Published in final edited form as:

Curr Mol Pharmacol. 2017 ; 10(3): 172–178. doi:10.2174/1874467208666150817111846.

Alcoholic Liver Disease: from CYP2E1 to CYP2A5

Tung Ming Leung* and Yongke Lu#

*Graduate Program in Public Health, Graduate School of Biomedical Sciences, Icahn School of Medicine at Mount Sinai

#Department of Health Sciences, College of Public Health, East Tennessee State University

Abstract

This article reviews recent studies on CYP2E1-mediated alcoholic liver injury, the induction of CYP2A5 by alcohol and the mechanism for this upregulation, especially the permissive role of CYP2E1 in the induction of CYP2A5 by alcohol and the CYP2E1-ROS-Nrf2 pathway, and protective effects of CYP2A5 against ethanol-induced oxidative liver injury. Ethanol can induce CYP2E1, an active generator of reactive oxygen species (ROS), and CYP2E1 is a contributing factor for alcohol-induced oxidative liver injury. CYP2A5, another isoform of cytochrome P450, can also be induced by ethanol. Chronic feeding of ethanol to wild type mice increased CYP2A5 catalytic activity, protein and mRNA levels as compared to pair-fed controls. This induction was blunted in CYP2E1 knockout (*cyp2e1*^{-/-}) mice but was restored when human CYP2E1 was reintroduced and expressed in *cyp2e1*^{-/-} mice. Ethanol-induced CYP2E1 co-localized with CYP2A5 and preceded the elevation of CYP2A5. The antioxidants N-acetyl cysteine and vitamin C lowered the alcohol elevation of ROS and blunted the alcohol induction of CYP2A5, but not CYP2E1, suggesting ROS play a novel role in the crosstalk between CYP2E1 and CYP2A5. The antioxidants blocked the activation of Nrf2, a transcription factor known to upregulate expression of CYP2A5. When alcohol-induced liver injury was enhanced in Nrf2 knockout (*Nrf2*^{-/-}) mice, alcohol elevation of CYP2A5 but not CYP2E1 was also lower in *Nrf2*^{-/-} mice. CYP2A5 knockout (*cyp2a5*^{-/-}) mice exhibited an enhanced alcoholic liver injury compared with WT mice as indicated by serum ALT, steatosis and necroinflammation. Alcohol-induced hyperglycemia were observed in *cyp2a5*^{-/-} mice but not in WT mice.

Keywords

Alcohol; antioxidants; CYP2A5; CYP2E1; interactions; mouse liver; Nrf2; reactive oxygen species

CYP2E1 AND ALCOHOLIC LIVER DISEASE

Chronic alcohol abuse causes many disorders including alcoholic fatty liver (steatosis).

Alcoholic fatty liver had been considered benign for a while, but now it is generally accepted

#Address correspondence to this author at the Department of Health Sciences, College of Public Health, East Tennessee State University, 1276 Gilbreath Drive, Box 70673, Johnson City, TN, USA; Tel: 423-439-4575; Fax: 423-439-4562; luy004@etsu.edu.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

that alcoholic fatty liver can progress to alcoholic steatohepatitis (inflammation), fibrosis and cirrhosis, even liver cancer [1]. Absorbed ethanol is principally oxidized in the liver. Many of the toxic effects of ethanol have been linked to its metabolism in the liver [2]. The major pathway for ethanol disposition involves alcohol dehydrogenase (ADH) [2]. ADH oxidizes ethanol to acetaldehyde in the cytosol; the resulting acetaldehyde enters mitochondria and is subsequently metabolized by aldehyde dehydrogenase (ALDH) to acetate/acetyl CoA for energy production in mitochondria [2]. The ADH system requires NAD^+ to serve as a co-factor to accept the hydrogens/reducing equivalents from alcohol and produce NADH, which increases the ratio of NADH/NAD^+ and contributes to the development of alcoholic fatty liver. Alcohol can also be metabolized by the microsomal ethanol oxidizing system (MEOS). Micorosomes are mainly derived from endoplasmic reticulum (ER). Cytochrome P450 enzymes (CYP), which are mainly located in smooth endoplasmic reticulum (SER), constitute the major components of MEOS. Chronic alcohol consumption results in a proliferation of SER and induction of CYPs especially CYP2E1 [1, 2]. CYP is a superfamily of hemeproteins that metabolize various endogenous substrates such as steroids and fatty acids, and xenobiotics including drugs, toxins and carcinogens [3]. Three CYP isoenzymes are able to oxidize ethanol: CYP2E1, CYP1A2, and CYP3A4; CYP2E1 plays the major role in the ethanol oxidation by microsomes and has been studied extensively in the field of alcoholic liver disease (ALD) [2]. CYP2E1 has a relatively high K_m for ethanol (8–10 mM for CYP2E1 vs 0.2–2 mM for hepatic ADH), so CYP2E1 only accounts for a small amount (about 10%) of the liver capacity to oxidize ethanol, but induction of CYP2E1 may play an important role in the metabolic tolerance found after chronic ethanol treatment [3]. Unlike the ADH system that requires NAD^+ to serve as a co-factor, CYP2E1 efficiently activates oxygen requiring NADPH provided by the NADPH-cytochrome P450 reductase, and this results in the production of reactive oxygen species (ROS) [3]. Due to its poor coupling with NADPH-cytochrome P450 reductase, CYP2E1 exhibits enhanced NADPH oxidase activity and generates more ROS. Microsomes from ethanol treated animals displayed elevated rates of production of ROS and lipid peroxidation (LPO). At the microsomal level, increased generation of ROS by ethanol was thought to be through the induction of CYP2E1 [4, 5]. Microsomes from ethanol-treated animals with a high level of CYP2E1 displayed elevated rates of generation of hydrogen peroxide and superoxide radical. Increases in production of ROS after ethanol treatment are prevented by anti-CYP2E1 IgG, thus linking generation of ROS to induction of CYP2E1.

