Oxidative Medicine and Cellular Longevity 3:4, 238-253; July/August 2010; © 2010 Landes Bioscience

Deleterious effects of reactive metabolites

Sabry M. Attia

Department of Pharmacology and Toxicology; College of Pharmacy; King Saud University; Riyadh, Saudi Arabia

Key words: metabolism, reactive metabolites, adverse drug reactions, drug design

A number of drugs have been withdrawn from the market or severely restricted in their use because of unexpected toxicities that become apparent only after the launch of new drug entities. Circumstantial evidence suggests that, in most cases, reactive metabolites are responsible for these unexpected toxicities. In this review, a general overview of the types of reactive metabolites and the consequences of their formation are presented. The current approaches to evaluate bioactivation potential of new compounds with particular emphasis on the advantages and limitation of these procedures will be discussed. Reasonable reasons for the excellent safety record of certain drugs susceptible to bioactivation will also be explored and should provide valuable guidance in the use of reactive-metabolite assessments when nominating drug candidates for development. This will, in turn, help us to design and bring safer drugs to the market.

Introduction

The World Health Organization defines adverse drug reactions as "a response to a drug that is noxious, unintended and occurs at doses normally used in man for the prophylaxis, diagnosis, or therapy of disease or for modification of the physiological function." Adverse drug reactions can be generally classified into type-A and type B reactions. Type-A reactions are associated with the primary pharmacology of the drug (e.g., risk of hypotension with antihypertensives) and are responsible for 80% of all side effects. Type-A adverse drug reactions can be detected in animal models of pharmacology and/or toxicology; they exhibit simple dose-response relationships and are usually avoided in the clinic via dose adjustments. In contrast, type-B (bizarre or idiosyncratic) adverse drug reactions are unrelated to known drug pharmacology, and although they are dose dependent in susceptible individuals, they do not occur at any dose in most patients. Because the frequency of occurrence of idiosyncratic adverse drug reactions is very low these reactions are often not detected, until the drug has gained broad exposure in a large patient population. Importantly, standard regulatory animal toxicity studies have traditionally shown a poor concordance with occurrence of idiosyncratic adverse drug reactions in humans.[1]

*Correspondence to: Sabry M. Attia; Email: attiasm@ksu.edu.sa
Submitted: 06/28/10; Revised: 08/03/10; Accepted: 08/04/10
Previously published online:
www.landesbioscience.com/journals/oximed/article/13246
DOI: 10.4161/oxim.3.4.13246

Type B adverse drug reactions can affect any organ, but the most common organs involved are liver, skin, and bone marrow. The life-threatening type B adverse drug reactions noted for drugs include hepatotoxicity,[2] severe cutaneous reactions,[3-5] aplastic anaemia[6] and blood dyscrasias.[7] Amongst these, hepatotoxicity is the most frequent reason for drug withdrawal and is also the major cause of attrition in drug discovery/development.[8] Manifestations of liver injury can range from mild, asymptomatic changes in serum transaminases, which occur at high frequency with a number of drugs, to fulminant liver failure, which, although rare, can be potentially life-threatening and may necessitate a liver transplant. Considering that the liver is exposed to high concentrations of drug/metabolite(s) after oral administration, it is not altogether surprising that the organ is particularly vulnerable to damage by xenobiotics including drugs. The role of drug metabolism and reactive metabolites that cause these serious reactions have been investigated over the past 25 years and will be the focus of this review. Clinical management is still empirical, but recognition of a drug induced disease is important for future management of the patient.

Reactive Metabolites

The basic principle of drug metabolism is to convert a lipophilic drug or xenobiotic to hydrophilic metabolites that can be more readily excreted from the body. Sometimes during this process of biotransformation some of the drug or xenobiotic may be activated to chemically reactive species, i.e. reactive metabolites. This biotransformation of relatively inert chemicals to highly reactive intermediary metabolites is commonly referred to as metabolic activation or bioactivation, and it is known to be the initial event in many chemically induced toxicities. Some toxicants are direct acting and require no activation, whereas other chemicals may be activated nonenzymatically.[9] The focus of this review, however, is on xenobiotics requiring metabolic activation and to those processes involved in activation.

In the 1940s and 1950s the pioneering studies of James and Elizabeth Miller provided early evidence for in vivo conversion of chemical carcinogens to reactive metabolites. They found that reactive metabolites of the aminoazo dye N,N-dimethyl-4-aminoazobenzene (DAB), a hepatocarcinogen in rats, would bind covalently to proteins and nucleic acids. The term, metabolic activation, was coined by the Millers to describe this process. Moreover they demonstrated that covalent binding of these chemicals was an essential part of the carcinogenic process.[10] The overall scheme of metabolism for potentially toxic xenobiotics is outlined in **Figure 1**. As illustrated by this diagram, xenobiotic

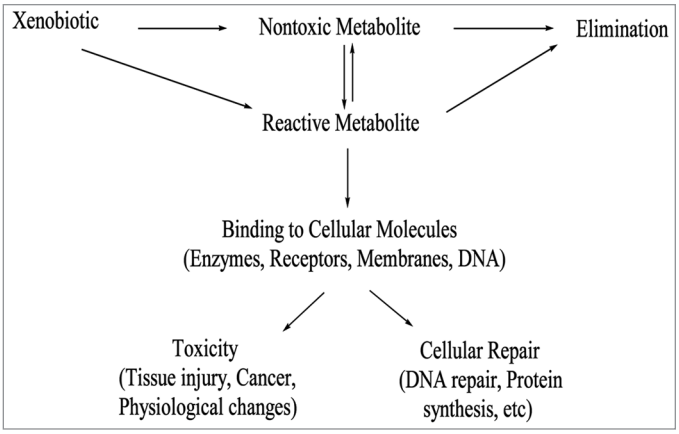

Figure 1. The relationship between metabolism, activation, detoxication, and toxicity of a xenobiotic.

metabolism can produce not only nontoxic metabolites, which are more polar and readily excreted (detoxication), but also highly reactive metabolites, which can interact with vital intracellular macromolecules, resulting in toxicity. In addition reactive metabolites can be detoxified for example, by interaction with glutathione (GSH).

Within the tissue a variety of reactions may occur depending on the nature of the reactive species and the physiology of the organism. Reactive metabolites are usually electron deficient molecules and are referred to as electrophiles (molecules containing positive centers). If not detoxified properly, these electrophiles can react with electron rich species, i.e. nucleophiles (molecules containing negative centers), through covalent bond formation. The nucleophiles usually contain atoms such as S, N, or O that have a lone pair of electrons, which can form a new bond to the electrophile. Such nucleophiles are present on macromolecules such as proteins, nucleic acids and lipids. Chemically reactive metabolites can directly react with proteins causing changes in protein structure or protein folding.[11,12] These modified proteins are processed by antigen presenting cells and can look "foreign" to the immune system leading to an immune response. Chemically reactive electrophiles can also covalently react with nucleic acids on the DNA thereby causing changes in DNA structure or gene expression. Changes in DNA can lead to mutagenicity, teratogenicity or carcinogenicity.[13-16]

Types of reactive metabolites. Reactive metabolites include such diverse groups as epoxides, quinones, free radicals, reactive oxygen species, and unstable conjugates. As a result of their high reactivity, reactive metabolites are often considered to be short-lived. This is not always true, however, because reactive intermediates can be transported from one tissue to another, where they may exert their deleterious effects.[9] For example, carcinogenic aromatic amines are metabolized in the liver to the N-hydroxylated derivatives that, following sulfation and/or acetylation conjugation, are transported to the bladder, where the N-hydroxy derivative is released under the acidic conditions of urine.[16]

As mentioned above, reactive metabolites are usually electrophiles or free radicals. Electrophiles can be further subdivided into hard or soft depending on how concentrated or diffuse their electron deficient site is. Soft electrophiles are generally uncharged and are less electrophilic. Example of soft electrophiles are Michael acceptor, quinine methide and iminoquinone. Hard electrophiles are generally small and charged such as alkyl carbocation, carbonyl carbocation and nitrenium ion.[17] Soft electrophiles react with soft nucleophiles through orbital interactions, while hard electrophiles react with hard nucleophiles to a large degree through electrostatic interactions. Examples of soft nucleophiles are RSH, GSH, RS^-, I^-, RSe^-, alkenes and R_3P (soft because the nucleophilic site is a sulfur atom, which is relatively large and hence its electron cloud is more polarizable). In contrast, R-NH_2, R-OH, RO^-, $SO_4^{2-}$and Cl^- are hard nucleophiles because nitrogen is small and much less polarizable relative to sulfur. Several factors play a role in the formation and reactivity of theses reactive metabolites. These factors include the presence of a good leaving group (such as sulfate, sulfonate, chloride, and acetate), ring strain, polarization of a double bound by a carbonyl group (Michael acceptor), and the presence of electron withdrawing groups.[11]

Free radicals are another kind of reactive metabolite that can be formed by xenobiotics.[18] Free radical refers to compounds having an unpaired electron. Since electrons "like" to be paired to form a chemical bond, a free radical cannot react covalently with nucleophiles. Rather they react with another free radical to form a covalent bond, abstract a hydrogen atom from a neutral molecule to generate a new radical, or abstract an electron to form an anion and generate a radical cation. An example of a free radical mechanism is the ring opening of cyclopropyl ring of tertiary amine as in trovofloxacin (**Fig. 2**). It has been reported that trovafloxacin-induced hepatotoxicity may be mediated through the oxidation of the cyclopropylamine substructure to reactive intermediates that may form covalent adducts to hepatic proteins, resulting in damage to liver tissue.[19]

Free radicals can occur, for example, in lipids, amino acids, nucleotides, and oxygen compounds. Oxygen radicals are of particular importance as they can trigger the formation of all other radicals. Other oxygen containing species that are chemically not radicals also have high reactivity with biological substances. Jointly, these substances are known as reactive oxygen species (ROS) (**Fig. 3**). In addition to ROS, there are radicals that contain an additional nitrogen atom for example, nitric oxide (NO). NO can be generated enzymatically by the so-called NO synthases (NOS) or non-enzymatically by nitrite ($NO2^-$). NO has important signalling and protective functions; in 1998, a Nobel Prize was awarded for their discovery. NO/NO2- and ROS, in turn, can react with each other. This generates peroxynitrite (ONOO-), the most reactive compound of all ROS, which can oxidize and nitrite proteins, lipids, and nucleic acids.[20]

It is worthwhile to note that free radicals are a two-edged sword. On the one hand, they have important physiological functions. In addition to NO, which is an important protective factor in the vasculature and a neurotransmitter in the nervous system,[21] oxygen radicals are, for example, essential in the immune defence, as well as in the regulation of cellular growth and gene expression.[22] But too much of a good thing can literally be harmful,

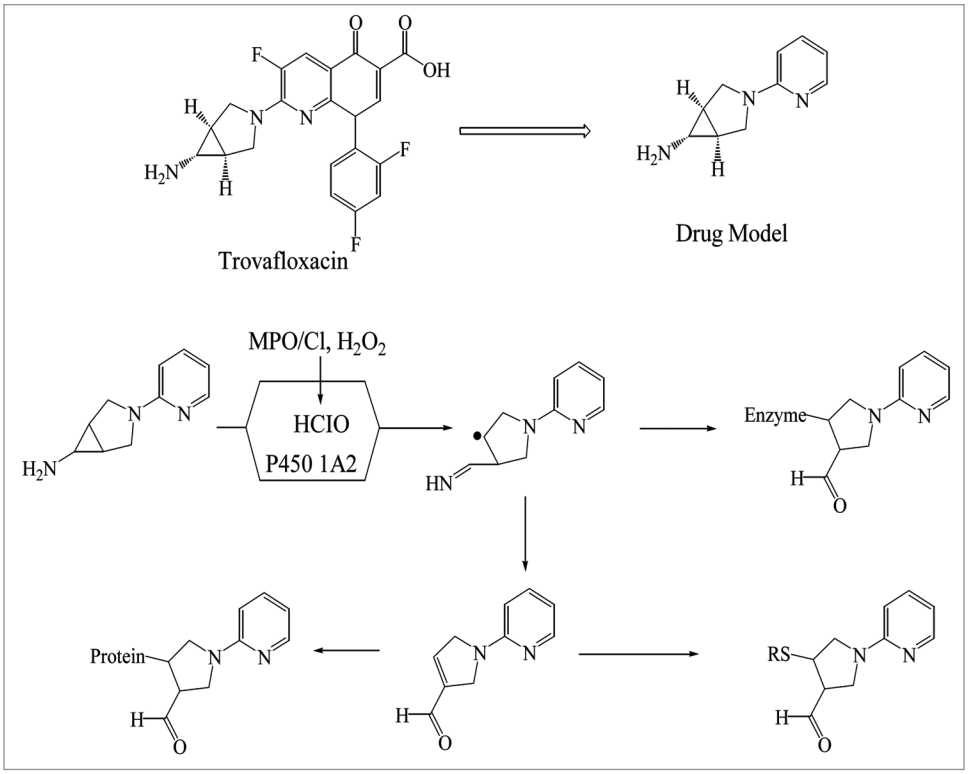

Figure 2. Free radical mechanism of cyclopropylamine ring opening: insights into trovafloxacin-induced hepatotoxicity.

because radicals are also highly dangerous by-products of the cellular metabolism.[23] An oxidative imbalance, i.e., a disturbance in the balance between the production of ROS (especially free radicals) and antioxidant defenses, has been described as an oxidative stress status.[24] This can result in several kinds of cell damage, leading to a loss of function and integrity. Undesirable effects include inactivation of NO as a result of a direct chemical reaction with ROS and oxidative damage of cell components such as DNA and proteins.[20,25] These effects are potentially involved in the development of a large number of pathological conditions,[26,27] including cardiovascular diseases, neurological disorders, cancer and aging process.[28-33]

