Healthy animals and animal models of human disease(s) in safety assessment of human pharmaceuticals, including therapeutic antibodies

Rakesh Dixit[1] and Urs A. Boelsterli[2]

[1] Toxicology-Translational Sciences, MedImune Inc., 1 MedImmuneway, Gaithersburg, MD 20878, USA
[2] Molecular Toxicology Laboratory, Department of Pharmacology and Department of Pharmacy, National University of Singapore, Singapore 117 597

Although the predictability of untoward drug effects in humans has improved in recent years, certain new drugs, with new pharmacological mechanisms, still pose a considerable challenge. This holds particularly true for biotherapeutics and their drug-related immune reactions, idiosyncratic drug hepatotoxicity and systemic toxicity. The selection of the 'right' animal models remains crucial; the species selected must be relevant (to humans) and sensitive with regard to three basic variables: pharmacodynamics, pharmacokinetics (including metabolism) and the mechanisms underlying the toxicity in the target human diseases. Furthermore, normal healthy animals might be a poor model in certain cases because the underlying disease in patients can be an important determinant of susceptibility to adverse effects. Therefore, we suggest that, where appropriate, new animal models of human disease (s) are introduced into drug safety assessment.

Introduction

Drug development is an extensive process involving drug discovery and preclinical and clinical development. To meet regulatory requirements, a sponsor of a clinical trial must submit preclinical animal toxicology data showing that the drug is reasonably safe for use in initial, small-scale clinical studies under an Investigational New Drug Application (IND). During preclinical drug development, the sponsor of the IND evaluates the toxic and pharmacological effects of the investigational drug through a combination of *in vitro* and *in vivo* laboratory testing. Preclinical studies typically include an assessment of genotoxicity, adverse pharmacology, general toxicity and target organ toxicity in two species, in addition to drug absorption, distribution, metabolism and excretion. Depending on the intended clinical use of the therapeutics, long-term toxicity studies, fertility and developmental toxicity and two-year carcinogenicity evaluations are conducted to support subsequent Phase II–III clinical development and the regulatory filing of the new drug application. Clinical development typically involves three phases of study, which are as follows: Phase I studies are conducted typically in healthy human volunteer subjects; however, diseased patients can be included with appropriate justification. These studies are conducted in a small number of human subjects (typically fewer than 50) to assess the pharmacokinetic, pharmacological and general adverse effects and, if possible, early evidence on efficacy-indicating biomarkers or surrogate endpoints. Phase II studies are typically conducted to assess the effectiveness and safety of the drug for a particular disease indication or in multiple diseases where a particular pharmacology might be involved. Phase III studies (pivotal drug registration studies) are essentially expanded controlled trials and typically involve several hundred to several thousand patients. Phase III clinical trials are conducted to assess the full efficacy and safety of a drug in a large number of patients exposed to the drug candidate for several months to several years. These data are crucial for assessing the benefits and risks of the drug before its regulatory approval for marketing.

With increasing numbers of blockbuster drug withdrawals, enhanced black-box warnings due to safety concerns, and a steep rise in the cost of drug discovery and development, pharmaceutical companies are facing unprecedented challenges. It has been estimated that as many as 50% of all new molecular entities (NMEs) that enter preclinical animal testing fail to advance to human trials, and as much as 30% of all drugs that enter clinical testing are terminated owing to unexpected safety problems [1–5].

Corresponding author: Dixit, R. (Dixitr@medImmune.com.com)

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NMEs of chemical origin are generally referred to as new chemical entities, whereas NMEs of biological origin are referred to as new biological entities (NBEs). High failure rates of NMEs or NBEs due to preclinical and/or clinical safety issues pose serious challenges for drug development.

Traditional animal toxicology tests predict in the range of less than 10% to ~70% of all human adverse effects [1–5]. The most predictable adverse effects are gastrointestinal, cardiovascular and hematological toxicities. Conversely, certain target organ toxicities, such as drug-induced adverse immune reactions, dermatological reactions or central nervous system adverse effects, are often poorly predictable from traditional animal toxicology tests [1–5].

