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Cytochrome P450s and Other Enzymes in Drug Metabolism and Toxicity

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Abstract

The cytochrome P450 (P450) enzymes are the major catalysts involved in the metabolism of drugs. Bioavailability and toxicity are 2 of the most common barriers in drug development today, and P450 and the conjugation enzymes can influence these effects. The toxicity of drugs can be considered in 5 contexts: on-target toxicity, hypersensitivity and immunological reactions, off-target pharmacology, bioactivation to reactive intermediates, and idiosyncratic drug reactions. The chemistry of bioactivation is reasonably well understood, but the mechanisms underlying biological responses are not. In the article we consider what fraction of drug toxicity actually involves metabolism, and we examine how species and human interindividual variations affect pharmacokinetics and toxicity.

Keywords: Cytochrome P450, drug metabolism, toxicity, reactive metabolites

Introduction

This review will focus on issues in the development of pharmaceuticals, particularly metabolism. The problems involving mechanisms of chemical toxicity are not unique to this area; they are also encountered with chemical carcinogens, toxicants in the environment, drugs of abuse, and alcohol. Human studies with pharmaceutical agents and even drug candidates have some advantages in that these are administered to humans under controlled conditions and toxicity is monitored. However, other experimental approaches are also needed to define mechanisms of toxicity of drugs and other chemicals. The term *chemical toxicity* will be used here in a broad sense to encompass pharmaceuticals, carcinogens, alcohol, drugs of abuse, and chemicals of issue in the environment, in a global sense.

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Challenge of Metabolism in Drug Development

The numbers related to success in producing new medicines are very challenging. Even with some discrimination in the selection of chemicals for leads, ~10^4 compounds are tested to produce one compound that reaches the market. The average cost of taking a drug to the market stage is $800 million to $1 billion, and only 1 in 3 drugs that does reach the market is profitable. Compounding the problems are social and political pressures to both reduce the costs of drugs and provide nearly absolute safety.

One strategy adopted in the pharmaceutical industry in the past 20 years is to make chemical prospects more "druggable." A cycle of biology (usually efficacy tests in animals) and chemistry (synthesis) has been used in the past. Unfortunately, many of the candidates developed in this manner had excellent properties in preclinical animal models but did not show efficacy in humans. Two of the major problems have been bioavailability and toxicity, both of which can be related at least in part to metabolism. A version of the current general paradigm used in most companies today involves a "subloop" termed *developability screening* and predictions, largely made in vitro with human enzyme systems of one sort or another (Figure 1). The issues are toxicology, pharmaceutics, and the process of absorption, distribution, metabolism, and excretion. The goal is to do a better job of selecting candidate drugs early in the development process (even as part of discovery) to avoid problems later in clinical trials, when more resources (including time) have been invested, by restricting the development process to the compounds with the highest likelihood for success.

Cytochrome P450: Background

Cytochrome P450 (P450) enzymes are found throughout nature. For our purposes, those of most interest with drugs (and with other chemicals for which toxicity is an issue) are the 57 human enzymes (Table 1). Many of these are used in the metabolism of sterols and vitamins A and D.[2] About one quarter of the 57 P450s are generally considered to be involved primarily in the metabolism of "xenobiotic" chemicals (not normally in the body).[3] In contrast to the P450s

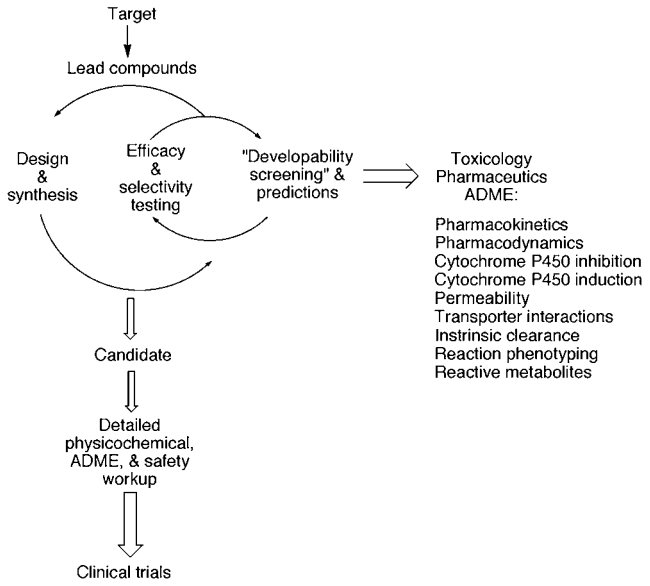

Figure 1. General strategy used in preclinical drug development. ADME indicates absorption, distribution, metabolism, and excretion.[1]

Table 1. Classification of Human Cytochrome P450s Based on Major Substrate Class[2,3]

Sterols	Xenobiotics	Fatty Acids	Eicosanoids	Vitamins	Unknown
1B1	1A1	2J2	4F2	2R1	2A7
7A1	1A2	4A11	4F3	24A1	2S1
7B1	2A6	4B1	4F8	26A1	2U1
8B1	2A13	4F12	5A1	26B1	2W1
11A1	2B6		8A1	26C1	3A43
11B1	2C8			27B1	4A22
11B2	2C9				4F11
17A1	2C18				4F22
19A1	2C19				4V2
21A2	2D6				4X1
27A1	2E1				4Z1
39A1	2F1				20A1
46A1	3A4				27C1
51A1	3A5				
	3A7				

involved in sterol metabolism, which are important in normal physiology,[4] the levels of the "xenobiotic-metabolizing" P450s can vary widely (Figure 2), and individuals can be completely devoid of some of these because of genetics.

