CURRENT TREND

Increased expression of cytochrome P450 2E1 in nonalcoholic fatty liver disease: Mechanisms and pathophysiological role

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Summary  Due to the worldwide surge in obesity and type 2 diabetes, the increased incidence of nonalcoholic fatty liver disease (NAFLD) is a major concern for the public health. Indeed, NAFLD encompasses a large spectrum of conditions ranging from fatty liver to nonalcoholic steatohepatitis (NASH), which can progress to cirrhosis in some patients. A better understanding of the mechanisms involved in fatty liver and its progression into NASH is important in order to develop efficient drugs able to alleviate these liver diseases. Although numerous investigations pointed to reactive oxygen species (ROS) as key players in the progression of fatty liver to NASH, their exact source is still uncertain. Besides the mitochondrial respiratory chain, cytochrome P450 2E1 (CYP2E1) has recently emerged as another potentially important cause of ROS overproduction. Indeed, higher hepatic CYP2E1 expression and activity have been frequently observed in the context of obesity and NAFLD. It is currently unknown why CYP2E1 is enhanced in these dysmetabolic diseases, although increased hepatic levels of fatty acids and insulin resistance might play a role. Nonetheless, higher hepatic CYP2E1 could play a significant role in the pathophysiology of NASH by inducing lipid peroxidation and oxidative damage of key cellular components. Moreover, CYP2E1-mediated overproduction of ROS could promote hepatic insulin resistance, which can further aggravate fatty liver. Since a significant amount of CYP2E1 can be located within liver mitochondria, higher levels of CYP2E1 in NAFLD could also have detrimental effects on mitochondrial function. Finally, increased CYP2E1 activity during NAFLD could enhance the susceptibility of some patients to the hepatotoxicity of different xenobiotics through the CYP2E1-mediated generation of harmful reactive metabolites. © 2011 Elsevier Masson SAS. All rights reserved.
It is currently estimated that up to 70% of obese and diabetic people could present hepatic steatosis, whereas its prevalence is between 15 and 25% in the general population [1,2]. Although simple fatty liver is a benign condition, it can evolve in the long term to nonalcoholic steatohepatitis (NASH) in 10 to 20% of patients [1,2]. In some individuals, NASH can further progress into cirrhosis and hepatocellular carcinoma [3,4]. Collectively, the large spectrum of conditions ranging from fatty liver to NASH is referred to as nonalcoholic liver disease (NAFLD). With the increasing prevalence of obesity and related metabolic disorders, NAFLD is the most frequent cause of chronic liver disease in the US [4,5].

NAFLD pathophysiology is still incompletely understood, although several key mechanisms have been identified. Firstly, insulin resistance and hyperinsulinemia undoubtedly play a major role in hepatic fat accumulation [6-8]. Whereas insulin resistance in the adipose tissue increases lipolysis and the hepatic entry of free fatty acids, insulin resistance-associated hyperinsulinemia favours de novo synthesis of fatty acids and triglycerides (i.e. lipogenesis). Secondly, different mechanisms have been involved in fatty liver progression to NASH, including oxidative stress, mitochondrial dysfunction, lipotoxicity and overproduction of deleterious cytokines promoting cell death, inflammation and fibrosis [2,5,9]. In NAFLD, enhanced cytochrome P450 2E1 (CYP2E1) expression and electron leakage from the mitochondrial respiratory chain (MRC) seem to be important sources of reactive oxygen species (ROS), which trigger oxidative stress [2,6,8,10]. The present review deals with the mechanisms whereby CYP2E1 can be overexpressed in NAFLD and its potential role in the pathophysiology of this disease. Moreover, as CYP2E1 can be located within liver mitochondria, we discuss the possible relationship between CYP2E1 overexpression and mitochondrial dysfunction in NAFLD.