Oxidative stress is a major risk factor of ALD. Studies in rodents with the intragastric ethanol infusion model showed that significant alcoholic liver injury evidently paralleled with induction of CYP2E1 by chronic alcohol consumption [6–8], and alcohol-induced liver injury was attenuated by inhibition of CYP2E1 [9, 10]. CYP2E1 inhibitors such as diallyl sulfide (DAS) [11], phenethyl isothiocyanate (PIC) [12, 13] and chlormethiazole (CMZ) [14], blocked hepatic lipid peroxidation (LPO) and ameliorated alcohol-induced hepatic pathological changes in rats. Polyenylphosphatidylcholine (PPC), which had inhibitory effect on CYP2E1 [16], was also effective in suppressing alcohol-induced oxidative stress [15]. Bardag-Gorce *et al* found that chronic ethanol-induced liver injury, oxidative stress and decline in activity of the proteosome were blunted in CYP2E1 knockout (*cyp2e1*^{-/-}) mice [17]. However, with an intragastric infusion model and *cyp2e1*^{-/-} mice, Kono *et al* did not

observed any differences in liver injury including steatosis between wild type (WT) mice and *cyp2e1*^{-/-} mice [18]. The intragastric infusion model may produce endotoxemia, which might mask differences in alcoholic liver injury between WT and *cyp2e1*^{-/-} mice. Compared with the intragastric alcohol infusion model, voluntary oral alcohol feeding model usually induces a milder alcoholic liver injury with minimal or no endotoxemia. We applied an oral feeding model instead of the intragastric infusion model to examine the role of CYP2E1 in ALD [19]. After 4 weeks of ethanol feeding, we found that alcoholic fatty liver were observed in WT mice but not in the *cyp2e1*^{-/-} mice. CYP2E1 was induced by ethanol in WT mice but not in *cyp2e1*^{-/-} mice, and ethanol-induced oxidative stress was higher in WT mice than in *cyp2e1*^{-/-} mice. Peroxisome proliferator-activated receptor alpha (PPAR α), a regulator of fatty acid oxidation, was up-regulated by ethanol in *cyp2e1*^{-/-} mice but not in WT mice. A PPAR α target gene, acyl CoA oxidase, was decreased by ethanol in WT but not in *cyp2e1*^{-/-} mice. CMZ, an inhibitor of CYP2E1, blunted alcoholic fatty liver in WT mice; when CYP2E1 was reintroduced into *cyp2e1*^{-/-} mice by injection with adenovirus expressing CYP2E1, alcoholic fatty liver was restored [19].

CYP2E1 knockin (*cyp2e1*^{-/-} KI) mice in which human CYP2E1 gene was reintroduced and expressed in *cyp2e1*^{-/-} mice were generated in the laboratory of Dr. Fank Gonzalez, National Cancer Institute. We applied *cyp2e1*^{-/-} KI mice to further examine the role of CYP2E1 in the development of ALD. We found that after chronic ethanol feeding, liver injury and oxidative stress were blunted in the *cyp2e1*^{-/-} mice but restored in the *cyp2e1*^{-/-} KI mice. Significant liver injury with elevated serum transaminases, necrosis, increased levels of collagen type I and smooth muscle actin were produced in the ethanol-fed *cyp2e1*^{-/-} KI mice but not in WT mice, which was associated with elevated oxidative stress and elevated levels of the human CYP2E1 compared to levels of the mouse CYP2E1 in WT mice, suggesting the essential role of CYP2E1 in alcoholic liver disease [20]. Ethanol can induce hypoxia [21]. Hypoxia as indicated by an increased area of staining using the hypoxia-specific marker pimonidazole was higher in the ethanol-fed *cyp2e1*^{-/-} KI mice than in the ethanol-fed WT mice and lowest in the ethanol-fed *cyp2e1*^{-/-} mice, and pimonidazole and CYP2E1 were co-localized to the same area of injury in the hepatic centrilobule [22], suggesting that CYP2E1 contributes to ethanol-induced hypoxia. Ethanol also elevates hypoxia inducible factor 1 α (HIF-1 α) in the liver [23, 24]. Protein levels of HIF-1 α and downstream targets of HIF-1 α activation were elevated in the ethanol-fed *cyp2e1*^{-/-} KI mice compared to the WT and *cyp2e1*^{-/-} mice; levels of HIF prolyl hydroxylase 2, which promotes HIF-1 α degradation, were decreased in the ethanol-fed *cyp2e1*^{-/-} KI mice in association with the increases in HIF-1 α [22]. These results suggest that CYP2E1 contributes to activation of HIF-1 α *in vivo*. As HIF-1 α is a transcription regulator rather than a receptor ligand or enzyme, the results of increasing HIF-1 α activity may be dependent on other signals the hepatocyte is receiving at the same time [25]. The *in vitro* results with CYP2E1 over-expressing HepG2 cells showed that 2-Methoxyestradiol, an inhibitor of HIF-1 α , blunted the ethanol-related toxic effects in association with inhibition of HIF-1 α , suggesting that HIF-1 α contributes to CYP2E1-dependent ethanol-induced toxicity [22].

INDUCTION OF CYP2A5 BY ETHANOL AND CYP2E1-CYP2A5 INTERACTIONS

Coumarin 7-hydroxylase (COH) is encoded by the mouse *cyp2a5* gene and the human *cyp2a6* gene (for details about CYP2A5, please refer to recent reviews [26–29]). Human CYP2A6 is expressed predominantly in the liver [27]. Liver tissue CYP2A6 expression was increased in patients with alcoholic and nonalcoholic fatty liver and co-localized with lipid droplets [30]. *In vitro* studies showed that ethanol treatment up-regulated CYP2A6 expression in human U937 monocytes [31]. CYP2A5, the mouse ortholog to human CYP2A6, is also expressed in liver, although the highest CYP2A5 is expressed in olfactory mucosa [26]. Does ethanol induce COH (CYP2A5) in mice? When mice were subjected to oral alcohol feeding in a Lieber-DeCarli liquid diet, hepatic CYP2A5 (COH) activity and protein expression were strikingly upregulated [32, 33]. In rats, CYP2A3, the ortholog to mouse CYP2A5 and human CYP2A6, is not detectable in the liver, even after treatments with known CYP2A5 inducers [34, 35]. Most studies on microsomal ethanol metabolism were performed with rat microsomes, but COH activity (CYP2A3) is absent in rat liver. This might be the major reason why ethanol induction of COH was not detected before. Induction of CYP2E1 by chronic ethanol is largely a posttranscriptional mechanism involving stabilization of the CYP2E1 protein by the ethanol substrate/ligand against proteasome-mediated degradation [36, 37]. Indeed, after 3 weeks of ethanol feeding, ethanol did not increase CYP2E1 mRNA levels in WT mice (Fig. 1B); however, ethanol did increase CYP2A5 mRNA levels approximating 5-fold in WT mice (Fig. 1A). Thus, while chronic ethanol feeding increases two important CYPs in the liver, the mechanism of induction for each CYP appears to differ.

