



Published in final edited form as:

*J Dig Dis.* 2012 March ; 13(3): 133–142. doi:10.1111/j.1751-2980.2011.00569.x.

## Oxidative stress and redox signaling mechanisms of alcoholic liver disease: updated experimental and clinical evidence

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### Abstract

Alcoholic liver disease (ALD) is a major cause of morbidity and mortality worldwide. The spectrum of ALD ranges from fatty liver to alcoholic hepatitis and cirrhosis, which may eventually lead to the development of hepatocellular carcinoma. In developed countries as well as many developing nations, ALD is a major cause of end-stage liver disease that requires liver transplantation. The most effective therapy for ALD is alcohol abstinence; however, for individuals with severe forms of ALD and those in whom alcohol abstinence is not achievable, targeted therapies are absolutely necessary. In this context, advances of our understanding of the pathophysiology of ALD over the past two decades have contributed to the development of therapeutic modalities (e.g., pentoxifylline and corticosteroids) for the disease though the efficacy of the available treatments remains limited. This article is intended to succinctly review the recent experimental and clinical findings regarding the involvement of oxidative stress and redox signaling in the pathophysiology of ALD and the development of mechanistically-based antioxidant modalities to target the oxidative stress and redox signaling mechanisms of ALD. The biochemical and cellular sources of reactive oxygen and nitrogen species (ROS/RNS) and the dysregulated redox signaling pathways associated with alcohol consumption are particularly discussed to provide insight into the molecular basis of hepatic cell dysfunction and destruction as well as tissue remodeling underlying ALD.

### Keywords

alcoholic liver disease; oxidative stress; redox signaling; inflammation; antioxidants

### INTRODUCTION

In chemistry, an alcohol is any organic compound in which a hydroxyl functional group (-OH) is bound to a carbon atom, usually connected to other carbon or hydrogen atoms. Although alcohol is a general term for any alcoholic compound, here in this article alcohol

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refers specifically to ethyl alcohol or ethanol ( $\text{CH}_3\text{CH}_2\text{OH}$ ). Alcohol has been a part of human culture since the beginning of recorded history. Excessive use of alcohol contributes substantially to the global burden of disease (4% of the total mortality) and is thus one of the largest avoidable risk factors<sup>1</sup>. In the United States, alcoholism is also a major public health problem, and it is estimated that over 100 million Americans are alcoholic. Alcohol abuse accounts for 100,000 to 200,000 deaths annually in the United States, of which over 20,000 are attributable directly to end-stage hepatic cirrhosis and many more are the result of automobile accidents ([www.cdc.gov/nchs/fastats/alcohol.htm](http://www.cdc.gov/nchs/fastats/alcohol.htm)). Because of the above, it is recommended that alcohol consumption be moderate:  $\leq 2$  drinks daily in men and  $\leq 1$  drink in women. In the United States, a “standard” drink is any drink that contains about 0.6 fluid ounces or 14 grams of “pure” ethanol. It is of note that ethanol contains 7 calories per gram.

Although excessive alcohol consumption is associated with a variety of disorders, alcoholic liver disease (ALD) is of the great health impact. As depicted in Fig. 1, ALD encompasses a spectrum of hepatic disorders including: (1) fatty liver (also known as steatosis, due to abnormal accumulation of lipids in hepatic tissue), (2) alcoholic hepatitis (due to inflammation and necrosis), and (3) cirrhosis (due to excessive fibrosis)<sup>2</sup>. These are not necessarily distinct stages of evolution of the disease, but rather multiple stages that may be present simultaneously in a given individual. Over 90% of heavy drinkers develop fatty liver, and of those, 10-35% develop alcoholic hepatitis. About 8-20% of chronic alcoholics develop liver cirrhosis, which may eventually lead to hepatocellular carcinoma<sup>3</sup>.

## **PATHOPHYSIOLOGY OF ALD**

Multiple pathways are involved in the genesis and progression of alcoholic fatty liver, alcoholic hepatitis, and cirrhosis. The spectrum of ALD is illustrated in Fig. 1 above and the pathophysiology summarized individually below. Alcoholic fatty liver results from excessive accumulation of triglycerides in hepatocytes.