The significance of variability in levels of a single P450 is demonstrated in Figure 3. In this instance, the drug of interest is cleared primarily by a single P450. In most of the populations administered a particular dose of the drug, the pharmacokinetic pattern shown in the upper trace (extensive metabolizer) is observed, with the level of the drug in the plasma (and target organ) maintained in a range that yields the desired pharmacological effect. However, in the lower panel an individual deficient in the particular P450 (poor metabolizer) shows very limited metabolism of the same dose of drug, with the result that after a few doses a much higher level of the drug will accumulate in the patient. The most obvious side effect that would be expected would be an exacerbated pharmacological response.

P450s are the enzyme systems most commonly involved in drug metabolism (Figure 4A), with the other major contributors being uridine dinucleotide phosphate (UDP) glucuronosyl transferases and esterases.[5,6] Of the P450s, ~90% of the metabolism can be accounted for by 5 human P450s (Figure 4B). Thus, studies on metabolism and toxicity are simpler than they would be if all of the 57 human P450s (Table 1) had similar roles in drug metabolism.

Thus far we have only used "metabolism" in a broad sense, indicating all transformations of a drug by an enzyme(s). The majority of these changes deactivate the drug or other xenobiotic, attenuating its biological activity and perhaps accelerating its clearance from the body. However, P450s and other enzymes can also "bioactivate" chemicals,

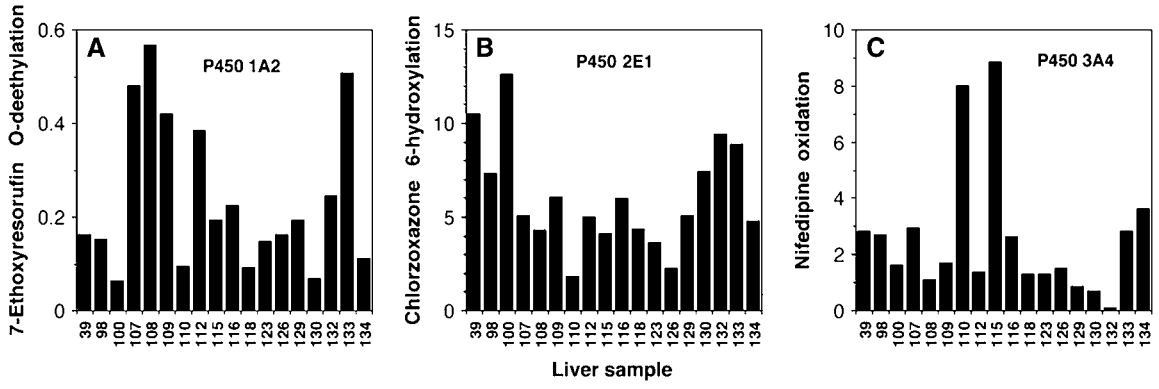

Figure 2. Variability in levels of individual P450s in 18 human liver samples (designated with code numbers). Marker activities are used for each P450; immunochemical assays yield similar patterns. P450 indicates cytochrome P450.[2]

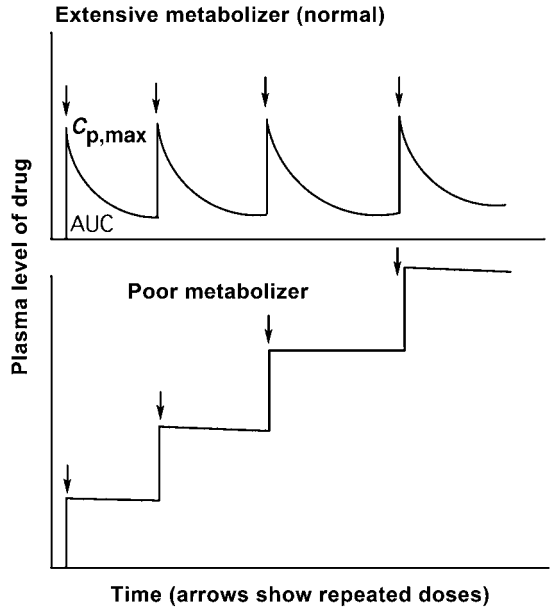

Figure 3. Effect of variation in human P450 activities on pharmacokinetics. P450 indicates cytochrome P450; AUC, area under the curve.[2]

converting them to reactive products that modify cellular constituents and produce damage (*vide infra*).

Other Enzymes Involved in Bioactivation

P450s are probably involved in more bioactivation processes than other enzymes because (1) the P450s collectively have more substrates than any other enzymes, at least among drugs (Figure 4A); and (2) several of the reaction products (eg, epoxides, hydroquinones) have reaction chemistry because of their inherent instability.[7]

However, P450s are not the only enzymes involved in the metabolism of drugs and other xenobiotic chemicals. Several enzymes catalyze conjugations of chemicals with low M_r compounds already in the cells, or they catalyze hydrolysis reactions. These enzymes have often been collectively referred to as phase II reactions (P450 and other oxidations being phase I), although such a convention is no longer tenable and we have argued that it should be eliminated.[8]

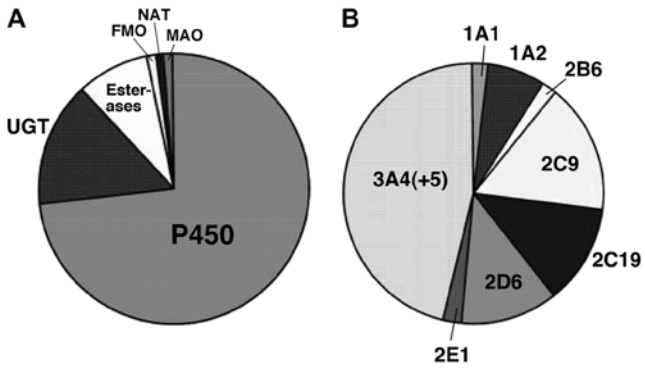

Figure 4. (A) Contribution of individual enzyme systems to metabolism of marketed drugs; (B) contribution of individual P450s in metabolism of drugs. UGT indicates uridine dinucleotide phosphate (UDP) glucuronosyl transferase; FMO, flavin-containing monooxygenase; NAT, *N*-acetyltransferase; MAO, monoamine oxidase; P450, cytochrome P450.[5,6]