The lack of predictability of immune responses by animal toxicology tests has been demonstrated by the recent disaster of the Phase I clinical trial of TGN1412 in the UK in March 2006 [6,7]. TGN1412 is a CD28-SuperMAB (monoclonal antibody), which binds to the human CD28 receptor on T cells; these cells normally require both signal 1 (the antigen) and signal 2 (co-stimulation) to become fully activated. The superagonistic properties of this MAB meant that it could fully activate T cells without the need for additional antigen-receptor stimulation. It was hypothesized that MABs with these properties can activate all T cells simultaneously and can be used to treat B cell lymphocytic leukemia and rheumatoid arthritis, diseases that predominantly involve the patient having abnormal T cells or abnormal T cell functions. On the first day of the Phase I clinical trials, TGN1412 caused severe adverse effects, including a near-fatal massive 'cytokine release syndrome' in six healthy human volunteers [6,7]. This was surprising, considering that a 500-fold higher dose was used in macaques using the same type of dosing schedule and did not show any adverse effects. The safety of TGN1412 had been assessed in rats and monkeys using 1000-fold or greater dose levels than the lowest dose used in humans. This drug had not shown any significant adverse effects on the immune system in either rats or monkeys, and had not exhibited any target organ toxicity. The monkey was considered to be an animal model relevant to humans because TGN1412 was able to bind to the CD-28 receptor protein and induce the expected T cell proliferation, albeit to a much lesser extent. It remains unknown whether the cytokine storm in humans was caused by the direct ligation of CD28 on T cells or by other mechanisms, including the ligation and activation of other immune cells or off-targets by TGN1412. The species differences (humans versus animals) in potency, affinity and pharmacological responses (related to CD28, or other immune cells or off-targets) of TGN1412 might have been responsible for the differences in adverse effects. The failure of preclinical toxicology studies to predict the adverse effects of TGN1412 in humans is a major concern and has emphasized the need to search for better animal models for use in preclinical toxicology studies.

For the small-molecule pharmaceuticals, hepatobiliary toxicity is one of the poorly predicted toxicities from the animal studies. It is also one of the most frequently encountered target organ toxicities in preclinical, clinical and postmarketing settings [1–5]. It has been estimated that in ~50% of all cases, human hepatobiliary toxicity (both idiosyncratic and non-idiosyncratic) is not predictable from preclinical toxicology studies [1–5]. Although the reasons for this are not known, it is believed that certain hepatic adverse effects might be associated with factors unique to certain individuals. These susceptibility factors include idiosyncratic immune responses (through drug-induced hapten formation), genetic polymorphisms or infrequent genetic abnormalities in drug-bioactivating or detoxifying enzymes, intrinsic or acquired diseases having an impact on the hepatic toxic response, nutritional or lifestyle factors (e.g. excessive alcohol, tobacco smoking), other environmental factors and combined use of other drugs that modulate the toxicokinetics or toxicodynamics. Animal models often do not reveal this pattern of hepatobiliary liability, which might be specific to humans and related to intrinsic and extrinsic factors, as outlined earlier. Moreover, there can be animal strain-specific responses, whereby certain strains of a species develop an adaptive response (tolerance) to a drug, masking the drug toxicity. For example, tolcapone (Tasmar®), a drug used to treat Parkinson's disease, was found to cause rare hepatotoxicity in humans, despite the absence of hepatotoxicity in preclinical tests in rodents and non-rodents [8]. However, a follow-up study using a different strain of rats revealed that tolcapone produced strain-specific liver injury. Thus, the use of a more sensitive rat strain could have revealed the hepatic adverse effects. This example highlights the fact that certain toxicities might have a strong genetic predisposition, which might not be easily identifiable from standard preclinical toxicology studies.

Recommended criteria for selecting animal models for preclinical safety assessment

Prior to initiating human clinical trials, preclinical safety data are needed to ensure the safety of initial clinical doses, the proposed dose escalation and the duration of clinical dosing in both healthy volunteers and patients. The first goal is to 'Do No Harm' to healthy volunteers and patients, especially when there is no assurance of health benefits in early clinical trials.

Depending on the stage of drug development, the preclinical objectives might change slightly; however, an adequate characterization of drug safety is prerequisite at all stages of drug development, and preclinical safety data are needed before the intended human exposure (e.g. dose, schedule and duration). Ideally, the relevant animal model should be sensitive enough to reveal all potential toxicities in healthy volunteers and targeted patients. Typically, one rodent and one non-rodent species are required for toxicity evaluation for small-molecule pharmaceuticals, and this is based on the known combined strength of two mammalian species in predicting human toxicities (Figure 1). For biological pharmaceuticals, a single relevant rodent or non-rodent species might be sufficient, although two-species toxicology is preferred. This is because biological pharmaceuticals are specific to their targets and tend to produce effects related solely to their mechanism of action, so one relevant species might be sufficient to identify mechanism-based toxicity. By contrast, small-molecule pharmaceuticals tend to have many off-target effects (nonspecific binding to tissue components), which are best evaluated using two mammalian species [1–5] (Figure 1).

