ELSEVIER

The heterozygous *Sod2*$^{+/-}$ mouse: modeling the mitochondrial role in drug toxicity

Urs A. Boelsterli and Chin-Ju J. Hsiao

University of Connecticut, School of Pharmacy, Department of Pharmaceutical Sciences, Storrs, CT 06269-3092, United States

Mitochondria have been increasingly implicated in being a crucial subcellular target and amplifying oxidative injury induced by many drugs. Among the major cytoprotective antioxidants is the mitochondrial matrix protein, superoxide dismutase-2 (SOD2). Genetic modification of the expression of SOD2 by transgenic techniques or gene silencing has generated a number of distinct animal models with SOD2 deficiency including the heterozygous *Sod2*$^{+/-}$ knockout mouse model. These mice display a discreet underlying mitochondrial stress but are otherwise phenotypically normal and thus model a variety of clinically silent mitochondrial abnormalities. The model has found application in oxidative stress and age-related research, but it is only recently that it has been successfully used to study mechanisms of idiosyncratic drug-induced liver injury.

Introduction: role of mitochondria in drug toxicity

Mitochondrial dysfunction is a frequent off-target effect of a large number of drugs, a well-known finding that has gained renewed appreciation in recent years [1–6]. The most sensitive organs of mitochondrial toxicity are cardiac muscle and the central nervous system, but skeletal muscle, liver, and kidney can also be affected. These adverse drug effects are, however, not always obvious in preclinical studies, as most cells harbor hundreds or thousands of individual mitochondria and, until the majority of mitochondria are affected, the effects do not readily become apparent as tissue dysfunction or overt pathologic manifestations *in vivo*. By contrast, this mitochondrial hazard can be more readily detected in cell culture models where a clear concentration-dependent toxicity has been demonstrated for many drugs [7,8].

Traditionally, mitochondrial dysfunction has been associated with impaired bioenergy production. This may, however, not be the predominant mode of action that leads to toxicity. For example, cells can activate the cytosolic glycolytic pathway to compensate for insufficient mitochondrial oxidative phosphorylation (OXPHOS). Furthermore, there is a threshold for insufficient ATP generation below which a given tissue does not develop obvious pathology from bioenergy impairment [9]. Therefore, another equally important mode of action is based on the fact that mitochondria are not only targeted by drugs but that they mediate chemically induced lethal cell injury. One of the key mechanisms is drug-associated increased generation of reactive oxygen species (ROS) in mitochondria. Not only are mitochondria the cell's major source of ROS under physiological conditions but certain drugs can greatly increase the steady state levels of mitochondrial ROS by a number of distinct mechanisms [10,11]. This increased oxidant stress has dual consequences; it can directly oxidize sensitive molecular targets including mitochondrial proteins or mtDNA, leading to abnormal function, and/or it is involved in signaling, initiating highly regulated death-inducing cascades such as the opening of the mitochondrial permeability transition (mPT) pore and/or the induction of mitochondrial outer membrane permeabilization (MOMP), thus triggering apoptosis and/or necrosis by caspase-dependent or caspase-independent pathways [12–14]. Again, depending on the extent and duration of exposure, this may not necessarily become manifested as toxicity in preclinical animal models, as there are normally a host of antioxidant defense mechanisms in place. It is possible, however, that these mechanisms may become clinically relevant in certain susceptible individuals with compromised antioxidant defense systems, or in patients featuring an overproduction of mtROS owing to genetic or acquired abnormalities [15].

Corresponding author: Boelsterli, U.A. (urs.boelsterli@uconn.edu)

1359-6446/06/$ - see front matter doi:10.1016/j.drudis.2008.08.002

In view of the growing importance of the role of mitochondria in drug toxicity, recent attempts in drug discovery and development have aimed at recognizing this hazard and developing strategies to significantly reduce the attrition due to mitochondrial toxicity [16,17]. While drug-induced impairment of mitochondrial function can be effectively studied in isolated mitochondria or cell cultures, there are, however, few animal models available that lend themselves as predictive tools to detect, understand, and predict drug-induced mitochondrial toxicity.

Animal models of drug-induced oxidant stress and mitochondrial injury

There are three types of animal models that have been used to study the role of mitochondrial dysfunction in organ damage [18]. Firstly, in certain cases, it has been possible to use standard drugs and induce an organ-selective toxic response in rodents that is clearly due to mitochondrial injury. Well-known examples include high-dose acetaminophen-induced hepatic necrosis in sensitive mouse strains [19–21] or doxorubicin-induced cardiac toxicity in rats [22]. Besides these isolated cases, however, normal healthy laboratory animals are largely refractory to the apparent mitochondrial toxicity of most drugs, at least at therapeutically relevant doses [23].

Secondly, certain underlying diseases in animals can compromise mitochondrial function and prime the host to developing pathological manifestations. For example, diabetes mellitus or advanced age has been associated with increased oxidative damage to mtDNA or proteins of the electron transport chain (ETC) complexes [24–26]. While such models have been widely used for diabetes-related or age-related research, they have, however, not found any appreciable application in drug toxicology.