**Role of cytochrome P450 2E1 in the biotransformation of drugs and endogenous compounds**

CYP2E1 catalyses the biotransformation of numerous drugs used in the current pharmacopeia including acetaminophen (paracetamol), salicylic acid, halothane, isoflurane, isoniazid, theophylline and dapsone. Moreover, this enzyme metabolises ethanol, several industrial chemicals (e.g. carbon tetrachloride and vinyl chloride) as well as contaminants (e.g. nitrosamines and acrylamide) [11,12]. Importantly, CYP2E1-mediated biotransformation of these compounds can generate toxic reactive metabolites. For instance, halothane and acetaminophen are respectively transformed into trichloroacetyl chloride and N-acetyl-p-benzoquinone imine (NAPQI). The latter reactive metabolite is able to induce major oxidative stress, mitochondrial dysfunction and cell demise [13,14]. Consequently, CYP2E1-mediated generation of NAPQI is a key event in acetaminophen-induced hepatotoxicity [13]. Thus, CYP2E1 plays a major role in drug metabolism and the pathophysiology of drug-induced liver injury.

CYP2E1 also catalyses the biotransformation of acetone, glycerol and different fatty acids [11,15]. Regarding the latter molecules, CYP2E1 metabolises saturated C_{10}-C_{20} fatty acids and the unsaturated arachidonic and linoleic acids [15]. However, it is still unclear whether CYP2E1-mediated biotransformation of these endogenous compounds has biological significance.

Inside the hepatocyte, CYP2E1 is mostly located within the endoplasmic reticulum (ER). However, studies performed in rodents have shown that it is also present in significant amounts in mitochondria [16,17]. Mitochondrial CYP2E1 (mtCYP2E1) seems to be more stable than its microsomal counterpart, possibly as a consequence of lesser proteasomal degradation [17]. It is still unclear whether mtCYP2E1 metabolises the very same substrates than its ER counterpart, although recent investigations indicate that mtCYP2E1 transforms ethanol and acetaldehyde into cytotoxic metabolites [18].

**Cytochrome P450 2E1-mediated production of reactive oxygen species and other deleterious endogenous derivatives**

In contrast to many other cytochromes P450, CYP2E1 is able to produce significant amounts of ROS (Fig. 1). Indeed, CYP2E1 generates superoxide anion and hydrogen peroxide during its catalytic cycle [15]. These ROS can subsequently damage unsaturated fatty acids, thus leading to lipid peroxidation and the generation of highly reactive aldehydes such as malondialdehyde and 4-hydroxynonenal (Fig. 1). ROS can also attack nucleic acids and proteins and induce mitochondrial membrane permeabilization, thus initiating cell death. Hence, CYP2E1-mediated ROS production can directly (or indirectly) damage numerous cellular and mitochondrial components including mitochondrial DNA and cytochrome c oxidase, a key MRC enzyme [19,20].

CYP2E1-mediated biotransformation of fatty acids can generate ω-hydroxylated fatty acids (Fig. 1). These derivatives harbour a hydroxyl group on the terminal carbon (i.e. the farthest carbon from the carboxylic acid moiety). Interestingly, ω-hydroxylated fatty acids can be further transformed by alcohol and aldehyde dehydrogenases to dicarboxylic fatty acids that can have deleterious effects when present at high cellular levels. In particular, dicarboxylic fatty acids are able to uncouple oxidative phosphorylation and inhibit several electron transfer reactions within the MRC [21]. Hence, CYP2E1 can be involved in the generation of different endogenous compounds such as ROS, lipid-derived reactive aldehydes and dicarboxylic acids that can all be harmful for cell homeostasis and viability (Fig. 1). It is noteworthy that fatty acid ω-hydroxylation is also catalysed by CYP4A, a CYP isofrom that can be induced in NAFLD and other metabolic diseases [22]. However, CYP4A does not seem to produce ROS in significant amount.