Reactivity and toxicity of reactive metabolites. The relationship between drug metabolism and adverse drug reactions was first demonstrated with the analgesic agent paracetamol. Paracetamol is a major cause of drug-related morbidity and mortality in humans, capable of producing hepatic necrosis after a single toxic overdose.[34] At normal therapeutic doses, paracetamol is safe, but can be hepatotoxic at high doses. The major portion of paracetamol is conjugated with either sulfate or glucuronic acid to form water-soluble, readily excreted metabolites and only small amounts of the reactive intermediate, believed to be *N*-acetyl-*p*-benzoquinonimine (NAPQI), are formed by the cytochrome P450 enzymes (**Figure 4**). When therapeutic doses of paracetamol are ingested, the small amount of reactive intermediate forms is efficiently deactivated by conjugation with GSH. When large doses are ingested, however, the sulfate and glucuronide cofactors (PAPS and UDPGA) become depleted, resulting in more of the paracetamol being metabolized to the reactive intermediate.[35-37]

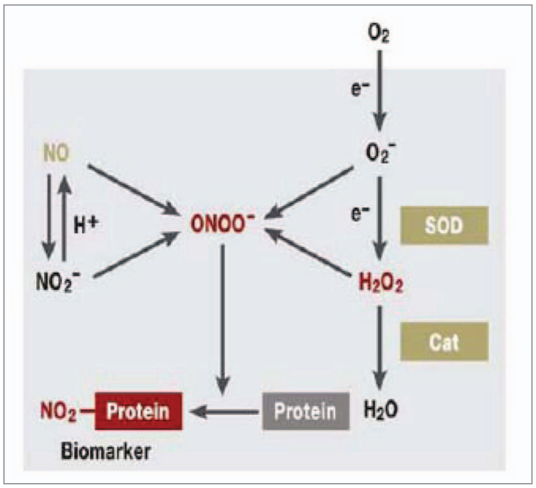

Figure 3. Reactive oxygen species (ROS). O_2- superoxide radical, H_2O_2 = hydrogen peroxide, SOD = superoxide dismutase, Cat = catalase, NO = nitric oxide, NO_2-nitrite, $ONOO^-$ = peroxynitrite, Nitrated proteins are biomarkers for oxidative stress. Red indicates disease promoting proteins or compounds; green, protective factors; arrows, reactions or transformations; a box indicates a protein for example, an enzyme or receptor. The processes shown within the grey area occur naturally in the body.

As long as GSH is available, most of the reactive intermediate can be detoxified. When the concentration of GSH in the liver also becomes depleted, however, covalent binding to sulfhydryl (-SH) groups of various cellular proteins increases, resulting in

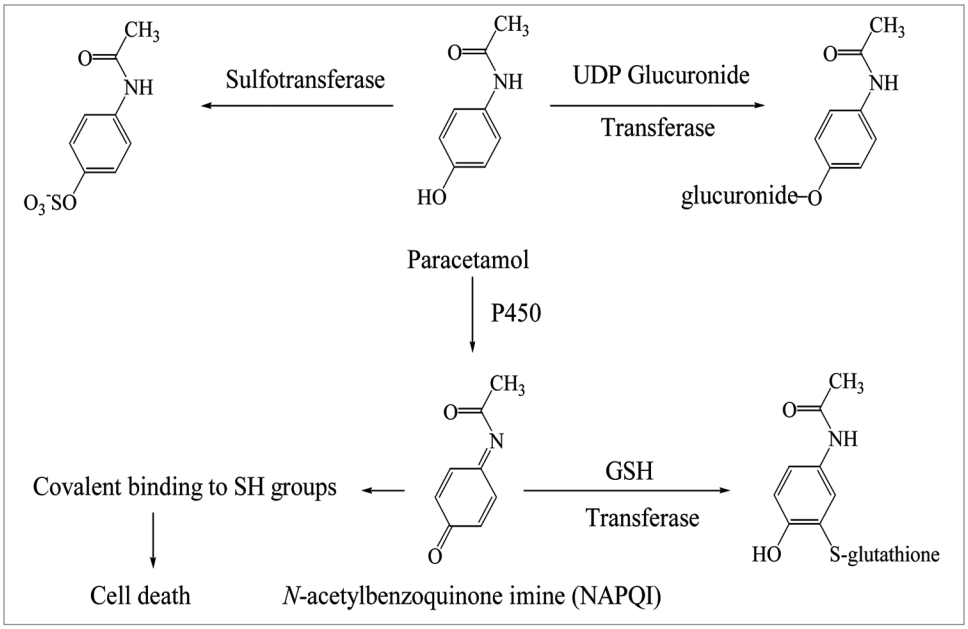

Figure 4. Metabolism of paracetamol and formation of reactive metabolites.

hepatic necrosis. If sufficiently large amounts of paracetamol are ingested, as in drug overdoses and suicide attempts, extensive liver damage and death may result.

Following the studies with paracetamol, there have been myriad examples of drugs associated with idiosyncratic adverse drug reactions for which reactive-metabolite formation has been demonstrated.[38] These examples provide a circumstantial link between reactive-metabolite formation and toxicity. It is very important to make a distinction here between drugs that exhibit dose-dependent and dose-independent adverse drug reactions. The hepatotoxic effects of paracetamol in humans can hardly be considered as idiosyncratic as they are dose dependent and can be replicated in animals.[35-37] In contrast, drugs like the antidiabetic agent troglitazone, which exhibits dose-independent hepatotoxicity in a very small segment of the population, is a true example of an idiosyncratic toxin, given that this compound received a 'clean bill of health' in conventional animal toxicological assessments.

In certain cases the metabolites are so reactive that they do not escape the enzyme that formed them and covalently bind to the enzyme leading to irreversible inhibition.[39,40] Because metabolism precludes enzyme inactivation, these compounds fall into the category of mechanism-based inactivators. Inactivation can occur via coordination of the reactive species with the heme prosthetic group (formation of a metabolite-inhibitor (MI) complex) or via covalent adduction of the reactive intermediate with heme and/or with an amino acid residue on the apoprotein. Cytochrome P450 inactivation can translate into clinical drug-drug interactions, some of which can be potentially deleterious, and can lead to the withdrawal of the perpetrator drug. For instance, co-administration of the calcium-channel blocker and potent P450-3A4 inactivator mibefradil and simvastatin in patients with hypertension has been associated with increased cases of myopathy including rhabdomyolysis. The biochemical mechanism for the clinical drug-drug interactions involves the mechanism-based inactivation of the P450-3A4-catalyzed simvastatin metabolism process by mibefradil; a consequence which results in elevated plasma concentrations of the statin.[41,42] Myopathy or rhabdomyolysis is a rare side effect common to the statin class of compounds and is usually associated with high levels of HMG-CoA reductase inhibitory activity in susceptible target tissue. Given the potential for such life-threatening drug-drug interactions, the manufacturer of mibefradil announced a voluntary withdrawal of the drug from the market worldwide.

Dietary supplements containing *Piper methysticum* (kava) have been implicated in multiple cases of liver injury in humans following the usage of kava-containing products. Its sedatives and anxiolytic benefits have been hampered by several reports of clinically significant herb-drug interactions upon concomitant use with benzodiazepines and barbiturates,[43,44] and several cases of idiosyncratic hepatotoxicity have also been reported.[45] Consequently, over-the-counter sales of kava herbal preparations have been banned in several countries in the European Union. Studies on P450 inhibition with kavalactone derivatives methysticin and 7,8-dihydromethysticin (**Fig. 5**), the major constituents of kava extract,[46] have revealed potent mechanism-based inactivation of multiple human P450 enzymes.[47] The time- and NADPH-dependent formation of the 455-nm absorbing MI complex is consistent with a bioactivation mechanism involving the metabolism of the 1,3-benzodioxole group to the corresponding carbene intermediate followed by its coordination with the heme iron.[47]

Consistent with this finding, the kavalactone kawain (**Fig. 5**), which differs from methysticin in that it does not contain the 1,3-benzodioxole group is not a P450 inactivator.[47] GSH conjugates of electrophilic *ortho*-quinone intermediates obtained via the biotransformation sequence (1,3-benzodioxole→catechol→*ortho*-quinone) in methysticin and 7,8-dihydromethysticin have also been identified in rat and human liver microsomes (**Fig. 5**), and the involvement of these reactive quinonoid intermediates in the immunoallergic hepatotoxic effects of kava extract has been speculated.[48] Overall, given the potential for drug-drug interactions via enzyme inactivation, mechanism-based inactivation of major human P450 enzymes by new compounds is routinely assessed in a drug-discovery paradigm.

Reactive metabolites which covalently bind to the DNA have a dominant role in the mutagenicity and carcinogenicity. The concept of genotoxic/mutagenic response arising from metabolism was first proposed in the 1930s and the 1940s to account for the carcinogenicity of chemically inert polycyclic aromatic hydrocarbons, aminoazo dyes, and nitroso compounds.[49,50] All pharmaceutical companies utilize a standard battery of genetic

toxicology assays to test the mutagenic potential of drug candidates.[51] These assays measure several different types of genetic damage in a variety of cell types to increase the probability of detecting a mutagenic response. The endpoints routinely monitored include the induction of gene mutations and chromosomal aberrations in bacteria and mammalian cells, respectively, as well as the production of DNA strand breaks, DNA intercalation, and covalent modification.

An ariclor-1254-induced rat liver S-9/NADPH system has been adopted in these in vitro tests for detecting pro-mutagens capable of forming DNA-reactive metabolites.[52] Genetic toxicology assessments have become an integral part of drug safety evaluation and are required by regulatory agencies for drug approvals worldwide. Because a good correlation has been established between in vitro metabolism dependent mutagenic response and the outcome of rodent carcinogenicity evaluations, drug candidates intended for non-life-threatening indications are generally discontinued from development, when they exhibit a positive response in the in vitro assays in the presence of S-9/NADPH. An example of this phenomenon was highlighted with a study on the anti-obesity agent and 5-hydroxytryptamine $(5\text{-HT})_{2C}$ agonist 2-(3-chlorobenzyloxy)-6-(piperazin-1-yl)pyrazine (**1; Figure 6**).[40] The attractive in vitro/in vivo pharmacology and pharmacokinetic attributes of **1** were offset by its S-9/NADPH-dependent genotoxic effects in the bacterial Salmonella Ames assay, which led to its discontinuation from clinical development. Studies with (^{14}C)-**1** revealed the irreversible and concentration-dependent incorporation of radioactivity in calf thymus DNA in an S-9/NADPH-dependent fashion confirming that **1** was bioactivated to a DNA-reactive metabolite.

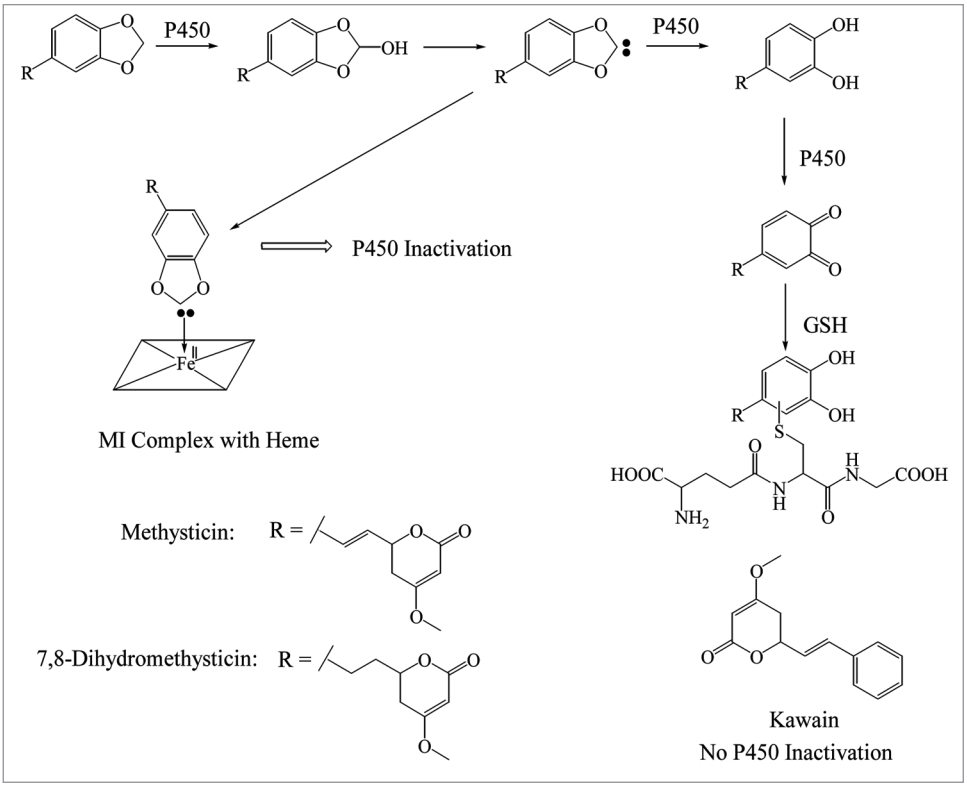

Figure 5. Proposed mechanisms of P450 inactivation and hepatotoxicity by components of kava extract.

