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Mechanisms of Drug Toxicity and Relevance to Pharmaceutical Development

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Abstract

Toxicity has been estimated to be responsible for the attrition of ~ 1/3 of drug candidates and is a major contributor to the high cost of drug development, particularly when not recognized until late in the clinical trials or post-marketing. The causes of drug toxicity can be organized 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 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 > US $1.7 billion.[1)] However, a graph of the number of new approved drugs per year is relatively flat.[2)] Some reasons for the problem include the difficulties of target validation—in approaching increasingly complex disease areas—and the 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,

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toxicity issues have increased (Figure 1). Together, pre-clinical toxicity (animal) and adverse events (human toxicity) account for ~ 1/3 of the cases of attrition.[2] If one excludes the “non-scientific” issues (e.g. commercial, financial) then the fraction is even higher.

The real issue is the expenditure of resources (of 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 (Figure 2). If compounds with toxicity issues are not dropped until a very late time, then the loss may run into hundreds of millions of dollars and years of research. Thus, earlier 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 here.

Contexts of Drug Toxicity

All compounds are toxic at high doses and all are safe at very low doses, using the axiom of Paracelsus.[3] 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. What the context of toxicity is 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. What is presented here is a systematic one previously described (Table 2).[4] Others have presented alternate but similar classifications.[5]

The first context of toxicity is **on-target** (or mechanism-based) toxicity. That is, the toxicity is 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 is the same one that produces both the efficacious and the 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 the toxicity. Changing the target for the disease may be necessary. However, another strategy is exemplified in the case of statins. All statins produce hypercholesterolemic properties by inhibiting 3-hydroxy-3-methylglutaryl CoA (HMG CoA) reductase in the liver, i.e. the target. The adverse effects of statins are also due to inhibition of HMGCoA reductase in muscle and possibly other tissues, i.e. geranylgeranylation of proteins[6] 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.[7]

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,[8] is that drugs (or their metabolites) react with proteins in the body (as haptens) to induce antibodies and immune responses. In this example (penicllins) the chemical 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 H_1 receptor (eliciting the desired antihistaminic response) but also to the hERG channel and thus causing arrhythmias. In principle, this liability can be addressed by more screening and development of drug candidates with lower IC_{50} and K_d values, in that 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 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 ($1/10^3$ to $1/10^4$ 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 (Figure 3).

Theories regarding mechanisms of idiosyncratic reactions

This topic has been reviewed by others[11–14] (Table 4). At least five theories have been proposed to explain idiosyncratic reactions, and these may not be exclusive of each other in considering all drugs for which idiosyncrasies have been reported (Table 5). The first theory is 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 metabolism of a drug, including a lack of detoxication. For instance, an individual might be the ~1% of a (Caucasian) population with a high propensity to activate a drug (e.g., the ultra-rapid metabolizers in the P450 2D6 group[17]) 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.[13]

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.[11] There is some support in the cases of tienilic acid[18] and hydralazine,[19] which are activated by human P450s and bound covalently to P450s, generate anti-P450 antibodies in humans, and cause hepatitis. The deficiency is that it has not been possible to prove that these events are causal for the hepatotoxicity or simply all happening at the same time but unrelated.[20]

The third theory is sometimes referred to as the **inflammagen** model.[21] 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.[22] However, exactly how representative this is as a model for human idiosyncrasies is subject to debate.[12]

The fourth (theory) is the **danger** hypothesis.[23,24] Here the injured tissue produces danger signals (e.g. lipid oxidation products, cytokines) that evoke a toxic response, not the drug or its metabolites. As Uetrecht has pointed out,[12] 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 idiosyncracies.

The fifth theory is the **pharmacological intervention** model.[25,26] 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 ximelegatran, a peptide-like substance, might act in this manner.[27]

Where does covalent binding fit in?

An overall scheme of drug toxicity includes many aspects, some of which are related to metabolism (Figure 4). Covalent binding of drugs to proteins has been with us at least since 1973, with the classic papers of Gillette and Brodie on acetaminophen.[29,30] Even before then the covalent binding of carcinogens to proteins had been demonstrated by the Millers.[31] However, two important questions remain unanswered. One is how important this process really is with drug toxicity, in that the evidence remains highly correlative. 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 hepatoxicity, which is both a pre-clinical and clinical issue (Tables 3, 6). In a seminal review, Walgren *et al.*[35] listed 14 drugs which have been withdrawn from the market due to hepatoxicity (Table 7). Of these, nine (64%) have been shown to be activated to reactive products. Another list of drugs includeds those that had been withdrawn in other countries due to hepatotoxicity (and never introduced into the United States) (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 “Black Box” warnings for hepatoxicity had ten (71%) that showed reactive metabolites (out of all 14 examined) (Table 9).

Collectively, two conclusions can be reached in examining these retrospective studies.[35] One is that a large fraction of problematic drugs produce reactive products, which may be an issue. However, there are caveats: one is that any relationship is not necessarily causal, and another being that the binding of non-toxic drugs was not compared here. The other point to make about the review of Walgren *et al*.[35] is that all of the drugs producing idiosyncratic hepatotoxicity were used at high doses. Uetrecht[56] has made the point that idiosyncratic problems are seldom seen with drugs used at doses of ≤ 10 mg/day. (Some drugs certainly can be hepatoxic at lower doses, e.g. cerivastatin at sub-mg per day doses, but in this case the situation is explained by the high potency, on-target toxicity, and some cytochrome P450 polymorphisms.[7]) 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.

Recently several studies have made careful comparisons of the extent of covalent binding of drugs (in animals) and correlated these with hepatoxicity. Masubuchi *et al*.[57] showed good correlation between rat and human liver microsomes with a series of drugs. Reasonably good *in vitro*/*in vivo* correlations were observed[57] (Figure 5). In other studies, the degree of covalent binding was higher for hepatotoxic drugs than non-hepatotoxic drugs (Figures 6–8). Nevertheless, the variation in covalent binding was considerable for both the hepatoxic and nonhepatotoxic drugs, and the two sets showed considerable overlap.

