Idiosyncratic hepatotoxicity is a rare and unpredictable event of liver injury affecting generally less than 1 in 10,000 patients treated with certain drugs. However, it is a serious clinical problem as it accounts for 10% of all drug-induced liver failure cases (Kaplowitz, 2005). Since idiosyncratic drug reactions are not detected in preclinical testing and in most cases not even during clinical trials, the problem surfaces generally after the drug is approved and hundreds of thousands of patients are being treated. Idiosyncratic hepatotoxicities are currently the main cause for Food and Drug Administration-mandated warnings, restrictions of use or even withdrawals of drugs from the market (Kaplowitz, 2005). As such, this is a considerable problem for the pharmaceutical industry and for regulatory agencies worldwide. One of the recent examples of drugs causing idiosyncratic hepatotoxicity and liver failure was the antidiabetic drug Rezulin (troglitazone).

Troglitazone, a peroxisome proliferator–activated receptor gamma agonist, which enhances insulin sensitivity, was approved for the treatment of type 2 diabetes in 1997. Troglitazone was an effective antidiabetic drug with a fundamentally new mechanism of action. However, within a year after its widespread use, individual cases of liver injury and failure were reported (Watkins, 2005). The mounting evidence for the idiosyncratic hepatotoxicity of troglitazone in the following years and the development of rosiglitazone and pioglitazone, drugs with a similar mechanism of action but presumably without the liver liabilities, led to the withdrawal of troglitazone from the market in the year 2000. Since then, a considerable effort has been made to elucidate the mechanism of troglitazone-induced hepatotoxicity. A number of hypotheses were brought forward to explain troglitazone-induced cell injury including the formation and accumulation of toxic metabolites, mitochondrial dysfunction and oxidant stress, inhibition of the bile salt transporter and bile acid toxicity, and the induction of apoptosis (Choijker, 2005). However, virtually all the studies were performed with cultured cell lines using concentrations of troglitazone 1–2 orders of magnitude above pharmacological levels. Thus, these in vitro studies could not provide a relevant mechanistic explanation for troglitazone hepatotoxicity in patients (Choijker, 2005). Nevertheless, some of these experiments demonstrated that high concentrations of troglitazone can induce mitochondrial dysfunction in these cell lines (Haskins et al., 2001; Tirmenstein et al., 2002). Furthermore, troglitazone, but not rosiglitazone or pioglitazone, was able to induce the mitochondrial membrane permeability transition pore opening in isolated rat liver mitochondria (Masubuchi et al., 2006). Although these mechanisms appear not to be relevant for damage in normal hepatocytes in vivo, it raises the possibility that under certain conditions troglitazone has the potential to cause a mitochondrial stress.

The currently favored concept of idiosyncratic hepatotoxicity assumes that the injury is caused by a combination of certain genetic and environmental factors, which sufficiently enhance an individual’s susceptibility to otherwise clinically silent adverse effects of a drug (Watkins, 2005). This may more likely develop into a problem when the cellular stress caused by the drug superimposes with the stress of the disease to be treated (Boelsterli, 2003). For example, obesity, diabetes, and steatosis result in a chronic oxidant stress and mitochondrial dysfunction (Pessayre et al., 2001), which may enhance the toxicity of drugs that target mitochondria, e.g., acetaminophen (Jaeschke and Bajt, 2006). In fact, acetaminophen hepatotoxicity is significantly aggravated in obese and diabetic mice (Kon et al., 2005). Mitochondrial dysfunction is generally accompanied by enhanced reactive oxygen formation. A critical defense against mitochondrial oxidant stress is the mitochondrial enzyme manganese superoxide dismutase (MnSOD, SOD2). SOD2-deficient mice (SOD2−/−) die within a few days after birth underscoring the critical role of MnSOD.
importance of continuously detoxifying mitochondria-derived reactive oxygen species. However, mice partially deficient in SOD2 (SOD2+/−) are viable and initially without pathology. However, the lifelong reduction of mitochondrial SOD2 levels causes a gradual accumulation of mitochondrial and nuclear oxidative DNA damage, which results in a higher tumor rate compared to wild-type animals (VanRemmen et al., 2003). Thus, younger SOD2+/− mice can be considered a model of a chronic, subclinical mitochondrial stress.