The generalization that these conjugations and hydrolysis reactions are detoxicating is often seen in the literature, and some "chemoprevention" strategies are focused on induction of these enzymes.[9] But it should be remembered that many of these enzymes also catalyze the bioactivation of chemicals in many settings.[10] A few examples include the important role of epoxide hydrolase in the overall activation of the polycyclic hydrocarbon benzo[*a*]pyrene and 7,12-dimethylbenz[*a*]anthracene,[11,12] the conversion of the pesticide ethylene dibromide to a half-mustard/episulfonium ion by glutathione transferases,[13] and the modification of aryl hydroxylamines and benzylic alcohols by sulfonylation to generate good leaving groups and form reactive nitrenium ions and carbocations.[14]

Why Are Drugs Toxic?

The challenge of developing drugs without toxic side effects has been mentioned above. We will now consider the reasons for toxicity, because knowing these reasons can help pharmaceutical companies attempt to produce drugs that avoid these types of side effects. It is important to remember that almost all drugs will show some adverse reactions at very high doses, and accidental overdoses are generally considered a separate problem in drug development. In general, the US Food and Drug Administration (FDA) finds the occurrence of some kind of toxicity (in experimental animal models) at high doses useful, in that parameters are established for searches for end points of toxicity at typical doses.

Recently we suggested 5 contexts of drug toxicity (Table 2).[15] This classification is not absolute, and alternate systems could certainly be proposed.

On-target toxicity is based on the drug's mechanism of interaction with its intended target. An example is the statins. As a class, these drugs have been very safe, and almost all of the toxicities have been documented to result from inhibition of the enzyme target 3-hydroxymethyl glutaryl CoA reductase in muscle instead of liver.[16]

Table 2. Contexts of Drug Toxicity[15]

On-target toxicity
Hypersensitivity and immunological reactions
Off-target pharmacology
Bioactivation to reactive intermediates
Idiosyncratic drug reactions

Hypersensitivity and immunological reactions are associated with penicillins and other β-lactum antibiotics. The reaction of these compounds with proteins can lead to autoimmune responses, in which the body's immune system begins to respond to the compounds as foreign entities. This phenomenon is the basis of the "hapten" and "danger" hypotheses, in which enough of a load triggers adverse responses.[17,18]

Off-target pharmacology is a drug's interaction with a system other than that for which it was intended. One example is terfenadine, which can accumulate and block human ether-a-go-go (hERG) channels in addition to the target H_1 (histamine) receptor[19-21] (this example also involves metabolism, in that the accumulation of the parent drug terfenadine is associated with impaired metabolism to fexofenadine, which is the normal circulating product[22]).

The fourth context of toxicity involves bioactivation of drugs to reactive intermediates that bind covalently to macromolecules, a concept that has already been introduced. This aspect will be discussed in more detail later. The pathways relevant to bioactivation of acetaminophen, a classic example, are shown in Figure 5.

The fifth and final classification is idiosyncratic drug reactions. These are more sporadic (~$1/10^4$ cases) and often unrepeatable, *apparently* dose-independent, and very problematic in that prediction has been difficult and these problems do not appear until late clinical trials or after a drug enters the market. As their name implies, the nature of idiosyncratic toxicities is not understood. As more knowledge develops, many of these toxicities should fit into the other 4 contexts of drug toxicity. For example, the hepatitis seen with dihydralazine may involve bioactivation and immune components (Figure 6). Although idiosyncratic toxicities are often considered dose-independent, the data to support this view are very limited and the point has been correctly made that very few of these problems occur with drugs administered at daily doses of 20 mg or less.[17,24]

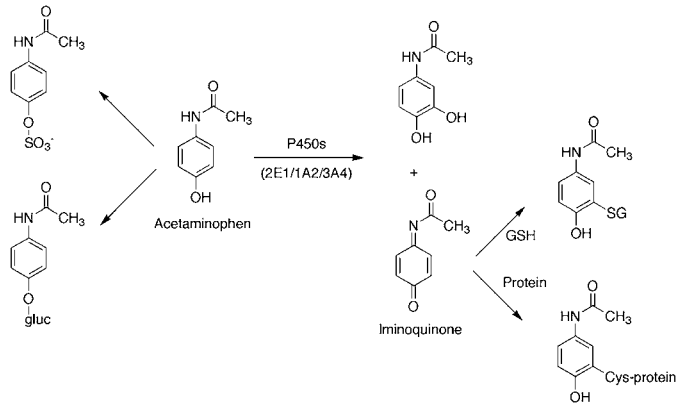

Figure 5. Metabolism of acetaminophen. P450 indicates cytochrome P450; GSH, glutathione.

