CYP2E1 and oxidant stress in alcoholic and non-alcoholic fatty liver disease

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Summary

Alcoholic (ALD) and non-alcoholic fatty liver diseases (NAFLD) are clinical conditions leading to hepatocellular injury and inflammation resulting from alcohol consumption, high fat diet, obesity and diabetes, among others. Oxidant stress is a major contributing factor to the pathogenesis of ALD and NAFLD. Multiple studies have shown that generation of reactive oxygen species (ROS) is key for the progression of fatty liver to steatohepatitis. Cytochrome P450 2E1 (CYP2E1) plays a critical role in ROS generation and CYP2E1 is also induced by alcohol itself. This review summarizes the role of CYP2E1 in ALD and NAFLD.

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Cytochrome P450 and oxidant stress

The cytochrome P450 super family is a group of heme-containing proteins with multiple functions including the metabolism of xenobiotics such as drugs, toxins, carcinogens, and endogenous substrates, such as fatty acids and steroids. The cytochrome P450 enzymes catalyze a number of chemical reactions such as peroxidation, dealkylation, mono-oxygenation, reduction, epoxidation, and dehalogenation (reviewed in [3,14]). A major function of the cytochrome P450 system is to convert non-polar to polar compounds for conjugation by phase II enzymes or for direct excretion.

Toxic metabolites are generated by cytochrome P450-mediated metabolism, which in turn cause significant cellular injury. The catalytic activity of the cytochrome P450 enzymes requires oxygen activation, which results in the generation of ROS such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (·OH).

ROS are produced by the mitochondrial respiratory chain, the cytochrome P450 system, auto-oxidation of heme proteins, the NADPH oxidase complex, xanthine oxidase, oxidative enzymes, and other cellular systems (reviewed in [3,19]). ROS are toxic to cells since they react with macromolecules, denature proteins, inactivate enzymes, and cause RNA and DNA damage. NADPH oxidase-containing macrophages and neutrophils produce ROS to remove foreign micro-organisms. ROS are also important in signal transduction, cellular physiology and are involved in critical metabolic pathways. However, a high concentration of ROS is definitively harmful.

A number of enzymatic and non-enzymatic mechanisms in the body maintain physiological levels of ROS and prevent cellular damage by ROS. These mechanisms include superoxide dismutase, catalase, glutathione peroxidase, glutathione transferase, heme oxygenase, ceruloplasmin, ferritin, glutathione (GSH), vitamin E, vitamin A, vitamin C, uric acid, and bilirubin (reviewed in [3,19]). Under normal physiological conditions, there is a balance between the rate of ROS generation and the rate of ROS removal along with repair from ROS-induced cellular damage. Oxidant stress is caused by excess ROS production, which leads to apoptosis and necrosis. ROS also lead to a free radical chain reaction with unsaturated fatty acids generating toxic lipid intermediates, a reaction magnified by the presence of free iron. Lipid peroxidation modifies the integrity of cellular membranes and damages proteins and DNA. There are three major sites within hepatocytes for ROS generation, i.e., mitochondria, peroxisomes, and the smooth endoplasmic reticulum.

Oxidant stress in ALD and NAFLD

Oxidant stress is a pathogenic factor for the onset of ALD and NAFLD. In vivo models of alcohol infusion induce lipid peroxidation because of increased free radical formation and decreased hepatic antioxidants such as GSH [15,22]. GSH is synthesized in the cytosol and is translocated to the mitochondria. The liver is the major organ excreting GSH into the plasma and the bile. GSH levels decrease in both ALD and NAFLD animal models suggesting either reduced endogenous antioxidant availability or consumption of GSH to cope with the large amount of pro-oxidants generated or perhaps both.

Keywords: Cytochrome P450; Alcoholic liver disease; Non-alcoholic fatty liver disease.
Clinical Application of Basic Science

Key Points

- Oxidant stress is key in the pathogenesis of ALD and NAFLD
- Alcohol metabolism via cytochrome P450 2E1 generates reactive oxygen species
- Polyunsaturated fatty acids further enhance alcohol-induced liver injury
- Positive feedback loop between cytochrome P450 2E1 activation and insulin resistance contributes to the progression of steatosis into steatohepatitis

In addition to GSH, other hepatic antioxidants such as vitamin A, vitamin C, bilirubin, and enzymes such as superoxide dismutase and catalase remove ROS. In vitro studies showed more oxidant stress and cellular injury in hepatocytes isolated from chronic alcohol-fed rats and in ethanol-treated HepG2 cells overexpressing CYP2E1 when compared with the corresponding controls [30]. Moreover, treatment with an inhibitor of alcohol oxidation such as 4-methylpyrazole or an antioxidant such as trollox, effectively prevented or reduced alcohol-induced toxicity, pointing one more time at the importance of oxidant stress in the pathogenesis of ALD [30].