Alcohol is metabolized in hepatocytes through oxidation to acetaldehyde and subsequently from acetaldehyde to acetate catalyzed by various enzymes or enzymatic systems, including the alcohol dehydrogenase pathway, cytochrome P4502E1 system, and catalase (Fig. 2)<sup>4</sup>. The oxidative metabolism of alcohol generates an excess of NADH, resulting in an increased ratio of NADH to  $\text{NAD}^+$  in hepatocytes. This altered NADH/ $\text{NAD}^+$  ratio in hepatocytes leads to inhibition of fatty acid oxidation and promotion of lipogenesis. In addition, alcohol promotes lipid metabolism through inhibition of peroxisome proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ ) and AMP kinase, and via stimulation of sterol regulatory element-binding protein 1, a membrane-bound transcription factor<sup>5,6</sup>. In combination, these effects result in a fat-storing metabolic remodeling of the liver, which is manifested as excessive accumulation of lipids in hepatocytes. Although fatty liver resolves with abstinence, it predisposes the individuals who continue to drink excessively to more serious forms of ALD, i.e., alcoholic hepatitis and cirrhosis.

Alcoholic hepatitis is characterized by inflammation and necrosis<sup>7</sup>. It is believed that alcohol compromises the intestinal barrier, which leads to increased permeability and the subsequent presence of gut bacteria-derived lipopolysaccharide (LPS) in the portal blood. LPS stimulates the Kupffer cells and possibly other types of cells in the liver, resulting in excessive release of inflammatory cytokines and reactive oxygen and nitrogen species (ROS/RNS). The dysregulated inflammatory responses are thought to primarily contribute to the hepatocyte injury and tissue necrosis. In addition, accumulation of lipids also promotes inflammation. Progression of these detrimental processes may eventually cause excessive accumulation of collagen in extracellular matrix, resulting in liver fibrosis and cirrhosis. The crucial role of inflammation in ALD is supported by the demonstrated therapeutic efficacy

of anti-inflammatory drugs (e.g., glucocorticosteroids) in animal models and certain patient subgroups<sup>7, 8</sup>. In addition to dysregulated inflammation, recent work demonstrated that oxidative stress also plays an important role in the development of alcoholic hepatitis and cirrhosis as well as fatty liver. Since the general aspects of oxidative stress mechanism of ALD have been reviewed previously<sup>5, 9</sup>, this article is intended to focus on discussing the new findings from both animal experiments and human studies with an emphasis on novel oxidative stress and redox signaling pathways and the mechanistically-based modalities targeting these molecular pathways for the intervention of ALD. To set a stage for the discussion of the new findings, previously well-established data on oxidative stress in ALD are briefly outlined wherever pertinent.

## ROLE OF OXIDATIVE STRESS IN ALD IN EXPERIMENTAL ANIMALS

Studies using animal models have contributed greatly to our understanding of how ALD develops and how the severity of liver injury is influenced by factors other than alcohol, such as nutrition, oxygen deprivation (as occurs with sleep apnea and smoking), and gene regulation. In this regard, the intragastric feeding model results in liver lesions that mimic human ALD and has been widely used<sup>10, 11</sup>. In addition, chronic feeding of liquid diet containing ethanol is also a commonly used model of ALD<sup>12</sup>. Animal experiments have provided important information on the pathophysiology of ALD including the causative involvement of oxidative stress and also contributed to the development of new therapeutic approaches.

### Evidence for a causative role of oxidative stress in experimental ALD

As discussed below, there are four lines of experimental evidence that support a causative involvement of oxidative stress in the development of ALD in animal models.

**Increased formation of ROS/RNS**—The ability of acute and chronic alcohol treatment to increase the production of ROS/RNS as well as other free radical species (e.g., 1-hydroxyethyl radical) has been demonstrated in a variety of systems, including cell cultures and experimental animals, as well as in human subjects (see refs. 5 and 9; also see later sections for the discussion of human evidence). The detailed cellular sources and mechanisms of ROS/RNS formation are also described later in the manuscript.

**Depletion of antioxidants and increased biomarkers of oxidative damage**—Although acute exposure to alcohol may cause transient induction of certain antioxidant genes, excessive chronic exposure usually results in decreased levels of antioxidant defenses both in hepatic tissue and in blood. Of note, an early change after alcohol treatment is the depletion of mitochondrial glutathione in hepatocytes, which appears to play an important part in alcohol-induced liver injury<sup>13</sup>. In line with the augmented formation of ROS/RNS and decreased levels of antioxidant defenses, increased levels of oxidative stress biomarkers are observed in experimental animals exposed to alcohol. These biomarkers include lipid peroxidation products (e.g., malondialdehyde, 4-hydroxy-2-nonenal, F<sub>2</sub>-isoprostanes), oxidative DNA base modifications (e.g., 8-hydroxy-2'-deoxyguanosine) and the protein adducts derived from reactive aldehydes, as well as 1-hydroxyethyl radical. Notably, the levels of the oxidative stress biomarkers are correlated with the severity of alcohol-induced liver injury. As such, oxidative stress biomarkers are frequently used to assess the liver injury as well as the protection by potential therapeutic agents, including antioxidant compounds<sup>5, 9</sup>.

