ANIMAL MODELS OF HUMAN DISEASE IN DRUG SAFETY ASSESSMENT

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ABSTRACT — Animal models of human disease have been widely used in drug discovery, but they are rarely utilized in toxicological research and screening (except for transgenic models in carcinogenicity testing). Although genetic and/or acquired pathophysiological alterations associated with a particular disease may greatly exacerbate toxic responses to drugs in certain patient subsets, these pre-existing pathological conditions are usually not considered in preclinical safety assessment. Examples of disease-related determinants of susceptibility include disruption of the cytokine network in pro-inflammatory conditions, mitochondrial alterations and oxidative stress in certain neurodegenerative diseases, altered antioxidant defense in certain viral infections, and altered gene expression and mitochondrial dysfunction in type 2 diabetes. Hence, if cellular stress caused by drugs or metabolites and the disease-related effects are superimposed, then an individual can become sensitized to potential drug toxicity. Animal models of modest inflammation indeed can potentiate the toxicity of certain drugs. Similarly, rodent models of type 2 diabetes predispose the animals to hepatotoxic effects of thiazolidinediones antidiabetics. In conclusion, it is suggested that tailor-made and simplified models be adopted and increasingly used, in spite of clear limitations, as optimal substrates for satellite toxicity studies to facilitate candidate selection, help predict rare and unexpected toxicity, and identify new biomarkers.

KEY WORDS: Animal models, Disease models, Idiosyncratic drug toxicity, Susceptibility factors, Toxicity testing, Type 2 diabetes

INTRODUCTION

Animal models of human disease have been widely used to study the pathogenesis of a disease or to test the efficacy of therapeutic treatment. In contrast, such models have rarely been adopted to investigate drug toxicity and to explore the underlying molecular mechanisms. The reasons why toxicologists have been reluctant to utilize non-classical animal models in drug safety assessment are manifold. For example, the use of non-conventional animal models is neither standardized nor required by regulatory authorities. Furthermore, inclusion of novel animal models within the existing test batteries might create new and unexpected findings which are difficult to interpret. Finally, animal models ideally should closely mimic the human disease in both its etiology, clinical-pathological manifestations, and mechanisms, and this is not always the case.

There are two basic types of animal models, in addition to the conventional use of healthy animals, that are currently used and which lend themselves ideally for application in drug safety assessment. The first type encompasses animals that are genetically modified, typically with respect to a single target gene, in order to increase the sensitivity for a specific and known pathway of toxicity. One example is the use of transgenic knock-in or knock-out models in carcinogenicity assessment. Some of these models (e.g., the heterozygous p53 knockout, rasH2 overexpression, or XPA-deficiency models) have proved of equal or even superior value as compared to the traditional second bioassay species (Cohen et al., 2001). However, although designed to enhance the incidence rate of tumor formation and thus to serve as hazard identification screens, these models do not definitely prove a mechanism or mode of action, as the upstream molecu-
lar events (e.g., bioactivation pathways) are not defined. Furthermore, the models are not related to the therapeutic indication for which a particular drug is being used, nor do they reflect a particular disease state in humans.

In contrast, the second type of animal models is not focused on one gene but rather on a complex, often polygenetically controlled disease state. The overall concept is based on the assumption that an underlying pathologic state in a certain disease might alter the response of an individual to the potential adverse effects of a drug. Hence, an animal model is chosen that ideally mimics a disease, often without possessing a detailed knowledge of all the underlying pathophysiological and structural alterations. Examples include inherited or acquired forms of diabetes mellitus, neurodegenerative disorders, viral infections, or rheumatoid disorders. Ideally, the therapeutic indication in the patient is reproduced in the animal model in which potential adverse drug effects (ADRs) are being investigated. The rationale for using such models is to increase the sensitivity, too, and to unveil possible determinants of susceptibility.

The aim of this review, which is focused on this second type of disease models, is to analyze some of the key factors that are associated with a specific disease and that might alter the toxic response to drugs. If such disease models indeed react differentially to drugs, then they could offer new insights into underlying molecular mechanisms of toxicity, and they could provide new predictive tools for safety assessment, in particular for compounds that cause rare and unexpected ADRs in certain subsets of patients.