Interestingly, a time course study showed that elevation of CYP2E1 activity was earlier than CYP2A5 elevation. Indeed, after one week of ethanol feeding CYP2E1 protein levels and catalytic activity (with para-nitrophenol as substrate) were elevated 2–3 fold as compared to the dextrose controls and this level of increase was maintained over the 3 week feeding period. CYP2A5 protein and catalytic activity (coumarin hydroxylation) was not altered after 1 week of ethanol feeding, relative to the pair-fed dextrose controls. However, both CYP2A5 protein and activity doubled by 2 weeks of ethanol feeding and both were elevated about 4-fold after 3 weeks feeding. CMZ, an inhibitor of CYP2E1 that can blunt the induced activity of CYP2E1, also inhibited CYP2A5 activity (Fig. 2). Interestingly, those zones of the liver from the ethanol-fed mice with the highest levels of CYP2E1 as detected by immunohistochemical staining also contained the highest amounts of CYP2A5, i.e CYP2E1 and 2A5 co-localized in the liver [32]. With respect to human liver, the levels of CYP2A6 paralleled the levels of CYP2E1, being elevated in ALD and cirrhotic patients with the higher CYP2E1. Those livers with lower CYP2E1 content also had lower CYP2A6 content [32]. Ethanol up-regulation of CYP2A6 in human U937 monocytes was blunted by the inhibition of CYP2E1 [31]. These studies suggest a close association between induction of CYP2E1 and induction of CYP2A5/2A6.

To examine possible association between CYP2E1 and 2A5 more directly, we determined if chronic ethanol feeding induced CYP2A5 in *cyp2e1*^{-/-} mice. Whereas 3 weeks of ethanol

feeding elevated CYP2A5 protein, mRNA and activity by 4-fold in WT mice, there was no increase in CYP2A5 protein, mRNA or activity in the *cyp2e1*^{-/-} mice; basal levels of CYP2A5 mRNA and activity in the dextrose-treated mice were similar in the WT and the *cyp2e1*^{-/-} mice (Fig. 1A,C). Thus, ethanol failed to induce CYP2A5 in the *cyp2e1*^{-/-} mice implicating a role for CYP2E1 in the induction of CYP2A5 by chronic ethanol feeding [32, 33]. These experiments were repeated in *cyp2e1*^{-/-} KI mice, in which the human CYP2E1 is added back into the *cyp2e1*^{-/-} mice to reestablish expression of human CYP2E1 [32, 33]. Indeed, feeding the *cyp2e1*^{-/-} KI mice ethanol chronically for 3 weeks resulted in an induction of CYP2A5 protein, mRNA and catalytic activity. Ethanol elevated CYP2E1 protein and activity (but not mRNA) in the WT and the *cyp2e1*^{-/-} KI mice [32]. These results indicate that while CYP2A5 is expressed in the absence of CYP2E1 e.g the *cyp2e1*^{-/-} mice, induction of CYP2A5 by chronic ethanol requires CYP2E1.

Is ethanol induction of CYP2E1 influenced by CYP2A5? CYP2A5 KO (*cyp2a5*^{-/-}) mice were provided as a generous gift from Dr. Xinxin Ding (SUNY College of Nanoscale Science and Engineering, Albany, NY) and fed ethanol chronically along with their genetic control WT mice. The ethanol feeding produced a comparable 3-fold increase in CYP2E1 protein and catalytic activity in the WT and the *cyp2a5*^{-/-} mice as compared to dextrose-fed controls. The ethanol feeding elevated CYP2A5 protein and activity in the WT mice and not in the *cyp2a5*^{-/-} mice (Fig. 3B). Thus, the increase in CYP2E1 by ethanol is independent of the presence of CYP2A5, in contrast to the increase in CYP2A5 by ethanol that requires CYP2E1.

CYP2E1 is an effective generator of ROS during its catalytic cycle and CYP2E1 plays an important role in the oxidative stress produced by ethanol treatment. Therefore, the antioxidants N-acetyl-cysteine (NAC) and vitamin C (Vc) were applied to study the possible role of ROS for the potentiation of ethanol induction of CYP2A5 by CYP2E1. The treatments with either NAC or Vc prevented the increases in thiobarbituric acid-reactive substances (TBARS) as a reflection of LPO and the decline in GSH, indices suggestive of enhanced oxidative stress, thus blunting the acute ethanol- elevated oxidative stress. Simultaneously, the ethanol-induced activity of COH and the protein levels of CYP2A5 were also blunted by treatment with the antioxidants. However, the antioxidants had no effect on the induction of CYP2E1 activity or protein by the ethanol treatment. Thus, the ethanol induction of CYP2A5 but not CYP2E1 involves, at least in part, a role for ROS [33].

How can the elevated ROS potentiate ethanol induction of CYP2A5? CYP2A5 and 2A6 are regulated by the redox –sensitive nuclear factor erythroid 2 (NFE2)-related factor 2 (Nrf2) [38–41]. Pyrazole induced CYP2A5 in WT mice but not in Nrf2 KO (*Nrf2*^{-/-}) mice [42]. We hypothesize that ethanol induction of CYP2E1 leads to an increase in ROS and subsequent activation of Nrf2, which then activates expression of CYP2A5. Ethanol feeding resulted in an increase in nuclear levels of Nrf2 and Nrf2-DNA binding activity in WT mice but not in the *cyp2e1*^{-/-} mice. Thus, CYP2E1 was required for the ethanol activation of Nrf2. The ethanol activation of Nrf2 was blocked by the pretreatment with NAC or Vc, indicating that CYP2E1-generated ROS is important in the ethanol activation of Nrf2 [33]. Alcohol-induced liver injury was enhanced in *Nrf2*^{-/-} mice, and alcohol induced CYP2E1 comparably in the WT and *Nrf2*^{-/-} mice, suggesting that Nrf2 did not significantly modulate

CYP2E1 induction by alcohol [43]. CYP2A5 induction by alcohol was not evaluated in these mice. Samples of liver from these mice were kindly provided to us by Dr. Vogel of the study in [43]. Levels of CYP2E1 as well as CYP2E1 activity were elevated to the same extent by alcohol in the WT and Nrf2^{-/-} mice, confirming the findings of Lamle *et al* [43]. However, induction of CYP2A5 protein by alcohol was lower in the Nrf2^{-/-} mice than in the WT mice and CYP2A5 catalytic activity was increased 9-fold by alcohol in the WT mice as compared to a 4-fold increase in the Nrf2^{-/-} mice. Thus, maximal induction of CYP2A5 by alcohol requires Nrf2.

