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REVIEW ARTICLE

Role of oxidative stress in the pathogenesis of alcohol-induced liver disease

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Abstract
Chronic alcohol consumption is a well-known risk factor for liver disease, which represents a major cause of morbidity and mortality worldwide. The pathological process of alcohol-induced liver disease is characterized by a broad spectrum of morphological changes ranging from steatosis with minimal injury to more advanced liver damage, including steato-hepatitis and fibrosis/cirrhosis. Experimental and clinical studies increasingly show that the oxidative damage induced by ethanol contribute in many ways to the pathogenesis of alcohol hepatotoxicity. This article describes the contribution of oxidative mechanisms to liver damage by alcohol.

Keywords: alcohol, liver disease, oxidative damage, reactive oxygen species

Introduction

The pathological process of alcohol-induced liver disease (ALD) is characterized by a broad spectrum of morphological features ranging from steatosis with minimal injury to more advanced liver damage, including steato-hepatitis and fibrosis/cirrhosis. Moreover, approximately 15% of patients with established alcoholic cirrhosis develop hepatocellular carcinoma (HCC). HCC accounts for between 85% and 90% of primary liver cancer, the sixth most common cancer and the third leading cause of cancer mortality [1–3]. Progression of ALD is a multifactorial process that involves a number of genetic, nutritional and environmental factors [4]. Among the mechanisms implicated in the pathogenesis of ALD, oxidative stress has received growing interest [5–7]. This article will focus on the contribution of oxidative mechanisms to liver damage by alcohol.

Oxidative stress

Prooxidant species

Reactive oxygen species (ROS), which are highly reactive oxygen-containing molecules, is a collective term used to designate oxygen radicals, such as superoxide anion ($O_2^{\bullet -}$) and hydroxyl radical ($HO^{\bullet}$), and also derivatives of oxygen that do not contain unpaired electrons, such as hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and hypochlorous acid (HOCl). ROS can be generated by the mitochondrial respiratory chain [8], cytochrome P450 [9], and auto-oxidation of endogenous substrates, such as heme proteins, catecholamines and quinones [10,11]. Moreover, there are enzymes which produce ROS, such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex (Nox) [12] (Figure 1). ROS are produced by normal cellular metabolism with beneficial effects such as cytotoxicity against bacteria. However, they also may affect cells of the host organism. They can lead to the oxidation of cellular macro-molecules, such as lipids, protein or DNA, inhibiting normal function [13]. Peroxidation of lipids can result in destruction of biological membranes [14]. ROS may affect different signaling pathways modulating gene expression, cell metabolism, cell cycle and cell death. These events may induce oxidative DNA damage, which in turn increases chromosomal aberrations associated with cell transformation [15,16].

Antioxidant molecules

In contrast, other enzymes such as superoxide dismutase (SOD), which remove $O_2^{\bullet -}$, catalase and glutathione (GSH) reductase (GRed) and GSH peroxidase (GPx) system, which remove H_2O_2, are determinants of antioxidant defense [17,18] (Figure 1). Another family of antioxidant enzymes is the peroxiredoxins (Prx), which can detoxify H_2O_2 with the use of electrons provided by a physiological thiol-like thioredoxin (Trx) [19]. At last, glutaredoxins (Grxs) are thiol-disulfide GSH-dependent oxidoreductases that catalyze the GSH-dependent reduction of disulfide and GSH-mixed disulfides [20]. Moreover, non-enzymatic low molecular weight antioxidants such as GSH itself, vitamin E, ascorbate, vitamin A, ubiquinone and bilirubin have evolved to protect cells against ROS [21,22].