Reactive-metabolite trapping studies in S-9/NADPH incubations containing exogenously added hard and soft nucleophilic trapping agents methoxylamine and GSH, respectively, led to the detection of conjugates of **1** and its downstream metabolites. Structural elucidation of these conjugates by mass spectrometry allowed an insight into the bioactivation pathways leading to the formation of DNA-reactive metabolites. The mass spectrum of the methoxylamine conjugate of **1** was consistent with condensation of amine with an electrophilic, aldehyde metabolite derived from piperazine ring scission in **1** (**Fig. 6, Pathway a**), whereas, the mass spectrum of the GSH conjugate suggested a bioactivation pathway involving initial aromatic ring hydroxylation on the 3-chlorobenzyl motif in **1**, followed by β-elimination to a quinone-methide species that reacted with GSH (**Fig. 6, Pathway b**). The observation that methoxylamine and GSH reduced mutagenicity suggested that the trapping agents competed with DNA towards reaction with the reactive metabolites.

Perhaps the most important susceptibility factor for type-B adverse drug reactions is genetic variability. Genetic polymorphisms have a strong influence on drug metabolism and may increase risk of toxicity. For example, polymorphism of the N-acetyltransferase (NAT) 2 gene differentiates fast from slow acetylators; the latter have increased susceptibility to toxicity of certain aniline-containing drugs such as isoniazid, sulfamethoxazole, dapsone, and procainamide.[53,54] The major route of elimination of these drugs in humans involves *N*-acetylation of the aniline moiety by NAT2, resulting in the neutral amide metabolites. In a NAT2-deficient population, the aniline motif in isoniazid is hydrolyzed by amidases liberating hydrazine, which is toxic in its own right; likewise, sulfamethoxazole, dapsone, and procainamide are biotransformed by P450 enzymes to yield cytotoxic and protein-reactive metabolites that include *N*-hydroxyaniline derivatives and the subsequent two-electron oxidation products, i.e., the nitroso intermediates. The reactivity of the nitroso metabolites of these drugs with GSH and/or proteins in target organs has also been demonstrated.[55,56]

Genetic polymorphisms in glutathione-S-transferase (GST) isozymes, which catalyze GSH conjugation to reactive metabolites, are also considered risk factors for hepatotoxicity caused by several drugs such as troglitazone and carbamazepine.[57,85]

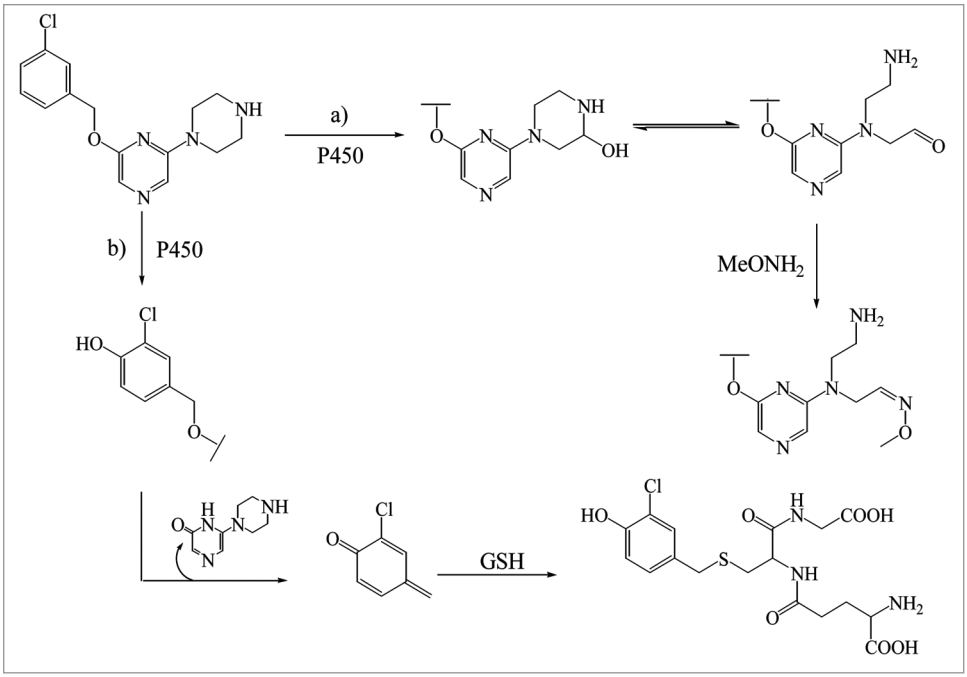

Figure 6. Postulated bioactivation pathways which explain the mutagenicity of the anti-obesity agent 2-(3-chlorobenzyloxy)-6-(piperazin-1-yl)pyrazine (1) in the Salmonella Ames test.

It is possible that patients deficient in GST isozymes are most at risk towards liver injury by reactive metabolites of troglitazone and carbamazepine because of ineffective scavenging of the reactive metabolites derived from the oxidative bioactivation of these drugs. There is also a strong possibility that components of ingested foods including herbal supplements can modulate drug metabolism and, therefore, increase idiosyncratic adverse drug reaction risk. For example, chronic alcohol abuse increases the risk of paracetamol hepatotoxicity by inducing P450-2E1, which predominantly catalyzes paracetamol bioactivation to NAPQI.[59]

Individual group summaries. Most reactive metabolites are formed by phase-I metabolic pathways; however, sometimes phase-II pathways can also generate reactive metabolites. Usually phase-II metabolism, such as the conjugation reactions (glucuronidation and sulfation), increases the polarity of a drug making it more polar and readily excreted from the body. In rare cases, these conjugates can be chemically reactive leading to toxicity. In the following section some reactive metabolites of drugs or xenobiotics are discussed with respect to their reactivity and deleterious effects. Elucidation of bioactivation pathways provides valuable information that can lead to designing safer drug candidates.

Quinones. Quinones represent one of the most frequently generated reactive intermediates. Quinones are known to cause a variety of toxicological effects in vivo including acute cytotoxicity, immunotoxicity, genotoxicity and carcinogenesis.[60-63] Quinones can be viewed as Michael acceptors and can cause cellular damage through alkylation of crucial cellular proteins and/or DNA. Quinones are also highly redox active molecules, which can redox cycle with their semiquinone radicals leading to formation of ROS including superoxide, hydrogen peroxide, and ultimately the hydroxyl radical. Production of ROS can cause severe oxidative stress within cells through the formation of oxidized cellular macromolecules, including lipids, proteins, and DNA (**Fig.** 7).

Benzene, a common solvent used in organic chemistry, is converted to hydroquinone by liver P450, and the subsequent peroxidase-catalyzed oxidation of this metabolite to *p*-benzoquinone in the bone marrow may explain the induction of leukaemia during chronic exposure to this solvent. The fact that *p*-benzoquinone is known to form DNA adducts strengthens this hypothesis.[64] It has been demonstrated that inhibition of human topoisomerases I and II by high concentrations of quinoid metabolites of benzene; however, a remarkably greater sensitivity of the enzymes was detected with phenolic metabolites of benzene in the course of their bioactivation using a peroxidase/H_2O_2 system. This suggests that free radical intermediates of phenolic oxidation (formed in the presence of peroxidase activity) may contribute to the clastogenic and carcinogenic effects of phenolic compounds through inhibition of topoisomerases.[65]

Etoposide has become one of the most widely used anticancer drugs in the world since its introduction.[66] However, etoposide is a somatic and germ-cell mutagen capable of inducing both numerical and structural chromosome aberrations in animals.[67-70] Moreover, numerous groups have reported that treatment schedules associated with the impressive efficacy of etoposide are also associated with an increased risk of secondary acute myeloid leukaemia.[71-73] This has prompted the removal of this highly effective anti-topoisomerases II agent from some treatment regimens. It is believed that the mechanisms for the extremely high susceptibility of myeloid stem cells to the leukemogenic effects of etoposide is due to etoposide phenoxyl radicals (etoposide-O$^{\bullet}$) formed from etoposide by myeloperoxidase.[74,75] Etoposide-O$^{\bullet}$ is reduced back to etoposide via oxidation of intracellular thiols (RSH), i.e., glutathione and sulfhydryl groups of proteins. Reduced etoposide is thus repeatedly available as a substrate for myeloperoxidase, at the expense of intracellular thiols, which undergo one electron oxidation to reactive thiyl radicals (RS$^{\bullet}$). RS$^{\bullet}$ can further react to generate disulfide anion-radicals (RS-S-$^{\bullet}$R), which can donate an electron to oxygen. Superoxide anion radical ($O2^{-\bullet}$) thus produced can form, in the presence of transition metal complexes, the extremely reactive hydroxyl radical (HO$^{\bullet}$). It is believed that accumulation of these radicals may cause damage to cell membrane leading to lipid peroxidation and also damage to cellular genome and other critical biomolecules, ultimately

inducing mutageneicity and leukaemia (**Fig. 8**).[13-16,76,77] It is worthwhile to note that several strategies have been developed to decrease the deleterious effects of etoposide in normal cells using nutritional antioxidants such as vitamin C, vitamin E homolog,[74,76] and the metal-chelator, dexrazoxane.[78] Ameliorations of these deleterious effects were associated with a concomitant alteration of the antioxidant potential.

Remoxipride is an atypical antipsychotic used in the treatment of schizophrenia. It is associated with rare cases of aplastic anaemia. It has been demonstrated that the metabolite of remoxipride, NCQ344, forms a reactive p-quinone,[79] which might be responsible for the aplastic anaemia associated with remoxipride (**Fig. 9**).

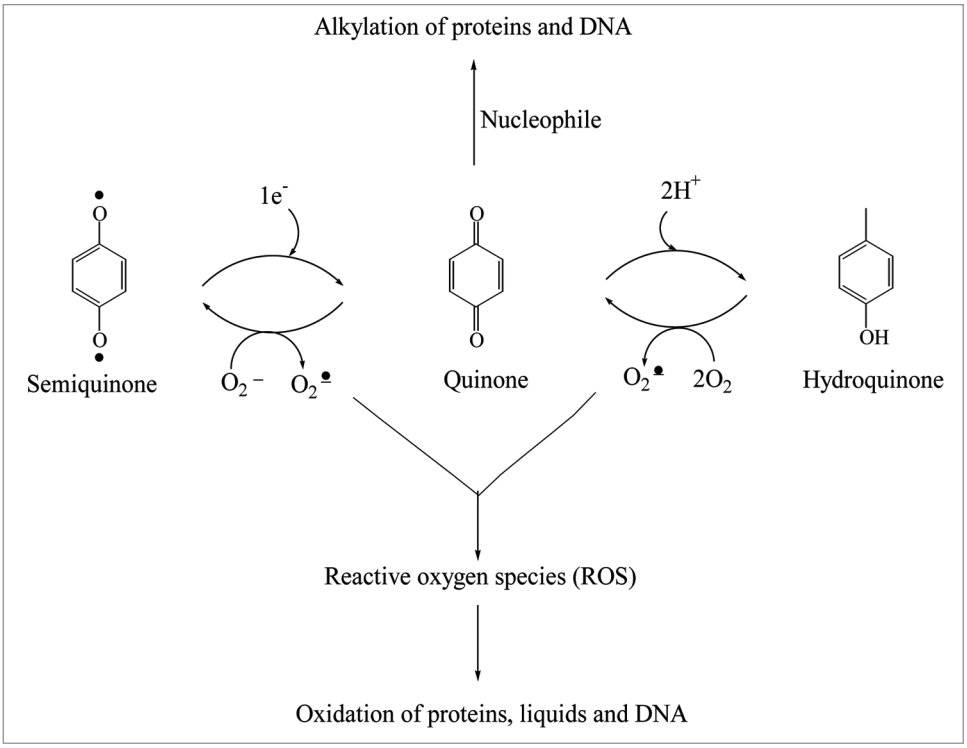

Figure 7. Alkylation by and redox cycling of quinones.

Iminoquinones. Iminoquinones are another class of reactive metabolites formed by the substitution of one of the oxygen atoms of the quinone with nitrogen; they can also act as Michael acceptors. Because nitrogen is less electronegative than oxygen, the reactivity of an iminoquinone will be relatively lower compared to a quinone. Once generated, an iminoquinone can covalently bind to protein nucleophiles and cause toxicity. A number of drugs and chemicals of environmental importance can be converted to reactive quinonimines. The 4-aminoquinoline antimalarial, amodiaquine, was withdrawn from the market because of idiosyncratic agranulocytosis and hepatotoxicity, and this was attributed to its bioactivation to a quinonimine metabolite.80 Iminoquinones preferentially react with thiol nucleophiles. In the case of the drug, acetaminophen, the reactive intermediate NAPQI, once formed, interacts with protein thiols, including that of the plasma membrane Ca^{2+}-ATPase, causing increased cytosolic calcium concentrations, adverse cytoskeletal effects, and cell death.[81] Lumaricoxib is a new COX-2 inhibitor used for the treatment of inflammatory diseases. It is associated with idiosyncratic hepatotoxicity in patients. It has been demonstrated that lumaricoxib is bioactivated to a reactive quinone (**Fig. 10**), which might be responsible for causing idiosyncratic hepatotoxicity.[82]

Quinonemethide. Quinonemethide is a class of reactive metabolite in which one of the oxygens of a quinone is substituted by carbon. Because of this, the double bond is polarized and can react in a Michael fashion with nucleophiles. The reactivity of the quinone methide depends on the other substituents. A series of o-methoxy-4-alkylphenols were used to investigate the electrophilicity and toxicity of quinonemethide intermediates.[83] It was observed that the reactivity of the corresponding quinone methide was influenced by the presence and nature of substituents on the benzylic carbon; increased steric hindrance of the exocyclic methylene group decreased the rate of nucleophilic attack.