Finally, a number of animal models have been developed in which mutations of genes coding for mitochondrial proteins have been introduced to make these animals more sensitive to mitochondria-mediated injury. Because the assembly of fully functional mitochondria requires the concerted action of both

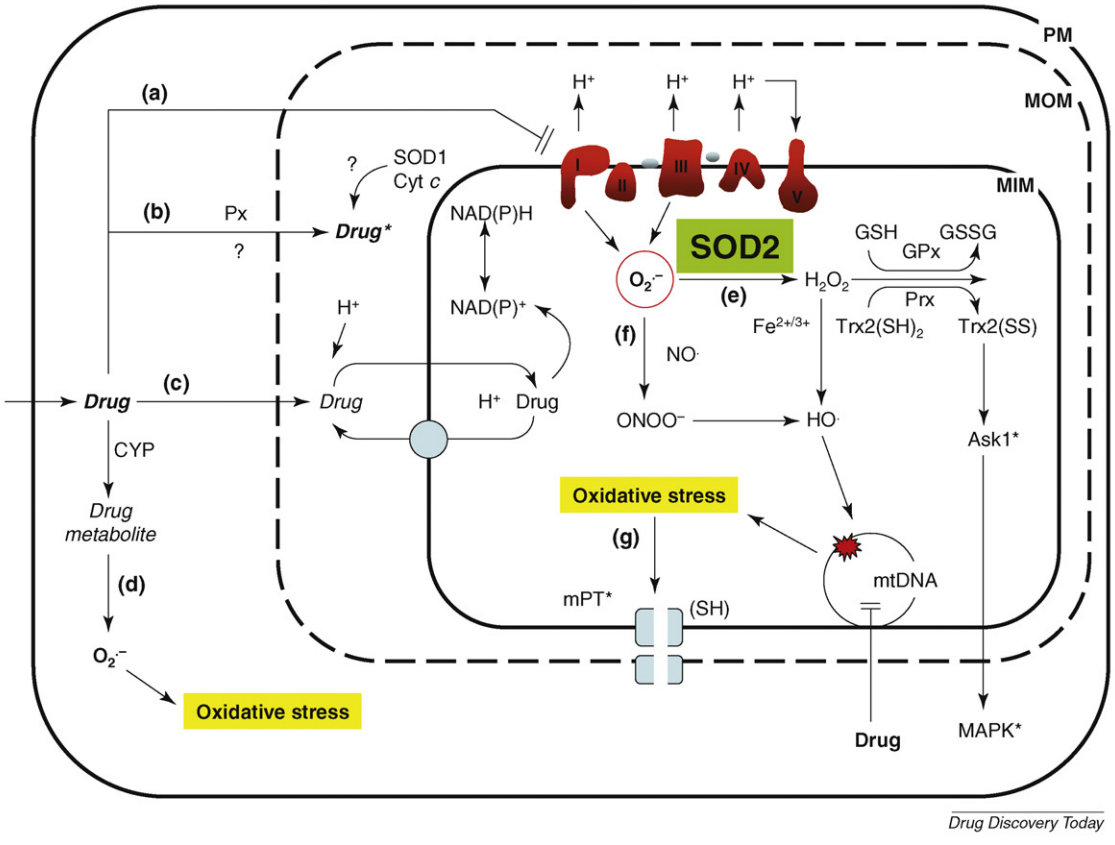

FIGURE 1

Drug-induced mitochondrial oxidant stress and key role of SOD2 in regulating the mitochondrial redox state. First, drugs can enhance oxidative stress by a number of mechanisms including **(a)** inhibition of components of the ETC, particularly complex I, **(b)** putative peroxidase-mediated bioactivation to drug radicals, **(c)** uncoupling of OXPHOS, thereby increasing respiration and depleting the mitochondrial reducing equivalents, and **(d)** redox cycling of drug metabolites. Second, superoxide can be generated in the mitochondrial matrix primarily through reduction of molecular oxygen at complex I and III; downstream consequences include **(e)** dismutation of superoxide to hydrogen peroxide, which, in the presence of catalytic amounts of iron can react to the extremely reactive hydroxyl radical, if hydrogen peroxide escapes removal by glutathione peroxidase (GPx); **(f)** in the presence of nitric oxide, conversion of superoxide to the highly toxic peroxynitrite, which in turn can form hydroxyl radicals and damage mtDNA, or cause the release of cytochrome *c*; **(g)** enhanced oxidative stress can also directly oxidize sulfhydryl groups of the mPT megapore, leading to opening of the pore and release of death proteins. *Third*, oxidative stress can also shift the redox state of thioredoxin-2 (Trx2) to the oxidized state, which releases Ask1 and activates a crucial MAPK cascade, eventually leading to cell death. *, Activation; PM, plasma membrane; Prx, peroxyredoxin; MOM, mitochondrial outer membrane; MIM, mitochondrial inner membrane; mPT, mitochondrial permeability transition.

mtDNA and nuclear DNA, different techniques were used to generate these models; while conventional knockout or knockin models were applied for the nuclear-encoded genes [27], novel techniques to introduce the mutations into the female mouse germ line had to be used for mitochondrial genes [28–30]. Among the panel of knockout mice where different antioxidant enzymes were targeted by recombinant deletion (e.g. *GPx1*, *Trx2*), superoxide dismutase (*Sod*)-deficient mice are one of the most widely used models. This review will focus on the characterization of the *Sod2*-deficient mouse model and, in particular, discuss recent advances of the heterozygous $Sod2^{+/-}$ model for studying molecular mechanisms and predicting organ-selective drug toxicity.

Mouse models with altered expression of SOD2

Superoxide dismutase-2

Superoxide dismutase (SOD) is an important enzyme that converts two superoxide molecules to hydrogen peroxide and molecular oxygen by dismutation (e.g. one molecule of $O_2^{\bullet}-$ is oxidized while the second one is reduced). There are several forms of this protein in mammals, SOD1, 2, and 3. SOD1 (Cu, ZnSOD) is abundant in the cytosol and in the intermembrane space of mitochondria [31]. In mitochondria, SOD1 has recently been shown to cooperate with cytochrome *c* to trigger apoptosis upon leaking out into the cytosol [32]. Besides being a sink for superoxide, SOD1 also possesses inherent non-specific peroxidase activity and may thus peroxidize compounds [33]. SOD3 is a non-inducible extracellular form.