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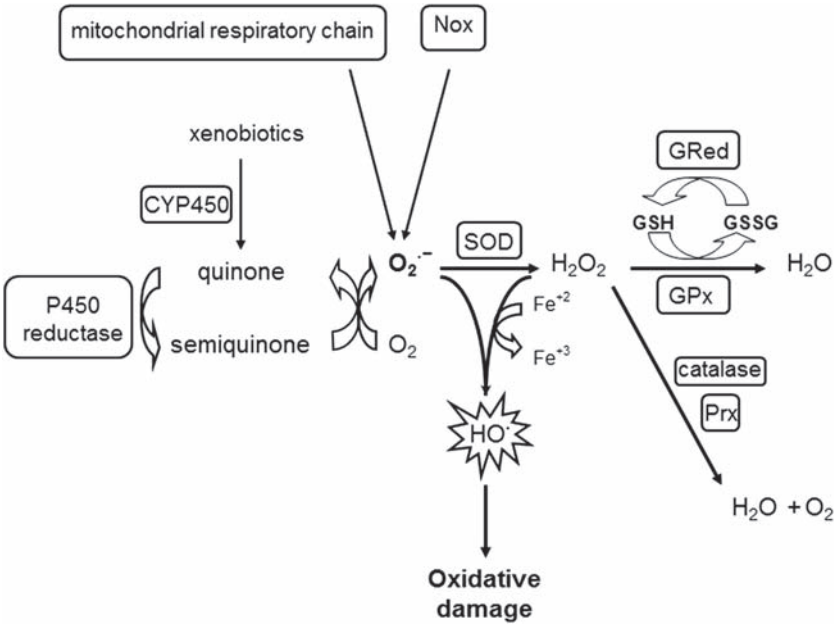

Figure 1. *Simplified scheme of oxidative and antioxidative systems in hepatocytes.* Normal cells generate ROS such as $O_2^{\cdot -}$ and H_2O_2 as a result of normal metabolism. Both $O_2^{\cdot -}$ and H_2O_2 can be converted to the highly reactive $OH^{\cdot}$ by iron (Fe^{2+})-catalyzed Haber–Weiss and Fenton reactions. Alternatively, ROS can be generated by mitochondrial respiratory chain or by Nox. Similarly, ROS can be formed as a result of exposure to environmental agents including chemicals (xenobiotics) like quinones. These quinones are subjected to redox-cycling leading to the formation of semihydroquinones/hydroquinones and ROS. Numerous intracellular enzymes serve to degrade ROS. Some of these enzymes are specific such as SODs, which detoxify $O_2^{\cdot -}$ to H_2O_2, whereas others have overlapping substrate affinities such as catalase, peroxyredoxins (Prx) and glutathione peroxidases (GPx), which can degrade H_2O_2 to water and O_2. Note that GPx can also degrade organic peroxides to relatively non-toxic alcoholic species. GPx require GSH during the course of peroxide degradation leading to the oxidation of GSH to GSSG, which can be reduced back to GSH by the enzyme GRed.

Thus, the balance between antioxidants and prooxidants is crucial for normal cell function. Oxidative stress exists when there is an imbalance between oxidants and antioxidant defenses in favor of the oxidants in the cell.

Alcohol-induced oxidative stress in liver pathogenesis

High alcohol consumption provokes liver steatosis, which returns to a physiological condition whenever this consumption is stopped [23]. Chronic alcohol exposure leads to progressive liver damage, represented pathologically by steato-hepatitis, and accompanied by inflammation and cytokine production. This is the basis for the initiation of liver fibrosis [24,25]. Nevertheless, it should be noticed that a significant proportion of alcoholics never develop liver disease. Indeed, healthy liver is resistant to the action of ethanol and most individuals consuming alcohol have steatosis but not steatohepatitis [26]. The progression of steatosis to steatohepatitis has been shown to be dependent on additional factors such as endotoxin, nutritional factors and underlying diseases like hepatitis C viral infection [27,28]. In this regard, binge drinking habit in chronic alcoholics is one of the most important factors contributing to the progression of alcoholic liver injury [29–31]. Moreover, a recent study indicated that the effects of alcohol consumption on liver pathogenesis may depend on the drinking pattern and the diet, such as vitamins content [32].