Thus, the proposals of Evans *et al*.[61] to utilize *in vitro* and *in vivo* 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, in that this 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).[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-hydroxyacaetanilide. Both compounds yield similar levels of total covalent binding, both *in vitro* and *in vivo*.[62,63] However, different reactive intermediates are produced from acetaminophen and the *meta*-isomer, an iminoquinone and an *ortho*-quinone respectively. More 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.[64]

Mitochondrial stress has since developed in terms of a major aspect of drug toxicity.[65] Some of the evidence suggest a combination of drug (or drug metabolite) promoting oxidative stress (a “direct” effect) and alteration of signal transduction systems result in further loss of mitochondrial function (an “indirect” effect).[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[64] and produces mitochondrial injury and reactive oxygen species, the latter which in turn activate cytoplasmic signal transduction pathways. Thioredoxin is one of the proteins oxidized by the reactive oxygen species, which then dissociates from ASK-1 and leads to ASK-1 activation and its 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 hepatotoxicty can be considered an active process, involving specific signaling molecules and net up-regulation of activity, as opposed to the older concepts of massive inactivation of cellular proteins by reactive metabolites. In addition, recent work has demonstrated that the fraction of a cytochrome P450 (2E1) localized in the mitochondria is much more uncoupled (than the fraction in the endoplasmic reticulum) and generates more reaction 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, to 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 inflammagen 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.[67] 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, directed toward individual elements of toxicity, and not necessarily relevant to human issues 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* (Figure 9). Ideally, this all could be done in a human cell line and be predictable for humans (Figure 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.[69)]

"Omics" applications have been useful, but on a more limited basis and in addressing specific mechanistic questions, rather than as a 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) (Figure 10), in collaboration with several pharmaceutical companies.[70–72)]

Recently proteomics efforts have identified a number of candidates that have potential as biomarkers of toxicity.[72)] In particular, a panel of biomarkers has been evaluated in preclinical studies on nephrotoxicity, and urinary cystatin C, β2-microgloulin, trefoil factor 3, albumin, and kidney injury molecule-1 (Kim-1) have emerged as potentially useful biomarkers.[74–77)] 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.[78–81)] To date most of the success has come from correlative relationships as opposed to mechanistic ones. The difficulty with structure-based relationships is that these 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.

Conclusions

Although the field of drug toxicity is a difficult one it can also be viewed as one of great opportunities, both in terms of basic science and practical application. 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 can be summarized largely in these three items.

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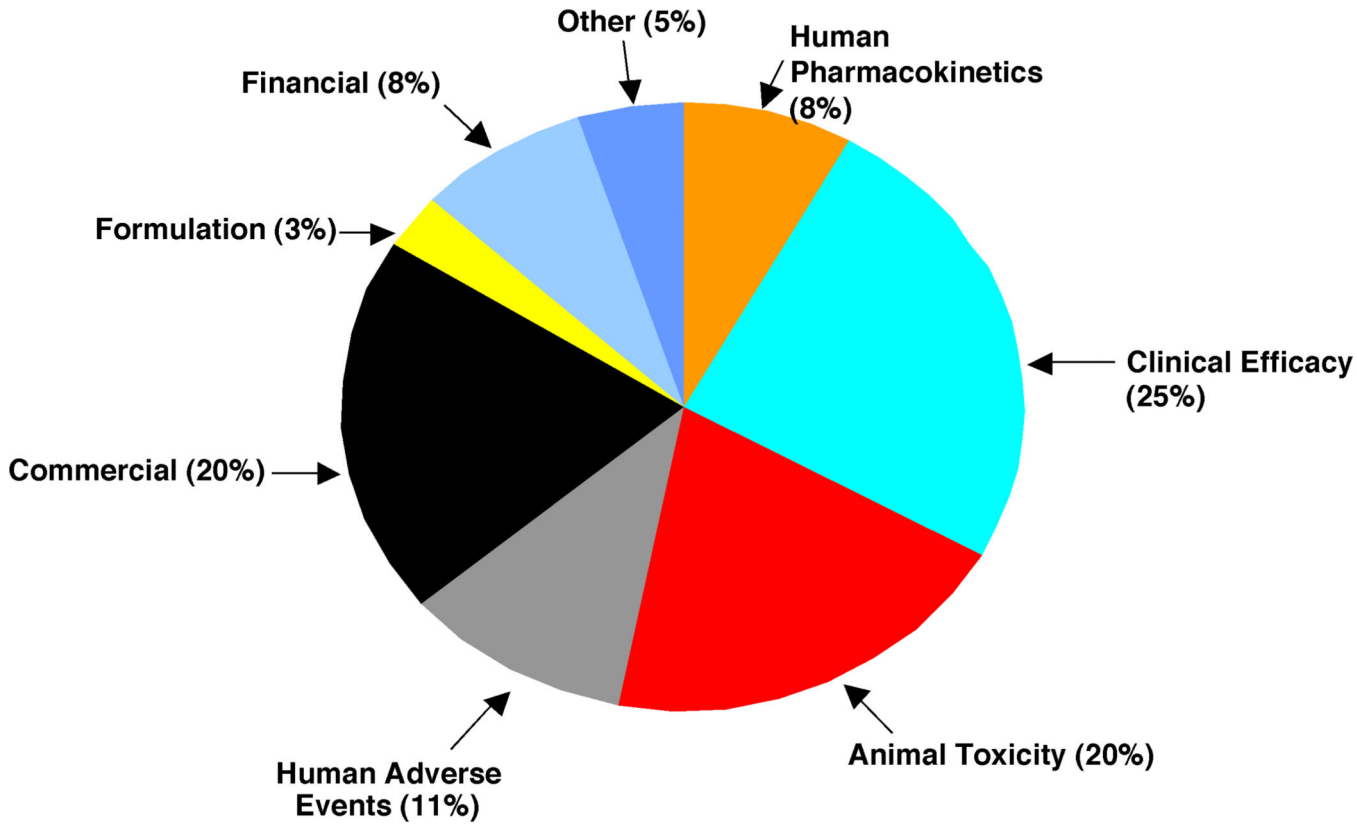

Fig. 1.
Estimates of fractions of reasons for attrition of drug candidates in pre-clinical and clinical development (ca. 2000).[2)]