SOD2 (MnSOD) resides in the mitochondrial matrix where it controls the cellular redox environment and regulates superoxide and hydrogen peroxide signaling [34]. SOD2 is a homotetramer containing one manganese ion in each subunit. The distribution of SOD2 across organs reflects energy consumption rates; therefore, it is abundant in heart, brain, liver, and kidney [35].

Figure 1 summarizes the key role of SOD2 in regulating cellular redox processes. If SOD2 is deficient, there will be increased steady state levels of mitochondrial superoxide anion radical. Superoxide can either react with molecular targets or combine with nitric oxide to form the highly reactive peroxynitrite, which can nitrate crucial proteins or be degraded to the extremely toxic hydroxyl radical. The pivotal role of SOD2 as a cytoprotective enzyme is illustrated by the findings that knockout mice deficient in *Sod2* do not survive, while, by contrast, *Sod1*-null or *Sod3*-null mice are viable. This shows that the presence of the mitochondrial SOD form is crucial for cell homeostasis. In the presence of certain mitochondria-targeting drugs, however, the physiological redox balance can be severely disrupted (Figure 1).

Because of the key role of SOD2, mouse models have been generated in which the *Sod2* gene is either overexpressed (by transgenic techniques) or fully or partially deleted (by knockout or gene silencing techniques) [36]. These animal models, however, have not yet found wide application in drug discovery and development.

Overexpression of SOD2

In the majority of transgenic mouse models that overexpress SOD2, this condition successfully protected against mitochondrial injury induced by drugs including doxorubicin [37], oxidative challenge from ischemia/reperfusion [38] or stress from diabetes [39]. SOD2 overexpression was associated with decreased superoxide net levels and increased mitochondrial tolerance in several tissues (as expected), but superoxide-mediated regulation of uncoupling protein and mitochondrial ATP production rates were not affected [40]. Importantly, overexpression of SOD2 does not necessarily lead to increases in H_2O_2 production, although this is often assumed [41]. Instead, the net production of H_2O_2 depends on the presence of other determinants of the cellular redox state; for example, if a reductant of superoxide is abundant (e.g. aconitase), additional SOD will decrease the yield of H_2O_2 [42]. Therefore, too much SOD2 is not always beneficial. In fact, overexpression of SOD2 did not afford a survival advantage against intrinsic irradiation exposure of the lung [43], and there were no significant differences in the amount of increased ROS produced during muscle contraction between wild-type and SOD2-overexpressing mice [44]. Collectively, although the available data suggest that transgenic mouse models overexpressing

TABLE 1

Animal models of *Sod2* deficiency (recombinant deletion or gene silencing)

Model	Reference
1. Mouse *Sod2* gene knockout, $Sod2^{-/-m1BCM}$ C57BL/6x129 mixed background; Phenotype: mice die around postnatal day 21	[46]
2. Mouse *Sod2* gene knockout, $Sod2^{-/-tm1Cje}$	[47]
(a) C57BL/6J background; Phenotype: fetal form of dilated cardiomyopathy, die around day 15 of gestation *in utero*	
(b) DBA/2J background; Phenotype: metabolic acidosis, mice die around day 8 postnatally	[47]
(c) F1 mice from both backgrounds; Phenotype: mice survive three weeks	[75]
3. Mouse (C57BL/6CrSlc)—tissue specific (liver) knockout (via a conditional knockout allele using Cre-loxP recombinase system; liver-specific *Sod2* knockouts were generated with liver-specific Cre recombinase in the transgene)—no obvious morphological or biochemical alterations in liver despite deletion of *Sod2*	[52]
4. Mouse (C57BL/6 x 129/Ola)—tissue-specific (epidermis) knockout (using Cre-loxP recombinase system; human keratin 14 promoter-driven Cre expression)—$Sod2^{+/-}$ mice exhibited myocard injury, mitochondrial swelling, lipid droplets, signs of oxidative stress and apoptosis in the heart	[76]
5. Mouse knockdown of *Sod2* (via Pol II-expressed shRNA) in C57BL/6J; Phenotype: slow growth, fatty liver, dilated cardiomyopathy, premature death	[53]
6. Heterozygous *Sod2* gene knockout, $Sod2^{+/-}$ (different backgrounds); Phenotype: 50% of wild-type SOD activity; grossly normal (normal body weight, normal hepatic GSPx, catalase, GSH, and GSSG levels); decreased $\Delta\Psi_m$, reduced complex I and aconitase activities; decrease in state 3 respiration; increased basal levels of protein carbonyl in mt protein and 8-OH-deoxyguanosine in mtDNA; increased sensitivity to mPT-inducing agents	[36,55–57, 60,61,67,77]

SOD2 are a valuable tool to investigate the role of superoxide and the downstream consequences of oxidative stress, the overall cellular antioxidant/prooxidant balance needs to be carefully evaluated.

SOD2 deficiency

Mitochondrial SOD2 is encoded by the nuclear *Sod2* gene and biosynthesized outside mitochondria; the presence of a targeting sequence then directs the transport of the protein across the mitochondrial membranes and import into the matrix. To study the physiological role of this enzyme, a number of distinct lines of *Sod2* knockout mice were generated on different genetic backgrounds and with different techniques (Table 1). For example, $Sod2^{tm1Cje}/Sod2^{tm1Cje}$ mice, generated on a C57BL/6 background, died *in utero* after developing embryonic dilated cardiomyopathy [45]. $Sod2^{m1BCM}/Sod2^{1BCM}$ mice (where exons 1 and 2 were deleted) survived up to three weeks after birth [46]. They exhibited a specific pathogenic phenotype including neurodegenerative changes, motor disturbances, and enlarged and dilated hearts, all due to mitochondrial injury in cardiomyocytes and neurons. In addition, they developed fatty infiltration in the liver. The second line, $Sod2^{m1UCSF}/Sod2^{m1UCSF}$ (where exon 3 was deleted) exhibited a slightly different phenotype; the mice died already five days after birth [47]. They developed massive dilated cardiomyopathy and hepatic steatosis, and exhibited severe mitochondrial defects [48]. This latter strain, however, was generated on another background strain, CD1 mice, while the first line was generated on a mixed C57BL/6x129 background.