Great efforts have been undertaken to identify the 'right' (primary and secondary) animal species for nonclinical safety studies, to have an optimal model to predict safety in humans. By contrast, less consideration is given to the question of whether the animal model actually mimics the human disease situation (indication)

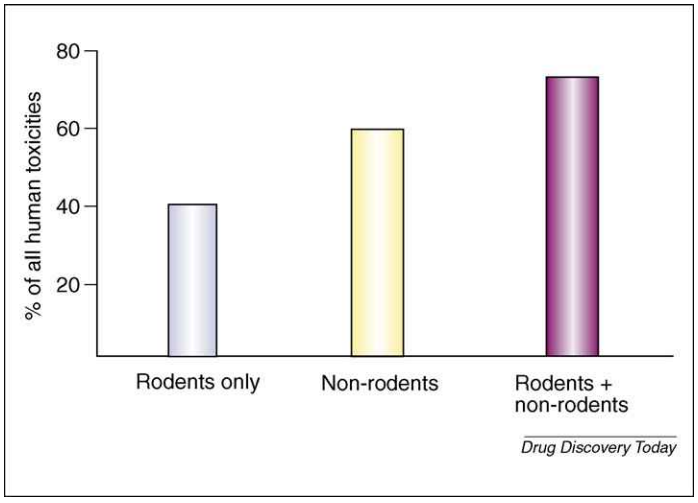

FIGURE 1

Prediction of all human toxicities from preclinical toxicology studies. Based on the review of 150 pharmaceuticals [1–5], it has been estimated that two-species (one rodent and one non-rodent) toxicology provides the best prediction of human toxicity for small-molecule pharmaceuticals.

against which the drug is being designed. In other words, non-clinical toxicity testing is normally performed in healthy animals with a rigorously controlled health status, whereas the approved drug is most likely to be used in patients that suffer from a particular disease. This indicates that, in some cases, toxicity testing using healthy animals might have limited value because the healthy animals are highly likely to be a poor surrogate for patients with a particular disease [9–15].

It is impossible to select the perfect surrogate species for humans. To assist toxicologists and drug developers, some of the important characteristics of the ideal animal models for toxicity testing are presented in the following sections (Figures 2,3).

Biological relevance to humans

It is now well established that laboratory animals used in toxicology studies, and humans share many important anatomical, physiological, pathological, pharmacological and biochemical features. Although important advances have been made towards identifying reliable *in vitro* systems to detect adverse toxic effects, most *in vitro* systems cannot reliably predict important human adverse effects because they lack complex whole-body *in vivo* pharmacokinetics; physiological, pharmacological and pathological processes; and their complex interactions with xenobiotics.

In principle, the selected animal model for preclinical toxicology studies should: (i) have normal health status with a low background incidence of pathological lesions and background diseases; (ii) be amenable to experimental laboratory settings; (iii) not be too sensitive to experimental stress; and (iv) be relevant with respect to potential drug-induced adverse effects in humans, including clinical signs of toxicity, clinical pathology and histopathology. A key to the detection of adverse effects using animal models is the similarity in mechanisms of toxicity across species, which are often based on similarity in anatomical, physiological, metabolic, pathological and pharmacological characteristics. For example, the vast majority of untoward effects associated with cytotoxic anticancer drugs are readily detected in preclinical toxicology studies because their basic mechanism of action, including mechanism-based target organ toxicity, is similar across species [1–5]. However, this might not be applicable to other pharmaceutical classes, for which effects specific to humans are encountered. For biological therapeutics, non-human primate species have been traditionally selected because of the similarity in the immunological response between these primates and humans. Additionally, many biological therapeutics show similar tissue crossreactivity across humans and non-human primates, and this enables an assessment of their pharmacological mechanism-based toxicity.

Recently, non-mammalian vertebrate species have been proposed to be a better predictor of human safety. A major advantage of some of these species is rapid testing during discovery and early drug development, requiring a small amount of test compound. One such model is the Z-tag, developed by Zygogen; this is a fluorescent zebrafish technology that can be used to design transgenic zebrafish with fluorescent organs [16]. Zebrafish offer many advantages, including rapid growth, a short life span, body transparency, small size, and having all important organ systems, including cardiovascular, digestive and nervous systems. Although the utility of the zebrafish Z-tag technology in predicting toxicity is still at a very early stage, it could be used to eliminate compounds that might be too toxic for further testing. Because the zebrafish is a phylogenetically primitive organism, its use is likely to be limited to early discovery toxicity screening and not for regulatory filing-driven toxicity testing.