Besides Nrf2, other transcription factors are known to be capable of upregulating the basal and the inducible expression of the CYP2A5 gene [28, 29] e. g. retinoid x receptor α (RXR α), the coactivator peroxisome proliferator-activated receptor γ coactivator-1 α (PGC-1 α), the aryl hydrocarbon receptor nuclear translocator [44–47]. The possible effects of alcohol on these transcriptional factors need to be assessed.

CYP2A5 AND ALCOHOLIC LIVER DISEASE

Ethanol induction of CYP2A5 is dependent on CYP2E1, an active generator of ROS and a risk factor for ALD, thus it is plausible that ethanol-induced CYP2A5 may act as a downstream target of CYP2E1 signaling pathway to promote alcohol-induced oxidative stress and liver injury. On the other hand, the CYP2E1-dependent induction of CYP2A5 by ethanol is regulated by Nrf2, which regulates a panel of anti-oxidant genes to protect against the development of ALD, thus the ethanol-induced CYP2A5 may be among the panel of Nrf2-regulated antioxidant genes to protect against ALD. WT and *cyp2a5*^{-/-} mice were treated with alcohol and biochemical assays of liver function were performed to assess the possible effects of CYP2A5 on the alcohol actions. Our results suggest that in contrast to CYP2E1, CYP2A5 protects against but not promotes alcohol-induced oxidative stress. After ethanol feeding, hepatic GSH was decreased comparably in *cyp2a5*^{-/-} mice and WT mice; however, TBARS, a marker of LPO, was increased by ethanol feeding more than 2-fold in *cyp2a5*^{-/-} mice, but it was increased only 50% in WT mice [48]. Formations of 9-hydroxy-nonanal adduct and 3-nitro-tyrosine adduct, markers of oxidative stress, were detected in *cyp2a5*^{-/-} mice to a greater extent than in WT mice [48]. These results suggest that CYP2A5 induction inhibits alcohol-induced oxidative stress, which is not surprising considering the facts that ethanol induction of CYP2A5 is regulated by the Nrf2 pathway and CYP2E1 up-regulates antioxidant genes. Besides generating oxidants including ROS, CYP2E1 can also upregulate antioxidants, which may reflect an adaptive mechanism to remove CYP2E1-derived oxidants [49]. In CYP2E1 over-expressing HepG2 cells, antioxidants including GSH, catalase and glutathione-S- transferase (GST) are upregulated [50, 51]. Due to the increase in ROS produced by the elevated levels of CYP2E1, Nrf2 is upregulated in CYP2E1 over-expressing HepG2 cells and livers from ethanol-fed rodents [52, 53]. Ethanol-induced liver injury was more severe in Nrf2^{-/-} mice compared with WT mice, suggesting that upregulation of Nrf2 may protect against alcoholic liver disease [43]. Nrf2 may antagonize CYP2E1-mediated liver injury via upregulating antioxidant enzymes such as heme oxygenase-1 (HO-1) and glutamyl cysteinylglycine synthase, a rate-limiting enzyme for GSH synthesis [54]. It was reported that chronic ethanol feeding does not induce HO-1 in rodent liver [55]. We observed a similar decrease in GSH levels in ethanol-treated WT

mice and *cyp2a5*^{-/-} mice (Fig. 3A) and a weak induction of HO-1 in WT mice and no induction in *cyp2a5*^{-/-} mice (Fig. 3B). Thus, other Nrf2-regulated antioxidant mechanisms may be involved in ALD protection.

Alcohol can cause an increase in levels of heme, and free heme can catalyze the production of ROS through Fenton chemistry [56, 57]. HO-1 exhibits antioxidant effects because HO-1 can decrease heme levels through degrading heme with the production of iron, carbon monoxide, and the bile pigments biliverdin and bilirubin [57, 58, 59]. Biliverdin can be reduced by ubiquitous biliverdin reductase to bilirubin [56, 57]. Bilirubin is an effective antioxidant at concentrations ranging from 0.01 to 10 μ M, but it is cytotoxic at concentrations >20 μ M [60, 61]. Thus, HO-1/bilirubin might have a dual role in antioxidant responses: elevated levels of HO-1 or its substrate heme can produce bilirubin to high concentrations that may be detrimental and not protective. Recently, bilirubin was identified as an endogenous substrate for CYP2A5, and CYP2A5 can oxidize bilirubin to biliverdin [60–62]. Both HO-1 and CYP2A5/6 are regulated by Nrf2, it is possible that HO-1 could cause induction of bilirubin, and CYP2A5 could control the intracellular concentration of bilirubin to a level with antioxidant instead of cytotoxic properties [61]. HO-1 and CYP2A5/6 may coordinate to maintain intracellular bilirubin at an antioxidant concentration. CYP2A5 is highly induced in liver [32, 33], but chronic ethanol feeding does not induce HO-1 (Fig. 3B). It will be interesting to address whether no or weak induction of HO-1 and highly induced CYP2A5 coordinate to protect against alcohol-induced oxidative liver injury.

