Review

Mechanisms of Drug Toxicity and Relevance to Pharmaceutical Development

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Summary: Toxicity has been estimated to be responsible for the attrition of approximately one-third of drug candidates and is a major contributor to the high cost of drug development, particularly when not recognized until late in clinical trials or post-marketing. The causes of drug toxicity can be classified in several ways and include mechanism-based (on-target) toxicity, immune hypersensitivity, off-target toxicity, and bioactivation/covalent modification. In addition, idiosyncratic responses are rare but can be one of the most problematic issues; several hypotheses for these have been advanced. Although covalent binding of drugs to proteins was described almost 40 years ago, the significance to toxicity has been difficult to establish; recent literature in this field is considered. The development of more useful biomarkers and short-term assays for rapid screening of drug toxicity early in the drug discovery/development process is a major goal, and some progress has been made using "omics" approaches.

Keywords: drugs; toxicity; mechanisms; idiosyncratic responses; covalent binding; short-term assays

Introduction

The cost of pharmaceutical development has been increasing for many years, and the estimated average cost of developing a profitable drug has been estimated at more than US$1.7 billion. However, the number of new drugs approved per year is relatively uniform. Some reasons for the problem include the difficulties of target validation—in approaching increasingly complex disease areas—and rising regulatory barriers.

It has been estimated that an average of between 10,000 and 25,000 individual chemicals are considered in the course of development of a new drug. What are the major reasons for attrition of lead compounds? A major issue 25 years ago was poor pharmacokinetics in humans. This aspect has been addressed through advances in the understanding of human cytochrome P450 (P450) and other enzymes, knowledge about transporters, and the development of predictive in vitro assays. However, as metabolic issues have been reduced, toxicity issues have increased (Fig. 1). Together, pre-clinical toxicity (animal) and adverse events (human toxicity) account for approximately one-third of the cases of attrition. If one excludes “non-scientific” issues (e.g., commercial, financial) then the fraction is even higher.

The real issue is the expenditure of resources (time and money) on compounds that have toxicity issues and ultimately have to be dropped from development. Toxicity and safety assessment are done at many steps in the drug discovery/development pathway (Fig. 2). If compounds with toxicity issues are not dropped until very late in the process, then the loss may run into hundreds of millions of dollars and years of research. Thus, early decisions are very important in drug development, and the initial decisions must be accurate. In this review, toxicity issues mostly relevant to drugs will be covered.

Contexts of Drug Toxicity

All compounds are toxic at high doses and all are safe at very low doses, using the axiom of Paracelsus. What we are considering here are not accidental drug overdoses but toxicity and adverse events at doses that are relevant to patients using a medicine. The context of toxicity will affect how one approaches the matter of circumventing toxicity or developing alternate compounds that will not have this liability. The most commonly encountered problems are with cardiovascular and hepatic toxicity (Table 1).

Several classifications are possible; presented here is a systematic one previously described (Table 2). Others...
have presented alternate but similar classifications. The first context of toxicity is on-target (or mechanism-based) toxicity, i.e., toxicity due to interaction of the drug with the same target that produces the desired pharmacological response. The concept is not one of competitive inhibition but rather that the biological response that the drug exhibits upon binding to its target produces both efficacious and toxic effects. In principle this type of toxicity is difficult to deal with because all classes of compounds developed to treat the disease will show this toxicity. Changing the target for the disease may be necessary. However, another strategy is exemplified in the case of statins. All statins produce hypercholesterolemic effects by inhibiting 3-hydroxy-3-methyl-glutaryl CoA (HMG CoA) reductase in the liver, i.e., the target. The adverse effects of statins are also due to inhibition of HMG CoA reductase, but in muscle and possibly other tissues, i.e., geranylgeranylation.
of proteins is inhibited. Fortunately, the distribution of statins between tissues can be modulated by various transport proteins, and although on-target toxicity is an issue, it can be controlled by inter-tissue distribution.

The second context of drug toxicity is **hypersensitivity** and **immune responses**. For instance, allergic reactions to penicillins have been recognized for many years. The concept, developed largely on the basis of the pioneering work of Landsteiner, is that drugs (or their metabolites) react with proteins in the body (as haptens) to induce antibodies and immune responses. In this example (penicillins), the drug is not completely stable and has the potential to bind covalently to proteins and initiate antibody production.