Pharmacodynamic and mechanistic relevance to humans

Despite major efforts by many drug discovery scientists to design selective molecules that hit only the desired target in human diseases, small molecules, depending on their pharmacokinetic properties, are often promiscuous and hit both desired as well as undesired targets. Therefore, it is important for safety assessment to know: (i) what effects are the result of on-target activity; (ii) what effects are related to the off-target activity of the molecule; and (iii) what is the nature of the dose–response relationship for the desired pharmacological effects versus the undesired adverse effects. Whereas off-target effects are often not species selective, the on-target effects can only be studied in a species expressing the desired pharmacological target and responding similarly to humans. Figure 2a presents a general guidance for the selection of species for preclinical toxicology studies for small-molecule pharmaceuticals. If the dose–response relationship for the desired and undesired effects is similar, it might be prudent not to develop such a drug because of an unfavorable risk-to-benefit assessment. For example, many old-generation cytostatic or cytotoxic anticancer drugs kill tumor cells and normal cells in rapidly dividing tissues by similar mechanisms, and, therefore, have a very narrow safety margin and a low therapeutic index. However, despite high risks of toxicities, anticancer benefits still favor the continued use of cytotoxic anticancer drugs.

Another example of the importance of pharmacodynamic mechanisms in causing adverse effects is the highly selective cyclooxygenase 2 (COX-2) inhibitors, which have provided significant health benefits to patients suffering from debilitating rheumatoid arthritis- and osteoarthritis-associated pain while improving gastrointestinal tolerability, compared with the older