**Protection by exogenous antioxidant compounds**—The ability of alcohol to cause oxidative stress makes it rational to use antioxidant compounds to protect against the liver

injury. Indeed, various compounds with antioxidant properties have been shown to be protective against alcohol-induced liver injury in animal models. These include antioxidant vitamins, glutathione-augmenting agents (e.g., glutathione esters, *N*-acetylcysteine, *S*-adenosyl-L-methionine), antioxidant mimetics, probucol, and dietary polyphenols<sup>14-18</sup>. In addition, induction of endogenous antioxidants by chemical inducers also ameliorated alcohol-induced liver injury<sup>19,20</sup>. These findings are consistent with the concept that oxidative stress plays a causal role in experimental ALD.

**Protection by genetic overexpression of endogenous antioxidant genes and sensitization by antioxidant gene knockout**—In line with the protection by exogenous antioxidant compounds, overexpression of endogenous antioxidant proteins (e.g., manganese superoxide dismutase, Cu, Zn superoxide dismutase, metallothionein) via transgenic approaches or gene delivery blunted alcohol-induced liver injury in experimental animals<sup>21-23</sup>. Of note, the protective effects were also observed with systemic administration of antioxidant proteins, such as thioredoxin<sup>24</sup>. Conversely, gene knockout of endogenous antioxidant proteins (e.g., Cu, Zn superoxide dismutase, catalase, glutathione peroxidase-1, metallothionein) sensitized the experimental animals to alcohol-induced liver injury<sup>25-28</sup>. More recently, it was reported that ablation of either sulfiredoxin or peroxiredoxin-1 in mice markedly aggravated alcohol-induced oxidative hepatic injury<sup>29</sup>. Notably, peroxiredoxin-1 was found to be closely associated with cytochrome P4502E1 on the cytosolic side of the endoplasmic reticulum membrane of the hepatocytes. This close proximity of peroxiredoxin-1 and P4502E1 was suggested to be responsible for peroxiredoxin-1-mediated removal of ROS generated by P4502E1<sup>29</sup>.

The above observations from studies using antioxidant gene overexpression and/or knockout models provide the most compelling evidence for a cause and effect relationship between oxidative stress and the development of ALD. The crucial beneficial function of endogenous antioxidant defenses in ALD is further strengthened by the finding that targeted disruption of Nrf2, a central regulator of antioxidant genes, markedly aggravated alcohol-induced liver injury in mice, as indicated by increased liver necrosis, inflammation, oxidative stress, and mortality<sup>30</sup>. As such, activation of Nrf2 signaling by pharmacologic agents or phytochemicals has been proposed as an important strategy for protecting against liver injury induced by alcohol as well as other hepatotoxicants<sup>31</sup> (see a later section for the further discussion of Nrf2 signaling in the molecular pathophysiology of ALD).

### Sources of ROS/RNS in experimental ALD

**Hepatocytes**—As oxidative damage is an event predominantly in hepatocytes following alcohol administration, hepatocytes may thus be a major source of the ROS/RNS as well as other free radical species. Indeed, several intracellular pathways have been shown to contribute to the increased production of the reactive species in hepatocytes. These include mitochondria, cytochrome P4502E1, NAD(P)H oxidase (NOX), and inducible nitric oxide synthase (iNOS).

- **Mitochondria:** As discussed earlier, metabolism of alcohol results in increased levels of NADH, which provide electrons for mitochondrial electron transport chain, leading to increased one-electron reduction of oxygen to superoxide. In addition, formation of acetaldehyde by alcohol was shown to cause mitochondrial damage, which may also lead to increased formation of superoxide from the electron transport chain<sup>32,33</sup>.

Recently, the redox-active protein p66<sup>SHC</sup> was found to be associated with mitochondria and generate ROS via electron transfer from cytochrome c<sup>34</sup>. This redox-active protein potentiated ethanol-induced oxidative stress and mitochondrial

damage in hepatocytes<sup>35</sup>. Ethanol treatment of mice also caused induction of p66<sup>SHC</sup> in hepatic tissue, and mice deficient in p66<sup>SHC</sup> showed increased resistance to ethanol-induced liver oxidative stress, steatosis, and necrosis as compared with the wild-type counterparts<sup>35</sup>. Interestingly, deletion of p66<sup>SHC</sup> led to marked augmentation of ethanol-mediated induction of manganese superoxide dismutase in hepatic tissue and cultured hepatocytes, suggesting p66<sup>SHC</sup> may also act as a transcriptional suppressor of liver manganese superoxide dismutase<sup>35</sup>. Hence, the mitochondria-associated, ethanol-inducible, redox-active protein p66<sup>SHC</sup> may function as a novel source of ROS generation during ethanol consumption, contributing to the oxidative liver injury underlying ALD.