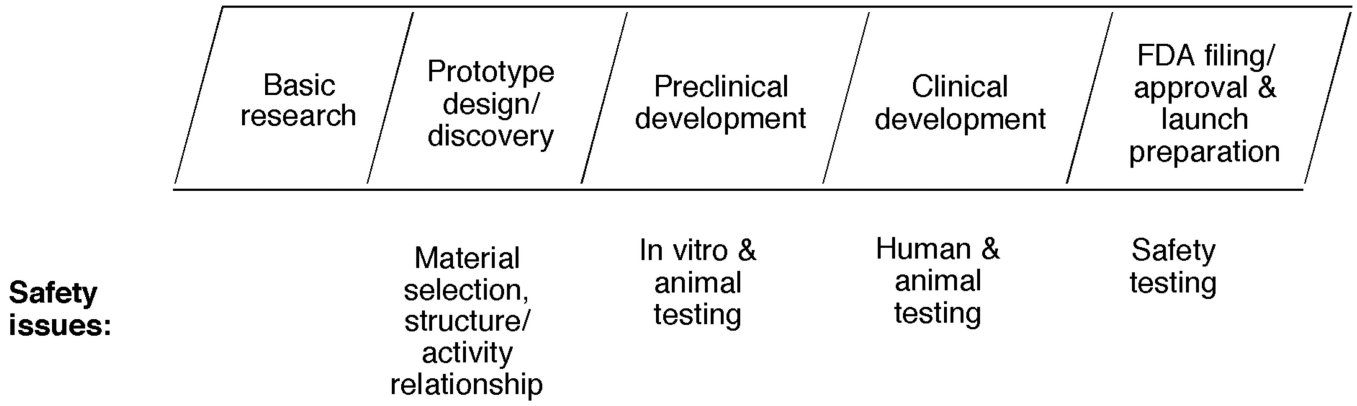

Fig. 2.
Safety issues at different stages of drug discovery and development.[1)]

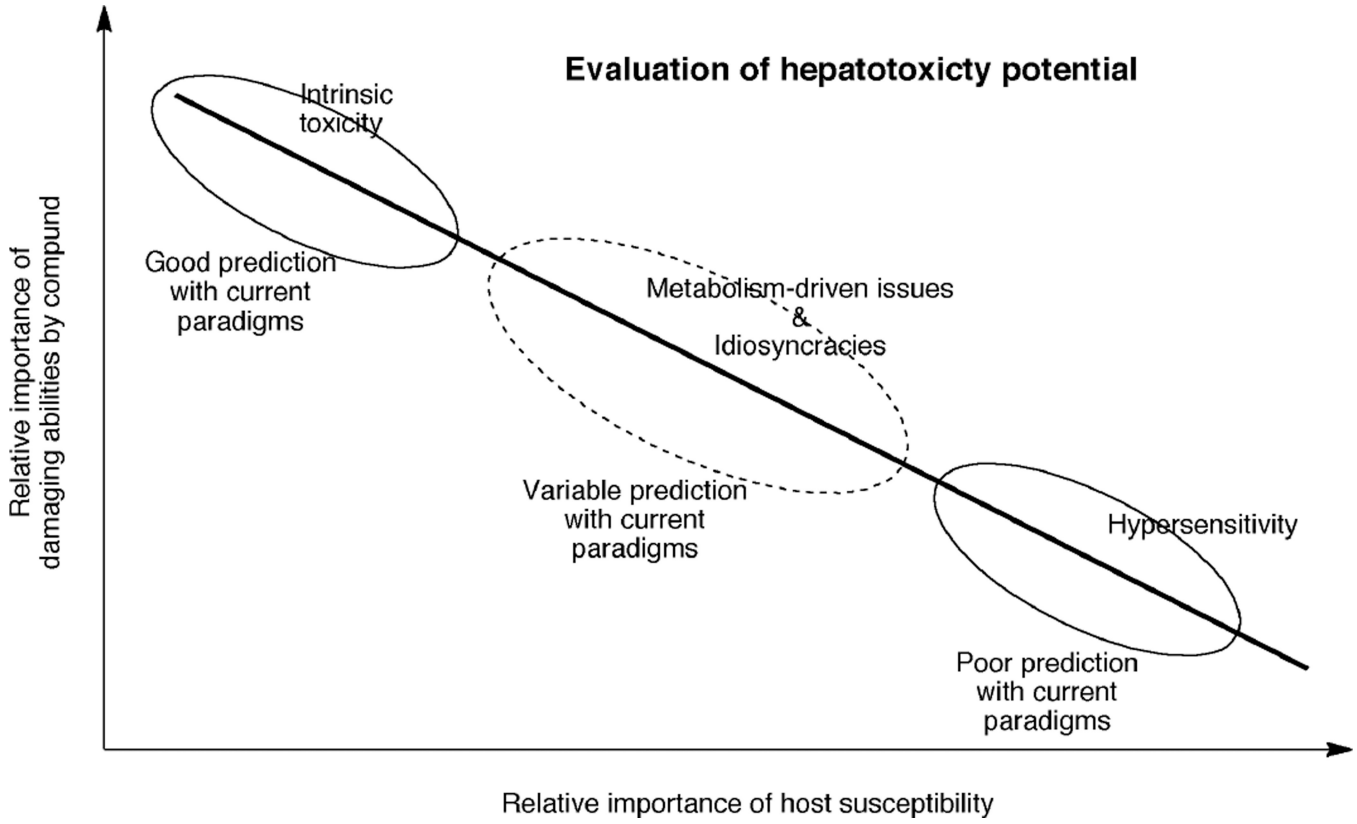

Fig. 3.

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.[9,10)] 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.