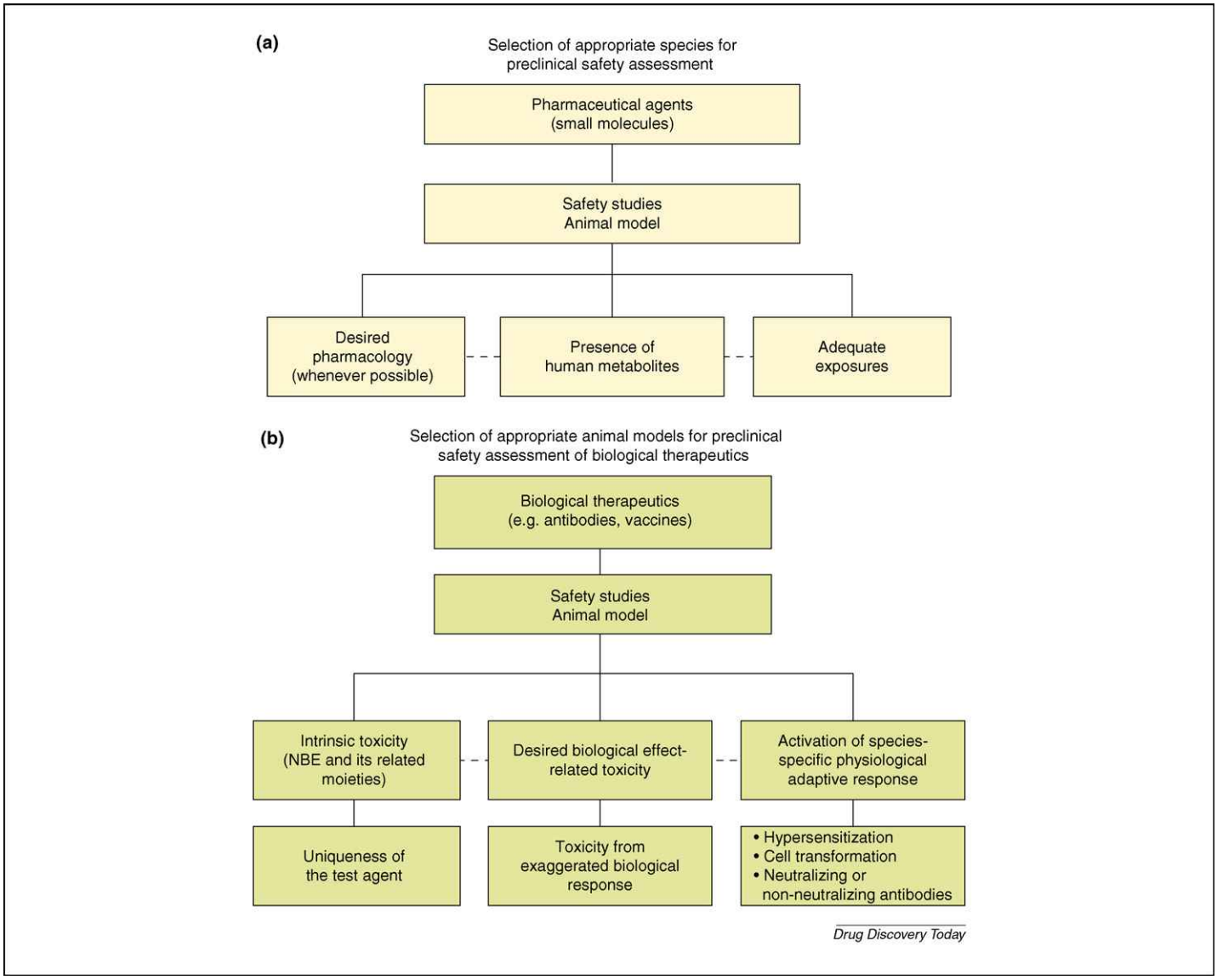

FIGURE 2

Fundamental principles applied to the selection of animal models for conducting preclinical toxicology studies of pharmaceuticals. **(a)** In selecting the preclinical species for toxicology studies of small-molecule pharmaceuticals, it is crucial to consider three important criteria: (i) the presence of the desired pharmacodynamics (whenever this is possible); (ii) the presence of all potential major human metabolites; (iii) adequate bioavailability and systemic exposure. **(b)** Because adverse effects associated with protein-based biotherapeutics tend to be mostly an extension of their pharmacological effects, it is important that the preclinical species selected have a desired pharmacodynamic activity. The intrinsic toxicity unique to a new biological molecule can be evaluated in a rodent or non-rodent species, although, traditionally, monkeys have been preferred. Various biological agents might have unique species-specific toxicity in animals, which can include species-specific physiological responses. The physiological response can include hypersensitization, cell transformation and the generation of neutralizing or non-neutralizing antibodies. The potential for a unique physiological response should be considered when extrapolating animal toxicology data to human clinical settings.

non-steroidal anti-inflammatory agents (NSAIDs). However, the cardiovascular risks associated with certain COX-2 inhibitors in a small number of susceptible patients have substantially altered the benefit-to-risk index of these drugs, and certain highly selective COX-2 inhibitors have been voluntarily withdrawn from the market. COX-2 is an inducible enzyme and is considered to be a mediator of inflammation and pain, and is therefore believed not to be expressed under physiological conditions [17]. However, COX-2 seems to be constitutively expressed in various organs, including the renal and cardiovascular systems, where it is involved in organ homeostasis. Studies in *COX-2* gene knockout mice [18] have shown that COX-2-null mice develop severe nephropathy (e.g. renal dysplasia with 100% penetrance), reduced viability and fertility, and cardiac fibrosis affecting both right and left ventricles (50% penetrance) [18]. The significance of these data and extrapolation to the adverse effects of COX-2 inhibitors in humans is unclear; however, these data provide some important insights into the beneficial role of COX-2 in the renal and cardiovascular systems under normal physiological conditions.

Pharmacological uniqueness of biotherapeutic humanized antibodies

Antibodies are unique in their structures and functions. The variable region provides the target specificity and crosslinking,