Kirby *et al* first raised the notion that CYP2A5 may involve energy metabolism [28]. Pyrazole, an inducer of CYP2A5, causes hepatic glycogen depletion and decreases serum glucose levels, and causes hepatic lipid accumulation [28]. We found that chronic ethanol feeding induced hyper-glycemia in *cyp2a5*^{-/-} mice but not in WT mice (Fig. 3C). Glucose tolerance test was carried out in naïve WT mice and *cyp2a5*^{-/-} mice. After glucose was injected i.p. at 2 g/kg, blood glucoses at different time points were elevated higher in *cyp2a5*^{-/-} mice than in WT mice (Fig. 3D). These results suggest that CYP2A5 may participate in regulation glucose homeostasis. Glucose homeostasis regulating factors such as glucagon, cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA) can induce CYP2A5 [63, 64]. Glucagon mediates cAMP production, and cAMP activates PKA, which causes cAMP response element-binding protein (CREB) phosphorylation at Ser133, subsequent translocation to the nucleus and binding to the cAMP response element (CRE) [65]. CREB cannot directly regulate CYP2A5 transcription because the *cyp2a5* promoter lacks CRE. However, CREB can act through the PGC-1 α [66] and PGC-1 α mediates CYP2A5 induction via hepatocyte nuclear factor 4 α (HNF-4 α) [45]. Interestingly, chronic ethanol increases cAMP levels in the liver [67, 68]. It is possible that ethanol induction of CYP2A5 is regulated, at least in part, by a cAMP-PKA-CREB pathway via PGC-1 α /HNF-4 α . It will be interesting to address whether CYP2A5/2A6 regulate glucose homeostasis via the cAMP-PKA-CREB pathway.

The previously published results obtained from WT and *cyp2a5*^{-/-} mice chronically fed ethanol [48] or from primary hepatocytes isolated from WT and *cyp2a5*^{-/-} mice incubated with ethanol, or from WT and *cyp2a5*^{-/-} mice gavaged with binge ethanol all show that

ethanol-induced steatosis was higher in *cyp2a5*^{-/-} mice or hepatocytes compared with WT mice or hepatocytes [33]. In another model, *cyp2a5*^{-/-} mice and WT mice were fed high fat diet, body weights were increased more rapidly in *cyp2a5*^{-/-} mice than in WT mice, and non-alcoholic fatty liver was more pronounced in *cyp2a5*^{-/-} mice than in WT mice (unpublished observation). These suggest that in contrast to CYP2E1, CYP2A5 protects against but not promotes alcohol-induced steatosis. In patients with alcoholic and nonalcoholic fatty liver, CYP2A6 was found to co-localize with lipid droplets [30], which thus can be extrapolated to be a compensatory or adaptive response to antagonize fat accumulation in the liver. Perhaps inhibitors of CYP2A5 may have therapeutic potential in ameliorating alcohol-induced liver injury.

Most alcoholics also smoke [69]. Heavy drinkers are also heavy smokers. Nicotine is a major addiction stimulating alkaloid in tobacco smoke. CYP2A5/2A6 are the major CYP for oxidation of nicotine [70, 71]. Therefore it is likely that alcohol induction of CYP2A5/2A6 will accelerate blood nicotine clearance and increase the amount of tobacco smoking to maintain blood nicotine levels, thus alcohol induction of CYP2A5/2A6 increase the amount of nicotine absorbed in the body. We recently reported that administration of nicotine increased chronic ethanol –induced steatosis [72]. While nicotine treatment or ethanol feeding alone failed to induce hypertriglyceridemia and hyperglycemia, the combination of ethanol feeding and nicotine administration caused increases in serum levels of TG and glucose [72]. Further studies on relationship of CYP2A5 and nicotine/alcohol-induced metabolic syndrome would be of importance.

CONCLUSION

Alcohol consumption induces both CYP2E1 and CYP2A5, and the alcohol induction of CYP2A5 is CYP2E1-dependent, i.e. CYP2E1-generated ROS up-regulates Nrf2, which in turn elevates CYP2A5 expression. CYP2E1 contributes to the development of ALD, however, unlike CYP2E1, CYP2A5 protects against ALD, which might be associated with Nrf2-mediated antioxidant effects..

Acknowledgments

cyp2a5^{-/-} mice and *cyp2e1*^{-/-} mice were generously provided by Dr. Xinxin Ding and Dr. Frank Gonzalez.

Grants: These studies were supported by ABMRF/The Foundation for Alcohol Research and National Institute on Alcohol Abuse and Alcoholism (AA020877).

References

1. Lieber CS. Alcoholic fatty liver: its pathogenesis and mechanism of progression to inflammation and fibrosis. *Alcohol*. 2004; 34:9–19. [PubMed: 15670660]
2. Lieber CS. Metabolism of alcohol. *Clin Liver Dis*. 2005; 9:1–35. [PubMed: 15763227]
3. Lu Y, Cederbaum AI. CYP2E1 and oxidative liver injury by alcohol. *Free Radic Biol Med*. 2008; 44:723–38. [PubMed: 18078827]
4. Gorsky LD, Koop DR, Coon MJ. On the stoichiometry of the oxidase and monooxygenase reactions catalyzed by liver microsomal cytochrome P450. *J Biol Chem*. 1984; 259:6812–6817. [PubMed: 6725272]