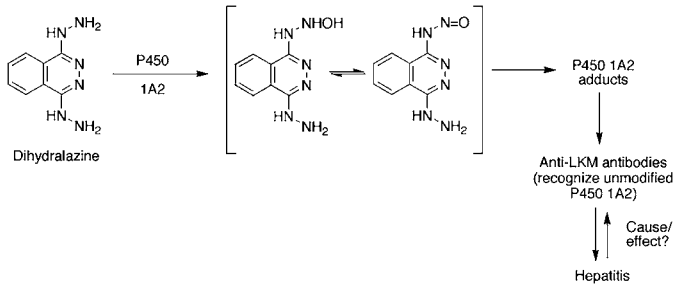

Figure 6. Activation of dihydralazine and possible relevance to hepatotoxicity. P450, cytochrome P450, LKM, liver kidney microsomal.[23]

BIOACTIVATION

Basic Principles

Several principles are involved in reactive species and toxicity.[15,25] The chemistry is reasonably well understood, although not completely. The basic reactions are governed by 2 chemical reactions: (1) the reaction of an electrophile (formed from the drug) and a nucleophile (in the tissue), and (2) free radical propagation. The initial reactive product formed (in a chemical *or* enzymatic reaction) may not be the one that undergoes reaction with the nucleophile. The stability and reactivity of the reactive species may vary considerably; even among epoxides,[7] the half-lives in water can range from 1 second to several hours for species known to be involved in biological damage. As a corollary, damage with some chemicals may be restricted to the cell in which activation occurs, but other chemicals may be distributed to other organs that are targets because of lack of repair and so forth.

In vitro systems are often good models for studying bioactivation and are critical for establishing basic mechanisms. The point should be made, however, that not all results will apply in vivo. The dose is an issue, and many times it is *the* issue in toxicity, an axiom going back 500 years to Paracelsus.[26] The extent of covalent binding of a drug or other chemical to tissue macromolecules can be used as a measure of potential toxicity,[27,28] but many exceptions exist. For instance, 3-hydroxyacetanilide is relatively nontoxic but yields as much covalent binding as 4-hydroxyacetanilide (acetaminophen).[29,30] The modified proteins differ for the 2 compounds,[31] but a satisfying mechanistic explanation for the differential toxicity is still not available. Other issues in toxicity are receptor-mediated and signaling events, cell proliferation, repair, and immune responses. A view of the complexity of events involved in cell toxicity is presented in Figure 7.

Oxidative Stress

The literature about oxidative stress is abundant and cannot be discussed in detail here. Clearly, oxidative stress is

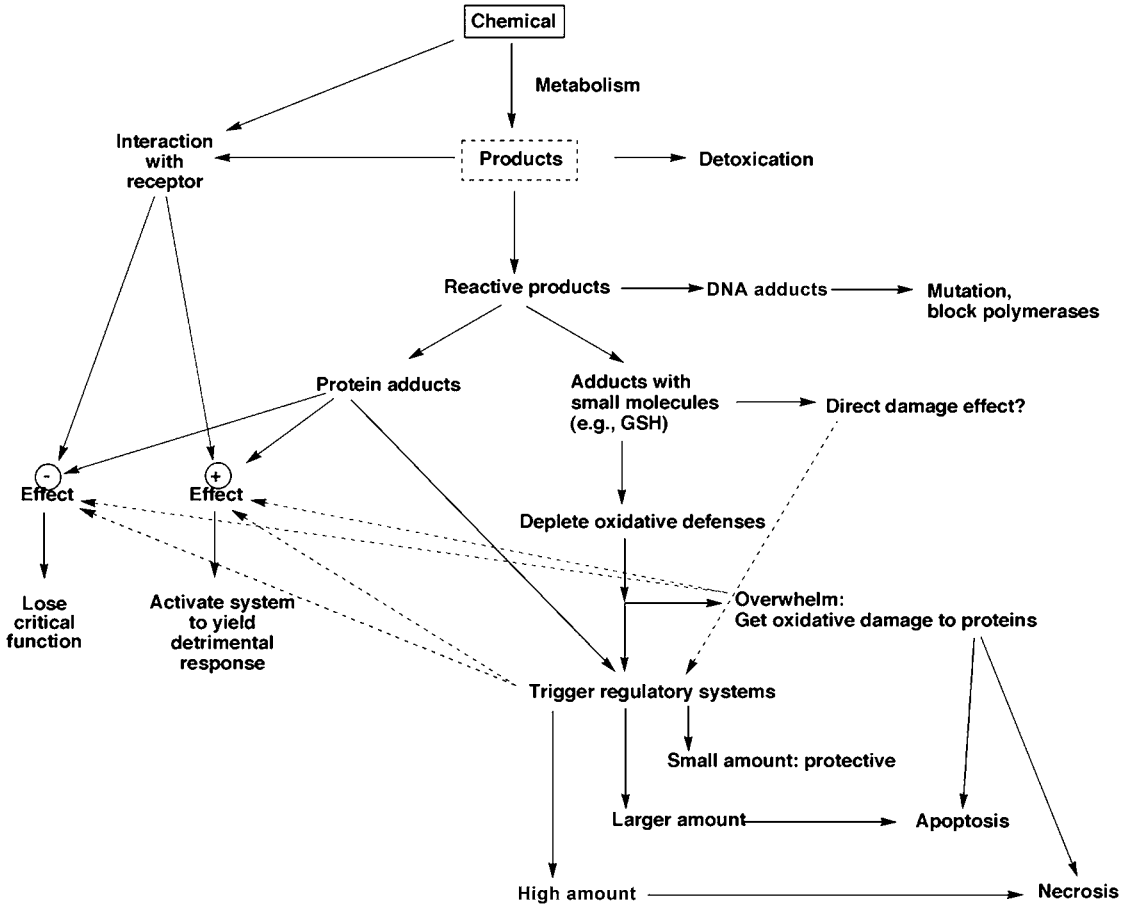

Figure 7. Biological events possibly relevant to chemical toxicity. GSH, glutathione.

a major issue with drugs capable of redox cycling (eg, daunorubicin). Beyond this, many investigators have proposed that oxidative stress is an important component of the toxicity caused by most chemicals that are activated to electrophiles. In the scheme shown in Figure 7, electrophiles also deplete reduced glutathione, one of the defenses against damage from reactive oxygen species such as hydroperoxides. Even more subtle changes in the redox balance of a cell may be critical, in that many transcription factors and other signaling systems are controlled by redox events.