HEALTHY ANIMALS VERSUS AILING PATIENTS

Preclinical safety studies are aimed at determining the dose-dependent hazard of a potential drug candidate, identifying the target organ(s) of toxicity, and assessing the type of toxicity. These studies are normally performed in highly standardized and well characterized strains of laboratory animals. In fact, rodent toxicity studies are usually conducted with a single outbred stock of animals, thereby neglecting the advantages of the parallel use of multiple defined isogenic (inbred) strains (Festing, 1997). Furthermore, and importantly, the animals are kept under specified pathogen-free (SPF) conditions, and their health status has to be rigorously controlled (Hickey, 1997). These measures facilitate interlaboratory comparison and guarantee optimal reproducibility of the results. However, extrapolation of these animal data are not only made across species (to humans) but, importantly, to ailing populations of humans (drug recipients, with a specific therapeutic indication). Hence, the possible impact of the disease is largely ignored. It has been recognized for many years that this might be a confounding factor in drug safety assessment. In fact, one of the reasons for incorrect prediction of toxic effects from preclinical toxicity studies has been attributed to the failure to consider pre-existing pathological conditions in certain populations. This obvious failure has even been called “one of the deadly sins of toxicology” (Zbinden, 1987).

One example (out of many similar other examples) is the mixed endothelin$_{A/B}$ receptor antagonist, bosentan (Breu et al., 1998). Bosentan is mainly used to treat pulmonary hypertension but has also been investigated for treatment of congestive heart failure (Ellahham et al., 2000). In a clinical trial, 18% of patients (versus 4% in the placebo group) exhibited clinical-chemical signs of liver injury (increases in aminotransferase activity $>3 \times$ ULN and/or cholestasis) (Fattinger et al., 2001). The underlying mechanism is not known, but as chronic heart failure has serious repercussions on the hepatic microcirculation, it is possible that repeated blockade of endothelin receptors in the liver modulates vascular changes, alters the expression of mediators, and adversely affects the oxidative stress response (Nishida et al., 1998; Tanaka et al., 1999). Nevertheless, the regulatory guidelines do not require pre-clinical safety studies with animal models of congestive heart failure, which would have more closely mimicked the hepatic microcirculatory disturbances. Similarly, other disease models (e.g., diabetic conditions, viral infections, inflammatory conditions) are not required as substrates for prolonged safety studies although appropriate models would be readily available. In fact, such disease models are widely utilized to assess the pharmacological efficacy.

If animal models of human disease were increasingly used in toxicity assessment, then additional information on possible adverse effects in humans might be obtained. Such disease models, as opposed to healthy animals, often exhibit a differential response to a drug, which ideally is similar to the adverse effects seen in humans. For example, administration of human recombinant erythropoietin, which caused hypertension in patients with chronic renal failure, caused hypertension in uremic dogs, but not in healthy normal dogs (Gad, 2002). Thus, animal models that are tailor-
Disease models in drug safety assessment.

made or selected to mimic a certain human disease, could answer specific questions with respect to safety aspects of certain therapeutics. One great advantage of this approach is that the toxic response can be directly compared in normal animals and in diseased animals. In many cases, one can assume that the variation between these two (or more) populations is higher than the interindividual variation seen within one single stock of outbred rats (Festing, 1997).

**DRUG-INDUCED LIVER INJURY AS A PARADIGM**

This review will focus on one type of organ-selective toxicity, which has attained increasing clinical significance, i.e., the rare, but severe cases of drug-induced hepatotoxicity. Potential liver injury induced by a new drug cannot always be predicted from preclinical safety studies conducted with normal healthy animals. In many cases, liver toxicity may occur unexpectedly and is recognized only in the postmarketing phase, upon exposure of a large number of patients. Hence, the prediction and understanding of this type of hepatotoxicity is a particularly challenging issue for the pharmaceutical industry and for the preclinical toxicologist (Boelsterli, 2003a). Although the incidence of serious liver injury is low for a single specific drug (typically 1:5,000 to 1:50,000 users), the total number of drugs that can potentially cause liver injury is quite high. Furthermore, the effects often occur in a delayed fashion and then can culminate in fulminant liver failure which might require transplantation. In fact, more than 50% of all cases of acute liver failure are caused by drugs, and drugs have been recognized to be the single major cause of acute liver failure (in the Western world, where viral infections are less important than in other parts of the world) (Ostopowicz and Lee, 2000; Kaplowitz, 2001). The unpredictable nature of these effects in combination with their severity have repeatedly led to the discontinuation of further development of a promising drug, or even to the withdrawal of efficacious drugs after successful launching.