- P4502E1: Cytochrome P450 enzymes, especially P4502E1 are known to produce ROS. The ability of P4502E1 to produce ROS is augmented during alcohol administration as this enzyme is highly inducible by alcohol. Both cell experiments and in vivo animal studies demonstrated P4502E1 as an important source of ROS that participate in alcohol-induced liver injury. Knockout of P4502E1 blunted alcohol-induced oxidative stress and liver injury in mice, whereas transgenic overexpression of the enzyme aggravated alcohol-induced hepatic damage, pointing to a causative involvement of P4502E1 in the pathophysiology of ALD<sup>36</sup>.
- NOX and iNOS: Although NOX and iNOS of inflammatory cells are primarily responsible for the augmented formation of ROS/RNS during inflammatory responses alcohol treatment also resulted in activation of both NOX and iNOS in hepatocytes, leading to increased production of both superoxide and nitric oxide<sup>37,38</sup>. Reaction of these two radical species generates the potent oxidant peroxynitrite, which has been suggested to cause mitochondrial damage underlying experimental ALD<sup>39,40</sup>.

**Inflammatory cells**—As stated above, activation of NOX and iNOS in inflammatory cells results in the formation of large amounts of ROS/RNS. Both hepatic Kupffer cells and infiltrated inflammatory cells were found to be activated to produce ROS/RNS following alcohol administration<sup>41</sup>. Activation of Kupffer cells by LPS derived from gut bacteria is an early event leading to ALD. In this regard, depletion or inactivation of Kupffer cells ameliorated alcohol-induced inflammation, oxidative stress, and liver injury in experimental animals<sup>41</sup>. Alcohol-induced oxidative stress and hepatic injury were also blunted in mice deficient in either p47<sup>phox</sup> or iNOS, indicating a causative role for NOX and iNOS in the pathophysiology of ALD<sup>42-44</sup>. p47<sup>phox</sup> is an essential component of the NOX enzyme complex.

**Hepatic stellate cells**—In addition to hepatocytes and inflammatory cells, hepatic stellate cells may also contribute to the formation of ROS during alcohol exposure. Treatment with alcohol or its metabolite acetaldehyde was shown to elicit increased ROS formation from NOX in stellate cells. Such formed ROS as well as the ROS derived from other cellular sources, such as P4502E1 may play an important part in the transformation of stellate cells into myofibroblasts as well as the increased production of collagen, a process critically involved in liver fibrosis<sup>5,45</sup>. Mechanistically, it was demonstrated that induction of collagen expression by acetaldehyde in stellate cells was dependent on PPAR- $\gamma$  phosphorylation induced by a hydrogen peroxide-mediated activation of the profibrogenic c-Abl signaling pathway<sup>46</sup>. This finding also highlighted the intimate association between acetaldehyde and ROS in the molecular pathophysiology of ALD.



## Mechanisms of oxidative stress-mediated injury in experimental ALD

The key molecular mechanisms underlying oxidative stress-mediated liver injury in ALD can be summarized into the following three schemes: (1) mitochondrial damage, (2) perpetuation of inflammation via dysregulated redox signaling, and (3) transformation of stellate cells and extracellular matrix remodeling associated with dysregulated redox signaling<sup>5, 47</sup>. In addition, recent studies suggested a crucial role for dysregulated Nrf2 signaling in molecular alterations leading to experimental ALD.

**Mitochondrial damage**—Alcohol administration causes increased formation of ROS in mitochondria<sup>32, 33</sup>. Such locally generated ROS as well as those derived from other cellular sources cause mitochondrial dysfunction, energy depletion, and ultimately cell death<sup>48</sup>. The increased production of ROS/RNS also results in damage of other cellular constituents, including membrane lipids, enzymes, and nucleic acids, contributing to cell injury. Notably, alcohol treatment increases hepatic iron accumulation, which may further facilitate the production of the highly reactive hydroxyl radical, causing damage to mitochondria and other cellular constituents<sup>49</sup>. The key role of mitochondrial oxidative stress injury in ALD is further supported by the finding that either overexpression of the mitochondrial antioxidant enzyme manganese superoxide dismutase or administration of exogenous antioxidant compounds targeted to mitochondria, such as mito-coenzyme Q (MitoQ), led to attenuation of oxidative liver injury underlying ALD<sup>22, 50</sup>.