Moreover, the quinine methides formed from *o*-methoxy-4-alkylphenols of intermediate reactivity were the most cytotoxic, presumably because quinone methides that do not exhibit this optimum reactivity are either too stable to react with critical cellular nucleophiles or so reactive with solvent so as to preclude reaction with critical cellular nucleophiles.[83] Acolbifene is a selective estrogen receptor modulator developed for the treatment of breast cancer. It has been demonstrated that acolbifene can be bioactivated to electrophilic quinonemethide and diquinonemethide reactive intermediates,[84] which could cause further toxicity (**Fig. 11**). It has also been shown that these reactive metabolites bind with the deoxynucleosides causing DNA damage.

Arene oxides and epoxides. Arene oxides are another class of reactive intermediates formed by numerous drugs that are associated with idiosyncratic drug reactions. Cytochrome P450 enzymes are capable of catalyzing this biotransformation in aromatic compounds. In the case of alkenes, the corresponding epoxides are formed. Both arene oxides and epoxides are reactive and are capable of reacting with nucleophilic proteins.[85] The major in vivo detoxification pathways for these arene oxides involve enzymes such as epoxide hydrolase and glutathione-S-transferase. An example of an epoxide is a metabolite of the drug carbamazepine. Carbamazepine is an anticonvulsant drug associated with a variety of idiosyncratic drug reactions such as skin rashes, aplastic anaemia, hepatitis, and generalized anticonvulsant hypersensitivity syndrome. Carbamazepine is biotransformed to a weakly

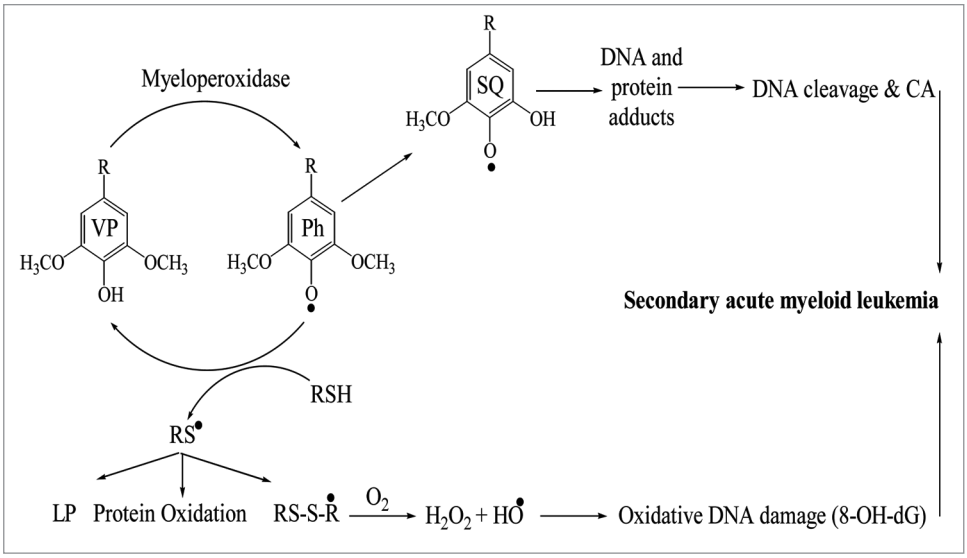

Figure 8. Potential cyto- and genotoxic pathways triggered by myeloperoxidase- catalyzed generation of etoposide phenoxyl radicals. VP = etoposide, Ph = phenoxyl radicals, sQ = semi-quinone free radical, CA = chromosomal aberration, LP = lipid peroxidation, RSH = intracellular thiols, RS· = thiyl radicals, RS-S-·R = disulfide anion-radicals, HO· = hydroxyl radical.

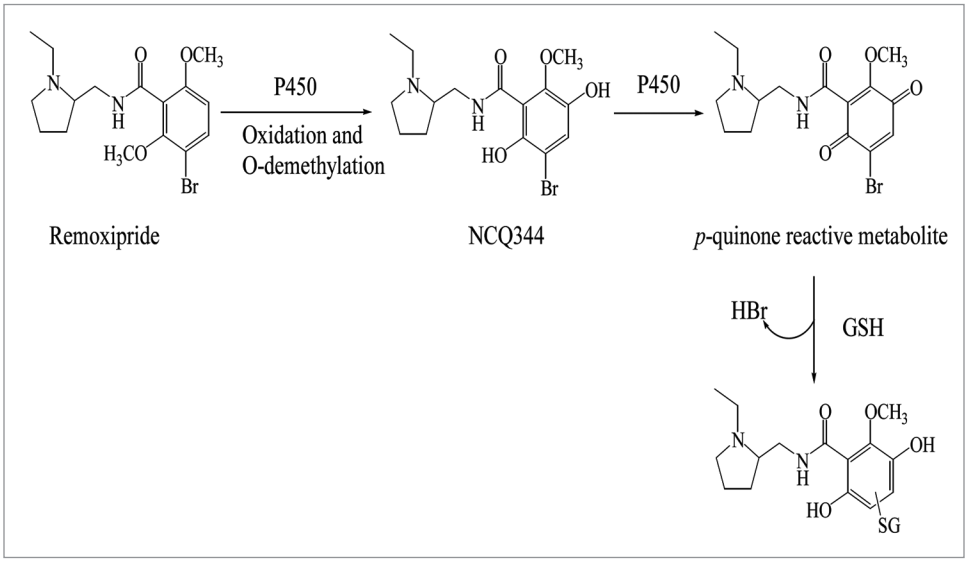

Figure 9. Bioactivation of the remoxipride metabolite, NCQ344, to a reactive p-quinone.

reactive 10, 11-epoxide by P450-3A4/P450-2C8 (**Fig. 12**). This is deactivated by epoxide hydrolase to dihydrodiols.[86]

Zomepirac is an anti-inflammatory drug (NSAID), which is associated with severe and fatal anaphylactic reactions because of which it was withdrawn from the market in 1983. It has been shown that zomepirac is bioactivated to arene oxide reactive intermediates, which could possibly cause these adverse reactions (**Fig. 13**).[87]

Glucuronidation. Glucuronidation is a process by which a drug or other substrate, which usually contain OH groups i.e., phenols, carboxylic acids, and alcohols, are cleared and detoxified. In the case of diclofenac, the carboxylic acid is converted to a glucuronide. This glucuronide is reactive and slowly reacts with protein amino groups leading to covalent binding.[88,89] In some cases, the acyl glucuronide also undergoes ring opening and an Amadori rearrangement that can lead to the formation of irreversible protein binding (**Fig. 14**). Carboxylic acids can also form Co-A esters, which are reactive because the Co-A group is a good leaving group. These esters may also contribute significantly to the covalent binding associated with carboxylic acids.[90]

Sulfation. Sulfation is another phase-II pathway in which the SO_3-functional group is transferred to the substrate through a sulfotransferase enzyme. The usual substrates for this type of reaction are hydroxyl or phenolic groups. Substrates containing nitrogen may also undergo this biotransformation. Usually these sulfate conjugates are nontoxic and can be excreted from the body. But in some cases, the sulfate group in the conjugate is reactive and can be displaced by a nucleophile or act as a leaving group to form a cation,[91] that can covalently bind to a protein leading to toxicity. An example is the bioactivation of an antifungal compound called N-(3,5-dichlorophenyl) succinimide (NDPS), which is known to cause nephrotoxicity. It was shown that its hydroxyl metabolite N-(3,5-dichlorophenyl)-2-hydroxysuccinimide (NDHS) is bioactivated to an activated O-sulfate that is likely responsible for the toxicity (**Fig. 15**).[92]

Detection and screening for reactive metabolites. Given the lack of availability of preclinical models as reliable predictors of idiosyncratic adverse drug reactions and the absence of relevant clinical safety biomarkers, it is currently impossible to accurately predict which new drugs will be associated with a significant incidence of idiosyncratic adverse drug reactions. Under the assumption that reactive metabolites, as opposed to the parent molecules from which they are derived, can be responsible for the pathogenesis of certain toxicities, most pharmaceutical companies have implemented assays to evaluate a compound's potential to undergo bioactivation with the goal of eliminating or minimizing reactive-metabolite formation by rational structural modification of the problematic chemical series.

Avoiding structural alerts. The initial step to avoid the formation of reactive metabolites is to identify functional groups that are known to form reactive metabolites and avoid these functional groups in the structure of drug candidates. For example, functional groups such as aromatic amines are known to form reactive nitroso species.[93] Another example is the hydrazine functional group that can form reactive carbocation species.[94] A good review of structural alerts is found in the publication of Kalgutkar et al.[38,95] Of course elimination of reactive metabolite formation will be of no benefit if it also eliminates the therapeutic effects of the drug.

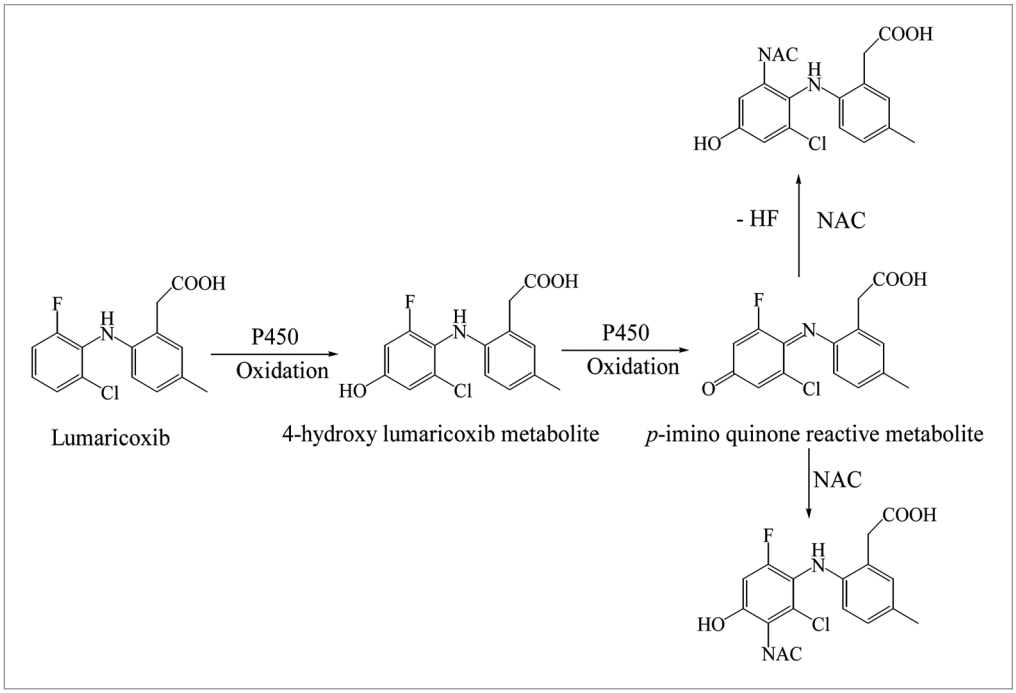

Figure 10. Bioactivation of lumaricoxib to a reactive p-iminoquinone.

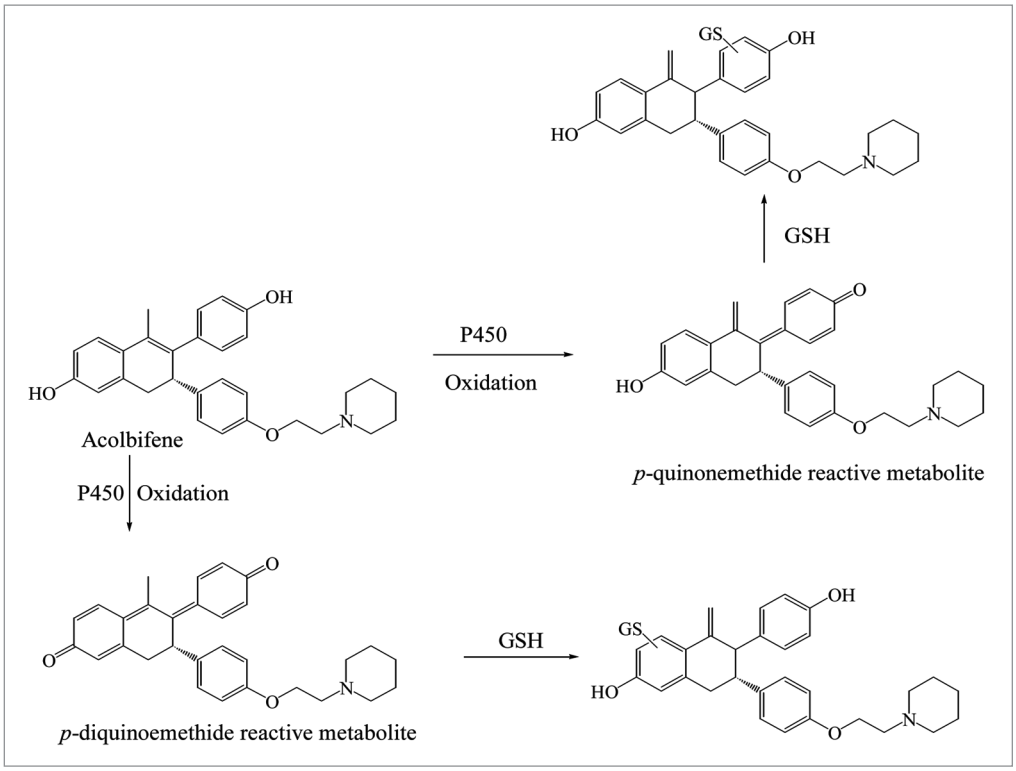

Figure 11. Bioactivation of acolbifene to reactive p-quinonemethide and p-diquinonemethide metabolites.