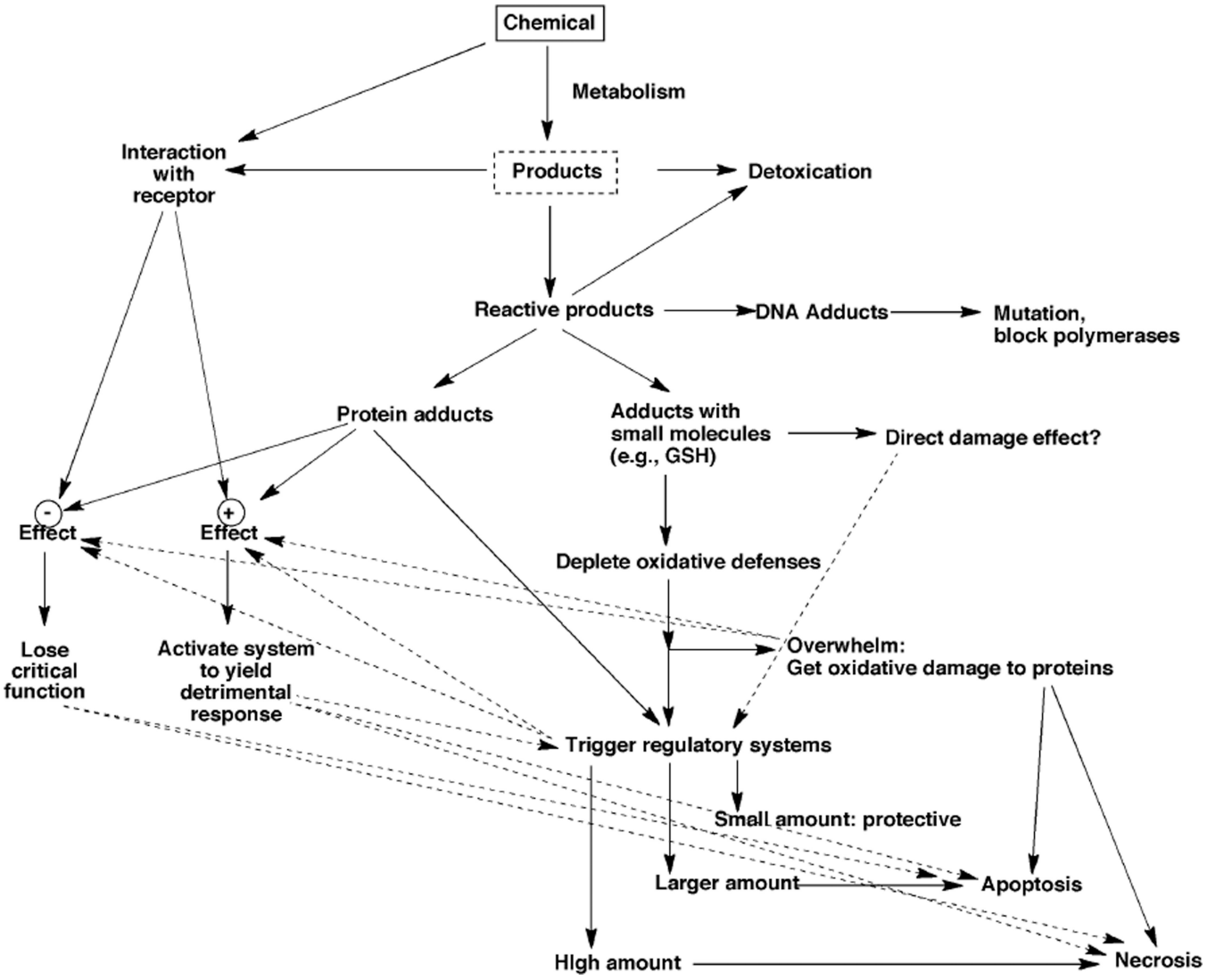

Fig. 4.

A general scheme of biological events related to the toxicity of drugs and other chemicals.[4,28)]

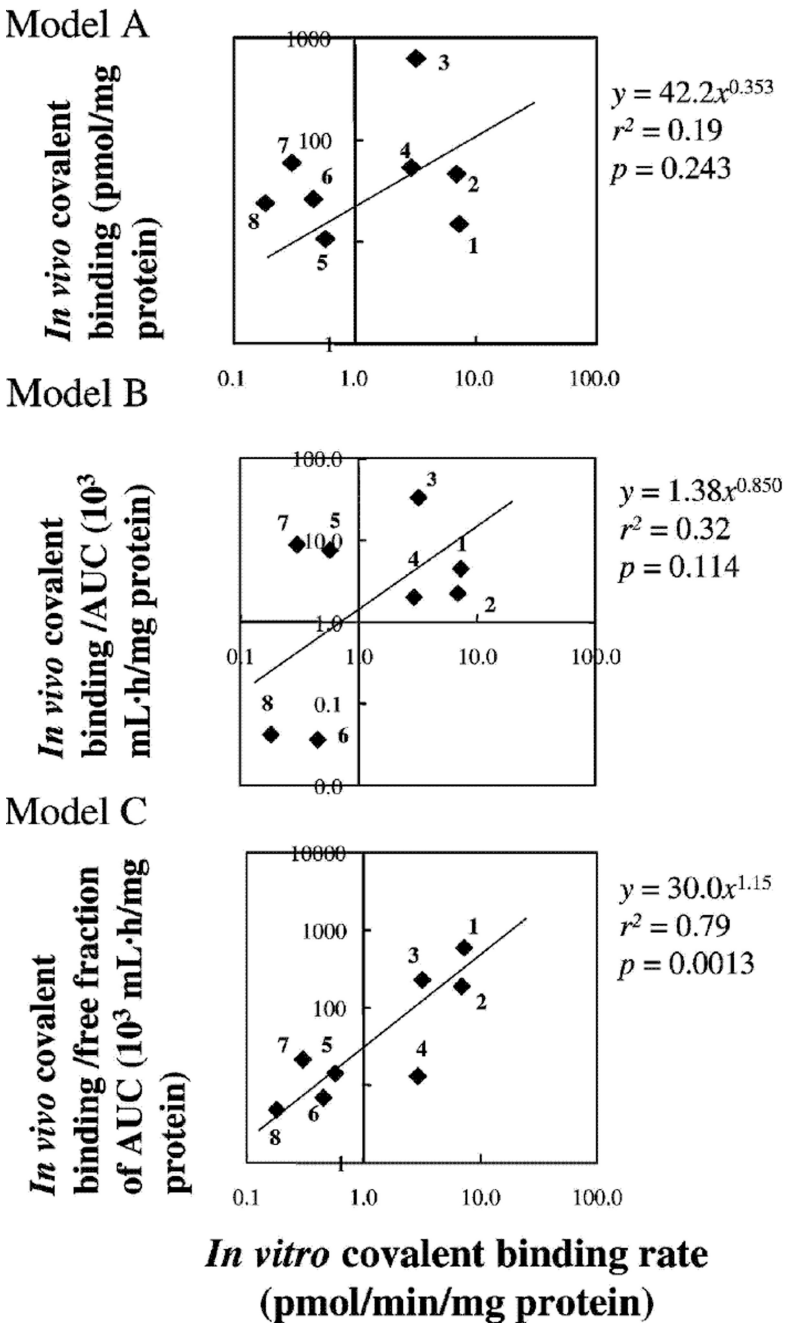

Fig. 5.

Relationship between *in vitro* covalent binding (rate of reactive metabolites to microsomal proteins (10 μM substrate) and *in vivo* covalent binding (rate) in rat liver tissue after administration of labeled compounds (at 20 mg/kg). Three different models were used.[57] 1, Furosemide; 2, tienilic acid; 3, clozapine, 4, imipramine; 6, acetaminophen; 6, indomethacin; 7, carbamazepine; 8, diclofenac.