**Perpetuation of inflammation via dysregulated redox signaling**—As noted earlier, activation of inflammatory cells results in the formation of large quantities of ROS/RNS. Such formed ROS/RNS also cause dysregulated redox homeostasis, leading to the activation of NF- $\kappa$ B and the subsequent overexpression of proinflammatory cytokines and adhesion molecules<sup>51, 52</sup>. Indeed, the NF- $\kappa$ B-regulated chemokine MCP-1 was recently shown to be essential for the continuous inflammatory responses in liver tissue and the development of hepatic steatosis in alcohol-treated mice<sup>53</sup>. Hence, the perpetuation of inflammation and inflammatory injury may be an important molecular mechanism underlying the development and progression of ALD. Since metabolism of alcohol also leads to the increased formation of ROS independent of the activation of inflammatory cells (for example, the P4502E1 and mitochondria-derived ROS) ROS may also contribute to the initiation of the inflammatory responses by activating NF- $\kappa$ B and other redox-sensitive signaling molecules. In this regard, oxidative stress may contribute to the genesis of ALD. This notion is supported by observations that administration of antioxidant compounds or overexpression of endogenous antioxidant proteins is frequently able to prevent the development of ALD in experimental animals (see earlier sections on protection by exogenous antioxidant compounds and genetic overexpression of endogenous antioxidant genes).

**Transformation of stellate cells and extracellular matrix remodeling associated with dysregulated redox signaling**—ROS/RNS are able to induce the transformation of hepatic stellate cells into myofibroblasts, leading to excessive production and deposition of collagen in extracellular matrix. Reactive aldehydes derived from lipid peroxidation, such as 4-hydroxy-2-nonenal have been shown to also stimulate collagen production from stellate cells<sup>54</sup>. In addition, ROS/RNS are capable of inducing proliferation and activation of other cells, such as periportal fibroblasts and fibrocytes. Furthermore, ROS/RNS may cause activation of matrix metalloproteinases, resulting in remodeling of the extracellular matrix<sup>55</sup>. Progression of these molecular and cellular events eventually leads to excessive hepatic fibrosis and cirrhosis<sup>56</sup>.

**Hepatic Nrf2 signaling**—Studies in the early 1990's demonstrated a crucial role for a cis-acting element, named antioxidant response element (ARE) in the regulation of antioxidant gene expression<sup>57</sup>. In 1997, M. Yamamoto and coworkers identified Nrf2 (nuclear factor-erythroid 2 p45-related factor 2) as a critical transcription factor that interacts with the ARE, leading to increased expression of various antioxidant and cytoprotective genes<sup>58</sup>. Subsequent studies from multiple research groups have established that Nrf2 plays a central role in regulating both constitutive and inducible expression of a wide variety of antioxidant and anti-inflammatory genes in mammalian tissues/cells<sup>59-61</sup>. As noted earlier, targeted disruption of Nrf2 in mice led to aggravation of ethanol-induced liver injury as evidenced by augmented hepatic oxidative stress, inflammatory responses, steatosis, and necrosis. The ethanol-mediated hepatic mitochondrial damage was also exacerbated in the Nrf2-knockout mice. These exacerbated deleterious responses led to accelerated liver failure and death caused by ethanol in the Nrf2-knockout mice<sup>30</sup>. Notably, short-term ethanol treatment resulted in induction of several antioxidant and anti-inflammatory enzymes, including heme oxygenase-1, NAD(P)H:quinine oxidoreductase-1, and glutathione S-transferase in the liver tissue in an Nrf2-dependent manner<sup>30</sup>. Hence, the induction of antioxidant and anti-inflammatory enzymes via Nrf2 signaling might act an early compensatory or adaptive mechanism to suppress ethanol-induced oxidative injury and inflammation in liver tissue, and ablation of this protective compensatory mechanism in Nrf2-knockout mice led to dramatic acceleration of ethanol-induced liver injury, hepatic failure, and death in mice<sup>30</sup>. In this context, a recent study reported that ethanol-induced upregulation of heme oxygenase-1 expression was mediated by both Nrf2 signaling and hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ), and the upregulation of heme oxygenase-1 attenuated inflammatory cytokine expression in ALD<sup>62</sup>. Nrf2 was also found to be induced by overexpression of P4502E1 in liver tissue and cultured hepatocytes, and the augmented Nrf2-dependent antioxidants attenuated P4502E1-mediated oxidative hepatic injury<sup>63</sup>.

Taken together, the above observations demonstrated a crucial role for Nrf2 signaling (and possibly other signaling pathways) in suppressing the oxidative and inflammatory pathophysiology of ALD (Fig. 3). Hence, disruption of the normal functionality of Nrf2 signaling may be a potential mechanism leading to increased susceptibility to ALD. Likewise, augmentation of the hepatic Nrf2 signaling by pharmacological approaches or gene therapy, as mentioned earlier, may represent an effective strategy for the intervention of the oxidative stress pathophysiology of ALD.