**Increased cytochrome P450 2E1 expression and activity in nonalcoholic fatty liver disease**

A key feature of CYP2E1 is its inducibility, and consequently liver CYP2E1 levels can be enhanced in different pathophysiological conditions. A well-known condition of CYP2E1 induction is chronic alcohol intake. In this
Figure 1  Possible causes and consequences of CYP2E1 induction in NAFLD. Different metabolic disturbances could explain higher expression and activity of hepatic CYP2E1 in NAFLD, including lipid overload, insulin resistance and/or high plasma levels of glucagon. Increased CYP2E1 activity induces ROS overproduction, whereas CYP2E1-mediated hydroxylation of some fatty acids could generate cytotoxic lipid intermediates. CYP2E1-generated ROS secondarily induce oxidative damage of cell components including lipids, proteins and nucleic acids. Oxidative alteration of lipids (i.e. lipid peroxidation) is able to generate deleterious aldehydes such as malondialdehyde and 4-hydroxynonenal. CYP2E1-mediated oxidative stress and lipid peroxidation subsequently alter key mitochondrial constituents including the mitochondrial respiratory chain (MRC). A significant amount of CYP2E1 can be located within mitochondria (mtCYP2E1), where it could also produce ROS and damage mitochondrial constituents. Impairment of MRC can further enhance ROS production, thus leading to a vicious circle. CYP2E1-mediated ROS production could also favour insulin resistance, which promotes fatty liver. Both oxidative stress and the cytotoxicity of toxic lipid intermediates are key players in the progression of fatty liver to NASH.

context, high CYP2E1 expression plays a key role in ethanol-induced oxidative stress, lipid peroxidation, mitochondrial dysfunction and hepatocyte injury [11,20,23]. CYP2E1 induction during chronic ethanol intake is most probably due to lower proteasomal degradation, thus inducing its stabilization [24,25]. In contrast, CYP2E1 mRNA levels are not increased during alcoholic liver diseases.

Hepatic CYP2E1 is also frequently induced in obesity and related metabolic disorders. Indeed, several investigations have reported enhanced hepatic CYP2E1 protein expression and/or activity in the context of obesity, fatty liver and NASH not only in rodents [26–32], but also in humans [33–39]. Some of these investigations and others reported higher CYP2E1 mRNA levels in NAFLD [28,40–43], thus suggesting increased rate of transcription and/or higher stability of the CYP2E1 transcripts. However, a stabilization of the CYP2E1 protein by endogenous substrate(s) cannot be excluded (see below). In the few investigations where CYP2E1 expression and activity have been compared between simple fatty liver and NASH, no significant difference was noticed [38,41,44]. However, increased CYP2E1 activity appeared to correlate with the degree of steatosis [37,44].

Nutritional and hormonal regulation of cytochrome P450 2E1

Fatty acids such as palmitic and oleic acids are able to increase CYP2E1 mRNA and/or protein levels in human hepatocytes or in differentiated human hepatoma HepaRG cells [45,46]. Moreover, high-fat diets enhance CYP2E1 expression and activity in rat liver [27,30,47]. In one of these studies, hepatic CYP2E1 expression was increased only with the diet containing the highest levels of lipids (65%), but not when rats were fed a diet containing only 24% of fat and enriched in sucrose [30]. Thus, the amount of fat intake could play an important role in hepatic CYP2E1 induction. This induction could be rapid, since increased CYP2E1 expression and activity were observed in vitro and in vivo after the first two to three days of fat overload [45,47]. In a recent study carried out in obese patients who underwent bariatric surgery for weight loss, the reduction of hepatic CYP2E1 expression correlated with the improvement of dyslipidemia [39]. Altogether, these data suggest that some lipid derivatives (possibly long-chain fatty acids) could augment hepatic CYP2E1 expression in NAFLD (Fig. 1). Actually, CYP2E1 induction in NAFLD could be an adaptive mechanism in order to
limit lipid overload since CYP2E1-mediated ω-hydroxylation of fatty acids is an alternative pathway to mitochondrial and peroxisomal β-oxidation. However, the precise mechanism of this induction is still unknown.

Besides the abnormal accumulation of lipids in liver, other metabolic disturbances could also play a role in higher hepatic CYP2E1 expression and activity in NAFLD.