The third context of drug toxicity is **off-target** toxicity. The issue here is that the drug is not specific in its interactions; binding to an alternate target is the cause of toxicity. With our current knowledge of the complexity of biological regulatory pathways and multi-gene families (e.g., protein kinases), it is not surprising that a drug might not be totally specific. The example in Table 2 is terfenadine, which binds not only to the H1 receptor (eliciting the desired antihistaminic response) but also to the hERG channel, thus causing arrhythmias. In principle, this liability can be addressed by more screening and development of drug candidates with lower IC50 and Kd values, because a lower dose might avoid the specificity issue.

The fourth context of drug toxicity is **bioactivation**. Many drugs are converted to reactive products (often termed “reactive” “metabolites”). These entities modify the proteins they react with and somehow cause toxicity, although the mechanisms have been evasive (vide infra). One theory is that important regulatory or other proteins are modified, with loss of function. Another possibility is that the modified proteins induce immune responses, linking with the second context of toxicity. An analysis of drugs at one company, Bristol-Myers Squibb, indicated that “metabolism” was an issue in 28% of cases in which drug candidates had been dropped from development (Table 3).

The fifth context of toxicity is **idiosyncratic** reactions. Idiosyncratic means “individual,” and these are rare events (1/10^4 to 1/10^6 individuals) which are not well understood. Such responses are highly problematic in that few (if any) animal models are very predictive. The low incidence makes such adverse events difficult to find even in large clinical trials. However, with widely used drugs for which millions of prescriptions may be written, even an incidence of 1/10^4 can yield hundreds of problems.

The context of toxicity has bearing on how difficult it is to predict safety problems (Fig. 3).

![Evaluation of hepatotoxicity potential](image)

**Fig. 3. Hypothetical relationship between the inherent toxicity of drugs and the variability of the response among hosts (e.g., test animals, humans)**

The dose is not a consideration in this treatment, adapted from Zimmerman. At toxic doses, the most readily understood compounds are those with high toxicity in all animal species. Variation among species introduces more uncertainty in extrapolation to humans. Predictions can be made if the issue is metabolism, but idiosyncratic problems are very difficult to understand with animal models.

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**Table 3. Mechanistic causes of toxicology attrition**

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>% of all advanced molecules'</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotransformation-related</td>
<td>27</td>
</tr>
<tr>
<td>Target-based</td>
<td>28</td>
</tr>
<tr>
<td>Single or multiple ion channel inhibition</td>
<td>18</td>
</tr>
<tr>
<td>Immune-mediated</td>
<td>7</td>
</tr>
<tr>
<td>All other mechanisms</td>
<td>36</td>
</tr>
</tbody>
</table>

'n = 88. Because categories are partially overlapping, the total is >100%.

Based on experience from DuPont-Merck and Bristol-Myers Squibb, 1993–2006. Information kindly provided by B. D. Car, Bristol-Myers Squibb.
In two polymorphisms at the 1% level would be multiplied to transferase or other enzyme to detoxicate the product. Thus, the danger hypothesis introduced. The concept is that some individuals will show metabolic reactions. Theories regarding mechanisms of idiosyncratic reactions

This topic has been reviewed by others (Table 4). At least five theories have been proposed to explain idiosyncratic reactions, and these may not be mutually exclusive in considering all drugs for which idiosyncrasies have been reported (Table 5).

The first theory involves polymorphisms or rare alleles of metabolism enzymes. (The term polymorphism may not be applicable in that this is generally reserved for incidences of >1–2%; otherwise the term “rare alleles” applies.) The concept is that the sensitivity is due to lack of (or too much) metabolism of a drug, including a lack of detoxication. For instance, an individual might be in the approximately 1% of the (Caucasian) population with a high propensity to activate a drug (e.g., ultra-rapid metabolizers in the P450 2D6 group) and also be deficient in a glutathione (GSH) transferase or other enzyme to detoxicate the product. Thus, two polymorphisms at the 1% level would be multiplied to yield an incidence of 1/10^4. This scenario is possible, but no solid examples exist to explain any observed idiosyncratic reactions.