Increased oxidative stress in hepatocytes is one of the mechanisms involved in steato-hepatitis. Under chronic ethanol exposure, ROS production is enhanced [33,34], whereas the level or activity of antioxidants is reduced [35–37]. Di Luzio [38] was the first to observe lipid peroxidation after prolonged ethanol exposure. The peroxidation of cellular lipids produces electrophiles, such as malondialdehyde (MDA) or 4-hydroxynonenal (4-HNE). These electrophiles are proposed to modify essential cellular proteins resulting in loss of protein function and cellular homeostasis. Increased lipid peroxidation-derived products have been shown in mitochondria isolated from chronic ethanol-fed rats [39], and MDA or HNE adducts have been found in human alcoholics [40]. Recent studies, using proteomic approaches to uncover molecular mechanisms of hepatic oxidative stress induced by chronic ethanol ingestion, have identified hepatic proteins modified with 4-HNE in rat and mouse models of ALD [41,31]. Among them, identification of ERK1/2 as an *in vivo* target for 4-HNE modification led to the identification of the specific site of adduction explaining the observed dysregulation of phosphorylation events potentially associated with impaired liver regeneration [41].

The capacity of alcohol to increase ROS production and oxidation of lipids, proteins and DNA has been demonstrated in a variety of systems, cells and species. The attack of ROS on proteins involves modulation of a protein's activity through nitrosylation, carbonylation, disulphide bond formation and glutathionylation. As a

consequence of excessive ROS production, site-specific amino acid modification, fragmentation of the peptide chain, aggregation of cross-linked reaction products, altered electric charge and increased susceptibility of proteins to proteolysis may occur [42]. For example, increase in ROS production by ethanol metabolism was associated with increases in mitochondrial protein carbonyl levels reflecting oxidized protein accumulation [43].

ROS can also cause oxidative damage to nuclear and mitochondrial DNA. Any damage to the DNA can result in changes in the encoded proteins, which may lead to malfunctions or complete inactivation of the encoded proteins. Among the various forms of oxidative DNA damage, 7,8-dihydro-8-oxoguanine (8-oxo-Gua) is frequently observed and can be a useful marker of cellular oxidative stress [44]. To examine the effects of alcohol consumption on liver redox homeostasis, both oxidative DNA damage and its repair activity were recently measured in the livers of rats fed with ethanol. An increase in the 8-oxo-Gua levels and its repair activity were observed in the livers of rats after long-term alcohol and vitamin-depleted feeding. These results indicated that the effects of alcohol consumption on oxidative DNA damage may depend on the drinking pattern and the diet [32].

Effects of alcohol on ROS production

During alcohol exposure, ethanol metabolism produces a concordant amount of ROS. Beyond a certain limit, ROS cannot be efficiently removed by antioxidant systems and they become important mediators of liver damage. Various cell types in the liver, including hepatocytes, Kupffer cells and hepatic stellate cells (HSC), may contribute to the formation of ROS [45,46]. Interestingly, hepatocytes are a major source of ROS and free radicals, and several intracellular pathways have been shown to contribute to the increased production of reactive intermediates in these cells. These include mitochondria, cytochrome P450 2E1 (CYP2E1) and NAD(P)H oxidase (Nox) [47]. In the cytosol, ethanol metabolism by alcohol dehydrogenase (ADH) leads to the formation of acetaldehyde and ROS [48] and to the generation of the reduced form of nicotinamide adenine dinucleotide (NADH). At this step, NADH can also interfere with the electron transfer system in the mitochondria, facilitating ROS generation [49]. In addition, the formation of acetaldehyde was shown to cause mitochondrial damage, which may also lead to the increased one-electron reduction of oxygen to superoxide [50,51]. More recently, the redox-active protein p66Shc was found to be associated with mitochondria to generate ROS via electron transfer from cytochrome c [52]. Moreover, the microsomal ethanol-oxidizing system (MEOS), located on the endoplasmic reticulum (ER), which primarily consists of ethanol-inducible CYP2E1, also converts ethanol to acetaldehyde and generates ROS [53]. During the course of the P450 catalytic cycle, P450s use NADPH to reduce O_2 leading to the production of hydrogen peroxide and superoxide anion radical. The process of uncoupling of the catalytic cycle can lead to escape of $O_2^{\cdot -}$ [54,55]. At last, alcohol treatment also resulted in the activation of Nox in hepatocytes, leading to an increased production of superoxide [56].