5. Ekstrom G, Ingelman-Sundberg M. Rat liver microsomal NADPH-supported oxidase activity and lipid peroxidation dependent on ethanol-inducible cytochrome P-450 (P-450III_{E1}). *Biochem Pharmacol.* 1989; 38:1313–1319. [PubMed: 2495801]
6. Morimoto M, Zern MA, Hagbjork AL, Ingelman-Sundberg M, French SW. Fish oil, alcohol, and liver pathology: role of cytochrome P450 2E1. *Proc Soc Exp Biol Med.* 1994; 207:197–205. [PubMed: 7938050]
7. Nanji AA, Zhao S, Sadrzadeh SM, Dannenberg AJ, Tahan SR, Waxman DJ. Markedly enhanced cytochrome P450 2E1 induction and lipid peroxidation is associated with severe liver injury in fish oil-ethanol-fed rats. *Alcohol Clin Exp Res.* 1994; 18:1280–1285. [PubMed: 7847620]
8. Tsukamoto H, Horne W, Kamimura S, Niemela O, Parkkila S, Yla-Herttuala S, Brittenham GM. Experimental liver cirrhosis induced by alcohol and iron. *J Clin Invest.* 1995; 9:620–630.
9. French SW, Morimoto M, Reitz RC, Koop D, Klopfenstein B, Estes K, Clot P, Ingelman-Sundberg M, Albano E. Lipid peroxidation, CYP2E1 and arachidonic acid metabolism in alcoholic liver disease in rats. *J Nutr.* 1997; 127:907S–911S. [PubMed: 9164261]
10. Kim ND, Kwak MK, Kim SG. Inhibition of cytochrome P450 2E1 expression by 2-(allylthio)pyrazine, a potential chemoprotective agent: hepatoprotective effects. *Biochem Pharmacol.* 1997; 53:261–269. [PubMed: 9065729]
11. Morimoto M, Hagbjork AL, Nanji AA, Ingelman-Sundberg M, Lindros KO, Fu PC, Albano E, French SW. Role of cytochrome P4502E1 in alcoholic liver disease pathogenesis. *Alcohol.* 1993; 10:459–464. [PubMed: 8123200]
12. Morimoto M, Reitz RC, Morin RJ, Nguyen K, Ingelman-Sundberg M, French SW. CYP-2E1 inhibitors partially ameliorate the changes in hepatic fatty acid composition induced in rats by chronic administration of ethanol and a high fat diet. *J Nutr.* 1995; 125:2953–2964. [PubMed: 7500173]
13. Albano E, Clot P, Morimoto M, Tomasi A, Ingelman-Sundberg M, French SW. Role of cytochrome P4502E1-dependent formation of hydroxyethyl free radical in the development of liver damage in rats intragastrically fed with ethanol. *Hepatology.* 1996; 23:155–163. [PubMed: 8550035]
14. Gouillon Z, Lucas D, Li J, Hagbjork AL, French BA, Fu P, Fang C, Ingelman-Sundberg M, Donohue TM Jr, French SW. Inhibition of ethanol-induced liver disease in the intragastric feeding rat model by chlormethiazole. *Proc Soc Exp Biol Med.* 2000; 224:302–308. [PubMed: 10964266]
15. Lieber CS. The discovery of the microsomal ethanol oxidizing system and its physiologic and pathologic role. *Drug Metab Rev.* 2004; 36:511–29. [PubMed: 15554233]
16. Aleynik MK, Leo MA, Aleynik SI, Lieber CS. Polyenyl-phosphatidylcholine opposes the increase of cytochrome P-4502E1 by ethanol and corrects its iron-induced decrease. *Alcohol Clin Exp Res.* 1999; 23:96–100. [PubMed: 10029208]
17. Bardag-Gorce F, Yuan QX, Li J, French BA, Fang C, Ingelman-Sundberg M, French SW. The effect of ethanol-induced cytochrome p4502E1 on the inhibition of proteasome activity by alcohol. *Biochem Biophys Res Commun.* 2000; 279:23–9. [PubMed: 11112412]
18. Kono H, Bradford BU, Yin M, Sulik KK, Koop DR, Peters JM, Gonzalez FJ, McDonald T, Dikalova A, Kadiiska MB, Mason RP, Thurman RG. CYP2E1 is not involved in early alcohol-induced liver injury. *Am J Physiol.* 1999; 277:G1259–G1267. [PubMed: 10600824]
19. Lu Y, Zhuge J, Wang X, Bai J, Cederbaum AI. Cytochrome P450 2E1 contributes to ethanol-induced fatty liver in mice. *Hepatology.* 2008; 47:1483–94. [PubMed: 18393316]
20. Lu Y, Wu D, Wang X, Ward SC, Cederbaum AI. Chronic alcohol-induced liver injury and oxidant stress are decreased in cytochrome P4502E1 knockout mice and restored in humanized cytochrome P4502E1 knock-in mice. *Free Radic Biol Med.* 2010; 49:1406–16. [PubMed: 20692331]
21. French SW. The role of hypoxia in the pathogenesis of alcoholic liver disease. *Hepatol Res.* 2004; 29:69–74. [PubMed: 15163427]
22. Wang X, Wu D, Yang L, Gan L, Cederbaum AI. Cytochrome P450 2E1 potentiates ethanol induction of hypoxia and HIF-1 α *in vivo*. *Free Radic Biol Med.* 2013; 63:175–86. [PubMed: 23669278]