The second is the hapten theory, which has already been introduced. The concept is that some individuals will show more activation of a drug to yield a hapten, and also the variations in the immune systems of individuals will dictate that only a few will show this response. There is some support for this theory in the cases of tienilic acid and hydralazine, which are activated by human P450s and bound covalently to P450s, generate anti-P450 antibodies in humans, and cause hepatitis. Unfortunately, it has not been possible to determine whether these events are causal for the hepatotoxicity or are simply all happening at the same time but are unrelated.

The third theory is sometimes referred to as the inflammannagen model. The concept is that bioactivation and other events occur in many people and that inflammation (or other predisposing episodes) render only some individuals more sensitive. It is possible to demonstrate this phenomenon in rats treated with lipopolysaccharide to cause oxidative stress. However, exactly how representative this is as a model for human idiosyncrasies is subject to debate.

The fourth theory is the danger hypothesis. Here, injured tissue produces danger signals (e.g., lipid oxidation products, cytokines) that evoke a toxic response, not the drug or its metabolites. As Uettrecht has pointed out, this hypothesis is not mutually exclusive from the hapten theory (if an immunological response is involved), although there is no hard evidence that this is a mechanism in clinical drug idiosyncrasies.

The fifth theory is the pharmacological intervention model. In this model, drugs elicit immunological responses by reversible binding to proteins, i.e., without covalent binding. One of the bases for this proposal was sulfamethoxazole, an arylamine prone to forming reactive metabolites. There is some evidence that ximelagatran, a peptide-like substance, might act in this manner.

Where does covalent binding fit in?

An overall scheme of drug toxicity includes many aspects, some of which are related to metabolism (Fig. 4). Covalent binding of drugs to proteins has been with us at least since 1973, with the classic papers of Gillette and Brodie on acetonophen. Even before then, the covalent binding of carcinogens to proteins had been demonstrated by the Millers. However, two important questions remain unanswered. One is how important this process really is for drug toxicity, in that the evidence remains highly correlational. The other question is, if covalent binding to proteins causes toxicity, what exactly is the mechanism (or does a general mechanism even exist)?

One of the major areas in which covalent binding has been studied is hepatotoxicity, which is both a pre-clinical and clinical issue (Tables 3, 6). In a seminal review, Walgren et al. listed 14 drugs that have been withdrawn from the market due to hepatotoxicity (Table 7). Of these, nine (64%) have been shown to be activated to reactive products. Another list of drugs includes those that have been withdrawn in other countries due to hepatotoxicity (Table 8). Of the 11 tested for generation of reactive products, all 11 (100%) were positive. Finally, a third list of 14 marketed drugs with

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**Table 4. Intrinsic vs. idiosyncratic hepatotoxicity**

<table>
<thead>
<tr>
<th>Intrinsic</th>
<th>Idiosyncratic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predictable (?)</td>
<td>Unpredictable</td>
</tr>
<tr>
<td>Relatively common occurrence</td>
<td>Rare occurrence (&lt;1/10^4)</td>
</tr>
<tr>
<td>Detected pre-clinically</td>
<td>Not detected until post-launch</td>
</tr>
<tr>
<td>Dose dependent</td>
<td>Occurs at any dose (?)</td>
</tr>
<tr>
<td>Acute or sub-acute onset</td>
<td>Delayed onset</td>
</tr>
<tr>
<td>No immune component</td>
<td>Immune/metabolic component (usually see fever, rash, eosinophilia)</td>
</tr>
<tr>
<td>Animal models useful</td>
<td>Few (any?) animal models available</td>
</tr>
<tr>
<td>Example: acetaminophen</td>
<td>Examples: isoniazid, halothane</td>
</tr>
</tbody>
</table>

**Table 5. Idiosyncratic toxicity: proposed mechanisms**

- Metabolic
  - [Rare allele] x [rare allele] = unusual metabolism
- Hapten hypothesis
  - Reactive metabolites act as haptens → immunological response
- Inflammannagen model
  - Inflammation or other predisposing episodes render some individuals more sensitive
- Danger hypothesis
  - Injured tissue → danger signals → toxicological response
- Pharmacological intervention
  - Drugs → immunological response by reversible binding (not electrophiles)

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"black box" warnings for hepatotoxicity includes ten (71%) that showed reactive metabolites (out of all 14 examined) (Table 9).