Furthermore, an increase of hepatic iron concentrations occurs in alcohol-dependent individuals and elevated hepatic iron uptake is observed in patients with alcohol-induced cirrhosis [57,58]. This mechanism can also participate in alcohol-induced oxidative stress since iron can catalyze the conversion of less reactive oxidants such as superoxide or H_2O_2 to more powerful oxidants such as hydroxyl radical [5].

Effects of alcohol on antioxidant defenses

The possible contribution of impaired antioxidant defenses to ethanol-induced oxidative stress has been extensively investigated. GSH is probably the most important non-enzymatic antioxidant present in cells. Diverse effects of ethanol on total hepatic GSH level have been reported [59–61]. Alcohol has been shown to deplete GSH levels in mitochondria, which are normally characterized by high levels of GSH needed to eliminate ROS generated during activity of the respiratory chain [62]. The selective depletion of the mitochondrial GSH pool is the consequence of a defect in GSH transport from the cytosol to the mitochondrial matrix [63]. Decreased fluidity of mitochondrial membranes from alcohol-fed rat livers, associated with cholesterol accumulation, seems to interfere with the activity of high-affinity GSH carriers. In agreement with this hypothesis, the fluidization of mitochondria by the fluidizing agent, 2-(2-methoxyethoxy) ethyl 8-(cis-2-n-octylcyclopropyl) (A(2)C), restored the initial transport rate of GSH [64]. Ethanol-induced decreases in mitochondrial GSH occur predominantly in the centrilobular hepatocytes, where most of the liver injury originates [65], and precede the development of mitochondrial dysfunction and lipid peroxidation [65,66].

Several studies have shown that antioxidant administration can ameliorate or prevent the toxic effects of ethanol. Indeed, administration of vitamin E, SOD and a precursor of GSH (L-2-oxothiazolidine-4-carboxylic acid, a cysteine prodrug that replenishes glutathione stores), prevented alcohol-induced hepatic damage in rats [59,67]. Vitamin E plays an important role as the main lipid-soluble antioxidant in the liver [68], and several studies have reported a reduction in liver and plasma vitamin E levels associated with ethanol consumption [69–71]. This vitamin E reduction resulted from either increased oxidative stress, differences in ethanol consumption or diet, altered lipoprotein status or enzymatic changes in the liver. However, although vitamin E-deficient rats have increased susceptibility to ethanol toxicity [72], α-tocopheryl acetate supplementation does not protect against liver injury [73]. Interestingly, when ethanol feeding is discontinued, vitamin E administration contributes to a reduction in the severity of hepatic lesions [74]. However, a randomized placebo-controlled clinical trial of patients with moderate alcoholic hepatitis did not demonstrate a marked effect of vitamin E supplementation [75].

Chronic alcoholism is able to induce CYP's expression and activity, which may also metabolize vitamins A and E. Indeed, many hepatic CYP isoforms have been identified to metabolize vitamin A-derived metabolites, including CYP2C8, CYP3A4 and CYP2C9 [76]. Likewise, vitamin E is metabolized similarly to xenobiotics, in that the lateral chain is ω-oxidized by CYPs (followed by β-oxidation), and hepatic CYP4F2 and CYP3A4 were suggested to be involved in α- and γ–tocopherol's degradation [77,78]. In this context, it has been shown that high doses of vitamin A supplementation over a longer period lead to the development of liver cirrhosis, and chronic alcohol consumption enhances this intrinsic hepatotoxicity [79,80]. In fact, chronic alcohol intake increases catabolism of vitamin A (retinol) into more polar metabolites in the liver [81]. This alcohol-induced polar retinol metabolites cause hepatocyte death through loss of mitochondrial membrane potential [82]. Moreover, work from Wang's laboratory has shown that CYP2E1 is the major enzyme responsible for the alcohol-enhanced catabolism of retinol in hepatic tissue after exposure to alcohol [83]. Hence, these mechanisms may limit the beneficial effects of antioxidant vitamins when administered in chronic alcoholism.