CYP2E1, oxidant stress in ALD

ALD results from increased steatosis, inflammation, oxidant, and nitrosative stress and mitochondrial dysfunction. Alcohol dehydrogenase (ADH) is the major enzyme oxidizing alcohol. The involvement of cytochrome P450 in alcohol metabolism was first identified by Charles S. Lieber in his studies on the microsomal ethanol-oxidizing system (MEOS) [18]. MEOS has a higher \( K_m \) than ADH in oxidizing alcohol and oxidizes alcohol to generate acetaldehyde. The activity of MEOS increases in chronic alcohol consumption partly due to the induction of cytochrome P450 enzymes.

Among the cytochrome P450 family, CYP2E1 has been identified as the most relevant for ALD as it is highly inducible and it has high catalytic activity for alcohol [18]. CYP2E1 is mainly expressed in the liver, with hepatocytes showing the highest expression, but it is also located in other organs such as the brain and intestine. CYP2E1 is mainly located within the endoplasmic reticulum (ER) although it is also expressed in the mitochondria [23]. CYP2E1 metabolizes a variety of substances including multiple drugs, polyunsaturated fatty acids, ethanol, acetaldehyde, and most organic solvents. Multiple factors such as insulin, adiponectin and cytokines regulate CYP2E1 mRNA and protein expression [19].

Many CYP2E1 substrates induce their own metabolism as it occurs with ethanol, which is particularly relevant to the development of ALD. Previous studies have shown that alcohol toxicity is reduced when CYP2E1 is inhibited using chemicals or in Cyp2e1 mice [5]. CYP2E1 expression and activity are higher after chronic ethanol feeding compared with pair-fed mice consuming a control diet on a pair-feeding regimen [19].

During the catalytic cycle of CYP2E1, significant amounts of ROS are generated, which subsequently cause cellular damage. Previous studies showed that increased cellular injury, lipid peroxidation, oxidant and nitrosative stress, and mitochondrial damage occurred in livers from chronic ethanol-fed mice compared with pair-fed mice. One of the reasons for the increase in CYP2E1 protein during chronic ethanol intake is decreased proteasomal degradation, which increases CYP2E1 protein stability [12,27].

Recent work has shown that CYP2E1 activity correlates with ethanol-induced liver injury and lipid peroxidation [7]. Inhibition of CYP2E1 effectively blocked the ethanol-mediated lipid peroxidation and reduced liver injury. In contrast, transgenic mice overexpressing CYP2E1 [8], mice infected with an adenovirus to overexpress Cyp2e1 and HepG2 cells transduced with an adenovirus encoding the human CYP2E1 gene, all exacerbated oxidant stress [4]. Endogenous GSH levels are a major factor in alcohol-induced oxidant stress and CYP2E1 overexpression in HepG2 cells induces GSH synthesis by transcriptional activation of gamma-glutamylcysteine synthetase, the rate-limiting enzyme in GSH synthesis [20].

CYP2E1 and NAFLD

In addition to ethanol, CYP2E1 also metabolizes polyunsaturated fatty acids such as linoleic acid and arachidonic acid to generate \( \omega-1 \)-hydroxylated fatty acids [3]. The \( \omega-1 \)-hydroxylated fatty acids are further metabolized to dicarboxylic fatty acids that are cytotoxic at high concentrations. Indeed, increased CYP2E1 protein expression and activity were found in obesity, fatty liver and non-alcoholic steatohepatitis (NASH) in both humans and rodents [reviewed in [3]]. Feeding rats with a high fat diet also increased CYP2E1, although some studies showed that fasting or prolonged starvation also elevated CYP2E1. The role of CYP2E1 in the pathogenesis of NAFLD is under active investigation.

Multiple studies have shown the significance of increased CYP2E1 expression and electron leakage from the mitochondrial respiratory chain in inducing oxidant stress in NAFLD [6,28]. CYP2E1 expression and activation are induced in fatty liver disease and ROS are generated by activated CYP2E1 resulting in oxidant stress. This contributes to the progression from steatosis to steatohepatitis. Chitioui et al. showed a significantly higher degree of steatosis in patients with NASH than in patients with NAFLD [9]. No significant difference in CYP2E1 expression was observed between simple fatty liver and NASH; however, CYP2E1 activity correlated with the extent of steatosis [9]. CYP2E1 is induced in rats fed a high fat diet compared to rats fed a low fat diet suggesting that the amount of fat consumed is critical for CYP2E1 induction [26]. These studies suggest that the amount of fat in the liver is important for the pathogenesis of NASH by increasing CYP2E1 and by rendering the liver more susceptible to noxious substances such as ROS. Since CYP2E1 is involved in the metabolism of fatty acids, inhibiting CYP2E1 may reduce oxidant stress but potentiate steatosis.

Correlation between CYP2E1 expression and lipid peroxidation was observed in obese patients [25]. Although there was increased serum GSH, significant hepatic GSH reduction occurred in patients with steatosis and steatohepatitis. This reduction in GSH may be the result of fatty acid toxicity. An in vitro study showed a dose-dependent increase in GSH content in HepG2 cells in response to increasing doses of oleic acid or palmitic acid. However, oleic acid but not palmitic acid sustained GSH levels due to the fact that palmitic acid reduced cell viability, indicating specificity of fatty acids in inducing liver toxicity and cell death.
Further studies are warranted to dissect how changes in specific fatty acids condition the development and progression of ALD, NAFLD and NASH.