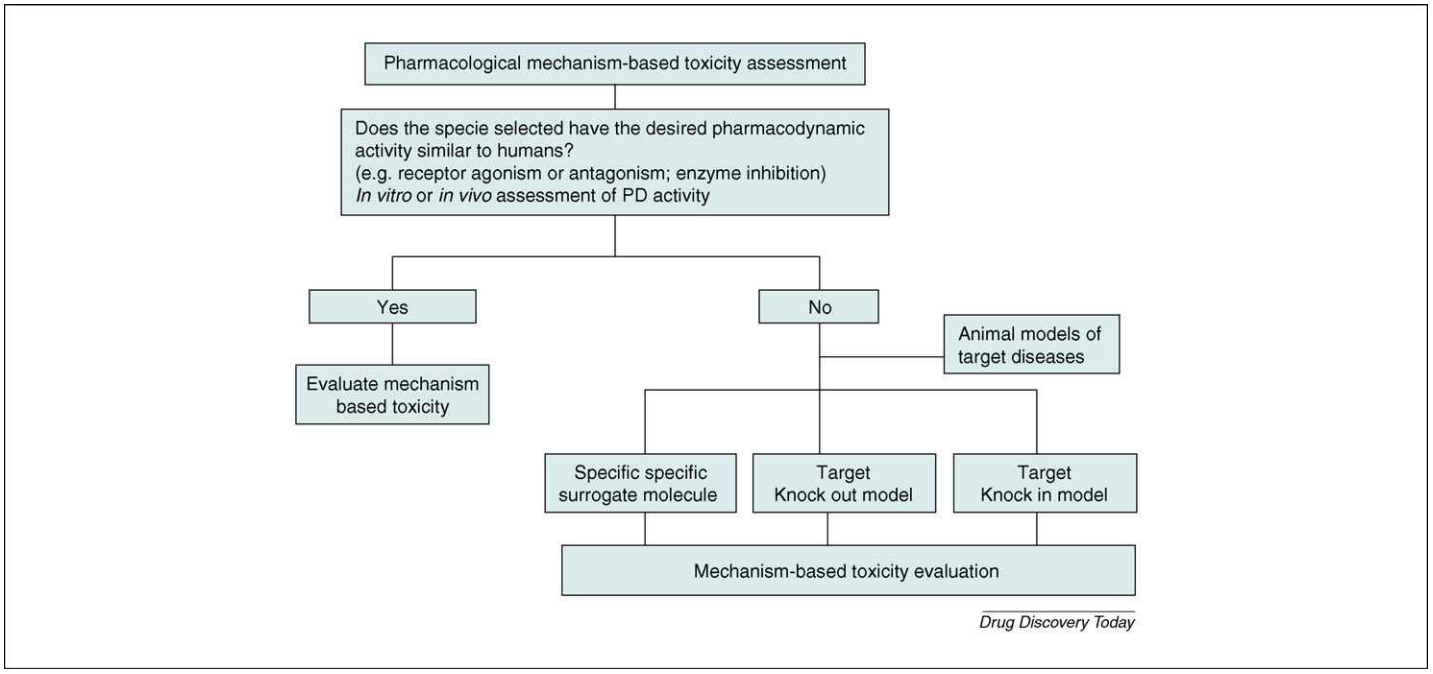

FIGURE 3

Pharmacological mechanism-based toxicity assessment. It is imperative that the toxicity associated with the desired pharmacological action is evaluated appropriately in the selected animal model. The desired pharmacodynamic activity can be assessed using a variety of *in vitro* and *in vivo* methods (see text). With recent trends in generating highly specialized humanized molecules, including small-molecule pharmaceuticals and biotherapeutic agents, it has become difficult to demonstrate the desired pharmacodynamic activity in standard laboratory species. When pharmacodynamic activity cannot be demonstrated, it might be necessary to develop surrogate molecules that produce the desired pharmacodynamic activity; alternatively, knockin or knockout transgenic models can be considered.

whereas the constant region is involved in multiple effector functions, including antibody-dependent cytotoxicity, complement activation, and facilitation and/or persistence of antibody in the systemic compartment. Antibodies also differ in their Fc portions. This enables their binding to different Fc regions on the innate immune cells. This binding can activate a cascade of effector mechanisms, including antibody-dependent cytotoxicity and complement activation, leading to unwanted effects. Antibody affinity and potency comparisons across species are an important consideration in species selection. The selection of a pharmacologically relevant animal model is crucially important for biotherapeutic agents, which tend to have species-specific characteristics (Figure 2b). Species used in standard toxicology studies (e.g. rats, mice and dogs) might not be adequate for the safety testing of biotherapeutics. This is because humanized biologics, including monoclonal antibodies, often lack the capacity to bind to the desired biological target in rats, mice and dogs, and, therefore, are poor surrogates for the assessment of mechanism-based toxicities. The pharmacodynamic or biological relevance of a species can be demonstrated by profiling receptor occupancy, receptor affinity and/or the expected pharmacological effects. It is also important to note that the humanized antibody might not bind to animal targets with the same affinity as it would bind to human targets. The off-target effects of biologics can be studied in any species used in standard toxicology studies, provided that the tissue cross-reactivity (TCR) profiles of the tissues are similar across preclinical species and humans. However, it should be emphasized that the unique human-specific TCR (absence of similar tissue binding in selected animal species) might undermine the value of animal toxicity tests for antibodies.