With the exception of the reasonably well-understood and clearly dose-dependent acute hepatoxic effects that may occur with certain drugs, e.g., acetaminophen (paracetamol), the determinants of susceptibility for the majority of the other drugs that are potentially hepatotoxic (at therapeutic dosage) are largely unknown. Typically, rodents and other animal species tolerate the compounds well even at supratherapeutic conditions. This is not surprising as rodents have an extremely high capacity for detoxifying and rapidly eliminating xenobiotics. Moreover, and importantly, the great majority of humans who are treated with these drugs equally tolerate them well (at therapeutic doses). It is only a small fraction of patients who exhibit a much lower threshold for the hepatotoxic response to these drugs. It is generally assumed that individual patient-specific factors (e.g., genetically determined or environmentally induced susceptibility factors) might predispose these patient subsets to the ADR. For example, the incidence rate of isoniazid hepatotoxicity correlates well with genetic polymorphisms for CYP2E1 and NAT2, both of which are involved in the sequential bioactivation steps of isoniazid to reactive metabolites, but also in a detoxication pathway (NAT2) (Huang et al., 2002, 2003). Although for most other drugs these factors have rarely been identified, it is currently believed that this idiosyncrasy (i.e., the specific genetic and acquired characteristics of a certain individual) is a major reason why the effects cannot be recognized and reproduced in laboratory animals. However, an additional important determinant of susceptibility comes into play, and this is often neglected. Key to this are susceptibility factors related to the underlying disease (usually the therapeutic indication, but also other disease states).

**SUSCEPTIBILITY FACTORS RELATED TO THE UNDERLYING DISEASE**

Clearly there is a multitude of pathophysiological alterations that are caused by or accompany a diseased state and which might greatly influence the toxic response to a drug (Boelsterli, 2003b). At one level, these factors can modulate drug toxicokinetics. For example, preexisting liver dysfunction can greatly affect the metabolism and disposition of a compound and alter its clearance. It seems logical that these conditions can lead to potentially higher plasma and/or tissue levels of the compound, which might result in toxicity. On the other hand, there are also toxicodynamic determinants which are less well understood. These include disease-altered expression of key genes, pathophysiological alterations in metabolic pathways, compromised energy homeostasis, or impaired antioxidant defense systems, all of which might dramatically change the toxic response to a compound or its reactive metabolite.

Pathologically altered conditions can render an individual with a specific disease, as opposed to a healthy individual, more sensitive to a drug’s adverse
effects. Well-known examples of increased risks for certain ADRs by disease factors are summarized in Table 1. The mechanisms underlying this increased susceptibility are not always known; however, for a number of disorders, certain key patterns have been emerging.

Proinflammatory conditions

Proinflammatory conditions can profoundly alter the hepatic response to drugs. This is due to a number of mediators released from inflammatory cells. For example, in rheumatoid arthritis, there is a dramatic increase in circulating proinflammatory cytokines. Furthermore, under these conditions membrane lipids are altered, and there is a severe impairment of energy homeostasis due to mitochondrial dysfunction of an unclear etiology (Ishizuki and Fujihira, 1984; Yamaguchi et al., 1989; Roubenoff et al., 1997).

Another relatively frequent proinflammatory condition is endotoxemia, i.e., increased release of bacterial lipopolysaccharide (LPS) as a consequence of gastrointestinal distress, liver disease, alcohol, or drug effects on the intestinal mucosa. While massive release of LPS can lead to circulatory shock and multiorgan failure, mild amounts cause clinically silent inflammatory changes, including increased release of TNFα, eicosanoids, ROS, or RNS (Hewett and Roth, 1993). Also, endotoxemia leads to the downregulation of key enzymes involved in oxidative drug metabolism (CYPs), which can lead to delayed metabolic clearance of certain drugs (Shedlofsky et al., 1994; Cheng and Morgan, 2001).

It seems, therefore, logical that certain drugs which are used to treat diseases that are characterized by such inflammatory conditions, and which themselves cause oxidative stress and/or mitochondrial damage, can exacerbate the toxic response. Experimental studies have indeed revealed critical interactions between LPS and a number of unrelated xenobiotics and identified some critical mediators including TNFα, eicosanoids, and neutrophil products (Roth et al., 1997; Barton et al., 2000).

Neurodegenerative disorders

Among the many pathophysiological changes that accompany certain neurodegenerative diseases, mitochondrial alterations play a pivotal role. For example, cells from patients with Alzheimer’s disease or Parkinson’s disease exhibit abnormalities in some proteins of the electron transport chain (Cassarino and Bennett, 1999). Specifically, mitochondria from Parkinson’s disease patients often have defects in complex I activity (NADH:ubiquinone oxidoreductase), while mitochondria from Alzheimer’s patients may exhibit abnormalities in complex IV activity (cytochrome c oxidase) (Parker et al., 1989; Davis et al., 1997). This invariably leads to compromised ATP production and increased formation of partially reduced oxygen species that leak from the electron transport chain. Subsequently other reactive oxygen species can be formed, and oxidative stress may ensue (Kumar et al., 1994; Swerdlow et al., 1996).