The issue of whether P450s contribute to oxidative stress is complex. H_2O_2 and $O_2^{\bullet -}$ are formed in the uncoupled reactions catalyzed by microsomes and purified P450s. Induction and heterologous expression of mammalian P450s lead to oxidative damage in cell culture systems.[32,33] Enhanced lipid peroxidation is associated with some P450 substrates but prevented by others. A major question is what happens in vivo, or even in perfused organs. The rates of oxygen reduction are very fast in vitro, and the question can be raised as to whether these are realistic in vivo. Few studies have tried to link oxidative stress indicators with P450s in vivo, and some possibilities exist with biomarkers, NAPDH-NADPH P450 reductase-deficient transgenic mice,[34,35] and so on.

Examples of Bioactivation

Aflatoxin B_1

Aflatoxin B_1 (AFB_1), a hepatocarcinogenic mycotoxin, provides a *case* history of a reactive metabolite and its role in the context of enzymes and reactivity (Figure 8).[36] The critical product of AFB_1 is the *exo* epoxide. All other oxidation products formed by P450s, including the *endo* 8,9-epoxide, are at least partially detoxicated products. The *exo* epoxide has a $t_{1/2}$ of only 1 second in neutral aqueous buffer,[37] but the binding and intercalation in DNA are so efficient that a high yield of DNA adducts is obtained.[38] Thus, even with a $t_{1/2}$ of 1 second, the product is stable enough to migrate from the endoplasmic reticulum to the DNA in the nucleus.

The epoxide can also be enzymatically conjugated with glutathione (GSH) (Figure 8). The hydrolysis product (dihydrodiol) is unstable and undergoes base-catalyzed rearrangement to a reactive dialdehyde, which appears to be a major species involved in reaction with proteins (but

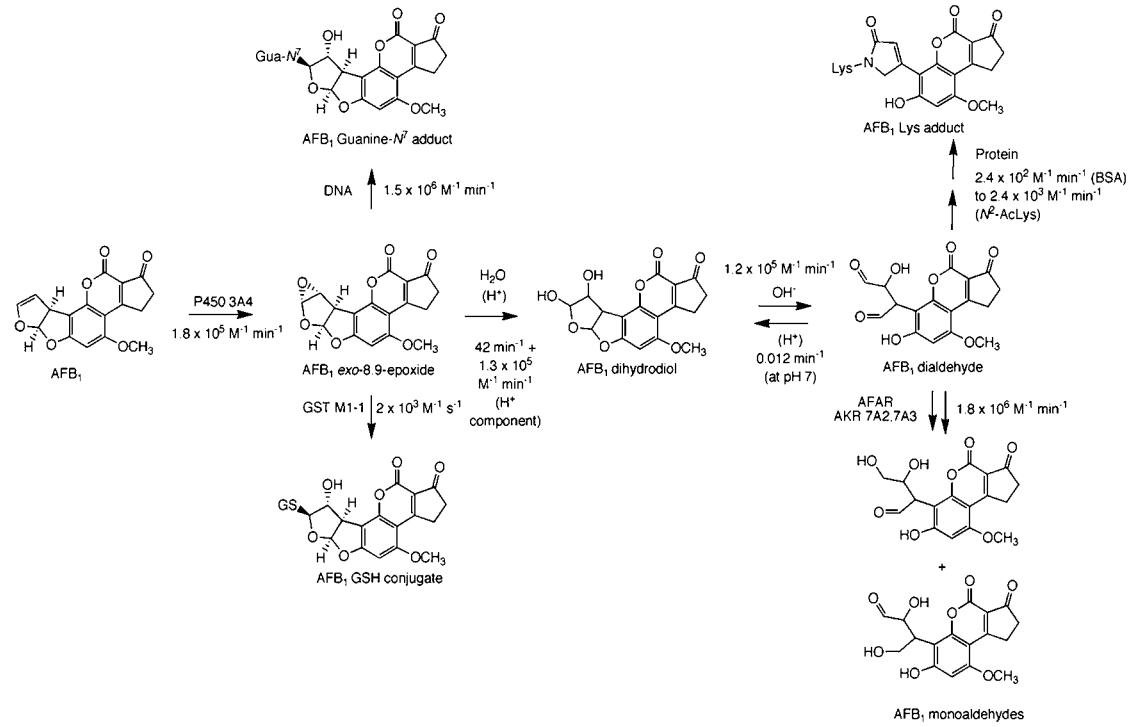

Figure 8. Major events in the metabolism of AFB_1.[36] The rates are either second-order rate constants for chemical steps or catalytic efficiencies (k_{cat}/K_m) measured for the major human enzyme involved in an enzymatic process. AFB_1 indicates aflatoxin B_1; BSA, bovine serum albumin; P450, cytochrome P450; GST, glutathione transferase; AFAR, aflatoxin aldehyde reductase; AKR, aldo-keto reductase; GSH, glutathione.

not DNA).[36] Another route of detoxication is reduction of the dialdehyde.[39]

Trichloroethylene

Although trichloroethylene (TCE) has been used as an anesthetic, its major use is as a solvent and degreasing agent. TCE is one of the chemicals most commonly found in water dump sites,[40,41] and major issues related to it are contamination of drinking water and several possible health effects.[42-44]

P450 oxidation of TCE yields chloral, a sedative, in a reaction that involves a 1,2-chloride migration[45] (Figure 9). A minor oxidation product is TCE oxide, which has a $t_{1/2}$ of 12 seconds in H_2O at neutral pH.[45] However, TCE oxide does not appear to react directly with proteins, at least not to yield stable adducts. A rearrangement gives glyoxal chloride, which can react with protein lysines via either of 2 pathways (*a*, *b*) to produce the observed 1- and 2-carbon conjugates. Dichloroacetyl chloride also reacts to form a third product.[46] A similar mechanism has been described for tribromoethylene.[47]

Historically, most covalent binding assays have used radioactivity and relatively slow methods. With the advent of mass spectrometry, it became possible to initiate a reaction of a reactive electrophile with a (small) protein and analyze the binding with minutes. An example with TCE oxide and the oligopeptide adrenocorticotrophic hormone of the pituitary gland (ACTH) is shown in Figure 10. With knowledge of the types of adducts formed (Figure 9) and the assumption that the responses of the modified forms of ACTH are similar in electrospray mass spectrometry, the amount of covalent binding can be estimated after integration of the peaks (Figure 10B).