### Summary of the role of oxidative stress in experimental ALD

Alcohol causes augmented formation of ROS/RNS from multiple sources, including hepatocytes, inflammatory cells, and other cell types in liver tissue. The augmented production of ROS/RNS and the consequent depletion of antioxidants together lead to marked oxidative stress that contributes to both the genesis and progression of ALD in animal models (Fig. 4). The causative involvement of oxidative stress in ALD is further supported by the demonstrated efficacy of both exogenous and endogenous antioxidants in protecting against alcohol-induced liver injury. These findings in experimental animals have prompted the investigation of the causative role of oxidative stress in human ALD and the antioxidant-based modalities for the disease intervention.

## ROLE OF OXIDATIVE STRESS IN ALD IN HUMANS

### Evidence for a role of oxidative stress in human ALD

**Increased ROS/RNS formation and biomarkers of oxidative stress**—Excessive consumption of alcohol led to increases in biomarkers of oxidative stress in plasma and urine, including the lipid peroxidation products F<sub>2</sub>-isoprostanes and 4-hydroxy-2-

nonenal<sup>64,65</sup>. The oxidative stress biomarkers were closely associated with the severity of liver injury. Notably, acute alcohol consumption in healthy individuals also resulted in increases in the oxidative stress biomarkers, suggesting that oxidative stress may precede the development of ALD<sup>66</sup>. In addition, levels of antioxidants, such as glutathione, vitamin C, and vitamin E were decreased in patients with ALD as compared with normal subjects. As discussed above, metabolism of alcohol forms 1-hydroxyethyl radical in experimental systems. The protein adduct of 1-hydroxyethyl radical was also detected in patients with alcoholic cirrhosis<sup>67</sup>.

**Antioxidant gene polymorphisms**—A number of studies have demonstrated that polymorphisms of glutathione S-transferase (GST), especially the null-genotypes of GSTM1 and GSTT1 were associated with increased risk of developing ALD as well as hepatocellular carcinoma<sup>68, 69</sup>. These findings are consistent with the notion that GST enzymes play an important role in the detoxification of reactive aldehydes, including those that participate in alcohol-induced liver injury. In addition to GST, a Val-Ala polymorphism in manganese superoxide dismutase (MnSOD) was associated with elevated risk of developing cirrhosis in French alcoholics and increased rates of hepatocellular carcinoma development and death in cirrhotic patients<sup>70</sup>. Notably, the polymorphisms in antioxidant genes may interact with those in other xenobiotic biotransforming enzymes, resulting in augmented risk of developing ALD. For example, combination of the GSTM1-null genotype and a polymorphism of P4502E1 (causing increased P4502E1 activity) resulted in increased risk of developing alcoholic liver cirrhosis<sup>71</sup>. Combination of GG-myeloperoxidase (MPO) genotype (leading to high MPO expression) and at least one Ala-MnSOD allele markedly elevated the risk of hepatocellular carcinoma occurrence and death in patients with alcoholic cirrhosis<sup>72</sup>.

**Antioxidant intervention**—Although the experimental evidence supporting a protective role of antioxidants in alcohol-induced liver injury is extensive, the effectiveness of antioxidant therapy remains unclear in human patients with ALD. Randomized controlled clinical trials on using antioxidant vitamin E or an antioxidant cocktail (containing antioxidant vitamins, *N*-acetylcysteine, and coenzyme Q among others) have failed to show an efficacy in improving the disease conditions in ALD patients<sup>73-75</sup>. The causes of the apparent discrepancy between the experimental studies and clinical trials are not clear. Potential reasons may include the doses of antioxidants used in the trials, the duration and time-window of the intervention, the patients' status of oxidative stress and antioxidants, as well as the lack of a profound understanding of the pharmacokinetic and pharmacodynamic activities of the antioxidant compounds in patients with ALD. In this context, *S*-adenosyl-L-methionine is a well-characterized antioxidant compound that functions as a methylating agent, a precursor of glutathione, and a modulator of proinflammatory cytokine expression. A previous randomized controlled trial demonstrated that patients with ALD treated with *S*-adenosyl-L-methionine showed improved mortality and decreased need for liver transplantation<sup>76</sup>. Although *S*-adenosyl-L-methionine showed promising effects, the exact contribution of the antioxidant activity of this compound to the therapeutic activity in ALD remains to be determined. Moreover, the clinical efficacy of *S*-adenosyl-L-methionine as a therapy for ALD needs to be further established in large-scale well-designed trials.