Again, it can be surmized that certain drugs, which are used to treat such neurodegenerative diseases (e.g., tolcapone or tacrine) and that themselves are known to potentially impair mitochondrial function (Berson et al., 1996; Robertson et al., 1998; Haasio et al., 2002; Smith et al., 2003), might exacerbate the underlying mitochondrial injury. This could become toxicologically relevant under two conditions; first, in tissues or cells with a high energy demand and a high mitochondrial density, and second, and importantly, in cells which have the capacity to enzymatically bioactivate the drug to a reactive metabolite that is ultimately responsible for the mitochondrial functional damage. Both is true for hepatocytes. Thus, if mitochondrial stress by a drug is superimposed on a preexisting and silent mitochondrial abnormality, then chronic exposure is more likely to eventually reach the critical threshold and result in overt cell demise and tissue injury.

Table 1. Examples of disease states associated with increased risk for developing adverse drug reactions (ADR).

<table>
<thead>
<tr>
<th>Disease</th>
<th>Type of ADR</th>
<th>Risk increase</th>
<th>Reference</th>
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<tbody>
<tr>
<td></td>
<td>fulminant liver failure</td>
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<tr>
<td>Rheumatoid disorders</td>
<td>Hepatotoxicity by NSAIDs</td>
<td>Rheumatoid arthritis : other indications 10 : 1</td>
<td>(Rodriquez et al., 1994)</td>
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<tr>
<td>Viral infections</td>
<td>Idiosyncratic reactions to sulfa drugs</td>
<td>HIV patients : Non-HIV patients 100 : 1</td>
<td>(Levy, 1997)</td>
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</table>
Disease models in drug safety assessment.

Viral infections

Underlying viral infections can sometimes increase the risk for certain ADRs in patients (Levy, 1997). The exact mechanisms are not known and are certainly multiple; however, in some cases, mitochondrial changes and/or increased oxidoreductive stress have been implicated in the increased sensitivity (Barbaro et al., 1999; Cai et al., 2003). For example, it is known that viral infections are accompanied by changes in the redox balance characteristic of oxidative stress (Peterhans, 1997). In fact, a number of different viruses cause decreases in intracellular GSH levels and increased production of reactive oxygen species. That oxidative stress is produced in the liver has been demonstrated both in cellular models and in mice (Hennet et al., 1992). Alternatively, virus-induced downregulation of drug-metabolizing enzymes has also been implicated in posing a higher risk for drug toxicity (Renton and Armstrong, 1994). This condition will result in reduced clearance and higher exposure to those drugs whose elimination depends on these particular enzyme forms.

Thus, it is likely that oxidant stress which is inflicted upon the liver by a given drug may exacerbate preexisting pro-oxidative conditions and augment possible mitochondrial abnormalities that are associated with the underlying viral infection. This is not only true for anti-viral drugs but may also apply for drugs used for other therapeutic indications. For example, a number of reported cases of hepatic toxicity attributed to ibuprofen (one of the hepatic-safest NSAIDs) have revealed that the patients who developed liver injury also had an underlying infection with hepatitis C virus. Because the severity of liver dysfunction changed in parallel with the discontinuation and rechallenge of the drug, it was concluded that it was the drug, and not the virus, that caused liver injury (Riley and Smith, 1998; Andrade et al., 2002).

Type 2 diabetes

Type 2 diabetes (T2D) can entail a multitude of pathophysiological effects, three of which might become particularly relevant in predisposing for hepatic ADRs. First, hepatic steatosis is frequently associated with the disease, and this invariability produces lipid peroxidation and inflammatory reactions in the liver, which can enhance a toxic response to certain drugs (Yang et al., 1997; Guebre-Xabier et al., 2000). Secondly, the expression of specific genes involved in glucose or fatty acid metabolism is altered. One of the key regulators is the ligand-activated transcription factor, peroxisome proliferator-activated receptor γ (PPARγ), which is highly upregulated in the liver in several murine models of T2D (Bedoucha et al., 2001). Ligands/activators of PPARγ (e.g., glitazone anti-diabetics) will elicit differential effects in normal animals (low expression) or diabetic animals (high expression). Finally, diabetic individuals feature distinct mitochondrial changes. For example, due to the chronic oxidative stress posed by hyperglycemia, mitochondrial DNA (mtDNA) gradually accumulates a greater extent of oxidative damage than normoglycemic individuals (Rehman et al., 1999; Suzuki et al., 1999). Also, uncoupling protein-2 (UCP-2) is upregulated in the liver of obese and diabetic mice, which leads to increased oxidative stress, impairment of energy production, and greater sensitivity to certain drugs that are known to interfere with mitochondrial function (Rashid et al., 1999).