Another interesting aspect of this work with TCE oxide was that brief treatment of the modified ACTH with mild base (pH 12) removed a large fraction of the adducts (Figure 10C). Adducts were also lost more slowly ($t_{1/2}$ ~1 hour) at neutral pH.[48] These results are interpreted to mean that many of the adducts are esters, not lysine amides. Studies with model enzymes having sensitive serine, tyrosine, and cysteine groups showed a recovery of catalytic activity ($t_{1/2}$) following partial inactivation with TCE oxide or model acylating agents.[48] These results indicate that proteins can be modified transiently. Obviously, such binding would have been missed with older methods. The results can be interpreted in 2 ways. One view is that some protein binding is not so serious as thought, in that function can be restored with time. An opposite view is that the levels of adducts estimated with many methods using slower assays may underestimate the true extent of protein modification, and that transient periods of high modification may provide a window for biological expression of damage.

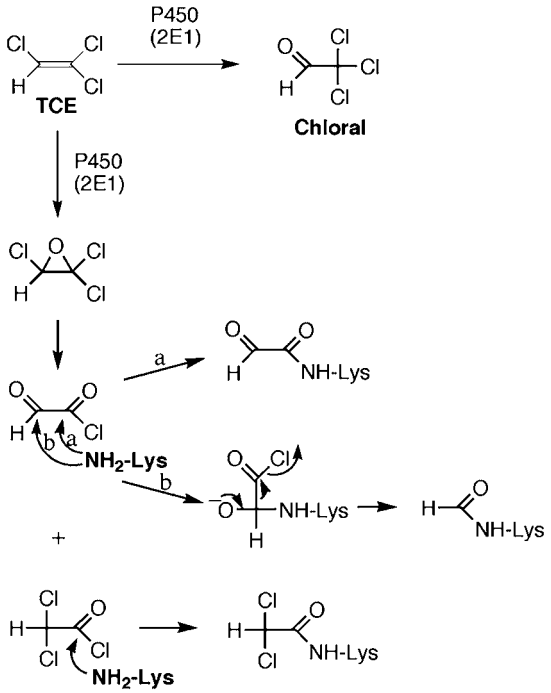

Figure 9. Oxidation of TCE and reaction with protein lysine groups. TCE oxide rearranges to TCE oxide, which either hydrolyzes, undergoes C-C bond splitting, or reacts to form the products shown. The mechanisms were established by ^{13}C labeling.[46] TCE indicates trichloroethylene; P450, cytochrome P450.

Finally, a study with perchloroethylene (tetrachloroethylene) oxide indicated that it has chemical mechanisms similar to those identified for TCE.[49] The study also found that oxalyl chloride, the rearrangement product derived from perchloroethylene oxide, reacts not only with proteins but also with phosphate. The product, oxalyl phosphate, is relatively stable and can be isolated ($t_{1/2}$ ~100 minutes); it does not react with proteins.[49] These results are of interest given the large number of bioactivation studies that have been done using high concentrations of phosphate buffer (eg, 100 mM).

Considerations of Variability

Species Variations

One inherent problem in all toxicology, including safety assessment in the pharmaceutical industry, is that experiments must be done in animal systems and extrapolated to humans. The choice of the most appropriate animal species is critical, and even in the best of circumstances the extrapolations are not trivial.

One example of the problems in extrapolation—from a system that is relatively well defined—is presented here.[50] Many heterocyclic amines, formed in meat during pyrolysis, are activated by P450 1A2-catalyzed *N*-hydroxylation, including 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) and2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline(MeIQx). The main enzyme involved in the (hepatic) activation in rats and humans is P450 1A2, although the point should be made that these 2 proteins are 75% identical. While rat and human P450 1A2 have nearly identical catalytic activities with the model substrate 7-methoxyresorufin, human P450 1A2 has ≥10-fold higher catalytic efficiency than rat P450 1A2 in activating both PhIP and MeIQx.[50] The level of P450 1A2 varies considerably among humans. When the genotoxicity of MeIQx was assayed in various liver microsomal samples, some humans were found to have much higher bioactivation than did rats in which P450 1A2 had been induced with 3-methylcholanthrene or polychlorinated biphenyls (Figure 11). These results are of interest because the rat cancer bioassays were done with "untreated" rats and used for risk assessment extrapolation to humans.

One way to address differences between species is to use pharmacokinetic modeling. An older approach involves allometric scaling, based on body size. However, critical pathways involved in bioactivation and detoxication show little relationship to body weight. A more popular approach involves physiologically based pharmacokinetic (PBPK) models. The basis of the approach is that most of the physical aspects of distribution can be established in different species (eg, blood flow, tissue extraction). What is important is the identification of the most critical pathways involved in bioactivation and detoxication and their characterization in terms of quantitative parameters, generally in in vitro settings. With this knowledge, appropriate predictions can be made for humans. An example of efforts from our laboratory, collaborating with Dow Chemical Co, involves the conversion of CH_2Cl_2 to a genotoxic species by glutathione transferase.[51] In this work, the conjugation rates in humans were found to be similar to those in rats and hamsters, and much slower than those for mice.[51] Of the animal species, tumors had been found in only mice, and the conclusion was that humans are at lower risk than previously assumed.