The differential survival time of the *Sod2*-null mice suggests that the genetic background is important and that there exist genetic modifiers. For example, the degree of mitochondrial oxidative damage caused by SOD2 deficiency is higher in C57BL/6 mice than in other strains (e.g. DBA/2 mice). Recently, it was found that C57BL/6 mice have a genetic defect in the gene coding for nicotinamide nucleotide transhydrogenase (NNT) [49]. NNT is a transmembrane protein in the inner mitochondrial membrane that functions as a proton-pumping transhydrogenase (pumping protons into the matrix while regenerating NADPH from NADH and $NAPD^+$). NADPH is used in the matrix to reduce glutathione disulfide (GSSG) and Trx2-SS to glutathione (GSH) and Trx2-$(SH)_2$, respectively. This may explain why C57BL/6J mice are more sensitive to oxidant stress than other mice, because these mice cannot sufficiently regenerate mitochondrial NADPH, especially under conditions of increased mitochondrial oxidant stress.

Taken together, *Sod2*-null mice represent a model of substantial endogenous oxidative stress that becomes obvious in a number of organs. For example, in the cerebral cortex, the oxidant stress was sufficient to cause defects in aconitase, complex I, II, III, and IV [50]. One reason for this sensitivity is that all these enzymes contain iron–sulfur clusters and are therefore particularly sensitive to oxidative injury. Accordingly, treatment of *Sod2*-null mice with the antioxidant and SOD mimetic, EUK189, attenuated the oxidative damage [51].

Because a homozygous deficiency in *Sod2* is linked with prenatal or early postnatal lethality in mice, this model cannot be utilized in pathophysiological studies in adult mice. A number of alternatives were therefore developed. As a first alternative approach, a liver-specific *Sod2* conditional knockout mouse was designed using the Cre-loxP system (under the control of the rat albumin promoter) [52]. These mice were viable and, although they lacked SOD2 in the hepatic parenchyma, did not exhibit any obvious morphological or biochemical abnormalities in the liver, suggesting that SOD2 is not essential for liver function in mice. Furthermore, these findings confirmed the notion that the liver may have a high degree of tolerance or resistance against oxidative stress.

Second, a transgenic mouse line was designed in which a polymerase II promoter drove the expression of a short hairpin (sh) RNA, efficiently knocking down the target gene, *Sod2* [53]. These mice were viable but had a deficiency of SOD2, coupled with increased oxidant stress and enhanced sensitivity to prooxidants. For example, fibroblasts from the transgenic mouse line showed greater sensitivity to *t*-butyl hydroperoxide than those from wild-type mice. This elegant approach allows for a more subtle modulation of SOD2 expression without causing too much damage that leads to lethality as seen in the null mice.

Finally, an alternative approach to using homozygously *Sod2*-deficient mice is to use heterozygous animals ($Sod2^{+/-}$). Heterozygous knockouts lend themselves as a potentially useful animal model to study drug-induced mitochondrial effects and oxidant stress because the phenotypical changes in these mice are much more subtle than in a homozygous knockout mouse.

The heterozygous $Sod2^{+/-}$ mouse model

Heterozygous animals have recently been recognized to provide alternative and novel genetic mouse models beyond knockouts [54]. As compared with the null-mice, heterozygous animals not only can display intermediate phenotypes but they can also exhibit principally new phenotypes. For *Sod2*, heterozygous deficiency leads to a much more discreet phenotype than that of the homozygously deficient mice. Mutant $Sod2^{+/-}$ mice proved to have a similar body weight and growth rate as wild-type controls and exhibited identical GSH and GSSG levels; furthermore, they had unchanged glutathione peroxidase, catalase, and SOD1 levels in liver and other organs (except in skeletal muscle) [46,55]. These mice exhibit, however, increased baseline levels of mitochondrial oxidative stress that results in age-related decline of mitochondrial function [56]. Furthermore, the heterozygous phenotype was shown to exhibit decreased aconitase activity, increased protein carbonyl levels, and a decrease in the respiratory control ratio, all indicative of underlying oxidative stress and mitochondrial functional damage [57]. This may become obvious in certain sensitive organs; for example, heterozygously deficient mice exhibited increased susceptibility to spontaneous seizures, which may reflect compromised neuronal mitochondrial function [58].

While this mouse model has found some application in experimental gerontology and neurodegenerative disease research, its use in toxicology has still been limited. A number of recent studies suggest, however, that the $Sod2^{+/-}$ mouse model could become a valuable tool in predictive drug safety studies in the future.

Application of the heterozygous $Sod2^{+/-}$ mouse model in drug safety studies

Sod2-transgenic and mutant mice have been used in the past to study the effects of increased ROS production [36,51]. For example, 50% deficiency in SOD2 exacerbates oxidant stress-dependent cerebral infarction following ischemia [59]. By implication, drugs or other chemicals that generate increased oxidant stress would