SENSITIZATION TO DRUG TOXICITY IN ANIMAL MODELS OF HUMAN DISEASE

While many of the pathophysiological changes associated with a disease state are well characterized, and despite the fact that these changes might alter dramatically the toxic response to drugs by an insult that is superimposed, this view is rarely taken into account in preclinical toxicity trials. However, with the advent of new molecular techniques, but also through the discovery and development of animal models that mimic such pathophysiological changes, new animal models of human disease lend themselves to increased use in pharmaco-toxicological research. A few selected examples are given in the following sections.

LPS model

Injection of small non-toxic doses of bacterial LPS is a widely used model to induce mild endotoxemia. The subsequent modest non-injurious inflammation can markedly reduce the threshold for several hepatotoxins in rats. For example, treatment of rats with the H-2 receptor antagonist ranitidine (which causes idiosyncratic liver injury in patients) produces hepatotoxicity only in rats pretreated (-2 hr) with nontoxic doses of LPS. In contrast, famotidine (which rarely causes liver injury in patients) in combination with LPS did not cause liver toxicity (Luyendyk et al., 2003). These data indicate that a response resembling human ranitidine idiosyncratic liver injury can be reproduced in animals with an underlying modest inflammatory response.
Similarly, the extent of liver injury induced by chlorpromazine (CPZ), a psychotropic drug which has been associated with idiosyncratic cholestatic liver injury, is greatly potentiated by concomitant administration of LPS (Buchwelt et al., 2002). Thus, pretreatment of rats with low doses of LPS, followed by injection of a non-toxic dose of CPZ, caused elevations of plasma aminotransferase activities and increases in the number of hepatic neutrophils. While the exact mechanisms of this sensitization are not known, one could speculate that activation by LPS of Kupffer cells and other phagocytes in the liver is involved, leading to massive release of proinflammatory cytokines, eicosanoids, and proteases.

It is not known whether similar mechanisms might by relevant in humans. However, in view of the fact that gastrointestinal injury induced by mixed COX-1/2 inhibitors (classical NSAIDs which are consumed by a large number of people) is relatively frequent and leads to increased permeability of the intestine as well as overt injury to the mucosa (Sigthorsson et al., 2002), this animal model of mild LPS release should be further explored in the future.

**Adjuvant-induced arthritis rat model**

Experimental arthritis has been induced in rats by intradermal injection of complete Freund’s adjuvant (heat-inactivated *Mycobacterium sp.*). This leads to a number of pathophysiological changes including renal dysfunction (Ibanez de Caceres et al., 2002) and disruption of hepatic glycogen metabolism (Fedatto-Junior et al., 2002). This underlying chronic inflammation can have profound effects on the extent of mitochondrial changes induced by certain drugs. For example, nimesulide, a sulfonanilide drug and preferential COX-2 inhibitor with the potential to dissipate the transmembrane potential in isolated mitochondria, induced greater uncoupling effects in mitochondria from arthritic rats than in those from normal control rats (Caparroz-Assef et al., 2001). This indicates that the underlying inflammatory conditions must have sensitized the mitochondria to the additional stress provoked by the drug. It is interesting to note that the mechanism of liver injury, a rare but potentially serious ADR associated with nimesulide in patients, most likely involves mitochondrial injury (Boelsterli, 2002).

**Models of viral infections**

Experimental models of virally infected cells or animals are rarely used to demonstrate abnormal path-ways or enhanced expression of drug toxicity. However, one example is an *in vitro* model with cultured human lymphoblasts infected with HIV. This model was used to investigate the role of viral infection in sensitizing for sulfamethoxazole toxicity (Rieder et al., 1995). A specific reactive metabolite of sulfamethoxazole caused in a concentration-dependent manner much higher cytotoxicity in HIV-infected cells than in non-virally infected cells or cells infected with HTLV-I. The underlying mechanism is not known but was speculated to be based on increased oxidoreductive stress in the virally infected cells (lower levels of GSH), which may lower the threshold for the toxic metabolites to exert an oxidoreductive/alkylating stress.