Testing for GSH conjugates. One of the methods to detect the formation of electrophilic intermediates is to look for GSH conjugates of the drug. GSH is a major scavenger of reactive metabolites and hence the detection of a GSH conjugate is an indication of the formation of a reactive intermediate. Formation of GSH conjugates can be detected by mass spectrometry, which, in turn, provides insight into the reactive metabolite structure.[96,97] With the possible exception of acyl glucuronides and cyclic iminium ions, most reactive metabolites are generally short-lived and are not usually detectable in circulation. Their formation can often be inferred from stable conjugates obtained via reaction with the endogenous anti-oxidant GSH. The presence of the soft nucleophilic sulfhydryl group in GSH ensures efficient conjugation with soft electrophilic centers on reactive species (e.g., Michael acceptors, epoxides, arene oxides, and alkyl halides) yielding stable sulfhydryl conjugates.[38,98]

Qualitative in vitro assessment of reactive-metabolite formation usually involve 'trapping' studies conducted with NADPH-supplemented human liver microsomes and GSH; analysis of the resulting metabolites by mass spectrometry is employed to characterize the structure of GSH conjugates, which, in turn, provides insight into the reactive metabolite structure.

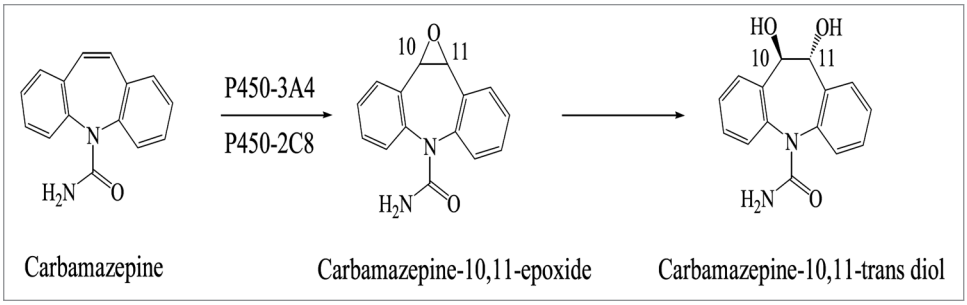

Figure 12. Bioactivation of carbamazepine to a reactive carbamazepine-10, 11-epoxide intermediate.

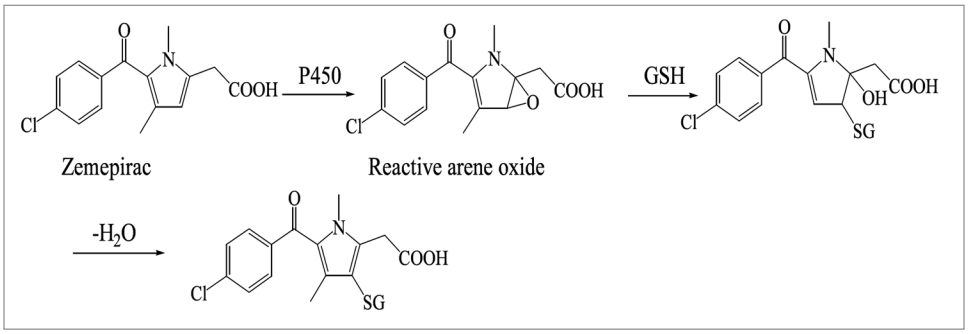

Figure 13. Bioactivation of zomepirac to a reactive arene oxide intermediate.

Considering that drug-metabolizing enzymes other than cytochrome P450 [e.g., monoamine oxidases, aldehyde oxidase, alcohol dehydrogenases, myeloperoxidase, uridine 5'-diphosphoglucuronosyl transferase (UGT), and sulfotransferases] are also capable of catalyzing bioactivation, due consideration must be given to the use of alternate metabolism vectors (e.g., S-9 fractions, hepatocytes, neutrophils, monocytes, etc.), which support the activity of these enzymes. This is especially important in cases where multiple enzymatic and/or chemical steps may be involved in the production of the reactive metabolite. It is noteworthy to point out that not all reactive metabolites can be trapped with GSH. Hard electrophiles including DNA-reactive metabolites (e.g., electrophilic carbonyl compounds) will preferentially react with hard nucleophiles such as amines (e.g., semicarbazide and methoxylamine), amino acids (e.g., lysine), and DNA bases (e.g., guanine and cytosine) affording the corresponding *Schiff* bases.[40] Likewise, the cyanide anion, N-acetyllysine and methoxylamine are 'hard' nucleophiles that can be used to trap hard electrophiles such as electrophilic iminium species that are generated via metabolism of tertiary amines.[99,100]

Usually GSH conjugate screening is performed on samples generated in vitro (using microsomes or hepatocytes), because in vivo, the conjugates might be transported to bile and destroyed by gut bacteria. This is more likely for high molecular weight drugs. Since non-microsomal drug metabolizing enzymes are also capable of catalyzing the bioactivation processes, due consideration must be given to the use of metabolic systems other than liver microsomes such as liver cytosol, the S-9 fraction, or hepatocytes. In the case of drugs causing hematological toxicity such as agranulocytosis or bone marrow toxicity, the myeloperoxidase enzyme system is likely to be more appropriate.[101]

In the case of drugs that form short-lived free radical intermediates,[102] the reactive intermediates can often be trapped using free radical trapping agents such as α-phenyl-N-*t*-butylnitrone, which is commonly used to trap nitrogen free radicals.[18,103] Other spin trapping agents such as 2-methyl-2-nitroso-propane (MNP) to trap carbon-centered free radicals,[104] 5,5-dimethyl-pyrroline-N-oxide (DMPO) to trap hydroxy, carbon-centered, and phenyl radicals,[105,106] and 5-diethoxyphosphoryl-5-methyl-1-pyrroline-N-oxide (DEPMPO) to trap S-centered radicals such as glutathionyl ($GS^{\bullet}$) and the sulfite radical anion ($SO3^{\bullet}$),[107] have also been used. The trapped radicals can then be studied using electron spin resonance (ESR). Drugs that possibly form free radicals such as aminoglutethimide,[108] procainamide,[109] and phenytoin,[18] can be detected using the above trapping agents.

Enzyme inactivation studies. As mentioned above, in certain cases the metabolites are so reactive that they do not escape the enzyme that formed them and covalently bind to the enzyme leading to irreversible inhibition.[39] This is referred to as mechanism-based inactivation as metioned above, and in the case of P450 enzymes, it may result from irreversible alkylation of an active site amino acid or the heme prosthetic group or a combination of both alkylation of heme inactivates P450, whereas amino acid alkylation does not always result in loss of catalytic activity. Inactivation of P450 enzymes often translates into clinically important drug-drug interactions. Enzyme kinetic studies can provide clues to the existence of mechanism-based inhibition.

Covalent binding studies. An important method for detecting and quantifying reactive metabolite formation is the use of radiolabelled drug to study the irreversible binding of the drug. Measurement of the amount of in vitro metabolism dependent covalent binding to biological tissue is possible if radiolabeled drug is available.[99] The assay provides quantitative estimates of radioactivity irreversibly bound to tissue but does not correctly provide information about the nature of covalently modified proteins as discussed below. Covalent-binding studies can be performed in vivo as well. Either tissue or blood/plasma can be examined for the degree of covalent binding. However, covalent binding may require multiple dosing to establish the true impact of the compound. Reactive metabolites formed after the first dose may be efficiently

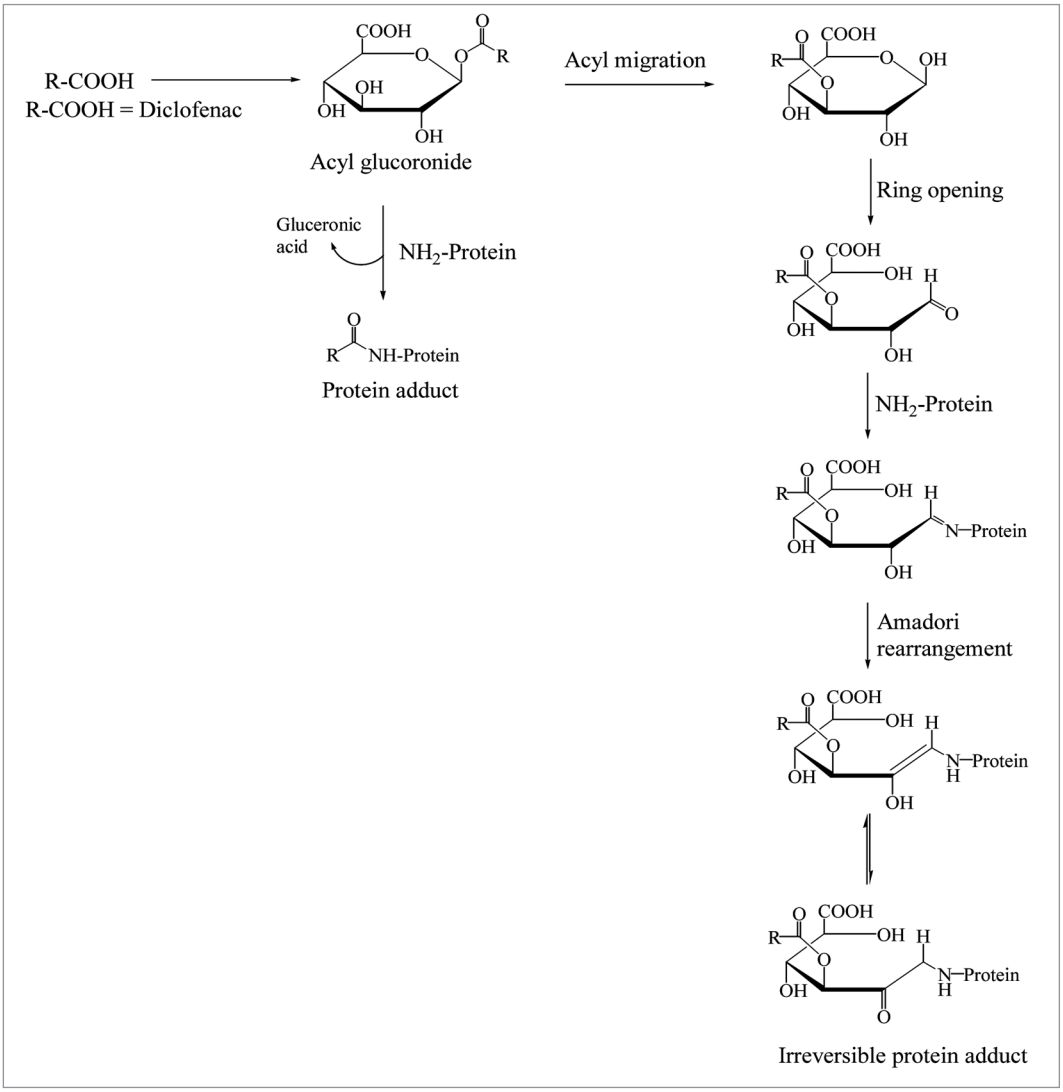

Figure 14. Bioactivation of diclofenac to a reactive acyl glucuronide.

trapped by GSH and eliminated from the body. Once GSH is depleted, the extent of covalent binding with cellular macromolecules may increase rapidly, resulting in toxicity. This is certainly the case with paracetamol where its reactive metabolite can cause direct hepatotoxicity upon an overdose, and yet paracetamol is rarely associated with idiosyncratic adverse drug reactions. This is because, at usual therapeutic doses, the phenolic group in paracetamol undergoes phase-II gluronidation and sulfonation, resulting in a small amount of NAPQI formed; most of which is scavenged by GSH before it binds to macromolecules. The hypothesis has been strengthened based on studies in mice which have shown that significant covalent binding does not occur until over 60–80% of the paracetamol overdose has been eliminated from the liver with concomitant reduction in GSH levels.[37]

An example of this overall approach is highlighted with studies on the potassium-channel opener, maxipost (BMS-204352) (**Fig. 16**), which undergoes P450-mediated bioactivation in rats, dogs, and humans to generate a reactive *ortho*-quinone-methide intermediate, which covalently binds to protein in vivo in animals and humans.[110-111] Acidic hydrolysis of plasma collected after intravenous administration of (^{14}C)-BMS-204352 to rats and human led to the characterization of a unique lysine conjugate of des-fluoro des-*O*-methyl BMS-204352 (**Fig. 16**).

Recent studies examining P450-mediated covalent binding of 18 drugs (nine hepatotoxins and nine non-hepatotoxins) to liver microsomes, S-9, and/or hepatocytes show no correlation between extent of covalent binding and toxicity.[112,113] Indeed, covalent binding study can also make it possible to determine to which proteins the reactive metabolite is bound but this method can also give false negatives if the wrong metabolism system is used. For example the drug trimethoprim is bioactivated by neutrophils or myeloperoxidase, which is the major oxidative enzyme in these cells. So this enzyme system was used to investigate the bioactivation potential of the drug. Trimethoprim is also associated with liver toxicity and hence its investigation of bioactivation by P450 was also carried out. So it is important to use the right enzyme system depending upon the site of toxicity. Although there are major advantages to an in vitro system, the

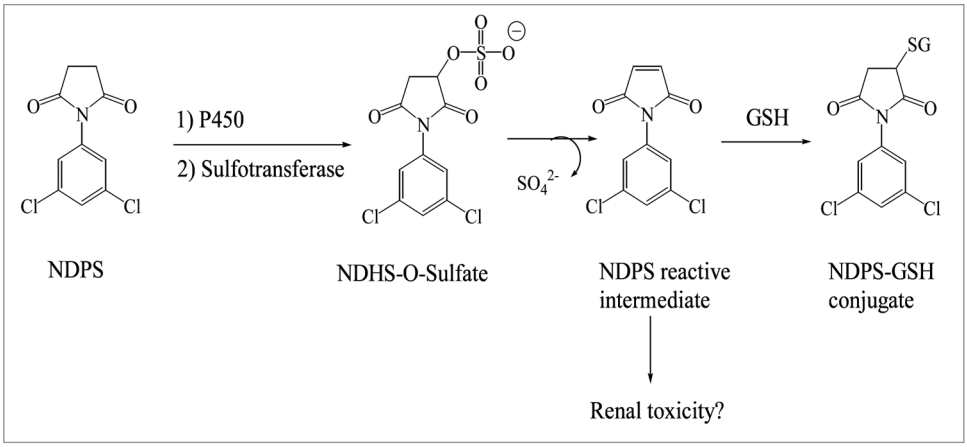

Figure 15. Bioactivation of the antifungal compound NDPS to a reactive O-sulfate.