It is worth mentioning that compensatory mechanisms could occur within the cytochrome P450 enzymes. Other cytochrome P450s, such as CYP4A10 and CYP4A14 are induced in Cyp2e1−/− mice with an increase in ROS and oxidant stress in a NASH murine model [17]. This suggests that CYP2E1 may not be the only cytochrome P450 enzyme promoting oxidant stress. This compensatory pathway was further clarified by treating wild type mice with CYP2E1 and CYP4A antibodies. Only the CYP2E1 antibody showed inhibition of lipid peroxidation, a hallmark of ALD. In contrast, the CYP4A antibody but not the CYP2E1 antibody protected from lipid peroxidation when treating Cyp2e1−/− mice with a methionine and choline-deficient diet compared to a methionine and choline-sufficient diet [17]. This indicates that CYP4A induction could be an alternative pathway when CYP2E1 is less available [28].

It is suggested that the induction of CYP2E1 in NAFLD is an adaptive response to prevent lipid overload as CYP2E1-mediated ω-hydroxylation of fatty acids is an alternative pathway to peroxisomal and mitochondrial β-oxidation. However, further studies are needed to clarify this theory.

Insulin resistance, CYP2E1 in ALD AND NAFLD

Insulin resistance and hyperinsulinemia play a key role in hepatic fat accumulation and are common to both ALD and NAFLD. Cyp2e1−/− mice showed protection from a high-fat diet-induced insulin resistance [1,31]. In contrast, mice knocked in for the human CYP2E1 transgene showed increased insulin resistance, oxidant stress, more hepatic steatosis and liver injury [16]. Indeed, insulin is known to decrease CYP2E1 expression [29]. Thus, insulin resistance may increase CYP2E1 expression and activity via the high concentration of ketone bodies produced from persistent mitochondrial fatty acid oxidation. Ketone bodies stabilize CYP2E1 and prevent its degradation. The increase in CYP2E1 and enhanced insulin resistance seem to promote each other by creating a positive feedback loop that may eventually make steatosis progress to steatohepatitis as oxidant stress increases. The mechanism by how CYP2E1 and insulin resistance interact requires further investigation. Both CYP2E1 activation and insulin resistance are common to ALD and NAFLD; thus, inhibiting CYP2E1 may disrupt this feedback loop and reduce insulin resistance and liver injury.

Clinical implications and perspectives

The toxicity induced by CYP2E1 in ALD and NAFLD should be further investigated. Indeed, it is necessary to identify the role of microsomal compared to mitochondrial CYP2E1 as they could contribute differently to these pathologies. The regulation of CYP2E1 expression and activity is of clinical importance since CYP2E1 plays a central role in ALD and NAFLD. CYP2E1 is triggered by both exogenous substrates such as ethanol, and endogenous substrates such as polyunsaturated fatty acids, and it induces injury by generating ROS and lipid peroxidation reactions. The reduction of endogenous antioxidants in ALD and NAFLD may further enhance CYP2E1-induced lipid peroxidation, oxidant stress and cellular toxicity. Replenishing the amount of antioxidants could target CYP2E1-induced oxidant stress by cleaning free radicals. Fig. 1 depicts the activation of CYP2E1 by alcohol and fatty acids in generating ROS, which induces oxidant stress and cellular damage. Furthermore, reduced endogenous antioxidants enhance oxidant stress.

The use of CYP2E1 inhibitors, such as chlorothiazole and polyenylphosphatidylcholine, showed partial but effective protection in ethanol-induced liver injury [2,13]. This partial protection suggested that CYP2E1 may not be the only source of pro-oxidants. Interestingly, CYP2E1-expressing cells showed an increase not only in GSH, but also in antioxidant enzymes such as glutathione-S-transferase, catalase and heme-oxygenase [19,21]. Treatment with antioxidants prevented the induction of these enzymes suggesting that CYP2E1-derived ROS may be responsible for the transcription and activation of the antioxidant genes [24]. The induction of CYP2E1 could also be protective in eliminating noxious substances and regulating various metabolic pathways in response to stress. However, some CYP2E1 inhibitors, for example YH439, a novel hepatoprotective agent, may inhibit other cytochrome P450 enzymes and affect other metabolic pathways, hence, causing cellular toxicity.

Concluding remarks

Evidence from experimental and clinical data shows that CYP2E1 is a multifunctional protein metabolizing various endogenous and exogenous substrates and generating ROS. CYP2E1 also induces other factors, for example Nrf2, to protect against CYP2E1-induced oxidant stress [11]. This suggests that CYP2E1 has adaptive responses to condition the oxidant stress induced during its metabolic activity. However, further studies are warranted to understand how CYP2E1 expression and activity regulate hepatotoxic and hepatoprotective pathways. Moreover, how endogenous antioxidants are depleted in ALD and NAFLD is not clear. Studies on various CYP2E1 inhibitors, antioxidant replenishment and alternative cytochrome P450 enzymes could provide relevant clinical information for the development of ALD and NAFLD treatments.
Conflict of interest

The Authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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