Firstly, insulin resistance could play a role. Although insulin down-regulates hepatocytic CYP2E1 expression by altering its mRNA stability [48,49], it is important to point out that while some metabolic pathways are resistant to insulin (i.e. gluconeogenesis), other pathways are intact or overactivated due to hyperinsulinemia (i.e. lipogenesis) [8,50]. Thus, insulin-mediated down-regulation of CYP2E1 mRNA levels might be impaired during insulin resistance, eventually leading to higher CYP2E1 expression (Fig. 1). Alternatively, high levels of glucagon in the obese insulin-resistant state [51,52] could play a role since this hormone is able to enhance CYP2E1 mRNA levels (Fig. 1) [53]. It is also noteworthy that acetone, like ethanol, is a potent CYP2E1 inducer by stabilizing the CYP2E1 protein [12,54]. During insulin resistance, CYP2E1 induction could thus be the consequence of its stabilization by the high levels of ketone bodies generated through sustained mitochondrial fatty acid oxidation [12]. Interestingly, a significant positive correlation between plasma β-hydroxybutyrate and hepatic CYP2E1 activity was found in patients with NASH [36].

Secondly, higher hepatic CYP2E1 levels in NAFLD could be related to altered secretion of some adipose hormones (also referred to as adipokines) such as leptin and adiponectin. Importantly, leptinemia is increased in obesity whereas plasma adiponectin levels are frequently reduced in obese individuals with insulin resistance [8,55]. Leptin was shown to increase hepatic CYP2E1 protein expression in ob/ob mice (that are fully deficient in leptin) by a mechanism that did not depend only on food intake reduction [56]. Another study showed higher hepatic CYP2E1 protein expression in adiponectin-knockout mice and conversely, adiponectin overexpression induced its down-regulation [57]. Hence, low adiponectinemia could favour CYP2E1 induction in some insulin-resistant patients with NAFLD. However, further investigations are required to confirm these data and to determine the exact mechanisms whereby leptin and adiponectin could regulate CYP2E1 expression.

Finally, it must be pointed out that several inflammatory cytokines such as TNF-α, IL-1β and IL-6 down-regulate CYP2E1 expression [58], whereas the anti-inflammatory IL-4 has the opposite effect [59]. As NASH is associated with inflammation, it is unlikely that these cytokines play a role in CYP2E1 induction in this liver disease.

Pathophysiological consequences of increased cytochrome P450 2E1 expression in nonalcoholic fatty liver disease

As mentioned previously, CYP2E1 generates significant amounts of ROS that can subsequently induce lipid peroxidation and other oxidative damages (Fig. 1). A recent study in obese patients showed a significant association between CYP2E1 protein expression and lipid peroxidation levels in liver [39]. Several recent reviews have dealt with the role of ROS and lipid peroxidation in the pathophysiology of NAFLD, and especially regarding the progression of simple steatosis into NASH [2,6,8,9]. It must be pointed out that, besides CYP2E1, the dysfunctional mitochondria could be a significant source of ROS in NAFLD. Indeed, MRC impairment favours abnormal electron leakage and overproduction of superoxide anion within the MRC complexes I and III [2,8,60,61].

A relationship could exist between CYP2E1 and mitochondrial dysfunction since a significant proportion of this enzyme can be found within liver mitochondria (Fig. 1) [16,17]. Indeed, mitochondrial CYP2E1-mediated ROS production could greatly favour oxidative damage of key MRC components [18,62]. Reduced mitochondrial importation of glutathione could also promote oxidative stress in the context of NAFLD by reducing ROS detoxication within mitochondria [8]. Thus, CYP2E1-mediated ROS overproduction, lower ROS detoxication and lipid peroxidation-derived reactive aldehydes could favour MRC alteration. This can trigger a vicious circle, which further increases ROS generation through abnormal electron leakage within the MRC complexes I and III (Fig. 1) [2,8].

Interestingly, two recent experimental studies carried out in vitro and in vivo showed that enhanced CYP2E1 activity can promote hepatic insulin resistance (Fig. 1), in particular through a c-Jun N-terminal kinase (JNK)-dependent mechanism [63,64]. Moreover, the in vivo investigations showed that liver CYP2E1 overexpression was associated with higher insulinemia and greater hepatic accumulation of lipids [64]. Thus, increased CYP2E1 expression could not only favour the progression of simple steatosis to NASH but it may also further increase lipid accretion through insulin resistance (Fig. 1).