have a much greater effect in the heterozygous *Sod2*$^{+/-}$ mouse model than in wild-type mice. Indeed, the heterozygous phenotype primed liver mitochondria to the prooxidant effects of *t*-butyl hydroperoxide, which resulted in an increased rate of induction of the permeability transition *in vitro*. Cardiomyocytes or isolated heart mitochondria from *Sod2*$^{+/-}$ mice were equally sensitized to oxidant stress induced by *t*-butyl hydroperoxide [60]. Similar potentiating effects were found *in vivo*; administration of paraquat, a redox cycling agent that produces oxidant stress, greatly increased mortality in heterozygous *Sod2*$^{+/-}$ mice or double knockout mice (*Sod2*$^{+/-}$; *GPx1*$^{-/-}$) as compared with wild-type controls [61,62]. In another model of SOD deficiency (caused by deletion of the *Tme* locus, which results in the removal of the coding region for SOD2) [63], treatment of *Tme*-deficient mice with the neurotoxicant 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) at low doses (15 mg/kg) caused massive brain damage in these animals by three weeks, while higher doses (100 mg/kg) were lethal in the mutant mice, but not in wild-type controls. Because the metabolite of MPTP selectively accumulates in certain dopaminergic neurons in the brain, these findings suggests that pharmacokinetic factors may dictate the organotropic effects of a chemical in this model. This led us to speculate that this may hold true for other organs and that the latent toxicity of drugs that are targeted and bioactivated in the liver may be exacerbated and unmasked by partial SOD2 deficiency.

Modeling drug-induced liver injury

Clinically relevant drugs that cause liver injury in humans principally fall into two categories, that is, drugs whose toxicity is predictable, clearly dose-dependent, and that would occur in the vast majority of patients if exposed to high doses (e.g. acetaminophen), and drugs whose toxicity is unpredictable, occurring in a small number of susceptible patients only, and clearly depending on host factors (the majority of idiosyncratic hepatotoxicants). The question arises why the liability of those drugs that cause idiosyncratic drug-induced liver injury (I-DILI) cannot be predicted from animal models. It seems, however, that in reality the current animal models are predictable in the sense that both normal healthy rats or mice and the vast majority of patients will not exhibit liver injury. The fact that only very few individuals are susceptible and develop overt injury suggests that they may possess a genetic or acquired abnormality in factors either favoring pro-toxicant action or compromising antioxidant defense [64,65]. Because many drugs that have been incriminated in causing I-DILI target mitochondria and induce mitochondrial injury or cell demise through mitochondrial pathways, we hypothesized that underlying mitochondrial abnormalities may be a key determinant of susceptibility in patients. Specific mitochondrial abnormalities, however, have not yet been identified.

On the basis of the above hypothesis, the *Sod2*$^{+/-}$ mouse would be ideally suited to model a diverse array of conditions of discreet and clinically silent yet potentially significant mitochondrial abnormalities in humans. The reasons are several-fold and include the consideration that many mitochondrial changes (e.g. abnormalities in the ETC protein subunits, and/or defects in complex assembly) would merge in a similar phenotype, that is, increased mitochondrial oxidant stress [11,66]. This is mimicked in the liver and other organs of *Sod2*$^{+/-}$ mice, which exhibit a slightly compromised antioxidant defense system. We reasoned that administration to *Sod2*$^{+/-}$ mice of certain drugs that cause I-DILI and that have been associated with oxidant stress and mitochondrial liability would potentiate the oxidant stress and unmask liver injury. Because oxidative damage to mitochondria, resulting in increasing heteroplasmy (a mixture of both normal and damaged mitochondria in a cell) is a cumulative effect reaching a crucial threshold, the mice were exposed to relatively low and clinically relevant doses of the drugs, but treated for a prolonged period of time (up to four weeks), to emulate the clinical situation. Indeed, administration of the non-steroidal anti-inflammatory drug nimesulide (10 mg/kg/day, ip, *bid*) for four weeks resulted in clear hepatic mitochondrial injury and oxidative damage to *Sod2*$^{+/-}$ mice, but not wild-type control mice [67]. Similarly, treatment of heterozygous *Sod2*$^{+/-}$ mice with the insulin sensitizer and antidiabetic drug troglitazone (30 mg/kg/day, ip) resulted in hepatic mitochondrial oxidative damage and the development of overt hepatic necrosis, while *Sod2*$^{+/+}$ control mice were refractory to even much higher doses of troglitazone [68]. A proteomics analysis revealed that liver injury proceeded as a two-stage process; first, the drug induced an adaptive response (after two-week treatment), which was followed by a clearly toxic response (after four-week treatment) [69]. A toxicokinetic analysis [70] revealed that the overall exposure to troglitazone in *Sod2*$^{+/-}$ mice was similar to the reported kinetic data in patients treated with troglitazone. Thus, the heterozygous *Sod2*$^{+/-}$ mouse for the first time provides a model to reproduce liver injury induced by certain drugs that have caused I-DILI in patients that would be extremely difficult to induce in normal healthy laboratory rodents. The model also lends itself to studying at the molecular level the pathogenesis of drug-induced mitochondrial injury and liver damage *in vivo*.

Genetic abnormalities of SOD2 in humans

Although the potential use of the *Sod2*$^{+/-}$ mouse goes far beyond merely emulating human abnormalities in SOD2 function, genetic abnormalities in *SOD2* have been identified. For example, there exist several *SOD2* polymorphisms in human populations [71]. For example, a genetic dimorphism (cytosine or thymine at position 1183 of the *SOD2* gene, encoding for either alanine (C) or valine (T), respectively, in the mitochondrial target sequence of SOD2) was found. This mutation modulates the import of the SOD2 protein into mitochondria [72]. Specifically, the presence of valine in the SOD2 protein hampers mitochondrial import, while the presence of alanine ensures efficacious transport into the matrix. The frequency distribution is about 1:1 in Caucasian populations. To determine whether the polymorphism could be one of the susceptibility factors in drug-induced liver injury, patient genotypes were correlated with the incidence of cirrhosis in alcoholic patients [73]. The authors found that the presence of at least 1 alanine allele increases the risk for developing cirrhosis and increased the rate of hepatocellular carcinoma. Although paradoxical at first, this increased SOD2 activity in the mitochondrial matrix was coupled to decreased GPx activity in alcoholics, which may have caused higher levels of hydrogen peroxide in the liver. Similarly, the presence of alanine (C/T or C/C form) increased the susceptibility to drug-induced liver injury, in particular anti-tuberculosis drugs [74]. Thus, the mutant C allele, and possibly other

SOD abnormalities, may contribute to increased susceptibility to I-DILI.