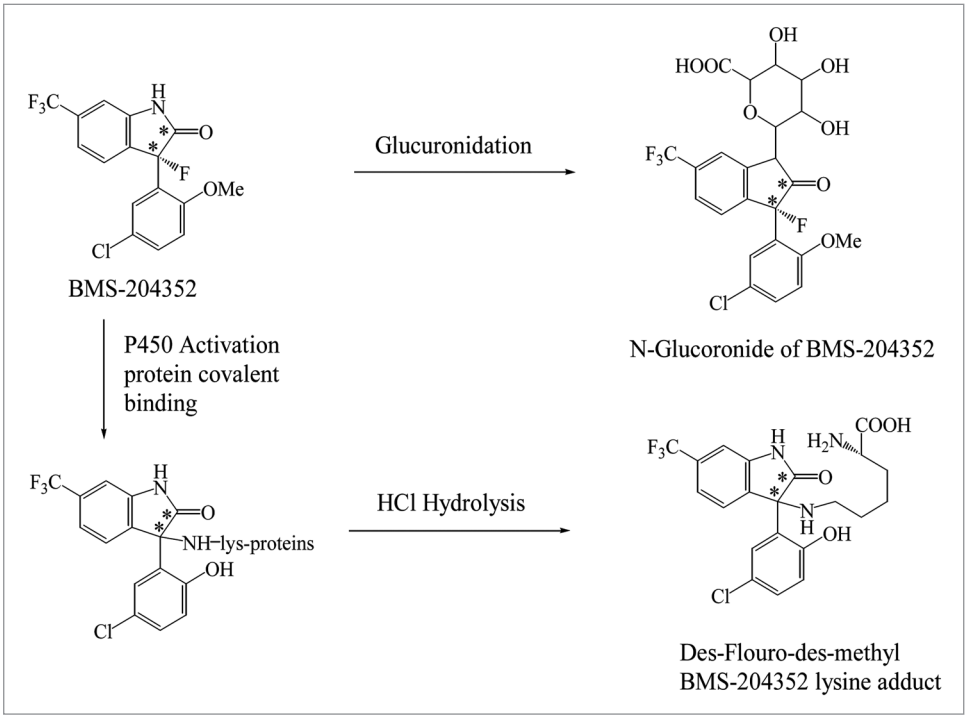

Figure 16. Proposed pathways for biotransformation of [14C]BMS-204352 in humans.

data cannot be extrapolated to predict what will happen in vivo because there are several in vivo detoxifying systems such as GSH and glutathione transferase that may not be present in the in vitro system.[11] Hence the amount of covalent binding observed in vivo might be a lower than that in vitro.

Merck has conducted covalent binding studies with various compounds and has proposed a benchmark of 50 pmole/mg of microsomal protein. If the amount of binding is greater than this amount, consideration would be given to modifying the structure to decrease the amount of covalent binding.[99] However, there is no firm limit because several other considerations enter into the final decision of how much covalent binding is permissible. One consideration is the daily dose of the drug because, if it is a very potent drug, even if it is efficiently converted to a reactive metabolite, the amount will still be too low to represent a significant liability.[114] It is noteworthy to point out that there are no examples of drugs that are dosed at <20 mg/day that cause idiosyncratic adverse drug reactions (whether or not these agents are prone to bioactivation). There are many examples of two structurally related drugs that possess identical toxicophore susceptible to bioactivation, but the one administered at the lower dose is safer than the one given at a higher dose. An illustration of this concept is evident with the antidiabetic thiazolidinedione drugs troglitazone (200–400 mg/day), rosiglitazone, and pioglitazone (<10 mg/day). Troglitazone was withdrawn from the United States market after numerous reported cases of liver failures requiring immediate liver transplantation or leading to death. In contrast, rosiglitazone and pioglitazone are devoid of the hepatotoxicity associated with troglitazone.[115] Another consideration is the structure of the drug and presumed reactive species and how hard it would be to eliminate reactive metabolite formation and still have an active drug. In some cases such as proton pump inhibitors, the pharmacological activity depends on covalent binding of the drug to the enzyme; therefore, it would be impossible to eliminate covalent binding. Other risk versus benefit considerations in using this data are discussed below.

Immunochemical studies. It is also important to know what proteins are modified by a reactive metabolite. Although radiochemical methods can be used, it is more common to use immunochemical methods to determine which proteins are modified. This involves synthesis of an immunogen (i.e. reactive metabolite adduct conjugated to protein), against which antibodies are raised via immunization of animals with the immunogen. The antiserum is screened for anti-drug antibodies using an enzyme-linked immunosorbent assay. The antibody then can be used to identify haptenized biomacromolecules via Western blot analysis.[116] The bands can then be cut out and the modified proteins identified by mass spectrometry. While this method can be a very powerful technique for the identification and characterization of cellular constituents that have undergone covalent modification by a

reactive metabolite, it would be impractical to use as a screening tool because an antibody would have to be generated to each drug candidate. It is also not quantitative.

Unfortunately, without a valid animal model, it is practically impossible to determine whether a specific reactive metabolite is responsible for a given idiosyncratic drug reaction. Therefore, we are left with trying to infer causality. Given their unpredictable nature, idiosyncratic drug reactions are generally not detected until the drug is released onto the market because clinical trials involve a limited number of subjects. If a drug is found to be associated with an unacceptable risk of serious idiosyncratic drug reactions it will cause a huge financial loss to the pharmaceutical company involved because drug development is a very expensive process. The current cost of drug development has been pegged at $1.3 billion US as per a 2009 report.[117] Even though one can minimize the ability of drugs to cause bioactivation, it is hard to predict which compounds will cause idiosyncratic drug reactions because not all covalent binding is associated with the same risk.

The existing in vitro tests are not able to mimic the complexity of in vivo biological systems, and our present mechanistic understanding of idiosyncratic drug reactions is quite superficial. Therefore, animal models are an essential tool for mechanistic studies. They might also be used to screen compounds in development with similar structures to predict which are most likely to cause idiosyncratic drug reactions. There are currently only two practical animal models that appear to involve the same mechanism as the idiosyncratic drug reactions that occur in humans. They are nevirapine-induced skin rash,[118,119] and D-penicillamine-induced autoimmunity.[11,120] Development of further animal models is necessary to understand the mechanisms underlying idiosyncratic drug reactions. It is unlikely that significant progress will be made in preventing idiosyncratic drug reactions until we have a better mechanistic understanding of these adverse reactions.

There are also other animal models of idiosyncratic drug reactions such as propylthiouracil-induced autoimmunity in cats;[121] however, cats are not a practical species to work with. Another model is halothane-induced liver injury in guinea pigs;[122] however, in this model halothane exposure never leads to significant damage and is milder on re-exposure rather than being worse as occurs in humans. A good discussion of these animal models is available in the review by Shenton et al.[123] Nevertheless, there are not many models of idiosyncratic drug reactions and developing more animal models would significantly contribute to a better understanding of underlying mechanisms associated with idiosyncratic drug reactions and ultimately help in the development of safer drugs.

Risk Versus Benefit

Testing of drug candidates for the formation of the reactive metabolites would likely result in safer drugs entering development. It can also result in false positives and false negatives. Nevertheless a decision has to be reached whether to advance compounds to development. Until we develop a better understanding of the risk of toxicity arising from the formation of reactive metabolites, a strategy for identifying and potentially minimizing their formation via rational and iterative medicinal chemistry efforts seems logical in certain cases.[124,125] It is noteworthy to point out that reactive-metabolite formation is only one aspect of the overall risk/benefit assessment for advancing a drug candidate into development. Consequently, bioactivation data (reactive metabolite trapping and/or covalent binding) needs to be placed in a broader context with due consideration given to the following points (**Fig. 17**):

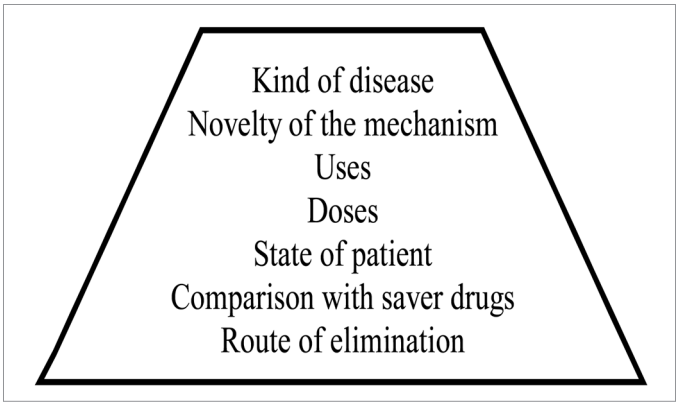

Figure 17. Schematic illustration of the risk and benefits for drug development. See text for explanation.

1. Is the drug intended to address a previously unmet medical need or a lifethreatening disease?
2. Is the drug candidate intended to provide proof-of-mechanism for a novel target?
3. Is the drug intended for acute or chronic use?
4. Is the clinical dose predicted to be low?
5. What is the intended patient population (e.g., would it be given to immunecomprised patients or patients with impaired liver functions)?
6. Are there alternate chemical series with comparable pharmacologic and pharmacokinetic attributes, wherein bioactivation liability is minimized or eliminated?
7. Is there an alternative (higher affinity but innocuous) route of metabolism within the drug candidate that minimizes bioactivation liability associated with the compound?
8. Is metabolism the exclusive route of elimination? What is the likelihood of nonmetabolic elimination processes (e.g., renal and/or biliary excretion of unchanged parent) in humans?

If the benefits outweigh the risk, the compounds can be advanced cautiously. In conclusions, a critical point of this review is the possibility that the reactive metabolites must be considered as "structural alerts" or "toxicophores" that should be avoided in drug development. Then the question is whether drug candidates that can form reactive metabolites must be totally avoided? There is no simple answer to this question. At least two major factors seem to be important: the dose of the drug and the amount of covalent binding. Any drug that is taken at a total dose of 20 mg/day or less is unlikely to be associated with a high incidence

of idiosyncratic drug reactions in humans.[114] Sometimes the marketed drug may contain a structural alert or toxicophore but still not cause a significant incidence of idiosyncratic drug reactions. Again, total exposure to a reactive metabolite is important. One possible reason for this is that the toxicophore is not the primary site of metabolism and therefore not much of the potential reactive metabolite is formed. Even if bioactivation is the major pathway in vitro, a microsomal incubation does not contain all of the metabolic enzymes, and there may be other major clearance pathways in vivo that do not lead to a reactive metabolite. An example of this is raloxifene; in vitro using microsome system, the major pathway is bioactivation of the phenolic metabolite leading to quinone intermediate whereas in vivo the principal mode of clearance is through the glucuronidation of phenolic metabolites rather than the bioactivation. It is associated with low incidence of idiosyncratic drug reactions presumably because of the protective effect of glucuronidation.[39,126] Another reason could be that the principle route of clearance is through a nonmetabolic pathway such as renal elimination.[38]

Examples of strategies that could be followed in drug design to minimize the metabolic liability associated with reactive metabolite formation are: (a) Replacement of the structural alert with substituents that are resistant to metabolism or can be metabolized to nonreactive species; (b) Blocking the functional groups that are known to undergo bioactivation by a functional group that does not undergo activation; (c) Incorporating a bulky substituent close to the site of metabolism so that metabolism could not occur at the site of metabolic activation. Of course elimination of reactive metabolite formation will be of no benefit if it also eliminates the therapeutic effects of the drug and, therefore, it is essential that the pharmacological effects of drug candidates be tested at each step in the optimization of the structure.

Finally, because it is now widely appreciated that reactive metabolites, as opposed to the parent molecules from which they are derived, are responsible for the pathogenesis of some idiosyncratic adverse drug reactions, it is essential to determine exactly how they induce idiosyncratic adverse drug reactions, and what factors determine which reactive metabolites are most likely to cause idiosyncratic adverse drug reactions, and which patients are at highest risk. This requires the development of animal models and the identification of which target proteins are most important for the induction of idiosyncratic adverse drug reactions. Knowledge gained through these processes will be useful to unravel the mysteries of the fascinating research area of idiosyncratic or bizarre adverse drug reactions. This will, in turn, help us to design and bring safer drugs to the market.

Acknowledgements

Editorial assistance by Prof. Kenneth Maiese is acknowledged.