Interindividual Variations

The variation of levels of individual human P450s has been already mentioned (Figure 2), along with the relevance to in vivo metabolism (Figure 3). Variability among humans is also seen in the MeIQx genotoxicity assays presented in Figure 11.

The PBPK modeling approach has already been mentioned for extrapolation from animals to humans. In early PBPK work the general concept was to extrapolate from animal models to an "average" human to properly evaluate risk. However, the concept of an average human is not very satisfying, as evidenced by Figures 2 and 11. An alternate approach to PBPK is to use in vitro (or possibly in vivo, if assays can be done) human parameters to estimate a range of expected data for humans in vivo.[52,53]

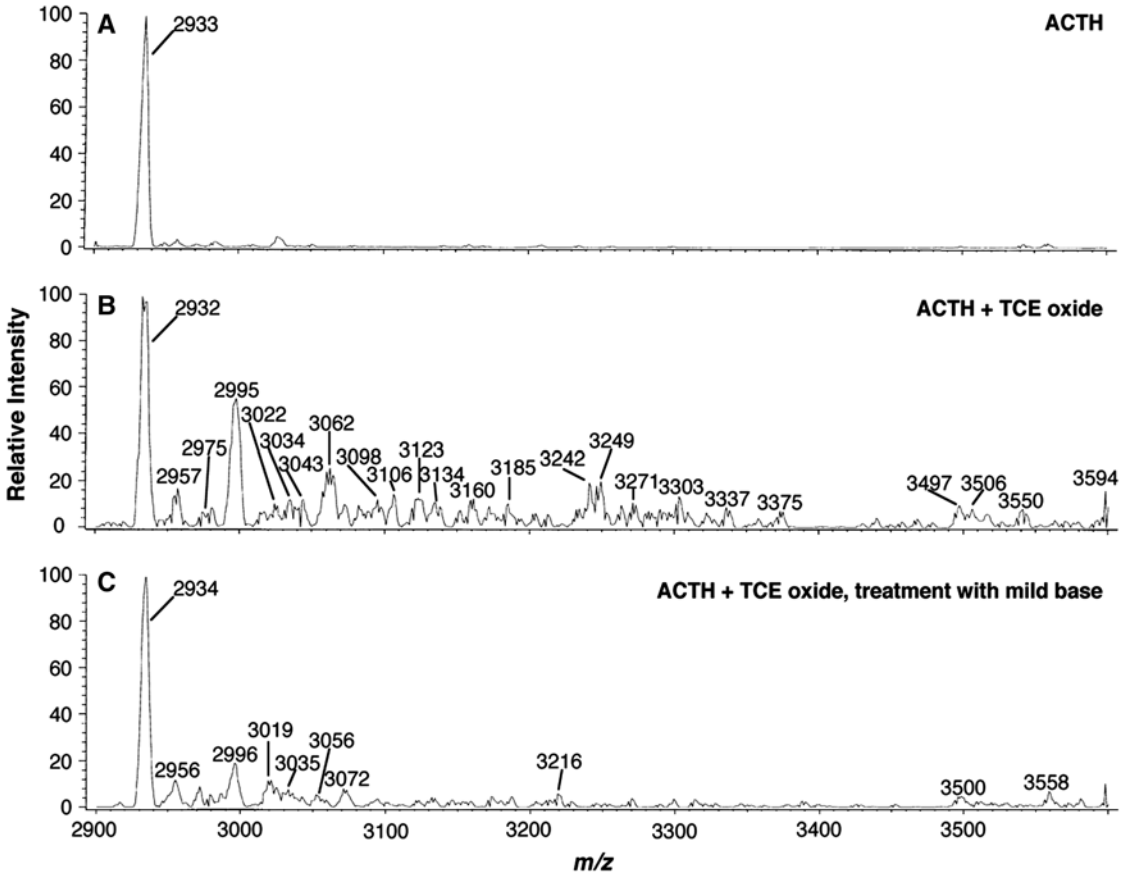

Figure 10. Mass spectra (LC-positive ion electrospray) of the reaction of ACTH with TCE oxide. (A) ACTH only; (B) reaction of ACTH with 4 mM TCE oxide, assayed directly after reaction; (C) reaction mixture from part B adjusted to pH 12 for 5 minutes and then neutralized prior to mass spectrometry. LC indicates liquid chromatography; ACTH, adrenocorticotrophic hormone of the pituitary gland; TCE, trichloroethylene.[48]

Another approach is to estimate the expected variation in a human population with an "uncertainty factor" for in vivo parameters related to individual enzymes. Examples have been published for P450 2D6 and 3A4.[54,55] Interestingly, the uncertainty factors are much higher in children than in adults (~4-fold). Also, the uncertainty with the P450 2D6 poor metabolizer phenotypic group was much higher than that for the extensive metabolizer group. The uncertainty factor for the clearance of an individual drug was found to increase exponentially as a function of the fraction of metabolism attributable to a single P450 enzyme.[55] For this reason, a common strategy in the pharmaceutical industry is to try to select drugs in which several P450s (or other enzymes) "share" the metabolism of the compound, to avoid problems in individuals who might be deficient in one of the enzymes.

How Important Are Metabolism Issues in Drug Toxicity?

Reactive Metabolites

Much has been written about the significance of reactive metabolites and covalent binding in toxicity. But what fraction of human drug toxicity problems are attributable to this phenomenon? A deficiency in any analysis is that we do not have demonstrated causal relationships; that is, the evidence is still very correlative because many events are happening, in addition to covalent binding (of several proteins) (Figure 7).