Another example is the woodchuck hepatitis B model, which has been used to assess the safety of a number of antiviral drugs. The woodchuck (*Marmota monax*) can become naturally infected with a hepatitis virus that resembles human HBV infection. During the development of fialuridine (developed to treat hepatitis B), unexpected and severe hepatotoxicity was observed in clinical phase II trials, which led to a discontinuation of further development. No indication that this might happen could be inferred from preclinical models (healthy rodents and non-human primates). Interestingly, fialuridine hepatotoxicity could later be reproduced and studied in the woodchuck hepatitis model (Tennant et al., 1998). The hallmarks of liver injury previously reported in the clinical trials (hyperbilirubinemia, lactic acidosis, hepatic steatosis, and the delayed onset of toxicity) could exactly be reproduced in the woodchuck model. However, and importantly, there was no significant difference in the toxic response between non-infected woodchucks and woodchucks infected with hepatitis B virus. Thus, in this example, it is probably not the viral infection alone that caused increased sensitivity to the drug but rather a biological feature of the particular species. Why the woodchuck is similar to humans in that it is much more sensitive to fialuridine hepatotoxicity than all the other species investigated is currently not known. One possibility is that there might be a differential rate of activation (phosphorylation) of the prodrug fialuridine to the ultimate nucleoside analog, which is then incorporated (in a desired manner) not only into viral DNA but also (as an undesired secondary effect) into mitochondrial DNA in hepatocytes. This drug-altered mtDNA will gradually cause damage to the mitochondria as mtDNA encodes for a number of the protein subunits of the electron transport
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chain. Ultimately, the accumulating mitochondrial functional damage will reach a critical threshold and precipitate the typically delayed liver injury. It is possible that other antiviral drugs which also damage mtDNA may pose a greater risk for becoming hepatotoxic in animals with an underlying viral infection as opposed to healthy animals.

Models of T2D and obesity

A number of rodent models of obesity and T2D have been developed and are widely used in drug discovery research. These include mutations in leptin (e.g., ob/ob mice), leptin receptor mutations (e.g., db/db mice), or mutations in the agouti gene that leads to overexpression of the agouti gene (e.g., in KKA' mice). Alternatively, aquired T2D can be induced experimentally in rats by feeding a high fat diet (40% of caloric intake as fat, vs. 12% in controls) coupled with a low dose of streptozotocin (STZ) on day 14 (Reed et al., 2000; Sawant et al., 2003). Although the etiology leading to obesity and T2D in these different models varies considerably and includes both genetically determined and environmentally induced factors, the phenotypes are strikingly similar and include hyperglycemia, hyperlipidemia, and insulin resistance. The high-fat/STZ model may prove to be superior to the genetic models as it more closely reflects the pathogenesis and metabolic characteristics of the human syndrome.

These models have been mostly utilized to explore therapeutic approaches or to study pathophysiological changes associated with the disease. It is rarely reported that these models have been used to address specific questions related to mechanisms of the toxic response of antidiabetic drugs. One exception are the glitazones (thiazolidinediones), which are insulin sensitizing drugs that mostly exert their therapeutic effects via binding to and activating PPARγ. Interestingly, the long-term effects seen with some glitazones given to diabetic animals are strikingly different from those seen in healthy lean controls, suggesting that the underlying pathophysiological alterations (biochemical changes, altered gene expression) associated with the diabetic phenotype may predispose the animals to develop these toxic responses. The hepatic changes induced by glitazones in several unrelated strains of diabetic mice include increases in liver weight, degeneration of hepatocytes, dramatic changes in lipid metabolism, and severe hepatic microvesicular steatosis (Weinstock et al., 1997; Sharyo et al., 2001; Boelsterli and Bedouca, 2002; Watkins et al., 2002). In one mouse model of T2D, rosiglitazone even induced mild increases (2-fold over controls) in plasma aminotransferase activities (Watkins et al., 2002). Whether this is only due to greatly upregulated PPARγ in the liver of these diabetic animals, or whether other factors (e.g., mitochondrial changes) contribute to these selective toxic responses, remains to be investigated. Some of these findings obtained with animal models of T2D are summarized in Table 2.

Interestingly, in a study that compared the mitochondrial toxicity of two glitazones (troglitazone and rosiglitazone) in human hepatocytes isolated either from normal control liver or from diabetic donors, a clear difference was seen between the healthy and the diseased liver. While normal hepatocytes tolerated troglitazone concentrations up to 100 μM without detectable mitochondrial effects, hepatocytes from one diabetic donor exhibited decreases in the mitochondrial membrane potential upon exposure to 50 μM and higher (in a second diabetic donor this was not observed) (Haskins et al., 2001). Whether liver cells from humans with T2D indeed react differently to potentially toxic drugs remains to be determined.