Other known abnormalities (in mitochondrial or nuclear genes coding for mitochondrial proteins) have not yet been correlated with increased susceptibility to I-DILI.

Conclusions

Murine models in which SOD2 has been modified by transgenic techniques, conditional knockout, or gene silencing approaches not only lend themselves for the study of mitochondrial oxidant stress, disease, or aging but also have been increasingly recognized as potential models that modulate drug-induced organ toxicity. Potential new applications of this paradigm will undoubtedly go beyond hepatotoxicity and include other organs such as the heart, brain, or skeletal muscle, but currently I-DILI is in the focus because unpredictable liver liability is one of the major causes of drug withdrawal from the market or discontinuation of development of a drug candidate. An additional application is the isolation and culture of primary hepatocytes from *Sod2*$^{+/-}$ mice. Comparison of the toxic response in these mutant cells has revealed that *Sod2*$^{+/-}$ hepatocytes respond differentially to certain drugs than their wild-type counterparts [68]. Clearly, more drugs that have caused I-DILI in patients, as well as 'negative comparators', will have to be tested in the heterozygous *Sod2*$^{+/-}$ mouse model before any firm conclusions about its suitability as a potentially new tool that would help predict liver injury of new drugs can be made.

Acknowledgements

This work was supported by the Boehringer Ingelheim Endowed Chair in Mechanistic Toxicology at the University of Connecticut and a research grant from Pfizer, Inc.

References

1 Wallace, K.B. and Starkov, A.A. (2000) Mitochondrial targets of drug toxicity. *Annu. Rev. Pharmacol. Toxicol.* 40, 353–388
2 Amacher, D.E. (2005) Drug-associated mitochondrial toxicity and its detection. *Curr. Med. Chem.* 12, 1829–1839
3 Chan, K. *et al.* (2005) Drug-induced mitochondrial toxicity. *Expert Opin. Drug Metab. Toxicol.* 1, 655–669
4 Kass, G.E.N. (2006) Mitochondrial involvement in drug-induced hepatic injury. *Chem. Biol. Interact.* 163, 145–159
5 Scatena, R. *et al.* (2007) The role of mitochondria in pharmacotoxicology: a re-evaluation of an old, newly emerging topic. *Am. J. Physiol. (Cell Physiol.)* 293, C12–C21
6 Wallace, K.B. (2008) Mitochondrial off targets of drug therapy. *Trends Pharmacol. Sci.* 29, 361–366
7 Wagner, B.K. *et al.* (2008) Large-scale chemical dissection of mitochondrial function. *Nat. Biotechnol.* 26, 343–351
8 Xu, J.J. *et al.* (2008) Cellular imaging predictions of clinical drug-induced liver injury. *Toxicol. Sci.* 105, 97–105
9 Rossignol, R. *et al.* (2003) Mitochondrial threshold effects. *Biochem. J.* 370, 751–762
10 Turrens, J.F. (2003) Mitochondrial formation of reactive oxygen species. *J. Physiol.* 552, 335–344
11 Orrenius, S. *et al.* (2007) Mitochondrial oxidative stress: implications for cell death. *Annu. Rev. Pharmacol. Toxicol.* 47, 143–183
12 Armstrong, J.S. (2006) The role of the mitochondrial permeability transition in cell death. *Mitochondrion* 6, 225–234
13 Chipuk, J.E. *et al.* (2006) Mitochondrial outer membrane permeabilization during apoptosis: the innocent bystander scenario. *Cell Death Different.* 13, 1396–1402
14 Kroemer, G. *et al.* (2007) Mitochondrial membrane permeabilization in cell death. *Physiol. Rev.* 87, 99–163
15 Boelsterli, U.A. and Lim, P.L.K. (2007) Mitochondrial abnormalities—a link to idiosyncratic drug hepatotoxicity? *Toxicol. Appl. Pharmacol.* 220, 92–107
16 Dykens, J.A. and Will, Y. (2007) The significance of mitochondrial toxicity testing in drug development. *Drug Discov. Today* 12, 777–785
17 Dykens, J.A. *et al.* (2007) Strategies to reduce late-stage drug attrition due to mitochondrial toxicity. *Expert Rev. Mol. Diagn.* 7, 161–175
18 Boelsterli, U.A. and Lee, Y.H. (2008) Development of animal models of drug-induced mitochondrial toxicity. In *Drug-Induced Mitochondrial Dysfunction* (Dykens, J.A. and Will, Y., eds), pp. 539–554, Wiley & Sons, Inc.
19 Gunawan, B. *et al.* (2006) c-Jun N-terminal kinase plays a major role in murine acetaminophen hepatotoxicity. *Gastroenterology* 131, 165–178
20 Latchoumycandane, C. *et al.* (2007) Mitochondrial protection by the JNK inhibitor leflunomide rescues mice from acetaminophen-induced liver injury. *Hepatology* 45, 412–421
21 Bajt, M.L. *et al.* (2008) Mitochondrial Bax translocation accelerates DNA fragmentation and cell necrosis in a murine model of acetaminophen hepatotoxicity. *J. Pharmacol. Exp. Ther.* 324, 8–14
22 Oliveira, P.J. and Wallace, K.B. (2006) Depletion of adenine nucleotide translocator protein in heart mitochondria from doxorubicin-treated rats—relevance for mitochondrial dysfunction. *Toxicology* 220, 160–168
23 Dixit, R. and Boelsterli, U.A. (2007) Healthy animals and animal models of human disease(s) in safety assessment of human pharmaceuticals, including therapeutic antibodies. *Drug Disc. Today* 12, 336–342
24 Suzuki, S. *et al.* (1999) Oxidative damage to mitochondrial DNA and its relationship to diabetic complications. *Diabetes Res. Clin. Pract.* 45, 161–168
25 Rolo, A.P. and Palmeira, C.M. (2006) Diabetes and mitochondrial function: role of hyperglycemia and oxidative stress. *Toxicol. Appl. Pharmacol.* 212, 167–178
26 Choksi, K.B. and Papaconstantinou, J. (2008) Age-related alterations in oxidatively damaged proteins of mouse heart mitochondrial electron transport chain complexes. *Free Radic. Biol. Med.* 44, 1795–1805
27 Wallace, D.C. (2001) Mouse models for mitochondrial disease. *Am. J. Med. Genet.* 106, 71–93
28 Nakada, K. *et al.* (2001) Mito-mice: animal models for mitochondrial DNA-based diseases. *Sem. Cell Dev. Biol.* 12, 459–465
29 Pinkert, C.A. and Trounce, I.A. (2002) Production of transmitochondrial mice. *Methods* 26, 348–357
30 Fan, W.W. *et al.* (2008) A mouse model of mitochondrial disease reveals germline selection against severe mtDNA mutations. *Science* 319, 958–962
31 Okado-Matsumoto, A. and Fridovich, I. (2001) Subcellular distribution of superoxide dismutase (SOD) in rat liver. Cu, Zn-SOD in mitochondria. *J. Biol. Chem.* 276, 38388–38393
32 Li, Q. *et al.* (2006) A possible cooperation of SOD1 and cytochrome *c* in mitochondria-dependent apoptosis. *Free Radic. Biol. Med.* 40, 173–181
33 Fridovich, I. (1995) Superoxide radical and superoxide dismutases. *Ann. Rev. Biochem.* 64, 97–112
34 Buettner, G.R. *et al.* (2006) A new paradigm: manganese superoxide dismutase influences the production of H_2O_2 in cells and thereby their biological state. *Free Radic. Biol. Med.* 41, 1338–1350
35 Beyer, W. *et al.* (1991) Superoxide dismutases. *Prog. Nucleic Acid Res. Mol. Biol.* 40, 221–253
36 Huang, T.T. *et al.* (1999) The use of transgenic and mutant mice to study oxygen free radical metabolism. *Ann. N.Y. Acad. Sci.* 893, 95–112
37 Chaiswing, L. *et al.* (2005) Manganese superoxide dismutase and inducible nitric oxide synthase modify early oxidative events in acute Adriamycin-induced mitochondrial toxicity. *Mol. Cancer Ther.* 4, 1056–1064
38 Jones, S.P. *et al.* (2003) Role of intracellular antioxidant enzymes after *in vivo* myocardial ischemia and reperfusion. *Am. J. Physiol. (Heart Circ. Physiol.)* 284, H277–H282
39 Goto, H. *et al.* (2008) Endothelial MnSOD overexpression prevents retinal VEGF expression in diabetic mice. *Biochem. Biophys. Res. Commun.* 366, 814–820
40 Silva, J.P. *et al.* (2005) SOD2 overexpression: enhanced mitochondrial tolerance but absence of effect on UCP activity. *EMBO J.* 24, 4061–4070
41 Liochev, S.I. and Fridovich, I. (2007) The effects of superoxide dismutase on H_2O_2 formation. *Free Radic. Biol. Med.* 42, 1465–1469
42 Gardner, R. *et al.* (2002) Why does SOD overexpression sometimes enhance, sometimes decrease, hydrogen peroxide production? A minimalist explanation. *Free Radic. Biol. Med.* 32, 1351–1357