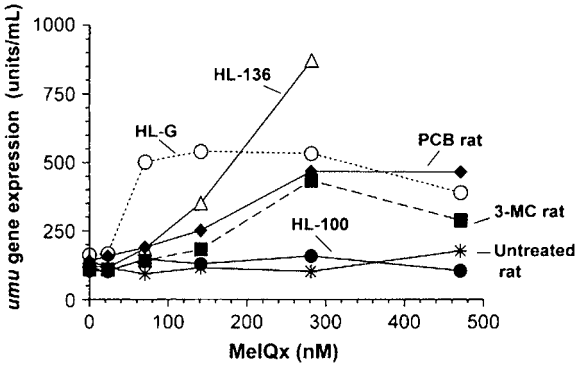

Figure 11. Genotoxicity of MeIQx in the *Salmonella typhimurium umu* test with liver microsomes (20 µg protein/mL) prepared from either untreated rats; rats treated with 3-MC or PCB; or HL-100, HL-136, or HL-G. MeIQx indicates 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; MC, methylcholanthrene; PCB, polychlorinated biphenyl mixture Aroclor 1254; HL, human livers.[50]

One recent analysis by a Pfizer group is of interest.[56] Of a set of 6 drugs withdrawn from the market (in the time frame examined), 5 (benoxaprofen, iproniazid, nefazodone, tienilic acid, and troglitazone) are known to have reactive products. Of another 15 drugs with "black box" warnings, 8 (dacarbazine, dantrolene, felbamate, flutamide, isoniazid, ketoconazole, tolcapone, and valproic acid) have reactive metabolites. Thus, 62% of the problem drugs in this group involve metabolism and reactive products.

Is 62% realistic? Perhaps. However, a caveat is that this analysis was based on drugs that reached the market. The fraction may be much lower if one considers the drug candidates that fail before going into or through clinical trials and safety assessment. Exactly what the fraction may be is impossible to estimate accurately. One issue is that most of the data are proprietary, and any analyses that reach the open literature would probably be based on experience within a single company. A large fraction of failures due to toxicity problems may be attributable to (uncharacterized) off-target pharmacology (Table 2) or even to previously unrecognized detrimental effects of downregulation of the main target itself (on-target, Table 2). The problem in analysis is that in most cases mechanisms are not determined; leads are simply scrapped, and there is no incentive to establish a mechanism, at least in the short term.

To return to the issue, there is another list of drugs with reactive products that have warnings for hepatoxicity: acetaminophen (Figure 5), carbamazepine, clozapine, diclofenac, disulfiram, halothane, leflunomide, methyldopa, rifampin, tacrine, tamoxifen, terbinafine, ticlopidine, and zileuton.[56] Another list in the same reference includes drugs with reactive metabolites that were used in other countries but never approved in the United States: alpidem, amineptine, amodiaquine, cinchophen, dihydralazine (Figure 6), dilevalol, ebrotidine, glafenine, ibufenac, isoxicam, niperotidine, perhexiline, pirprofen, and tilbroquinol.

The point has been made several times that the drugs associated with apparently idiosyncratic reactions are *not* really dose-independent. The pattern is also consistent with the hypothesis that activation to reactive products causes protein damage (and also oxidative stress), which has subsequent biological effects, and that reducing this level of covalent damage is an effective approach to avoiding toxicity. The means to this end are changing the chemistry of the molecule either to increase the biological efficacy (thus reducing the dose needed) or to block the enzyme activation.

Stable Metabolites

Thus far the discussion has centered on reactive metabolites and their potential for causing toxicity. However, the FDA has recently expressed concerns about stable metabolites, particularly those that are unique to humans (compared with test animals).[57] The FDA has proposed that unique human metabolites of drugs be tested to the same extent as the drug itself.

Stable metabolites are distinguished from reactive metabolites in several ways. Stable metabolites can be isolated and characterized. They can also be synthesized, at least in principle (although the cost may be much higher than that of the parent drug). With reactive metabolites, the chemical structures can only be inferred from trapped products. Even if reactive metabolites can be synthesized, they are generally stable in only aprotic solvents and cannot usually be administered to animals. One issue with the FDA draft is that the 4 examples provided are all reactive metabolites, although the purpose was to deal with stable metabolites. Another issue is that the draft is based on the fraction of a parent drug converted to unique metabolites and not the total amount, the area under the curve, and so on.

One can raise the issue of how hazardous stable metabolites really are. Most metabolites have attenuated biological effects toward the same target as the parent drug, with a few (prodrugs) showing enhanced activity after metabolism. However, there are few good examples of drugs being transformed to products with totally different pharmacology. Some examples are outside the realm of drugs, for instance, conversion of the solvent TCE (and an anesthetic) to the sedative chloral (Figure 9) and oxidation of the pesticide methoxychlor to an estrogen.[58] Although such transformations to products with unique biological activity are conceivable, these events will be rare among drugs, according to current information. Another point is that administration of a metabolite to an animal, even if the metabolite is stable, may not yield tissue distributions relevant to the parent drug.

CONCLUSIONS

Predicting toxicity is an important issue in the development of pharmaceuticals—as it is with carcinogens, environmental pollutants, and drugs of abuse. New findings in biology highlight the complexity of the mechanisms by which chemicals exert toxic effects. Many chemicals are inert unless converted to metabolites by P450s or other enzymes. Reactive electrophiles produced in this way are clearly important in toxicity, as demonstrated by the example of acetaminophen. However, exactly what fraction of drug toxicities are the result of this process is unclear. Stable metabolites could hypothetically be involved in drug toxicity, but few examples of this have been documented.

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