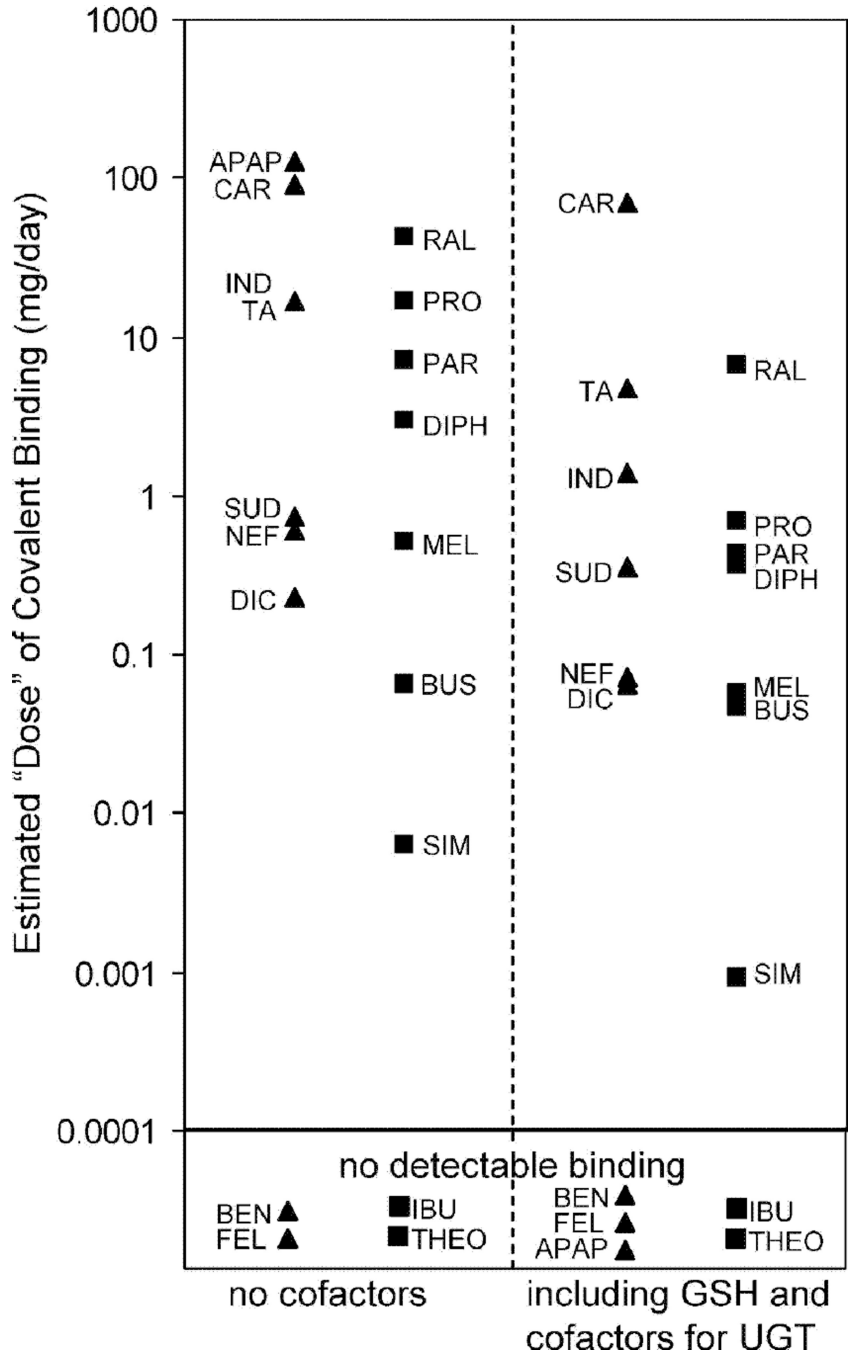

Fig. 6.

Comparisons of hepatoxins (▲) and non-hepatoxins (■) for estimates of total daily dose of covalently bound material extrapolated from *in vitro* liver microsomal covalent binding and doses. APAP, acetaminiophen; BEN, benoxaprofen; BUS, buspirone; CAR, carbamazepine; DIC, diclofenac; DIPH, diphenhydramine; FEC, felbamate; IBU, ibuprofen; IND, indomethacin; MEL, meloxicam; NEF, nefazodone; PAR, paroxetine; PRO, propranolol; RAL, raloxifene; SIM, simvastatin; SUD, sudoxicam; TA, tienilic acid; THEO, theophylline.[58)]

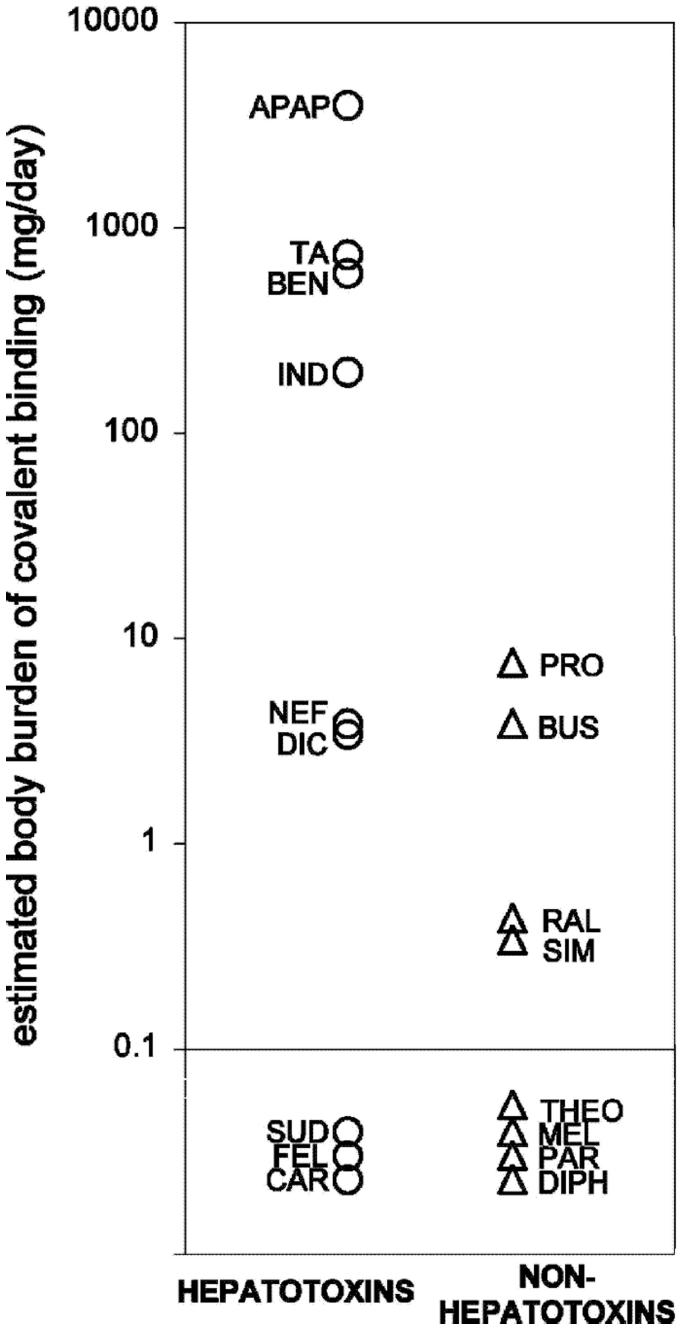

Fig. 7.