Higher susceptibility to drug-induced liver injury in patients with nonalcoholic fatty liver disease

CYP2E1 induction in NAFLD could also favour drug-induced liver injury for several compounds metabolised by this enzyme (Fig. 2) [65]. For instance, obesity and NAFLD could increase the risk of acute hepatitis induced by the volatile halogenated anaesthetic halothane [66,67] and the pain killer acetaminophen [68,69].

Regarding acetaminophen intoxication, investigations in obese animals have given conflicting results. Whereas some studies showed increased hepatotoxicity [70,71], others demonstrated an obvious protection [72,73]. A possible reason of this discrepancy is the presence of normal (or even reduced) hepatic CYP2E1 expression and activity in obese rodents used in the studies showing a lack of toxicity [65,74,75]. By using different murine models of obesity and type 2 diabetes, current investigations in our laboratory suggest that higher susceptibility to acetaminophen-induced liver injury is rather determined by elevated hepatic CYP2E1 activity, and not the degree of fat deposition. In intoxicated patients, NAFLD could enhance the risk of acetaminophen-induced acute liver injury [68], whereas obesity per se could be protective [76]. Actually, obesity and NAFLD seems to increase acetaminophen glucuronidation and clearance [77–79]. Thus, not all obese patients could be at risk for
acetaminophen-induced hepatotoxicity. Further investigations are needed in order to determine whether hepatic CYP2E1 induction is the main cause of this risk.

Besides drugs, experimental and clinical studies also suggest that NAFLD could favour ethanol-induced toxicity and liver diseases [74,75,80]. However, higher ethanol-induced liver injury was observed in ob/ob mice and fa/fa rats that do not present hepatic CYP2E1 induction. Thus, further investigations are needed in order to ascertain the exact role of CYP2E1 in such susceptibility.

Concluding remarks and perspectives

There is accumulating evidence for a physiopathological role of increased hepatic CYP2E1 expression in NAFLD. However, it is still unclear how this enzyme is deleterious for the liver in the absence of alcohol intoxication. Although a major mechanism could be ROS overproduction and subsequent oxidative alteration of key cellular components, CYP2E1 could also generate toxic derivatives through the oxidation of fatty acids. Moreover, it is still unknown whether CYP2E1 generates more ROS when compared to the MRC. If CYP2E1 is a major source of ROS in the context of NAFLD, it will be thus important to find molecules able to efficiently inhibit CYP2E1 activity and alleviate oxidative stress in liver. Since CYP2E1-mediated generation of ROS could favour insulin resistance, such molecules could also prevent fatty liver. However, it is noteworthy that some CYP2E1 inhibitors such as disulfiram and chlorimethiazole can be hepatotoxic [81]. Another interesting strategy to lessen oxidative stress in NAFLD could be antioxidants specifically designed to be targeted to mitochondria since mtCYP2E1 and the damaged MRC are both able to produce ROS in these organelles [2,82].

Clearly, future experimental investigations are needed in order to find efficient drugs able to alleviate oxidative stress and mitochondrial dysfunction in the context of NAFLD.

Although recent investigations revealed that CYP2E1 can be located within mitochondria, the different factors involved in its mitochondrial targeting are yet to be identified. Since protein kinase A-dependent phosphorylation of CYP2E1 favours its mitochondrial translocation [83], it is conceivable that glucagon could play a role. Thus, glucagon-mediated increased protein kinase A activity could not only enhance CYP2E1 expression [53], but also its targeting to mitochondria. A previous work in our laboratory also suggest that mitochondrial translocation of CYP2E1 could be leptin-dependent in liver [17]. Like glucagon, leptin could thus favour both hepatic CYP2E1 protein expression [56] and its mitochondrial translocation. Because fatty acids enhance CYP2E1 expression, it will be interesting to determine whether this induction involves both the microsomal and mitochondrial compartments. A better characterization of the factors involved in mitochondrial CYP2E1 localization may help in the future to find strategies to reduce mtCYP2E1 levels and subsequent mitochondrial dysfunction in NAFLD.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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