43 Epperly, M.W. *et al.* (2001) Overexpression of manganese superoxide dismutase (MnSOD) in whole lung or alveolar type II cells of MnSOD transgenic mice does not provide intrinsic lung irradiation protection. *Int. J. Cancer (Radiat. Oncol. Invest.)* 96, 11–21

44 Vasilaki, A. *et al.* (2006) Genetic modification of the manganese superoxide dismutase/glutathione peroxidase 1 pathway influences intracellular ROS generation in quiescent, but not contracting, skeletal, muscle cells. *Free Radic. Biol. Med.* 41, 1719–1725

45 Huang, T.T. *et al.* (2001) Genetic modification of prenatal lethality and dilated cardiomyopathy in Mn superoxide dismutase mutant mice. *Free Radic. Biol. Med.* 31, 1101–1110

46 Lebovitz, R.M. *et al.* (1996) Neurodegeneration, myocardial injury, and perinatal death in mitochondrial superoxide dismutase-deficient mice. *Proc. Natl. Acad. Sci. U. S. A.* 93, 9782–9787

47 Li, Y. *et al.* (1995) Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. *Nat. Genet.* 11, 376–381

48 Melov, S. *et al.* (1999) Mitochondrial disease in superoxide dismutase 2 mutant mice. *Proc. Natl. Acad. Sci. U. S. A* 96, 846–851

49 Huang, T.T. *et al.* (2006) Genetic modifiers of the phenotype of mice deficient in mitochondrial superoxide dismutase. *Hum. Mol. Genet.* 15, 1187–1194

50 Hinerfeld, D. *et al.* (2004) Endogenous mitochondrial oxidative stress: neurodegeneration, proteomics analysis, specific respiratory chain defects, and efficacious antioxidant therapy in superoxide dismutase 2 null mice. *J. Neurochem.* 88, 657–667

51 Morten, K.J. *et al.* (2006) Mitochondrial reactive oxygen species in mice lacking superoxide dismutase 2. *J. Biol. Chem.* 281, 3354–3359