References

1. Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, et al. Concordance of the toxicity of pharmaceuticals in humans and in animals. Regul Toxicol Pharmacol 2000; 32:56-67.
2. Srivastava A, Maggs JL, Antoine DJ, Williams DP, Smith DA, Park BK. Role of reactive metabolites in drug-induced hepatotoxicity. Handb Exp Pharmacol 2010; 196:165-94.
3. Wolf R, Orion E, Marcos B, Matz H. Life-threatening acute adverse cutaneous drug reactions. Clin Dermatol 2005; 23:171-81.
4. Sevketoglu E, Hatipoglu S, Akman M, Bicer S. Toxic epidermal necrolysis in a child after carbamazepine dosage increment. Pediatr Emerg Care 2009; 25:93-5.
5. Svensson CK. Biotransformation of drugs in human skin. Drug Metab Dispos 2009; 37:247-53.
6. Handoko KB, Souverein PC, van Staa TP, Meyboom RH, Leufkens HG, Egberts TC, et al. Risk of aplastic anemia in patients using antiepileptic drugs. Epilepsia 2006; 47:1232-6.
7. Andrès E, Federici L, Weitten T, Vogel T, Alt M. Recognition and management of drug-induced blood cytopenias: the example of drug-induced acute neutropenia and agranulocytosis. Expert Opin Drug Saf 2008; 7:481-9.
8. Kaplowitz N. Drug-induced liver injury. Clin Infect Dis 2004; 38: S44-8.
9. Levi PE, Hodgson E. Reactive metabolites and toxicity, in Introduction to Biochemical Toxicology, 3rd ed., (Hodgson E, Smart RC, eds), Wiley, New York 2001; 199-220.
10. Conney AH. In memoriam: James A. Miller (1915-2000). Cancer Res 2001; 61:3847-8.
11. Uetrecht J. Idiosyncratic drug reactions: current understanding. Annu Rev Pharmacol Toxicol 2007; 47:513-39.
12. Ikehata K, Duzhak TG, Galeva NA, Ji T, Koen YM, Hanzlik RP. Protein targets of reactive metabolites of thiobenzamide in rat liver in vivo. Chem Res Toxicol 2008; 21:1432-42.
13. Amacher DE. Reactive intermediates and the pathogenesis of adverse drug reactions: the toxicology perspective. Curr Drug Metab 2006; 7:219-29.
14. Wells PG, McCallum GP, Chen CS, Henderson JT, Lee CJ, Perstin J, et al. Oxidative stress in developmental origins of disease: teratogenesis, neurodevelopmental deficits, and cancer. Toxicol Sci 2009; 108:4-18.
15. Wells PG, Lee CJ, McCallum GP, Perstin J, Harper PA. Receptor- and reactive intermediate-mediated mechanisms of teratogenesis. Handb Exp Pharmacol 2010; 196:131-62.
16. Skipper PL, Kim MY, Sun HL, Wogan GN, Tannenbaum SR. Monocyclic aromatic amines as potential human carcinogens: old is new again. Carcinogenesis 2010; 31:50-8.
17. Clayden J, Greeves N, Warren S, Wothers P. Organic Chemistry, 1th ed., Oxford University Press Inc.: New York, USA 2001.
18. Kubow S, Wells PG. In vitro bioactivation of phenytoin to a reactive free radical intermediate by prostaglandin synthetase, horseradish peroxidase, and thyroid peroxidase. Mol Pharmacol 1989; 35:504-11.
19. Sun Q, Zhu R, Foss FW Jr, Macdonald TL. In vitro metabolism of a model cyclopropylamine to reactive intermediate: insights into trovafloxacin-induced hepatotoxicity. Chem Res Toxicol 2008; 21:711-9.
20. Beckman JS. Understanding peroxynitrite biochemistry and its potential for treating human diseases. Arch Biochem Biophys 2009; 484:114-6.
21. Vanhoutte PM. How we learned to say NO. Arterioscler Thromb Vasc Biol 2009; 29:1156-60.
22. Bedard K, Krause KH. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. Physiol Rev 2007; 87:245-313.
23. Wingler K, Schmidt HH. Good stress, bad stress-the delicate balance in the vasculature. Dtsch Arztebl Int 2009; 106:677-84.
24. Gutteridge JM, Halliwell B. Antioxidants: Molecules, medicines, and myths. Biochem Biophys Res Commun 2010; 393:561-4.
25. McNally JS, Davis ME, Giddens DP, Saha A, Hwang J, Dikalov S, et al. Role of xanthine oxidoreductase and NAD(P)H oxidase in endothelial superoxide production in response to oscillatory shear stress. Am J Physiol Heart Circ Physiol 2003; 285:H2290-7.
26. Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human disease. Clin Chem 2006; 52:601-23.
27. Fisher-Wellman K, Bell HK, Bloomer RJ. Oxidative stress and antioxidant defense mechanisms linked to exercise during cardiopulmonary and metabolic disorders. Oxid Med Cell Longev 2009; 2:43-51.
28. Sawyer DB, Siwik DA, Xiao L, Pimentel DR, Singh K, Colucci WS. Role of oxidative stress in myocardial hypertrophy and failure. J Mol Cell Cardiol 2002; 34:379-88.
29. Elahi MM, Yu Xiang Kong YX, Matata BM. Oxidative stress as a mediator of cardiovascular disease. Oxid Med Cell Longev 2009; 2:259-69.
30. Afanas'ev I. Reactive oxygen species and age-related genes p66shc, Sirtuin, FOX03 and Klotho in senescence. Oxid Med Cell Longev 2010; 3:77-85.
31. Essick EE, Sam F. Oxidative stress and autophagy in cardiac disease, neurological disorders, aging and cancer. Oxid Med Cell Longev 2010; 3:168-77.
32. Jung-Hynes B, Reiter RJ, Ahmad N. Sirtuins, melatonin and circadian rhythms: building a bridge between aging and cancer. J Pineal Res 2010; 48:9-19.
33. Calabrese V, Cornelius C, Mancuso C, Lentile R, Stella AM, Butterfield DA. Redox homeostasis and cellular stress response in aging and neurodegeneration. Methods Mol Biol 2010; 610:285-308.
34. Larson AM. Acetaminophen hepatotoxicity. Clin Liver Dis 2007; 11:525-48.
35. Jollow DJ, Mitchell JR, Potter WZ, Davis DC, Gillette JR, Brodie BB. Acetaminophen-induced hepatic necrosis. II. Role of covalent binding in vivo. J Pharmacol Exp Ther 1973; 187:195-202.
36. Mitchell JR, Jollow DJ, Potter WZ, Davis DC, Gillette JR, Brodie BB. Acetaminophen-induced hepatic necrosis. I. Role of drug metabolism. J Pharmacol Exp Ther 1973; 187:185-94.

37. Dahlin DC, Miwa GT, Lu AY, Nelson SD. N-acetyl-p-benzoquinone imine: a cytochrome P-450-mediated oxidation product of acetaminophen. Proc Natl Acad Sci USA 1984; 81:1327-31.
38. Kalgutkar AS, Soglia JR. Minimising the potential for metabolic activation in drug discovery. Expert Opin Drug Metab Toxicol 2005; 1:91-142.
39. Chen Q, Ngui JS, Doss GA, Wang RW, Cai X, DiNinno FP, et al. Cytochrome P450 3A4-mediated bioactivation of raloxifene: irreversible enzyme inhibition and thiol adduct formation. Chem Res Toxicol 2002; 15:907-14.
40. Kalgutkar AS, Dalvie DK, Aubrecht J, Smith EB, Coffing SL, Cheung JR, et al. Genotoxicity of 2-(3-chlorobenzyloxy)-6-(piperazinyl)pyrazine, a novel 5-hydroxytryptamine2c receptor agonist for the treatment of obesity: role of metabolic activation. Drug Metab Dispos 2007; 35:848-58.
41. Schmassmann-Suhijar D, Bullingham R, Gasser R, Schmutz J, Haefeli WE. Rhabdomyolysis due to interaction of simvastatin with mibefradil. Lancet 1998; 351:1929-30.
42. Prueksaritanont T, Ma B, Tang C, Meng Y, Assang C, Lu P, et al. Metabolic interactions between mibefradil and HMG-CoA reductase inhibitors: an in vitro investigation with human liver preparations. Br J Clin Pharmacol 1999; 47:291-8.
43. Almeida JC, Grimsley EW. Coma from the health food store: interaction between kava and alprazolam. Ann Intern Med 1996; 125:940-1.
44. Clouatre DL. Kava kava: examining new reports of toxicity. Toxicol Lett 2004; 150:85-96.
45. Izzo AA, Ernst E. Interactions between herbal medicines and prescribed drugs: an updated systematic review. Drugs 2009; 69:1777-98.
46. Singh YN, Singh NN. Therapeutic potential of kava in the treatment of anxiety disorders. CNS Drugs 2002; 16: 731-43.
47. Mathews JM, Etheridge AS, Black SR. Inhibition of human cytochrome P450 activities by kava extract and kavalactones. Drug Metab Dispos 2002; 30:1153-7.
48. Johnson BM, Qiu SX, Zhang S, Zhang F, Burdette JE, Yu L, et al. Identification of novel electrophilic metabolites of piper methysticum Forst (Kava). Chem Res Toxicol 2003; 16:733-40.
49. Fieser LF. Carcinogenic activity, structure, and chemical reactivity of polynuclear aromatic hydrocarbons. Am J Cancer 1938; 34:37-I24.
50. Miller EC, Miller JA. The presence and significance of bound aminoazo dyes in the livers of rats fed p-dimethyl- aminoazobenzene. Cancer Res 1947; 7:468-80.
51. Thybaud V, Aardema M, Clements J, Dearfield K, Galloway S, Hayashi M, et al. Expert working group on hazard identification and risk assessment in relation to in vitro testing. Mutat Res 2007; 627:41-58.
52. Ku WW, Bigger A, Brambilla G, Glatt H, Gocke E, Guzzie PJ, et al. Strategy Expert Group, IWGT. Strategy for genotoxicity testing-metabolic considerations. Mutat Res 2007; 627:59-77.
53. Woosley RL, Drayer DE, Reidenberg MM, Nies AS, Carr K, Oates JA. Effect of acetylator phenotype on the rate at which procainamide induces antinuclear antibodies and the lupus syndrome. N Engl J Med 1978; 298:1157-9.
54. Wolkenstein P, Carrière V, Charue D, Bastuji-Garin S, Revuz J, Roujeau JC, et al. A slow acetylator genotype is a risk factor for sulphonamide-induced toxic epidermal necrolysis and Stevens-Johnson syndrome. Pharmacogenetics 1995; 5:255-8.
55. Uetrecht JP. Reactivity and possible significance of hydroxylamine and nitroso metabolites of procainamide. J Pharmacol Exp Ther 1985; 232:420-5.
56. Cribb AE, Miller M, Leeder JS, Hill J, Spielberg SP. Reactions of the nitroso and hydroxylamine metabolites of sulfamethoxazole with reduced glutathione. Implications for idiosyncratic toxicity. Drug Metab Dispos 1991; 19:900-6.
57. Park EY, Cho IJ, Kim SG. Transactivation of the PPAR-responsive enhancer module in chemopreventive glutathione S-transferase gene by the peroxisome proliferator-activated receptor-gamma and retinoid X receptor heterodimer. Cancer Res 2004; 64:3701-13.
58. Ueda K, Ishitsu T, Seo T, Ueda N, Murata T, Hori M, et al. Glutathione S-transferase M1 null genotype as a risk factor for carbamazepine-induced mild hepatotoxicity. Pharmacogenomics 2007; 8:435-42.
59. Buckley NA, Srinivasan J. Should a lower treatment line be used when treating paracetamol poisoning in patients with chronic alcoholism?: a case for. Drug Saf 2002; 25:619-24.
60. Bolton JL, Trush MA, Penning TM, Dryhurst G, Monks TJ. Role of quinines in toxicology. Chem Res Toxicol 2000; 13:135-60.
61. Monks TJ, Jones DC. The metabolism and toxicity of quinones, quinonimines, quinone methides, and quinone-thioethers. Curr Drug Metab 2002; 3:425-38.
62. Hayasaki Y, Itoh S, Kato M, Furuhama K. Mutagenesis induced by 12 quinolone antibacterial agents in Escherichia coli WP2uvrA/pKM101. Toxicol In Vitro 2006; 20:342-26.
63. Attia SM. Use of centromeric and telomeric DNA probes in in situ hybridization for differentiation of micronuclei induced by lomefloxacin. Environ Mol Mutagen 2009; 50:394-403.
64. Levay G, Pongracz K, Bodell WJ. Detection of DNA adducts in HL-60 cells treated with hydroquinone and p-benzoquinone by 32P-postlabeling. Carcinogenesis 1991; 12:1181-6.
65. Chen H, Eastmond DA. Synergistic increase in chromosomal breakage within the euchromatin induced by an interaction of the benzene metabolites phenol and hydroquinone in mice. Carcinogenesis 1995; 16:1963-9.
66. Hande KR. Etoposide: four decades of development of a topoisomerase II inhibitor. Eur J Cancer 1998; 34:1514-21.
67. Turner SD, Wijnhoven SW, Tinwell H, Lashford LS, Rafferty JA, Ashby J, et al. Assays to predict the genotoxicity of the chromosomal mutagen etoposide - focussing on the best assay. Mutat Res 2001; 493:139-47.
68. Attia SM, Schmid TE, Badary OA, Hamada FM, Adler I-D. Molecular cytogenetic analysis in mouse sperm of chemically induced aneuploidy: studies with topoisomerase II inhibitors. Mutat Res 2002; 520:1-13.
69. Attia SM, Kliesch U, Schriever-Schwemmer G, Badary OA, Hamada FM, Adler I-D. Etoposide and merbarone are clastogenic and aneugenic in the mouse bone marrow micronucleus test complemented by fluorescence in situ hybridization with the mouse minor satellite DNA probe. Environ Mol Mutagen 2003; 41:99-103.
70. Choudhury RC, Palo AK, Sahu PJ. Cytogenetic risk assessment of etoposide from mouse bone marrow. Appl Toxicol 2004; 24:115-22.
71. Felix CA. Leukemias related to treatment with DNA topoisomerase II inhibitors. Med Pediatr Oncol 2001; 36:525-35.
72. Felix CA, Kolaris CP, Osheroff N. Topoisomerase II and the etiology of chromosomal translocations. DNA Rep 2006; 5: 1093-8.
73. Sung PA, Libura J, Richardson C. Etoposide and illegitimate DNA double-strand break repair in the generation of MLL translocations: new insights and new questions. DNA Rep 2006; 5:1109-18.
74. Kagan VE, Yalowich JC, Borisenko GG, Tyurina YY, Tyurin VA, Thampatty P, et al. Mechanism-based chemopreventive strategies against etoposide-induced acute myeloid leukemia: free radical/antioxidant approach. Mol Pharmacol 1999; 56:494-506.
75. Kagan VE, Kuzmenko AI, Tyurina YY, Shvedova AA, Matsura T, Yalowich JC. Pro-oxidant and antioxidant mechanisms of etoposide in HL-60 cells: role of myeloperoxidase. Cancer Res 2001; 61:7777-84.
76. Ritov VB, Goldman R, Stoyanovsky DA, Menshikova EV, Kagan VE. Antioxidant paradoxes of phenolic compounds: peroxyl radical scavenger and lipid antioxidant, etoposide (VP-16), inhibits sarcoplasmic reticulum Ca(2+)-ATPase via thiol oxidation by its phenoxyl radical. Arch Biochem Biophys 1995; 321:140-52.
77. Siitonen T, Alaruikka P, Mantymaa P, Savolainen ER, Kavanagh TJ, Krejsa CM, et al. Protection of acute myeloblastic leukemia cells against apoptotic cell death by high glutathione and gamma-glutamylcysteine synthetase levels during etoposide-induced oxidative stress. Ann Oncol 1999; 10:1361-7.
78. Attia SM, Al-Anteet AA, AL-Rasheed NM, Alhaider AA, Al-Harbi MM. Protection of mouse bone marrow from etoposide-induced genomic damage by dexrazoxane. Cancer Chemother Pharmacol 2009; 64:837-45.
79. Erve JC, Svensson MA, von Euler-Chelpin H, Klasson-Wehler E. Characterization of glutathione conjugates of the remoxipride hydroquinone metabolite NCQ-344 formed in vitro and detection following oxidation by human neutrophils. Chem Res Toxicol 2004; 17:564-71.
80. Naisbitt DJ, Williams DP, O'Neill PM, Maggs JL, Willock DJ, Pirmohamed M, et al. Metabolism-dependent neutrophil cytotoxicity of amodiaquine: A comparison with pyronaridine and related antimalarial drugs. Chem Res Toxicol 1998; 11:1586-95.
81. Albano E, Rundgren M, Harvison PJ, Nelson SD, Moldeus P. Mechanisms of N-acetyl-p-benzoquinone imine cytotoxicity. Mol Pharmacol 1985; 28:306-11.
82. Li Y, Slatter JG, Zhang Z, Doss GA, Braun MP, Stearns RA, et al. In vitro metabolic activation of lumiracoxib in rat and human liver preparations. Drug Metab Dispos 2008; 36:469-73.
83. Thompson DC, Perera K, Krol ES, Bolton JL. o-Methoxy-4-alkylphenols that form quinone methides of intermediate reactivity are the most toxic in rat liver slices. Chem Res Toxicol 1995; 8:323-7.
84. Liu J, Liu H, van Breemen RB, Thatcher GR, Bolton JL. Bioactivation of the selective estrogen receptor modulator acolbifene to quinone methides. Chem Res Toxicol 2005; 18:174-82.
85. Pirmohamed M, Kitteringham NR, Breckenridge AM, Park BK. The effect of enzyme induction on the cytochrome P450-mediated bioactivation of carbamazepine by mouse liver microsomes. Biochem Pharmacol 1992; 44:2307-14.
86. Pearce RE, Lu W, Wang Y, Uetrecht JP, Correia MA, Leeder JS. Pathways of carbamazepine bioactivation in vitro. III. The role of human cytochrome P450 enzymes in the formation of 2,3-dihydroxycarbamazepine. Drug Metab Dispos 2008; 36:1637-49.
87. Chen Q, Doss GA, Tung EC, Liu W, Tang YS, Braun MP, et al. Evidence for the bioactivation of zomepirac and tolmetin by an oxidative pathway: identification of glutathione adducts in vitro in human liver microsomes and in vivo in rats. Drug Metab Dispos 2006; 34:145-51.
88. Hargus SJ, Amouzedeh HR, Pumford NR, Myers TG, McCoy SC, Pohl LR. Metabolic activation and immunochemical localization of liver protein adducts of the nonsteroidal anti-inflammatory drug diclofenac. Chem Res Toxicol 1994; 7:575-82.
89. Ware JA, Graf ML, Martin BM, Lustberg LR, Pohl LR. Immunochemical detection and identification of protein adducts of diclofenac in the small intestine of rats: possible role in allergic reactions. Chem Res Toxicol 1998; 11:164-71.
90. Li C, Benet LZ, Grillo MP. Studies on the chemical reactivity of 2-phenylpropionic acid 1-O-acyl glucuronide and S-acyl-CoA thioester metabolites. Chem Res Toxicol 2002; 15:1309-17.
91. Glatt H, Engelke CE, Pabel U, Teubner W, Jones AL, Coughtrie MW, et al. Sulfotransferases: genetics and role in toxicology. Toxicol Lett 2000; 112-113:341-8.
92. Cui D, Rankin GO, Harvison PJ. Metabolism of the nephrotoxicant N-(3,5-dichlorophenyl)succinimide in rats: evidence for bioactivation through alcohol-Oglucuronidation and O-sulfation. Chem Res Toxicol 2005; 18: 991-1003.