### Summary and future perspectives on studies of ALD in humans

Compared with the substantial experimental evidence supporting a causative role of oxidative stress in ALD and the benefits of antioxidant-based modalities in disease intervention in animal models, evidence from studies in human subjects is less compelling. Nevertheless, there is accumulating evidence indicating a possible causative involvement of oxidative stress in the pathophysiology of human ALD. Future studies should focus on



developing novel antioxidant compounds with well-characterized pharmacokinetic and pharmacodynamic profiles, and testing them in selected patient populations with overt oxidative stress and/or antioxidant deficiency. Such a subpopulation targeting strategy has been shown to be effective in the intervention of other oxidative stress-associated diseases, such as cardiovascular disorders and diabetes<sup>77-79</sup>. In addition to the therapeutic intervention, future efforts should also be devoted to the development of antioxidant-based strategies for primary prevention of ALD in susceptible or high risk individuals.

## Acknowledgments

ZH is supported by NIH grant DK81905; YL is supported by a grant from AICR and NIH HL93557.

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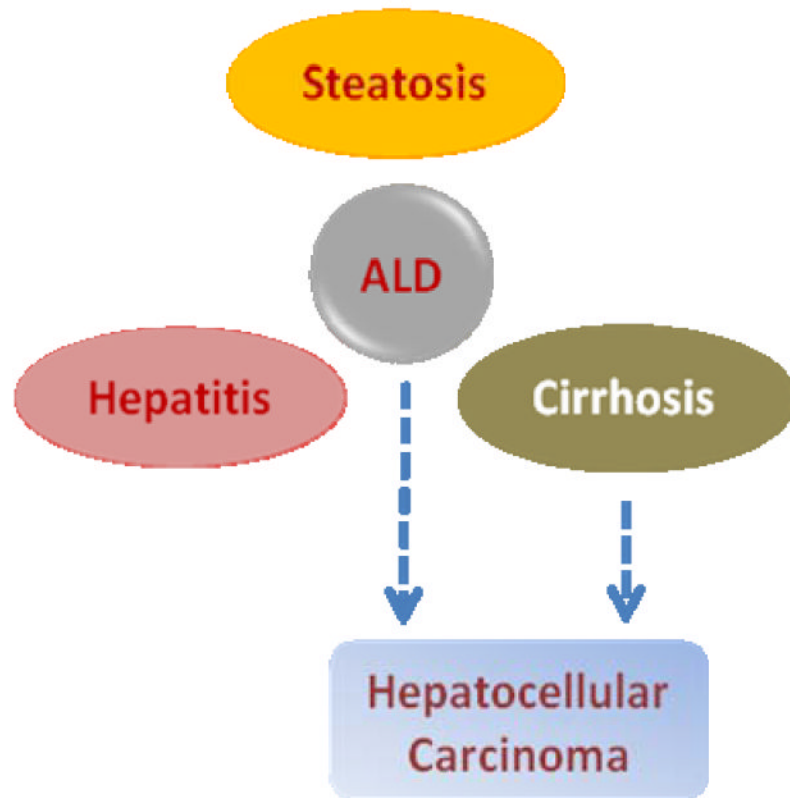
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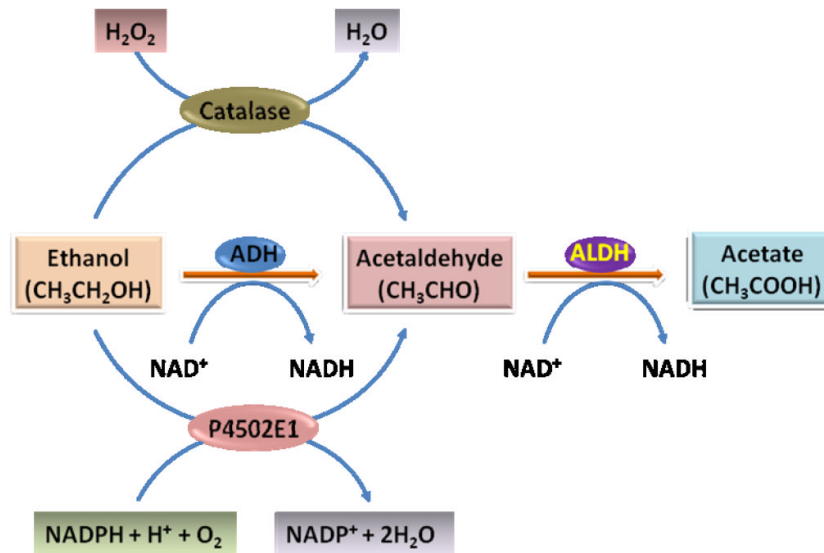
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**Fig. 1.** Spectrum of alcoholic liver disease (ALD). It is of note that although cirrhosis is usually the primary pathophysiological process that leads to the development of hepatocellular carcinoma, ALD may progress into liver cancer without necessarily going through cirrhosis.