For therapeutic protein products, demonstration of a lack of immunogenicity (e.g. an anti-drug antibody to the parent drug antibody) is an important criterion for species selection; however, this is often not readily achievable.

Immunogenicity issues

Human-specific therapeutic proteins often generate anti-drug antibodies in animal models, and this might be a confounding factor in toxicity evaluation. The immunogenicity of protein-based molecules typically might involve two important mechanisms. (i) The reaction of the human body to a neoantigen; this process generally occurs with proteins of animal origin, although certain recombinant fully-humanized proteins have also been found to function as neoantigens. The recognition of foreign antigenic sequences of neoantigens typically involves $CD4^+$ T helper cells. (ii) Impairment of self-tolerance, which might occur with human homologs or humanized proteins. This reaction can be provoked by impurities, protein aggregates or carbohydrate residues in the protein molecule. It is believed that nearly all humanized protein-based therapeutics induce an anti-drug antibody response, as discussed earlier, although this varies from less than 1% to greater than 80% of all treated patients or volunteers in clinical trials. However, in many cases this immunogenicity is not associated with harmful effects and immunogenicity *per se* is not a deterrent to the development of biological therapies. Drug-induced immunogenicity only rarely causes serious adverse effects. More often, the neutralizing antibodies to the parent protein molecule might have a negative impact on drug efficacy. This can occur when a neutralizing antibody response leads to a faster clearance of the parent

protein molecule, reducing its availability to produce the desired pharmacological effects.

Animals in preclinical studies often develop immunogenicity to humanized forms of biological therapeutics. Additionally, this response can be unique to a human protein sequence, epitope recognition and processing of the antigen. These unique species-related effects might not be relevant for humans because human-specific proteins might not induce an anti-drug antibody response in humans, owing to self-tolerance to human proteins.

In testing unique biological therapeutics, alternative approaches to conventional animal models include transgenic animals, use of homologous proteins that can target receptors in animals that are similar to those of humans, and animal models of disease. When using transgenic animals that are designed to express a humanized target receptor, it is important fully to characterize the interaction of an NBE with the intended biological target (receptor) and the expected pharmacological response. The goal is to fully understand the adverse effects associated with expected and/or exaggerated pharmacodynamics of an NBE.

Bioavailability and metabolic considerations (small-molecule pharmaceuticals)

Compared with humans, animals tend to clear drugs more quickly. This leads to a shorter drug exposure at equivalent doses (e.g. mg/kg or mg/m^2). It is desirable that the drug candidates in the selected animal species have an adequate plasma half-life and/or clearance to afford adequate bioavailability and systemic exposure. It is generally recognized that drugs that are well absorbed (no solubility-limiting processes) and that have a moderately long half-life (e.g. 5–10 hours) are likely to provide adequate systemic exposure. It is the authors' opinion that a minimum of 30% bioavailability and a tenfold exposure margin (relative to expected human exposure) are needed (at relevant doses) to ensure the adequacy of species selection for toxicity testing.

In assessing the systemic exposure, it is important to pay attention to drug-related effects of metabolic moieties because in many cases, both efficacy and toxicity are related to the properties of the metabolites. In the absence of specific safety data related to the metabolites, it becomes crucial to demonstrate that the species selected for safety testing is similar to humans with regard to exposure to all major drug-related moieties. Although it is uncommon to see species differences in metabolic pathways, certain drugs might be metabolized uniquely in humans. This can lead to qualitative and quantitative differences in exposure to metabolites, potentially undermining the safety risk assessment. Certain metabolites might be uniquely present in animal studies and responsible for the species-specific toxicity. For example, increased liver weights in dogs were related to the *N*-oxidation of the antipsychotic drug ketotifen, whereas humans were only minimally exposed to the *N*-oxide [8]. Another example is efavirenz, a potent non-nucleoside reverse transcriptase inhibitor that is marketed for the treatment of HIV infection. Efavirenz produced renal tubular epithelial cell necrosis in rats but not in cynomolgus monkeys or humans [19]. This unique species-specific nephrotoxicity in rats was proven to result from differences in the production (or processing) of reactive metabolites. A detailed comparison of the metabolites produced by rats, monkeys and humans revealed that rats produced a unique glutathione adduct, which was further biotransformed to a reactive intermediate. This glutathione adduct, as well as related reactive metabolites, was not produced in either monkeys or humans, both of which lacked renal toxicity.