Categorization of hepatoxins and nonhepatotoxins based on estimated total daily body burden covalent binding from human hepatocyte data.[59] See Figure 6 for drug abbreviations.

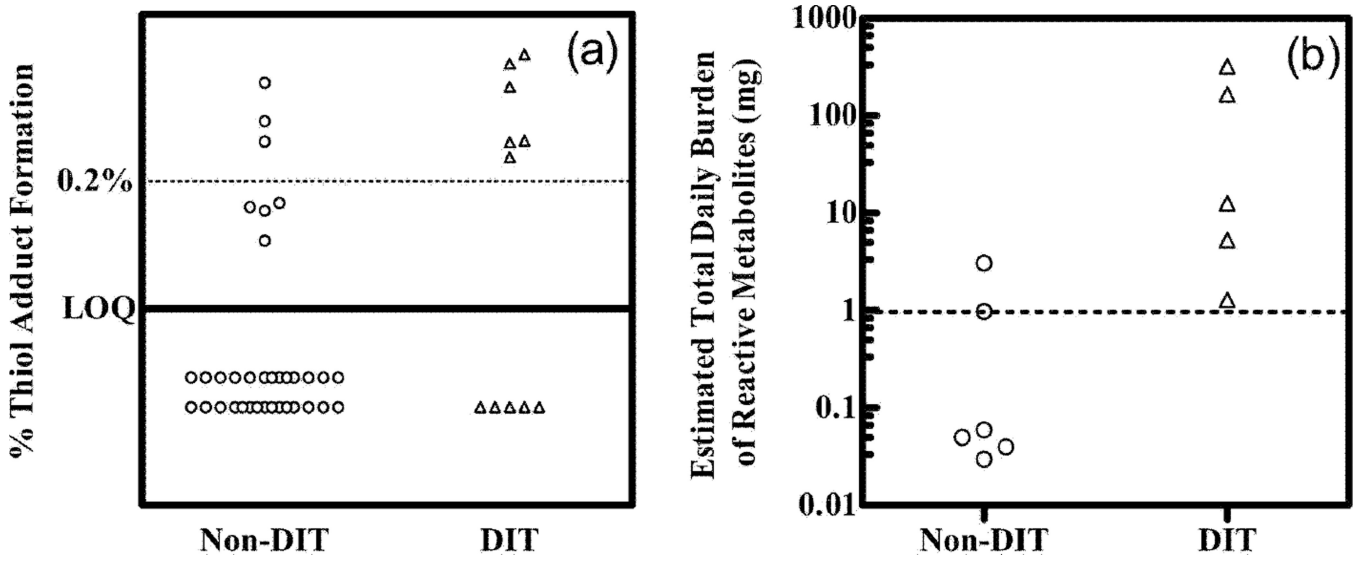

Fig. 8.

Scatter plots of (A) percentage GSH adduct formation and (B) estimated total daily covalent adduct burden in "drug-induced toxicity" (DIT, Δ) and non-drug-induced toxicity (Non-DIT, O) groups of chemicals.[60] Horizontal lines are drawn at a (A) 0.2% adduct level and (B) 1 mg body level.

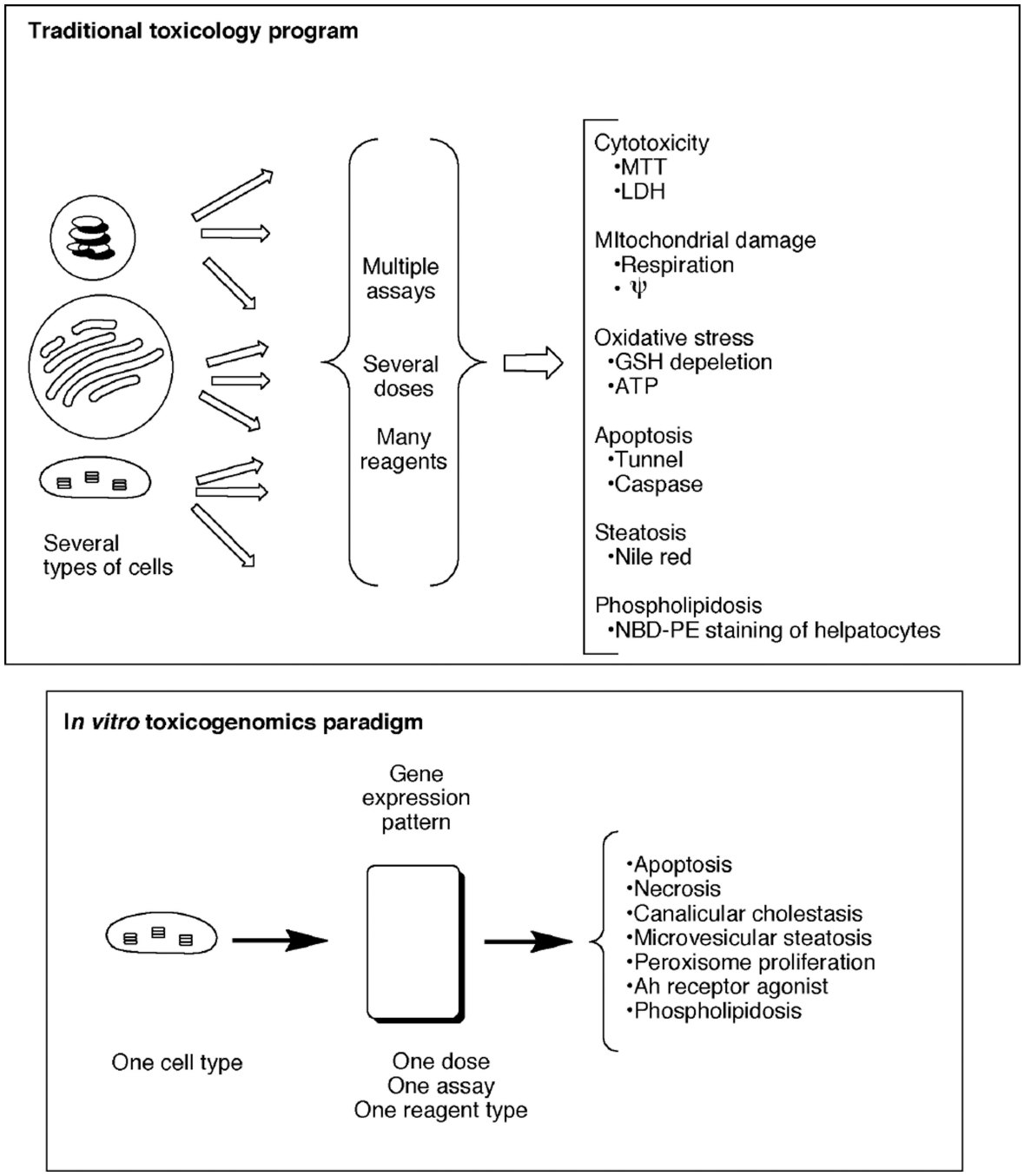

Fig. 9.