Collectively, two conclusions can be reached from examining these retrospective studies. One is that a large fraction of problematic drugs produce reactive products, which may be an issue. However, there are caveats, e.g., such a relationship is not necessarily causal, and the binding of non-toxic drugs was not compared here. The other conclusion that can be drawn from the review of Walgren et al. is that all of the drugs producing idiosyncratic hepatotoxicity were used at high doses. Uetrecht has made the point that idiosyncratic problems are seldom seen with drugs used at doses of ≤10 mg/day. (Some drugs certainly can be hepatotoxic at lower doses, e.g., cerivastatin at submilligram per day doses, but in this case the situation is explained by the high potency, on-target toxicity, and some cytochrome P450 polymorphisms.) The tendency for more hepatotoxicity with higher-dose drugs may be consistent with the view that a low-dose drug, even if extensively bioactivated to reactive products, might produce damage that would not exceed the usual threshold of protective systems in the body.
Table 8. Drugs withdrawn because of hepatotoxicity (non-U.S.)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Date</th>
<th>Dose (mg/day)</th>
<th>Reactive products</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpidem</td>
<td>1993 (France)</td>
<td>25–150</td>
<td>Yes</td>
<td>43</td>
</tr>
<tr>
<td>Aminopropine</td>
<td>1999 (France, Thailand)</td>
<td>200</td>
<td>Yes</td>
<td>44</td>
</tr>
<tr>
<td>Bendazac</td>
<td>1993 (Spain)</td>
<td>500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzarone</td>
<td>1992 (Greece)</td>
<td>300</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Benzodiazepine</td>
<td>1964 (U.K.)</td>
<td>300</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clomazone</td>
<td>1982 (U.K.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clometacin</td>
<td>1987 (France)</td>
<td>450</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclofenil</td>
<td>1987 (France)</td>
<td>200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dilevalol</td>
<td>1990 (U.K.)</td>
<td>200–400</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ebrotileno</td>
<td>1998 (Spain)</td>
<td>400–800</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exophone</td>
<td>1989 (France)</td>
<td>1200</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Piperoxide</td>
<td>1991 (France, Greece)</td>
<td>600</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Glafenine</td>
<td>1984 (France, Greece)</td>
<td>400</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>1968 (U.K.)</td>
<td>2400</td>
<td>Yes</td>
<td>45</td>
</tr>
<tr>
<td>Isonoxime</td>
<td>1984 (France)</td>
<td>1500</td>
<td>Yes</td>
<td>46</td>
</tr>
<tr>
<td>Mebanazine</td>
<td>1975 (U.K.)</td>
<td>30</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Moxisylyte</td>
<td>1993 (France)</td>
<td>480</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nipertidino</td>
<td>1995 (Italy)</td>
<td>230–460</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nitrofzone</td>
<td>1984 (Greece)</td>
<td>1200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perhexilene</td>
<td>1985 (U.K.)</td>
<td>300</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenoxypropazine</td>
<td>1966 (U.K.)</td>
<td>10–20</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Piperoxide</td>
<td>1990 (Europ. Union)</td>
<td>800</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Sulindac</td>
<td>1985 (Spain)</td>
<td>600</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tolrestat</td>
<td>1996 (Europ. Union)</td>
<td>400</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xenacetic acid</td>
<td>1965 (France)</td>
<td>Unknown</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ximelagatran</td>
<td>2006 (Europ. Union)</td>
<td>48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zimelidine</td>
<td>1985 (U.K.)</td>
<td>100–300</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

11/11 = 100%

Recently, several studies have made careful comparisons of the extent of covalent binding of drugs (in animals) and correlated these with hepatotoxicity. Masubuchi et al.\(^\text{57}\) showed good correlation between rat and human liver microsomes with a series of drugs. Reasonably good in \textit{in vitro} / in \textit{vivo} correlations were observed\(^\text{57}\) (Fig. 5). In other studies, the degree of covalent binding was higher for hepatotoxic drugs than non-hepatotoxic drugs (Figs. 6–8). Nevertheless, the variation in covalent binding was considerable for both the hepatotoxic and non-hepatotoxic drugs, and the two sets showed considerable overlap.