93. Uetrecht J. N-oxidation of drugs associated with idiosyncratic drug reactions. Drug Metab Rev 2002; 34:651-65.
94. Hofstra AH, Matassa LC, Uetrecht JP. Metabolism of hydralazine by activated leukocytes: implications for hydralazine induced lupus. J Rheumatol 1991; 18:1673-80.
95. Kalgutkar AS, Gardner I, Obach RS, Shaffer CL, Callegari E, Henne KR, et al. A comprehensive listing of bioactivation pathways of organic functional groups. Curr Drug Metab 2005; 6:161-225.
96. Mahajan MK. Evans CA. Dual negative precursor ion scan approach for rapid detection of glutathione conjugates using liquid chromatography/tandem mass spectrometry. Rapid Commun Mass Spectrom 2008; 22:1032-40.
97. Leblanc A, Shiao TC, Roy R, Sleno L. Improved detection of reactive metabolites with a bromine-containing glutathione analog using mass defect and isotope pattern matching. Rapid Commun Mass Spectrom 2010; 24:1241-50.
98. Baillie TA, Davis MR. Mass spectrometry in the analysis of glutathione conjugates. Biol Mass Spectrom 1993; 22:319-25.
99. Evans DC, Watt AP, Nicoll-Griffith DA, Baillie TA. Drug-protein adducts: an industry perspective on minimizing the potential for drug bioactivation in drug discovery and development. Chem Res Toxicol 2004; 17:3-16.
100. Argoti D, Liang L, Conteh A, Chen L, Bershas D, Yu CP, et al. 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 2005; 18:1537-44.
101. Furst SM, Uetrecht JP. Carbamazepine metabolism to a reactive intermediate by the myeloperoxidase system of activated neutrophils. Biochem Pharmacol 1993; 45:1267-75.
102. Mason RP, Kadiiska MB. In vivo spin trapping of free radical metabolites of drugs and toxic chemicals utilizing ex vivo detection in: Biomedical EPR, Part A: Free Radicals, Metals, Medicine, and Physiology 2006; 93-109.
103. Kubow S, Janzen EG, Bray TM. Spin-trapping of free radicals formed during in vitro and in vivo metabolism of 3-methylindole. J Biol Chem 1984; 259:4447-51.
104. Krings U, Andersen ML, Berger RG. In vivo ESR spin trapping detection of carbon-centered alpha-Farnesene radicals. J Agric Food Chem 2008; 56:4333-9.
105. Kadiiska MB, De Costa KS, Mason RP, Mathews JM. Reduction of 1,3-diphenyl-1-triazene by rat hepatic microsomes, by cecal microflora, and in rats generates the phenyl radical metabolite: nn ESR spin-trapping investigation. Chem Res Toxicol 2000; 13:1082-6.
106. Chou DS, Hsiao G, Shen MY, Tsai YJ, Chen TF, Sheu JR. ESR spin trapping of a carbon-centered free radical from agonist-stimulated human platelets. Free Radic Biol Med 2005; 39:237-48.
107. Liu KJ, Miyake M, Panz T, Swartz H. Evaluation of DEPMPO as a spin trapping agent in biological systems. Free Radic Biol Med 1999; 26:714-21.
108. Siraki AG, Bonini MG, Jiang J, Ehrenshaft M, Mason RP. Aminoglutethimideinduced protein free radical formation on myeloperoxidase: a potential mechanism of agranulocytosis. Chem Res Toxicol 2007; 20:1038-45.
109. Siraki AG, Deterding LJ, Bonini MG, Jiang J, Ehrenshaft M, Tomer KB, et al. Procainamide, but not N-acetylprocainamide, induces protein free radical formation on myeloperoxidase: a potential mechanism of agranulocytosis. Chem Res Toxicol 2008; 21:1143-53.
110. Zhang D, Ogan M, Gedamke R, Roongta V, Dai R, Zhu M, et al. Protein covalent binding of maxipost through a cytochrome P450-mediated ortho-quinone methide intermediate in rats. Drug Metab Dispos 2003; 31:837-45.
111. Zhang D, Krishna R, Wang L, Zeng J, Mitroka J, Dai R, et al. Metabolism, pharmacokinetics, and protein covalent binding of radiolabeled MaxiPost (BMS-204352) in humans. Drug Metab Dispos 2005; 33:83-93.
112. Obach RS, Kalgutkar AS, Soglia JR, Zhao SX. Can in vitro metabolism-dependent covalent binding data in liver microsomes distinguish hepatotoxic from non-hepatotoxic drugs? An analysis of 18 drugs with consideration of intrinsic clearance and daily dose. Chem Res Toxicol 2008; 21:1814-22.
113. Bauman JN, Kelly JM, Tripathy S, Zhao SX, Lam WW, Kalgutkar AS, et al. Can in vitro metabolism-dependent covalent binding data distinguish hepatotoxic from nonhepatotoxic drugs? An analysis using human hepatocytes and liver S-9 fraction. Chem Res Toxicol 2009; 22:332-40.
114. Uetrecht J. Screening for the potential of a drug candidate to cause idiosyncratic drug reactions. Drug Discov Today 2003; 8:832-7.
115. Alvarez-Sanchez R, Montavon F, Hartung T, Pähler A. Thiazolidinedione bioactivation: a comparison of the bioactivation potentials of troglitazone, rosiglitazone, and pioglitazone using stable isotope-labeled analogues and liquid chromatography tandem mass spectrometry. Chem Res Toxicol 2006; 19:1106-16.
116. Lai WG, Gardner I, Zahid N, Uetrecht JP. Bioactivation and covalent binding of hydroxyfluperlapine in human neutrophils: implications for fluperlapineinduced agranulocytosis. Drug Metab Dispos 2000; 28:255-63.
117. Collier R. Drug development cost estimates hard to swallow. CMAJ 2009; 180:279-80.
118. Shenton JM, Teranishi M, Abu-Asab MS, Yager JA, Uetrecht JP. Characterization of a potential animal model of an idiosyncratic drug reaction: nevirapine-induced skin rash in the rat. Chem Res Toxicol 2003; 16:1078-89.
119. Shenton JM, Popovic M, Uetrecht JP. In Drug Hypersensitivity. WJ. Pichler ED., Basel, Karger 2007; 115-28.
120. Donker AJ, Venuto RC, Vladutiu AO, Brentjens JR, Andres GA. Effects of prolonged administration of D-penicillamine or captopril in various strains of rats. Brown Norway rats treated with D-penicillamine develop autoantibodies, circulating immune complexes, and disseminated intravascular coagulation. Clin Immunol Immunopathol 1984; 30:142-55.
121. Peterson ME, Hurvitz AI, Leib MS, Cavanagh PG, Dutton RE. Propylthiouracil-associated hemolytic anemia, thrombocytopenia, and antinuclear antibodies in cats with hyperthyroidism. J Am Vet Med Assoc 1984; 184:806-8.
122. Furst SM, Luedke D, Gaw HH, Reich R, Gandolfi AJ. Demonstration of a cellular immune response in halothane-exposed guinea pigs. Toxicol Appl Pharmacol 1997; 143:245-55.
123. Shenton JM, Chen J, Uetrecht JP. Animal models of idiosyncratic drug reactions. Chem Biol Interact 2004; 150:53-70.
124. Kumar S, Kassahun K, Tschirret-Guth RA, Mitra K, Baillie TA. Minimizing metabolic activation during pharmaceutical lead optimization: progress, knowledge gaps and future directions. Curr Opin Drug Discov Devel 2008; 11:43-52.
125. Kumar S, Mitra K, Kassahun K, Baillie TA. Approaches for minimizing metabolic activation of new drug candidates in drug discovery. Handb Exp Pharmacol 2010; 196:511-44.
126. Kemp DC, Fan PW, Stevens JC. Characterization of raloxifene glucuronidation in vitro: contribution of intestinal metabolism to presystemic clearance. Drug Metab Dispos 2002; 30:694-700.