Animal models of obesity and T2D have also provided insights into mechanisms underlying drug toxicity. For example, when db/db mice are treated with carbon tetrachloride, they initially produce centrilobular necrosis similar in extent to lean control animals treated with the same dose. What is different in normal and obese mice, however, is the biological response secondary to the insult, in particular the regenerative tissue repair. Compensatory hepatocyte regeneration is impaired in obese and diabetic mice (Yang et al., 2001). It has become clear that intact leptin/leptin receptor signaling is crucial in mediating the normal regenerative response through induction of cyclin D1 (at G1/S transition of the cell cycle) (Leclercq et al., 2003). So, ultimately, it can be the effective tissue remodeling which determines whether a mouse or rat will rapidly recover from the injury and survive or succumb to the insult. Apart from increased oxidative stress in the liver of these mice (due to hepatic steatosis and lipid peroxidation), it is the impaired tissue regeneration capacity that makes such animals more susceptible to hepatic injury. In view of the fact that a very large proportion of the human population exhibits moderate to severe hepatic steatosis in developed countries, ADRs in the liver should be studied in models of this disease.
ALTERNATIVE ANIMAL MODELS 
FEATURING SPECIFIC BIOCHEMICAL 
OR MOLECULAR ALTERATIONS

Because there is a multitude of different diseases, it would be extremely difficult, impractical and unwise to develop or utilize so many different models for each major therapeutic indication. However, if one or several key factors pertinent to groups of diseases are known, then one could design models that mimic those diseases in just one or a small number of these pivotal factors. For example, viral infections do sensitize cells and laboratory animals to prooxidant stress and to prooxidant drugs, and this is generally assumed to be based on increased generation of ROS and greatly decreased antioxidant defense lines, in particular decreased GSH levels. Consequently, a greatly simplified but valuable alternative to using virus-infected cells would be to use cellular models or in vivo models with compromised GSH redox status or depleted antioxidant defense systems. This could be achieved by very simple and established experimental designs, e.g., inhibition of GSH synthesis in vivo (by buthionine sulfoximine), or GST-catalyzed conjugation of a substrate (e.g., phorone) to GSH, resulting in rapid depletion of the GSH pool. Such approaches could even be tailored to specific needs, e.g., selective depletion of the cytosolic GSH pool, or depletion of both cytosolic and mitochondrial pools.

Another example of constructing simple animal models that feature one key determinant that plays a role in many different forms of diseases is the use of animal models or cellular models with mitochondrial abnormalities. Mitochondrial abnormalities, reflected phenotypically by low ATP production, increased ROS generation due to inefficient electron transport, and eventually an energy crisis, is a common feature in diseases including neurodegenerative diseases and T2D. Cellular models or animal models with mitochondrial abnormalities include targeted disruption of specific genes coding for mitochondrial key proteins, or mitochondrial DNA mutations (mtDNA codes for 13 out of 80 subunits of the electron transport protein complexes I, III, IV and V) (Wallace, 2001; Inoue et al., 2002). Furthermore, in vivo models have been developed that feature an acquired mitochondrial alteration, e.g., carnitine deficiency (Spaniol et al., 2001). Such models certainly have a great potential for being used in drug