Drug toxicity in animal models of human disease

To take into account the effects of an underlying disease on the toxic response, it has been suggested to use animal models of human disease in safety assessment [10]. However, one could argue that ailing animals, owing to their pathophysiological alterations, might be sensitized to drug effects and display toxic responses which might not have a direct relevance for human safety. Although this could prove correct in certain cases, the opposite could also hold true. Because these animals are sensitized to various adverse effects, organ toxicity or other adverse drug effects might be revealed in these animal models which otherwise would remain masked and undetected in healthy animals.

An example of a disease-specific adverse effect is diabetes. The rat models of type 2 diabetes have demonstrated that these animals are indeed more sensitive to hepatotoxic compounds than are normal healthy rodents [19,20]. Other, more specific models of human diseases are currently being evaluated for their potential to differentiate between drugs that have caused rare, idiosyncratic (i.e. host-dependent) liver injury and congeners without the apparent potential to induce toxicity. Such animals provide tailor-made, genetically altered or chemically modulated models that are only used for specific applications. These animals are similar to the heterozygous p53-knockout mouse model, which is used in the assessment of chemical-induced carcinogenicity because the animals are much more sensitive than wild-type mice to the tumor-inducing capacity of a genotoxic chemical. Importantly, the aim of such models is not to mimic a human disease situation precisely. They only have the capacity to unmask a certain potential hazard of a drug under given pathophysiological conditions; they cannot be used to assess the human health risk.

The following two examples might illustrate the benefit of these models of human diseases in toxicity assessment. The first animal model – lipopolysaccharide-induced inflammation in the rat – is based on the assumption that underlying episodes of inflammation (which can be a common occurrence in a population) can greatly alter the toxic response to toxicants and could be one of the reasons for rare and unpredictable drug-induced liver injury. This working hypothesis, implying that inflammation is a major determinant of susceptibility, has been translated into an animal model, in which rats are treated with low, non-toxic doses of the bacterial endotoxin lipopolysaccharide before exposure to drugs [21,22]. Interestingly, several drugs that caused idiosyncratic hepatotoxicity in patients also produced liver injury in this rat model at otherwise non-toxic doses [23–25].

The second animal model is the heterozygous superoxide dismutase 2 (*Sod2*$^{+/-}$)-knockout mouse. This model has primarily been used in research into aging, and neurobiology [26]. However, recent evidence indicates that it can also be used for toxicological evaluation of various drugs. For example, the model proved to unmask the mitochondriotoxic potential of the NSAID nimesulide and the nucleoside reverse transcriptase inhibitor stavudine [26–28]. These compounds are typical examples of drugs causing idiosyncratic liver injury in a few patients, without causing apparent hepatic effects in the vast majority of recipients. Similarly,

these and other drugs do not cause overt liver injury in normal healthy rats or mice. The sensitivity of the *Sod2*$^{+/-}$-knockout mouse model lies in an underlying cumulative mitochondrial functional impairment (e.g. decreased mitochondrial membrane potential, decreased complex I activity, decreased ATP net production and increased basal superoxide levels) [26,28–31]. Importantly, these mice appear phenotypically normal, breed normally and unleash the increased risk for hepatic toxicity only when challenged with a drug that targets hepatic mitochondria. This model does not reflect a specific human genetic disease but rather stands for several inherited or acquired functional abnormalities in mitochondria that all merge in a similar phenotype.

Conclusions

The prediction of adverse effects using preclinical animal models remains a major goal in drug development. Despite efforts made over the past decade to improve the predictability of animal models, safety prediction has not improved. To improve the prediction of adverse effects in humans, animal models must be selected on the basis of pharmacological and pharmacokinetic similarities with humans. Figure 3 provides guidance for the selection of animal models to assess pharmacologically mediated toxicities. For biotherapeutic antibodies, efforts should be made fully to understand which adverse effects are based on pharmacological mechanisms (i.e. are target binding-based) and which are intrinsic to the biological entity or related to its effector functions. If mechanism-based toxicity cannot be evaluated, owing to the lack of the target in the animal species, or to human-specific molecules that are inactive (i.e. unable to induce the desired pharmacology) in animal species, efforts are needed to create surrogate molecules or transgenic animal species to assess mechanism(s)-based toxicities. Off-target toxicities can be assessed in any mammalian species, provided that the selected species have a metabolic profile or tissue crossreactivity similar to that in humans. In cases where there is evidence that diseases might influence toxicities, efforts are needed fully to understand both the impact of human diseases and the key pathways in toxicities.

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