(A) Traditional *in vitro* or *in vivo* toxicity program. (B) Idealized *in vitro* toxicogenomics system.

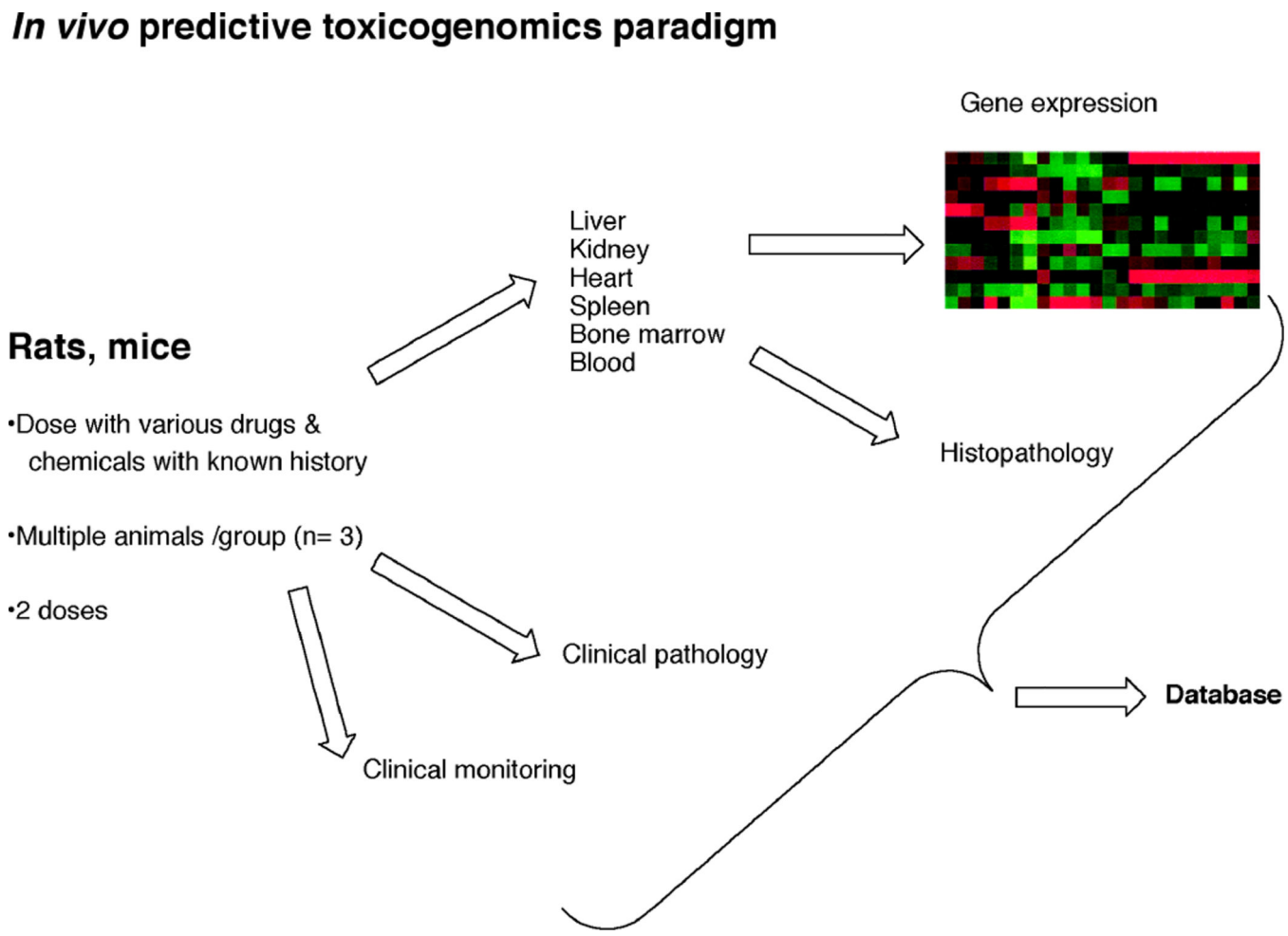

Fig. 10.

An *in vivo* predictive toxicogenomics paradigm for database development. The model shown here is the DrugMatrix® system developed by Iconix (now part of Entelos).[28)]

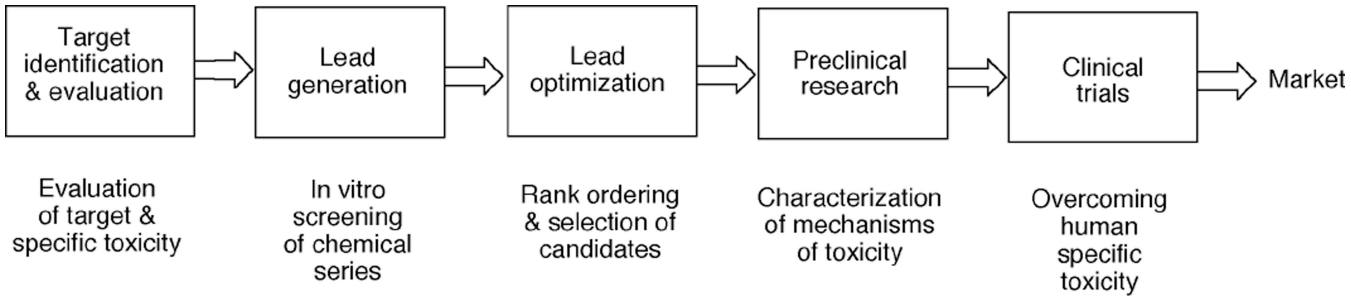

Fig. 11.

Uses of toxicity data at various stages of drug discovery and development. Major steps in the process are shown in boxes, with relevant screens listed below.[28)]

Table 1

Sites for toxicology attrition. Based on experience from DuPont-Merck and Bristol-Myers Squibb, 1993–2006. Information kindly provided by B. D. Car, Bristol-Myers Squibb.

