhang D, Ogan M, Gedamke R et al (2003) Protein covalent binding of maxipost through a cytochrome P450-mediated ortho-quinone methide intermediate in rats. Drug Metab Dispos 31:837–845

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 10:322–338