**Fig. 2.** Sequential metabolism of ethanol to form acetaldehyde and acetate. As illustrated, the metabolism of ethanol occurs in the liver (also in many other types of tissues) via three enzymatic systems, ADH, P4502E1, and catalase, which are primarily located in cytosol, endoplasmic reticulum membrane (microsomes), and peroxisomes, respectively. ALDH is located in mitochondria and cytosol. ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase.

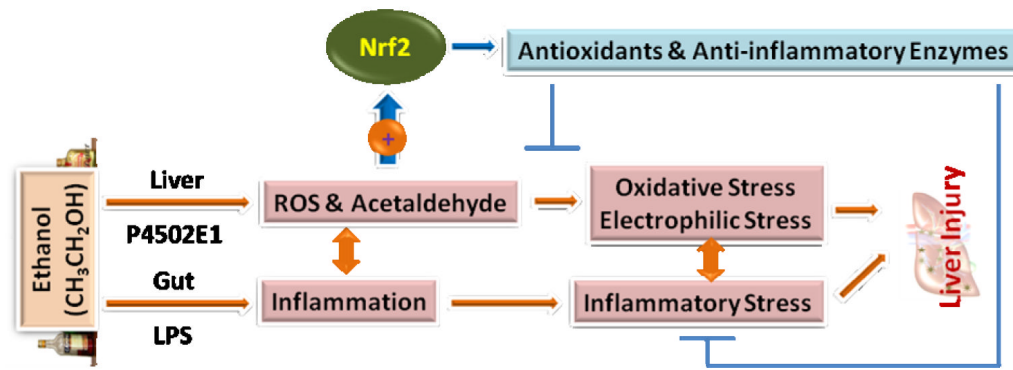
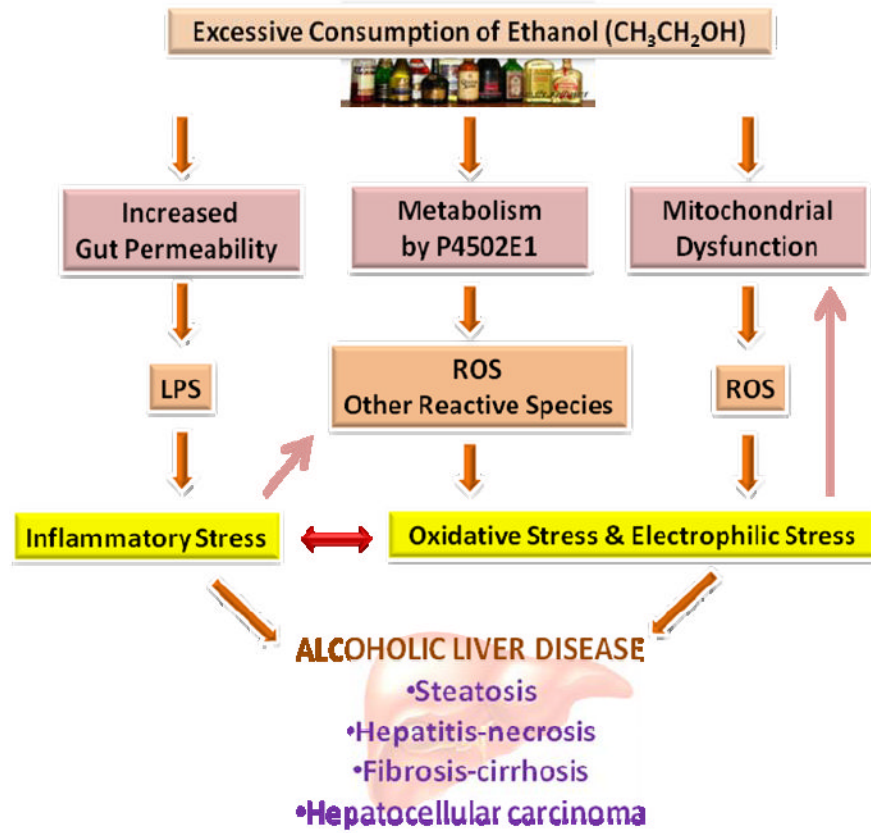


Fig. 3.

Involvement of Nrf2 signaling in the pathophysiology of alcoholic liver disease (ALD). Activation of Nrf2 signaling and Nrf2-dependent antioxidants and anti-inflammatory enzymes may act as a compensatory protective mechanism in ALD. Consequently, compromised Nrf2 functionality may aggravate the pathophysiology of ALD. See text for more detailed description. ROS, reactive oxygen species; LPS, lipopolysaccharide.



**Fig. 4.** Schematic illustration of the overall involvement of oxidative/electrophilic stress and dysregulated inflammation in the pathophysiology of alcoholic fatty liver disease. See text for more detailed description. LPS, lipopolysaccharide; ROS, reactive oxygen species.