<table>
<thead>
<tr>
<th>Animal model</th>
<th>Drug effects seen in T2D animals (but not in normal controls)</th>
<th>Reference</th>
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<tbody>
<tr>
<td>db/db mice (abnormalities in leptin synthesis)</td>
<td>Rosiglitazone induced changes in expression pattern of many proteins involved in peroxisomal fatty acid β-oxidation. Impaired hepatocyte proliferation and tissue regeneration after CCl₄ injury due to altered cytokine production</td>
<td>(Edvardsson et al., 1999)</td>
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<td></td>
<td>(Yang et al., 2001)</td>
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<tr>
<td>db/db mice (abnormalities in leptin receptor signaling)</td>
<td>Troglitazone, rosiglitazone, or pioglitazone caused slight increases in plasma aminotransferase activities and slight to moderate fatty change in hepatocytes</td>
<td>(Sharyo et al., 2001)</td>
</tr>
<tr>
<td>KKA¥ mice (mutation in Ratl gene, overexpression of Agouti gene)</td>
<td>Pioglitazone caused liver hypertrophy and fatty degeneration. Troglitazone or rosiglitazone caused microvesicular steatosis in liver with upregulated PPARγ</td>
<td>(Weinstock et al. 1997)</td>
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<td></td>
<td>(Bedoucha et al., 2001)</td>
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<tr>
<td>A-ZIP/F-1 mice</td>
<td>Rosiglitazone increased hepatic triacylglycerides and exacerbated hepatic steatosis</td>
<td>(Chao et al., 2000)</td>
</tr>
<tr>
<td>Otsuka Long-Evans Tokushima Fatty (OLETF) rats</td>
<td>Troglitazone prevented fatty change in liver (no histological changes)</td>
<td>(Jia et al., 2000)</td>
</tr>
<tr>
<td>NZO × NON/F1 mice</td>
<td>Rosiglitazone or troglitazone increased hepatic lipids and increased the expression of fatty acid synthase and fatty acid binding protein. Plasma ALT was increased</td>
<td>(Watkins et al., 2002)</td>
</tr>
<tr>
<td>High fat diet (20% fat) plus low-dose streptozotocin (45 mg/kg) on day 14</td>
<td>CCl₄ hepatotoxicity was greatly increased in diabetic rats through inhibition of tissue repair</td>
<td>(Sawant et al., 2003)</td>
</tr>
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Disease models in drug safety assessment.

CONCLUSIONS AND OUTLOOK

A large number of animal models of human disease exist and are currently used by the pharmaceutical industry for proof-of-concept studies in discovery research (Thomas, 1997; Rudmann and Durham, 1999; Rudolph and Moehler, 1999). These models include rodent strains derived from spontaneous mutations, but also transgenics, as well as physiologically/biochemically-induced animals. Although these animal models do not always closely emulate the human situation in every aspect, they have proven valuable as it is in the very nature of a model that there are clear limitations and simplifications.

Review of the current literature has revealed that while animal models have been developed for studying the pathogenesis of a variety of human diseases, including inflammatory conditions, neurodegenerative diseases, viral infections, and T2D, such animal models have not been applied in drug safety assessment. Exceptions are models of arthritis and endotoxemia, as well as models of obesity and diabetes, which are being increasingly utilized to address specific toxicological questions.

The use of animal models of human disease clearly offers some distinct advantages, as outlined above. On the other hand, adoption of these unconventional models for toxicology studies also bears some disadvantages, and it is probably some of these obvious pitfalls that have hindered toxicologists in using such models in preclinical safety assessment on a broader scale. Some of these points are summarized in Table 3. Importantly, while such models allow for recognizing possible hazards, and while the abnormalities associated with the particular model might set the stage and lower the threshold for a drug’s adverse effects, it is difficult to distinguish between truly relevant effects and “false positive” findings.

Finally, another advantage of the use of animal models of human disease in drug development is that both drug efficacy and drug toxicity can be studied in the same model, and efforts in improving the therapeutic index can be directly monitored. An example that illustrates this point is the evaluation of efficacy and toxicity of a number of antifungal drugs in mouse models of fungal infections (Stevens, 1996).

Taken together, animal models of human disease, designed or selected according to the specific type of drug to be tested, will be optimal substrates for toxicity studies both in screening and research. The ultimate goal of such novel and unconventional approaches is, apart from facilitated candidate selection, a means to better predict possible drug hazards to humans. Clearly, there is a need for extensive validation of these non-classical models with well-known compounds before new drugs can be tested. However, in combination with the new molecular technologies including the -omics techniques, such animal models of human disease may facilitate identification of novel mechanisms and the development of new biomarkers.

REFERENCES


Barbaro, G., Di Lorenzo, G., Asti, A., Ribersani, M., Belloni, G., Grisorio, B., Filice, G. and

Table 3. Advantages and disadvantages of including animal models of human disease in drug safety assessment.

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td>Improved sensitivity</td>
<td>False positive data</td>
</tr>
<tr>
<td>Closer to human situation (ailings vs. healthy population)</td>
<td>Lack of historical data</td>
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<tr>
<td>Model for screening</td>
<td>Additional costs/time</td>
</tr>
<tr>
<td>Provides biomarkers</td>
<td>Generation of pathology data that are neither required nor desired</td>
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<tr>
<td>Better understanding of mechanisms</td>
<td>Loss of efficacious drug</td>
</tr>
<tr>
<td>Explain clinical findings</td>
<td>Model may not be available at time of drug development</td>
</tr>
<tr>
<td>Improve candidate selection</td>
<td></td>
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<tr>
<td>Early recognition of hazard</td>
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<td>Therapeutic index can be directly estimated in the same animal model</td>
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</tbody>
</table>
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