Based on these previous observations, Ong et al. (2007) hypothesized that animals with a genetic defect in the mitochondrial antioxidant defense mechanisms may be susceptible to chronic exposure of troglitazone. To test their hypothesis, the authors treated wild type and SOD2+/− mice with 10 or 30 mg/kg troglitazone (ip) daily for up to 28 days. The higher dose in mice is equivalent to a human therapeutic dose. Consistent with previous studies in rodents and in primates (reviewed in: Haskins et al., 2001), troglitazone did not cause any measurable mitochondrial dysfunction, oxidant stress, or liver injury in wild-type animals. However, the authors observed a mild increase of plasma alanine aminotransferase (ALT) activities and hepatocellular necrosis in SOD2+/− mice after 4 weeks of treatment. Although the time course experiment was not very detailed, the lack of injury after 2 weeks of daily troglitazone injections indicated that liver injury developed during the second half of the treatment regimen (Ong et al., 2007). This suggests that cell injury does not develop gradually in this model but, similar to the human toxicity, there is a considerable lack phase between the initiation of treatment and the onset of cell injury. Mechanistically, the authors showed that the activities of aconitase and complex I of the mitochondrial electron transport chain were reduced and that there was an increase in protein carbonyl levels in mitochondria of troglitazone-treated livers. Further evidence for a mitochondrial oxidant stress was obtained in cultured hepatocytes, where only cells isolated from SOD2+/− animals generated more superoxide in mitochondria in response to troglitazone exposure in vitro (Ong et al., 2007). Together, these data convincingly demonstrate that prolonged treatment of SOD2+/− mice with 30 mg/kg troglitazone induced a mitochondrial oxidant stress and a partial loss of mitochondrial function, which correlated with cell injury.

The findings reported by Ong et al. (2007) are important for several reasons. First, the authors show for the first time in an animal model that troglitazone treatment can induce mild liver toxicity. In agreement with previous studies (reviewed in: Haskins et al., 2001), wild-type animals tolerated the drug without adverse effects. However, mice with a deficiency in mitochondrial antioxidant defense mechanisms were found to be susceptible. This response suggests that therapeutic doses of troglitazone cause a subclinical stress on liver mitochondria. This new mechanistic insight into troglitazone toxicity in vivo is the second novel finding of these studies. In addition, the results support the general concept that superimposed sub-clinical stresses from prolonged drug treatment and a genetic deficiency on the same organelle can lead to cell injury and organ damage. This represents an important paradigm shift from traditional toxicology studies where it is assumed that potential adverse reactions of drugs can be detected in normal animals by progressively increasing the dose. Since troglitazone did not cause liver cell injury in preclinical studies, this approach is obviously not always working. The study by Ong et al. (2007) suggests that using animals with endogenous antioxidant deficiencies may uncover potential liabilities of drugs even at therapeutic levels, which could provide a strong indication for possible idiosyncratic reactions in humans. Adding this type of testing to the traditional preclinical toxicity studies may be worth considering.

Can the findings by Ong et al. (2007) explain idiosyncratic liver failure in patients? This is definitively not the case, at least not at this point! The model generates only a mild liver injury and it remains unclear if further treatment would lead to more severe cell damage and liver failure. If the injury does not progress, the model may explain the fact that about 2% of patients treated with troglitazone but only 0.6% of patients receiving placebo experienced a significant increase of plasma ALT activities (Graham et al., 2003). A recent analysis of genetic polymorphism in Japanese patients treated with troglitazone revealed that a combined glutathione-S-transferase GSTT1-GSTM1 null genotype correlated with increased ALT levels (Watanabe et al., 2003). The emerging trend is clearly that a defect in cellular defense mechanisms against oxidant stress and reactive metabolites appears to enhance the risk for adverse effects of troglitazone. However, since only a very small subgroup of all treated patients (<0.005%) actually developed liver failure (Graham et al., 2003); one would expect that a single genetic defect may be insufficient to precipitate liver failure. Thus, it appears likely that a combination of genetic defects with potential adverse environmental conditions might be necessary to induce severe liver injury. Since different genetic defects can increase a person’s susceptibility to a drug, it will be a formidable challenge to identify these genetic deficiencies in livers of patients with drug-induced liver failure (Watkins, 2005). The animal model described by Ong et al. (2007) could be an important new approach to identify potential idiosyncratic liabilities of drugs already during preclinical development.

REFERENCES