Target organ or tissue	% of all advanced molecules[a]
Cardiovascular	27.3
Liver	14.8
Teratogenicity	8.0
Hematologic	6.8
Central and peripheral nervous system	6.8
Retina	6.8
Mutagenicity/clastogenicity	4.5
Reproductive toxicity	4.5
Gastrointestinal/pancreatic	3.4
Muscle	3.4
Carcinogenicity	3.4
Lung	2.3
Acute death (unspecified cause)	2.3
Renal	2.3
Irritant	2.3
Skeletal (arthritis/bone development)	1.1

[a]Total = 100%.

Table 2

Contexts of drug toxicity.[4)]

Type	Example
On-target (mechanism-based)	Statins
Hypersensitivity and immunological	Penicillins
Off-target	Terfenadine
Biological activation	Acetaminophen
Idiosyncratic	Halothane

Table 3

Mechanistic causes of toxicology attrition. Based on experience from DuPont-Merck and Bristol-Myers Squibb, 1993–2006. Information kindly provided by B. D. Car, Bristol-Myers Squibb.

	% of all advanced molecules[a]
Biotransformation-related	27
Target-based	28
Single or multiple ion channel inhibition	18
Immune-mediated	7
All other mechanisms	36

[a] $n = 88$. Because categories are partially overlapping, the total is > 100%.

Table 4

Intrinsic *vs*. idiosyncratic hepatoxicity.[4,12,15,16)]

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

Table 5

Idiosyncratic toxicity: proposed mechanisms.

- Metabolic

  [Rare allele] × [rare allele] = unusual metabolism

- Hapten hypothesis

  Reactive metabolites act as haptens —> immunological response

- Inflammagen 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)

Table 6

Drugs withdrawn because of hepatotoxicity.[32–35)]

Time period	Drug	Total number
Pre-1960	Cinchophen, iproniazid	2
1960–1969	Benziodarone, ibufenac, phenoxypropazine, pipamazine, xenazoic acid	5
1970–1979	Fenclozic acid, mebanazine, nialamide, oxyphenisatin	4
1980–1989	Benoxaprofen, clomacron, clometacin, cyclofenil, exifone, glafenine, isaxonine, nitrofazole, nomifensine, perhexiline, suloctidyl, tienilic acid, zimelidine	13
1990–1999	Alpidem, amineptine, bendazac, benzarone, bromfenac, chlormezanone, dilevalol, ebrotidine, fipexide, moxisylyte, niperotidine, pirprofen, tolrestat	13
2000–2006	Ximelagatran, pemoline, nefazodone, troglitazone	4

Table 7

Drugs withdrawn for hepatotoxicity (U. S.).[35)]

Drug	Date	Dose (mg/day)	Reactive products	Reference
Cincophen	1930	300	No	
Iproniazid	1959	25–150	Yes	36)
Pipamazine	1969	15	No	
Fenclozic acid	1970	300	Yes	37)
Oxyphenisatin	1973	50	No	
Nialamide	1974	200	Yes	38)
Tienilic acid	1980	250–500	Yes	20)
Benoxaprofen	1982	300–600	Yes	39)
Nomifensine	1986	125	Yes	40)
Chlormezanone	1996	600	No	
Bromfenac	1998	25–50	Yes	
Troglitazone	2000	400	Yes	41)
Nefazodone	2004	200	Yes	42)
Pemoline	2005	38–110	No	

9/14 = 64%

Table 8

Drugs withdrawn for hepatotoxicity (non-U. S.).[35)]

Drug	Date	Dose (mg/day)	Reactive products	Reference
Alpidem	1993 (France)	25–150	Yes	43)
Amineptine	1999 (France, Thailand)	200	Yes	44)
Bendazac	1993 (Spain)	500		
Benzarone	1992 (Greece)	300	Yes	
Benziodarone	1964 (U. K.)	300		
Clomacron	1982 (U. K.)			
Clometacin	1987 (France)	450		
Cyclofenil	1987 (France	200		
Dilevalol	1990 (U. K.)	200–400		
Ebrotidine	1998 (Spain)	400–800		
Exifone	1989 (France)	1200	Yes	
Fipexide	1991 (France, Greece)	600	Yes	
Glafenine	1984 (France, Greece)	400	Yes	
Ibufenac	1968 (U. K.)	2400	Yes	45)
Isaxonine	1984 (France)	1500	Yes	46)
Mebanazine	1975 (U. K.)	30	Yes	
Moxisylyte	1993 (France)	480		
Niperotidine	1995 (Italy)	230–460		
Nitrofazole	1984 (Greece)	1200		
Perhexiline	1985 (U. K.)	300		
Phenoxypropazine	1966 (U. K.)	10–20	Yes	
Pirprofen	1990 (Europ. Union)	800	Yes	
Suloctidyl	1985 (Spain)	600		
Tolrestat	1996 (Europ. Union)	400		
Xenazoic acid	1965 (France)	Unknown		
Ximelagatran	2006 (Europ. Union)	48		
Zimelidine	1985 (U. K.)	100–300		

11/11 = 100%

Table 9

Drugs with Black Box warnings for hepatotoxicity.[35] A "Black Box" warning is the strongest type of warning that the U. S. Food and Drug Administration can require for a drug and is generally reserved for warning prescribers about adverse drug reactions that can cause serious injury or death. At issue here is the benefit/risk ratio.

Drug	Dose (mg/day)	Reactive products	Reference
Acitretin	25–50	No	
Bosentan	125–250	No	
Dacarbazine	140–315	Yes	[47]
Dantrolene	300–400	Yes	[48]
Felbamate	1200	Yes	[49]
Flutamide	750	Yes	[50]
Gemtuzumab	(9 mg m^{-3})	Yes (?)	[51]
Isoniazid	300	Yes	[52]
Ketoconazole	200	Yes	[53]
Naltrexone	50	No	
Nevirapine	200	Yes	
Tolcapone	300	Yes	[54]
Trovafloxacin	100–500	No	
Valporic acid	1000–2400	Yes	[55]
		10/14 = 71%	

Table 10

Applying mechanisms of toxicity to human safety.

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

Table 11

Rodent carcinogenic responses not likely to apply to humans.

Tumor site	Illustrative chemical agents
Male rat kidney	*d*-Limonene, unleaded gasoline
Male bladder	Saccharin, nitrilotriacetic acid
Rat thyroid	Goitrogens, some alkylcarbamates, fungicides
Forestomach	Butylated hydroxyanisole, propionic acid, ethyl acrylate
Mouse liver	Barbiturates, peroxisome proliferators