23. Nath B, Levin I, Csak T, Petrasek J, Mueller C, Kodys K, Catalano D, Mandrekar P, Szabo G. Hepatocyte-specific hypoxia-inducible factor-1 α is a determinant of lipid accumulation and liver injury in alcohol-induced steatosis in mice. *Hepatology*. 2011; 53:1526–37. [PubMed: 21520168]
24. Nishiyama Y, Goda N, Kanai M, Niwa D, Osanai K, Yamamoto Y, Senoo-Matsuda N, Johnson RS, Miura S, Kabe Y, Suematsu M. HIF-1 α induction suppresses excessive lipid accumulation in alcoholic fatty liver in mice. *J Hepatol*. 2012; 56:441–7. [PubMed: 21896344]
25. Mehal WZ. HIF-1 α is a major and complex player in alcohol induced liver diseases. *J Hepatol*. 2012; 56:311–2. [PubMed: 21963521]
26. Su T, Ding X. Regulation of the cytochrome P450 2A genes. *Toxicol Appl Pharmacol*. 2004; 199:285–94. [PubMed: 15364544]
27. Raunio H, Rahnasto-Rilla M. CYP2A6: genetics, structure, regulation, and function. *Drug Metabol Drug Interact*. 2012; 27:73–88. [PubMed: 22706231]
28. Kirby GM, Nichols KD, Antenos M. CYP2A5 induction and hepatocellular stress: an adaptive response to perturbations of heme homeostasis. *Curr Drug Metab*. 2011; 12:186–97. [PubMed: 21395539]
29. Abu-Bakar A, Hakkola J, Juvonen R, Rahnasto-Rilla M, Raunio H, Lang MA. Function and regulation of the Cyp2a5/CYP2A6 genes in response to toxic insults in the liver. *Curr Drug Metab*. 2013; 14:137–50. [PubMed: 22497566]
30. Niemela O, Parkkila S, Juvonen RO, Viitala K, Gelboin HV, Pasanen M. Cytochromes P450 2A6, 2E1, and 3A and production of protein-aldehyde adducts in the liver of patients with alcoholic and non-alcoholic liver diseases. *J Hepatol*. 2000; 33:893–901. [PubMed: 11131450]
31. Jin M, Kumar A, Kumar S. Ethanol-mediated regulation of cytochrome P450 2A6 expression in monocytes: role of oxidative stress-mediated PKC/MEK/Nrf2 pathway. *PLoS One*. 2012; 7:e35505. [PubMed: 22530035]
32. Lu Y, Zhuge J, Wu D, Cederbaum AI. Ethanol induction of CYP2A5: permissive role for CYP2E1. *Drug Metab Dispos*. 2011; 39:330–336. [PubMed: 21051534]
33. Lu Y, Zhang H, Cederbaum AI. Ethanol induction of CYP2A5: role of CYP2E1-ROS-Nrf2 pathway. *Toxicol Sci*. 2012; 128:427–438. [PubMed: 22552773]
34. Su T, Sheng JJ, Lipinskas TW, Ding X. Expression of CYP2A genes in rodent and human nasal mucosa. *Drug Metab Dispos*. 1996; 24:884–890. [PubMed: 8869824]
35. Kimura S, Kozak CA, Gonzalez FJ. Identification of a novel P450 expressed in rat lung: cDNA cloning and sequence, chromosome mapping, and induction by 3-methylcholanthrene. *Biochemistry*. 1989; 28:3798–3803. [PubMed: 2751996]
36. Song BJ, Gelboin HV, Park SS, Yang CS, Gonzalez FJ. Complementary DNA and protein sequences of ethanol-inducible rat and human cytochrome P-450s. Transcriptional and post-transcriptional regulation of the rat enzyme. *J Biol Chem*. 1986; 261:16689–16697. [PubMed: 3782137]
37. Song B, Veech RL, Park SS, Gelboin HV, Gonzalez FJ. Induction of rat hepatic N-nitrosodimethylamine demethylase by acetone is due to protein stabilization. *J Biol Chem*. 1989; 264:3568–3572. [PubMed: 2914964]
38. Abu-Bakar A, Lämsä V, Arpiainen S, Moore MR, Lang MA, Hakkola J. Regulation of CYP2A5 gene by the transcription factor nuclear factor (erythroid-derived 2)-like 2. *Drug Metab Dispos*. 2007; 35:787–94. [PubMed: 17303623]
39. Lämsä V, Levenon AL, Leinonen H, Ylä-Herttuala S, Yamamoto M, Hakkola J. Cytochrome P450 2A5 constitutive expression and induction by heavy metals is dependent on redox-sensitive transcription factor Nrf2 in liver. *Chem Res Toxicol*. 2010; 23:977–85. [PubMed: 20402460]
40. Abu-Bakar A, Satarug S, Marks GC, Lang MA, Moore MR. Acute cadmium chloride administration induces hepatic and renal CYP2A5 mRNA, protein and activity in the mouse: involvement of transcription factor NRF2. *Toxicol Lett*. 2004; 148:199–210. [PubMed: 15041070]
41. Yokota S, Higashi E, Fukami T, Yokoi T, Nakajima M. Human CYP2A6 is regulated by nuclear factor-erythroid 2 related factor 2. *Biochem Pharmacol*. 2011; 81:289–294. [PubMed: 20887713]
42. Lu Y, Gong P, Cederbaum AI. Pyrazole induced oxidative liver injury independent of CYP2E1/2A5 induction due to Nrf2 deficiency. *Toxicology*. 2008; 252:9–16. [PubMed: 18721845]

43. Lamle J, Marhenke S, Borlak J, Von Wasielewski R, Eriksson CJP, Geffers R, Manns MP, Yamamoto M, Vogel A. Nuclear factor-erythroid 2-related factor 2 prevents alcohol-induced fulminant liver injury. *Gastroenterology*. 2008; 134:1159–1168. [PubMed: 18395094]
44. Cai Y, Konishi T, Han G, Campwala KH, French SW, Wan YJ. The role of hepatocyte RXR alpha in xenobiotic-sensing nuclear receptor-mediated pathways. *Eur J Pharmacol*. 2002; 15:89–96.
45. Arppianen S, Jarvenpaa SM, Manninen A, Viitala P, Lang MA, Pelkonen O, Hakkola J. Coactivator PGC-1 alpha regulated the fasting inducible xenobiotic metabolizing enzyme CYP2A5 in mouse primary hepatocytes. *Toxicol Appl Pharmacol*. 2008; 232:135–141. [PubMed: 18602936]
46. Arpiainen S, Lamsa V, Pelkonen O, Yim SH, Gonzalez FJ, Hakkola J. Aryl hydrocarbon receptor nuclear translocator and upstream stimulatory factor regulate cytochrome P450 2a5 transcription through a common E-box site. *J Mol Biol*. 2007; 369:640–652. [PubMed: 17466327]
47. Arpiainen S, Raffalli-Mathieu F, Lang MA, Pelkonen O, Hakkola J. Regulation of Cyp2a5 gene involves an aryl hydrocarbon receptor-dependent pathway. *Mol Pharmacol*. 2005; 67:1325–1333. [PubMed: 15657367]
48. Hong F, Liu X, Ward SS, Xiong H, Cederbaum AI, Lu Y. Absence of cytochrome P450 2A5 enhances alcohol-induced liver injury in mice. *Dig Liver Dis*. 2015; 47:470–7. [PubMed: 25804444]
49. Mari M, Wu D, Nieto N, Cederbaum AI. CYP2E1-dependent toxicity and up-regulation of antioxidant genes. *J Biomed Sci*. 2001; 8:52–8. [PubMed: 11173976]
50. Mari M, Cederbaum AI. Induction of catalase, alpha, and microsomal glutathione S-transferase in CYP2E1 overexpressing HepG2 cells and protection against short-term oxidative stress. *Hepatology*. 2001; 33:652–61. [PubMed: 11230746]
51. Mari M, Cederbaum AI. CYP2E1 overexpression in HepG2 cells induces glutathione synthesis by transcriptional activation of gamma-glutamylcysteine synthetase. *J Biol Chem*. 2000; 275:15563–71. [PubMed: 10748080]
52. Gong P, Cederbaum AI. Nrf2 is increased by CYP2E1 in rodent liver and HepG2 cells and protects against oxidative stress caused by CYP2E1. *Hepatology*. 2006; 43:144–153. [PubMed: 16374848]
53. Gong P, Cederbaum AI. Transcription factor Nrf2 protects HepG2 cells against CYP2E1 plus arachidonic acid-dependent toxicity. *J Biol Chem*. 2006; 281:14573–14579. [PubMed: 16551616]
54. Cederbaum A. Nrf2 and antioxidant defense against CYP2E1 toxicity. *Expert Opin Drug Metab Toxicol*. 2009; 5:1223–44. [PubMed: 19671018]
55. Mandal P, Pritchard MT, Nagy LE. Anti-inflammatory pathways and alcoholic liver disease: role of an adiponectin/interleukin-10/heme oxygenase-1 pathway. *World J Gastroenterol*. 2010; 16:1330–6. [PubMed: 20238399]
56. Gozzelino R, Jeney V, Soares MP. Mechanisms of cell protection by heme oxygenase-1. *Annu Rev Pharmacol Toxicol*. 2010; 50:323–54. [PubMed: 20055707]
57. Lämsä V, Levonen AL, Sormunen R, Yamamoto M, Hakkola J. Heme and heme biosynthesis intermediates induce heme oxygenase-1 and cytochrome P450 2A5, enzymes with putative sequential roles in heme and bilirubin metabolism: different requirement for transcription factor nuclear factor erythroid- derived 2-like 2. *Toxicol Sci*. 2012; 130:132–44. [PubMed: 22859313]
58. Bauer M, Bauer I. Heme oxygenase-1: redox regulation and role in the hepatic response to oxidative stress. *Antioxid Redox Signal*. 2002; 4:749–58. [PubMed: 12470502]
59. Maines, MD., Costa, LG., Reed, DJ., Sassa, S., Sipes, IG. Overview of heme degradation pathway Current protocols in toxicology. John Wiley & Sons; New York: 1999.
60. Abu-Bakar A, Moore MR, Lang MA. Evidence for induced microsomal bilirubin degradation by cytochrome P450 2A5. *Biochem Pharmacol*. 2005; 70:1527–35. [PubMed: 16183037]
61. Abu-Bakar A, Arthur DM, Aganovic S, Ng JC, Lang MA. Inducible bilirubin oxidase: a novel function for the mouse cytochrome P450 2A5. *Toxicol Appl Pharmacol*. 2011; 257:14–22. [PubMed: 21871474]
62. Kim SD, Antenos M, Squires EJ, Kirby GM. Cytochrome P450 2A5 and bilirubin: mechanisms of gene regulation and cyto-protection. *Toxicol Appl Pharmacol*. 2013; 270:129–38. [PubMed: 23628428]