Thus, the proposals of Evans et al.\(^\text{61}\) to utilize \textit{in vitro} and \textit{in vivo} data in making decisions about advancing drugs have support from these studies. Another question that one can ask is what fraction of the attrition of drug candidates is related to the five individual contexts of drug toxicity described earlier (Table 2). A definitive answer is not easy, because it would require information about proprietary compounds (and many compounds are probably not followed up after attrition). However, one estimate has been that 27% of (preclinical) candidates were dropped due to biotransformation-related issues and 28% due to on-target problems (from experience at DuPont-Merck and Bristol-Myers Squibb) (Table 3).\(^\text{28}\)

**Biological Mechanisms: Mitochondrial Toxicity**

One of the classic cases of utilizing structure-function relationships in understanding the toxicity of drugs involves the comparison of acetaminophen with its meta-isomer, 3-hydroxyacetanilide. Both compounds yield similar levels of total covalent binding, both \textit{in vitro} and \textit{in vivo}.\(^\text{62,63}\) However, different reactive intermediates are produced from acetaminophen and the meta-isomer, an iminoquinone and an ortho-quinone, respectively. Further careful analysis established that acetaminophen generated more mitochondrial binding and the meta-congener more cytosolic binding, apparently related to the stability of the reactive Michael acceptors produced in the two cases.\(^\text{64}\)

Mitochondrial stress has since developed in terms of being a major aspect of drug toxicity.\(^\text{65}\) Some of the evidence suggests a combination of the drug (or drug metabolite) promoting oxidative stress (a "direct" effect) and alteration of signal transduction systems resulting in further loss of mitochondrial function (an "indirect" effect).\(^\text{65}\) Oxidative stress can be defined as an imbalance of pro-oxidants and anti-oxidants in a cell or a cellular compartment. An example has been offered with acetaminophen, i.e., the reactive iminoquinone product reacts with mitochondrial proteins\(^\text{64}\) and produces mitochondrial injury and reactive oxygen species, and the latter, in turn, activate cytoplasmic signal transduction pathways. Thioredoxin is one of the
proteins oxidized by the reactive oxygen species; it then dissociates from ASK-1 and leads to ASK-1 activation and the phosphorylation and activation of KMM 4/7, which in turn phosphorylates and activates JNK. GSK-3β activity is also enhanced, and JNK and GSK-3β translocate to mitochondria and promote cell death, in part by binding to voltage-dependent anion channels and thus altering the mitochondrial permeability transition.65

Thus, acetaminophen hepatotoxicity can be considered an active process, involving specific signaling molecules and net up-regulation of activity, in contrast to the older concepts of massive inactivation of cellular proteins by reactive metabolites. In addition, recent work has demonstrated that the fraction of cytochrome P450 2E1 localized in the mitochondria is much more uncoupled than the fraction in the endoplasmic reticulum and generates more reactive oxygen species, as judged by both dye and isoprostane measurements.66

In part because of the above-mentioned role of oxidative stress, animal models with compromised anti-oxidant capacity have been utilized in efforts to gain insight into drug-induced liver injury and, by extension, insight into idiosyncratic hepatotoxicity.65,67 Heterozygous sod2 mice (missing one superoxide dismutase 2 allele) have been used and shown to be more sensitive to a number of drugs, with an initial adaptive response followed by a toxic response.68 This model is being utilized not so much for directly evaluating the role of human superoxide dismutase but as a probe for a role of impaired anti-oxidant capacity as a factor in idiosyncratic hepatotoxicity, in that regard resembling the inflammmagen model (Table 5). The model also relates to the hypothesis that underlying genetic or acquired mitochondrial abnormalities are a major determinant of susceptibility for a number of drugs that target mitochondria and cause drug-
induced liver injury. In the future, the availability of extensive genetic analysis methods with patients, i.e., DNA sequencing, may be used to critically test this hypothesis. High-throughput approaches

Traditional toxicology approaches are relatively slow, are directed toward individual elements of toxicity, and are not necessarily relevant to humans if done with experimental animals. A goal of many researchers in the field is to develop a very simple in vitro assay that will accurately predict multiple toxicities in vivo (Fig. 9). Ideally, this all could be done in a human cell line and be predictive for humans (Fig. 10). To some extent, the anticipation was that mRNA microarrays might be able to achieve such results, to the same extent that an Ames Salmonella typhimurium assay is used as a primary screen for genotoxicity.