52 Ikegami, T. *et al.* (2002) Model mice for tissue-specific deletion of the manganese superoxide dismutase (MnSOD) gene. *Biochem. Biophys. Res. Commun.* 296, 729–736

53 Xia, X.G. *et al.* (2006) Pol II-expressed shRNA knocks down *Sod2* gene expression and causes phenotypes of the gene knockout in mice. *PLoS Genet.* 2, 73–80

54 Kalueff, A.V. *et al.* (2007) The developing use of heterozygous mutant mouse models in brain monoamine transporter research. *Trends Pharmacol. Sci.* 28, 122–127

55 Van Remmen, H. *et al.* (1999) Characterization of the antioxidant status of the heterozygous manganese superoxide dismutase knockout mouse. *Arch. Biochem. Biophys.* 363, 91–97

56 Kokoszka, J.E. *et al.* (2001) Increased mitochondrial oxidative stress in the Sod2+/− mouse results in the age-related decline of mitochondrial function culminating in increased apoptosis. *Proc. Natl. Acad. Sci. U. S. A.* 98, 2278–2283

57 Williams, M.D. *et al.* (1998) Increased oxidative damage is correlated to mitochondrial function in heterozygous manganese dismutase knockout mice. *J. Biol. Chem.* 273, 28510–28515

58 Liang, L.P. and Patel, M. (2004) Mitochondrial oxidative stress and increased seizure susceptibility in *Sod2*$^{-/+}$ mice. *Free Radic. Biol. Med.* 36, 542–554

59 Murakami, K. *et al.* (1998) Mitochondrial susceptibility to oxidative stress exacerbates cerebral infarction that follows permanent focal cerebral ischemia in mutant mice with manganese superoxide dismutase deficiency. *J. Neurosci.* 18, 205–213

60 Van Remmen, H. *et al.* (2001) Knockout mice heterozygous for *Sod2* show alterations in cardiac mitochondrial function and apoptosis. *Am. J. Physiol. (Heart Circ. Physiol.)* 281, H1422–H1432

61 Van Remmen, H. *et al.* (2003) Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging. *Physiol. Genomics* 16, 29–37

62 Van Remmen, H. *et al.* (2004) Multiple deficiencies in antioxidant enzymes in mice result in a compound increase in sensitivity to oxidative stress. *Free Radic. Biol. Med.* 36, 1625–1634

63 Cortopassi, G. and Wang, E. (1995) Modelling the effects of age-related mtDNA mutation accumulation: complex I deficiency, superoxide and cell death. *Biochim. Biophys. Acta* 1271, 171–176

64 Boelsterli, U.A. (2003) Idiosyncratic drug hepatotoxicity revisited: new insights from mechanistic toxicology. *Toxicol. Mech. Methods* 13, 3–20

65 Boelsterli, U.A. and Kashimshetty, R. (in press) Idiosyncratic drug-induced liver injury: mechanisms and susceptibility factors. In *Hepatic and Gastrointestinal Toxicology*, (vol. 10) (Roth, R.A. and Ganey, P.E., eds,) Elsevier

66 Adam-Vizi, V. and Chinopoulos, C. (2006) Bioenergetics and the formation of mitochondrial reactive oxygen species. *Trends Pharmacol. Sci.* 27, 639–645

67 Ong, M.M.K. *et al.* (2006) Nimesulide-induced hepatic mitochondrial injury in heterozygous *Sod2*$^{+/-}$ mice. *Free Radic. Biol. Med.* 40, 420–429

68 Ong, M.M.K. *et al.* (2007) Troglitazone-induced hepatic necrosis in an animal model of silent mitochondrial abnormalities. *Toxicol. Sci.* 97, 205–213

69 Lee, Y.H. *et al.* (2008) Troglitazone-induced hepatic mitochondrial proteome expression dynamics in heterozygous *Sod2*$^{+/-}$ mice: two-stage oxidative injury. *Toxicol. Appl. Pharmacol* 231, 43–51

70 New, L.S. *et al.* (2007) Pharmacokinetic study of intraperitoneally administered troglitazone in mice using UPLC/MS/MS. *Rapid Commun. Mass Spectrom.* 21, 982–988

71 Zelko, I.N. *et al.* (2002) Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. *Free Radic. Biol. Med.* 33, 337–349

72 Shimoda-Matsubayashi, S. *et al.* (1996) Structural dimorphism in the mitochondrial targeting sequence in the human manganese superoxide dismutase gene. A predictive evidence for conformational change to influence mitochondrial transport and a study of allelic association in Parkinson's disease. *Biochem. Biophys. Res. Commun.* 226, 561–565

73 Nahon, P. *et al.* (2005) Genetic dimorphism in superoxide dismutase and susceptibility to alcoholic cirrhosis, hepatocellular carcinoma, and death. *Clin. Gastroenterol. Hepatol.* 3, 292–298

74 Huang, Y.S. *et al.* (2007) Genetic polymorphisms of manganese superoxide dismutase, NAD(P)H:quinone oxidoreductase, glutathione *S*-transferase M1 and T1, and the susceptibility to drug-induced liver injury. *J. Hepatol.* 47, 128–134

75 Lynn, S. *et al.* (2005) Selective neuronal vulnerability and inadequate stress response in superoxide dismutase mutant mice. *Free Radic. Biol. Med.* 38, 817–828

76 Strassburger, M. *et al.* (2005) Heterozygous deficiency of manganese superoxide dismutase results in severe lipid peroxidation and spontaneous apoptosis in murine myocardium *in vivo*. *Free Radic. Biol. Med.* 38, 1458–1470

77 Lee, Y.H. *et al.* (2008) Proteomics profiling of hepatic mitochondria in heterozygous *Sod2*$^{+/-}$ mice, an animal model of discreet mitochondrial oxidant stress. *Proteomics* 8, 555–568