63. Salonpää P, Pelkonen O, Kojo A, Pasanen M, Negishi M, Raunio H. Cytochrome P4502A5 expression and inducibility by phenobarbital is modulated by cAMP in mouse primary hepatocytes. *Biochem Biophys Res Commun*. 1994; 205:631–7. [PubMed: 7528014]
64. Viitala P, Posti K, Lindfors A, Pelkonen O, Raunio H. cAMP mediated upregulation of CYP2A5 in mouse hepatocytes. *Biochem Biophys Res Commun*. 2001; 280:761–7. [PubMed: 11162586]
65. He L, Sabet A, Djedjos S, Miller R, Sun X, Hussain MA, Radovick S, Wondisford FE. Metformin and insulin suppress hepatic gluconeogenesis through phosphorylation of CREB binding protein. *Cell*. 2009; 137:635–46. [PubMed: 19450513]
66. Herzig S, Long F, Jhala US, Hedrick S, Quinn R, Bauer A, Rudolph D, Schutz G, Yoon C, Puigserver P, Spiegelman B, Montminy M. CREB regulates hepatic gluconeogenesis through the coactivator PGC-1. *Nature*. 2001; 413:179–83. [PubMed: 11557984]
67. Nagy LE. Role of adenosine A1 receptors in inhibition of receptor-stimulated cyclic AMP production by ethanol in hepatocytes. *Biochem Pharmacol*. 1994; 48:2091–6. [PubMed: 7802699]
68. Nagy LE, DeSilva SE. Ethanol increases receptor-dependent cyclic AMP production in cultured hepatocytes by decreasing G(i)-mediated inhibition. *Biochem J*. 1992; 286:681–6. [PubMed: 1358061]
69. Wall TL, Schoedel K, Ring HZ, Luczak SE, Katsuyoshi DM, Tyndale RF. Differences in pharmacogenetics of nicotine and alcohol metabolism: review and recommendations for future research. *Nicotine Tobacco Res*. 2007; 9:S459–S474.
70. Benowitz NL, Swan GE, Jacob P, Lessov-Schlaggar CN, Tyndale RF. CYP2A6 genotype and the metabolism and disposition kinetics of nicotine. *Clin Pharmacol Ther*. 2006; 80:457–467. [PubMed: 17112802]
71. Zhou X, Zhuo X, Xie F, Kluetzman K, Shu YZ, Humphreys WG, Ding X. Role of CYP2A5 in the clearance of nicotine and cotinine: insights from studies on a CYP2a5-null mouse model. *J Pharmacol Exp Ther*. 2010; 332:578–587. [PubMed: 19923441]
72. Lu Y, Ward SC, Cederbaum AI. Nicotine enhances ethanol-induced fat accumulation and collagen deposition but not inflammation in mouse liver. *Alcohol*. 2013; 47:353–357. [PubMed: 23731694]

Fig. 1. Alcohol induces mRNA and activity of CYP2A5 in WT mice but not in *cyp2e1*^{-/-} mice. The WT mice and *cyp2e1*^{-/-} mice were fed ethanol-diet for 3 weeks. **(A)** CYP2A5 mRNA; **(B)** CYP2E1 mRNA; **(C)** CYP2A5 activity. * $P < 0.05$, compared with WT Control; # $P < 0.05$, compared with WT Ethanol.

Fig. 2.
CMZ, an inhibitor of CYP2E1, also inhibited CYP2A5 activity. The WT mice were fed ethanol-diet for 2 weeks. * $P < 0.05$, compared with Control; # $P < 0.05$, compared with Ethanol.

Fig. 3. Alcohol-induced oxidative stress and hyperglycemia in *cyp2a5*^{-/-} mice. The WT mice and *cyp2a5*^{-/-} mice were fed ethanol-diet for 3 weeks. **(A)** GSH and TBARS. **(B)** HO-1, CYP2A5 and CYP2E1; **(C)** serum glucose; **(D)** glucose tolerance test. * $P < 0.05$, compared with Control; # $P < 0.05$, compared with WT Ethanol; & $P < 0.05$, compared with KO.