Microarrays have not been that successful in this regard (at least in providing a single readout diagnostic of all potential toxicities), nor have any proteomics or metabolomics approaches. Such a goal may be unrealistic, in that even if hepatocyte cell mRNA analysis was successful it would probably have limited use in extrapolation to endocrine tissues, kidneys, etc. Moreover, in vitro and in vivo microarray results correspond, but the correlation is not perfect.

"Omics" applications have been useful, but on a more limited basis and in addressing specific mechanistic questions, rather than as broad sweep screens. For instance, microarrays allow for detailed pathway analysis of select sets of drug candidates. Considerable effort has been made to develop databases of mRNA responses to known toxic chemicals for which many aspects of toxicity are understood, with the goal of then being able to rapidly assess the potential toxicities of new drug candidates. This approach has been used by the Iconix company (now part of Entelos) (Fig. 10), in collaboration with several pharmaceutical companies. Recently, proteomics efforts have identified a number of candidates that have potential as biomarkers of toxicity. In particular, a panel of biomarkers has been evaluated in preclinical studies on nephrotoxicity, and urinary cystatin C, β2-microglobulin, trefoil factor 3, albumin, and kidney injury molecule-1 (Kim-1) have emerged as potentially useful biomarkers. An advantage to this proteomics approach is that it should be transposable to clinical studies.

In silico approaches are also under consideration and, in principle, may be the ultimate goal. Some insight has been obtained with such methods. To date, most of the success has come from correlative relationships as opposed to mechanistic ones. The difficulty with structure-based relationships is that they are not well established for toxicity, i.e., the targets are often not established, and the results are developed in the absence of basic biological knowledge. For example, structure-activity relationships are relatively well established for gross dioxin toxicity, even though there is no structural information available about the Ah receptor.

Fig. 7. Categorization of hepatotoxins (○) and non-hepatotoxins (△) based on estimated total daily body burden of covalent binding from human hepatocyte data. See Figure 6 for drug abbreviations.

Fig. 8. Scatter plots of (A) percentage GSH adduct formation and (B) estimated total daily covalent adduct burden in drug-induced toxicity (DIT, △) and non-drug-induced toxicity (Non-DIT, ○) groups of chemicals. Horizontal lines are drawn at a (A) 0.2% adduct level and (B) 1 mg body level. LOQ, limit of quantitation.
Fig. 9. (A) Traditional in vitro or in vivo toxicity program; (B) idealized in vitro toxicogenomics system

MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; LDH, lactate dehydrogenase; NBD-PE, N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine.

Fig. 10. An in vivo predictive toxicogenomics paradigm for database development

The model shown here is the DrugMatrix® system developed by Iconix (now part of Entelos).
Table 10. Applying mechanisms of toxicity to human safety

<table>
<thead>
<tr>
<th>What is toxic? (parent drug or metabolite(s))</th>
<th>How is it toxic?</th>
<th>What is the dose-response relationship?</th>
<th>Does toxicity occur in humans?</th>
<th>Can a screen be developed to assess the liability?</th>
<th>Can the liability be eliminated?</th>
</tr>
</thead>
</table>

Table 11. Rodent carcinogenic responses not likely to apply to humans

<table>
<thead>
<tr>
<th>Tumor site</th>
<th>Illustrative chemical agents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male rat kidney</td>
<td>d-Limonene, unleaded gasoline</td>
</tr>
<tr>
<td>Male bladder</td>
<td>Saccharin, nitroliotriacetic acid</td>
</tr>
<tr>
<td>Rat thyroid</td>
<td>Goitrogens, some alkylocarbonates, fungicides</td>
</tr>
<tr>
<td>Forestomach</td>
<td>Butylated hydroxynanoisole, propionic acid, ethyl acrylate</td>
</tr>
<tr>
<td>Mouse liver</td>
<td>Barbiturates, peroxisome proliferators</td>
</tr>
</tbody>
</table>

Conclusions

Although the field of drug toxicity is a difficult one, it can also be viewed as a great opportunity, both in terms of basic science and practical application (Fig. 11). To conclude, there are three major issues: (i) identifying useful biomarkers of toxicity, (ii) establishing in vitro/in vivo relationships, and (iii) linking animal models with human toxicity. There are still many known discrepancies in the effects of chemicals on experimental animals and humans (and between species of experimental animals) (Tables 10, 11). The challenges and opportunities that lie ahead can be summarized largely in terms of these three issues.

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References

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