Regarding antioxidant enzymes, a marked decline in protein concentration and enzymatic activity of liver SOD, catalase and GPx have been reported in animals that are fed with diets high in polyunsaturated fatty acids and ethanol [84,85]. The changes in these enzyme activities are inversely correlated with the extent of both lipid peroxidation and hepatic injury. Cederbaum and colleagues investigated the effect of a compromised antioxidant defense system, namely SOD1 KO mice, in an alcohol-induced hepatic damage model [86,87]. Moderate alcohol consumption induced oxidative stress and extensive centrilobular necrosis and inflammation in these animals, indicating that compromised antioxidant defense aggravates ethanol liver damage. Conversely, rodents overexpressing SOD1 are protected against the liver injury associated with enteral administration of a large amount of alcohol [88]. Studies with isolated hepatocytes from long-term ethanol-fed rats showed that ethanol metabolism via alcohol dehydrogenase led to increased ROS production, hepatocyte damage and apoptosis. These effects were prevented by antioxidants [89].

Nevertheless, the role of alcohol-induced changes in liver antioxidant enzymes in human pathology is still a subject of controversy [90]. In this context, extensive research has been conducted using SOD2 mutant mouse models to define various oxidative stress-induced disorders, including liver diseases [91]. The T to C nucleotide polymorphism (Val16Ala) has been identified in exon 2 of the human SOD2 gene, and the Ala variant is more efficiently imported into the mitochondria than the Val variant, thus resulting in increased mitochondrial SOD2 homotetramer activity derived from the Ala precursor variant [92]. The SOD2 Val/Val genotype has been proposed as a risk factor for susceptibility to non-alcoholic steatohepatitis [93]. However, a study performed on 281 patients with advanced ALD and 218 heavy drinkers without liver disease did not demonstrate any increased prevalence of SOD2 polymorphisms in ALD [94]. Moreover, the severity of liver injury is not increased in Ala-SOD2 homozygotes and the markers of oxidative stress remain unchanged [95].

Taken together, these results suggest a role of alcohol on antioxidant enzyme regulation but are not totally conclusive with regard to human pathology.

Mechanisms of ROS-mediated damage in ALD

Chronic ethanol treatment has long been known to depress mitochondrial function [96]. Indeed, ethanol-induced impairment of mitochondrial structure and function may increase the production of ROS and cause cell toxicity [97]. Moreover, under chronic ethanol exposure, ROS-induced cellular responses are strongly involved in innate immune cell activation within the liver and play a crucial role in the early pathogenesis of alcohol-induced liver injury. Finally, ROS appear to participate in the progression of ALD to liver fibrosis by stimulating the pro-fibrogenic pathway (Figure 2). These different effects of alcohol-induced oxidative stress are detailed below.

Oxidative stress and mitochondrial dysfunction

Although the molecular basis responsible for alcohol-dependent mitochondrial dysfunction remains to be identified, a key functional change to the mitochondria under conditions of fatty liver disease is its inability to maintain normal function of the respiratory chain and sufficient ATP levels [98–100].

Different hypotheses have been formulated to explain the observed alcohol-induced mitochondrial malfunction. A first hypothesis is linked to a loss of DNA integrity and impairment of protein synthesis. Indeed, mitochondria obtained from ethanol-treated rats show oxidative modifications of their DNA [101]. Deletions of mitochondrial DNA are eight-fold more frequent in the livers of patients with alcoholism compared with age-matched controls [102]. Moreover, inhibition of mitochondrial protein synthesis [103], linked to mitochondrial DNA damage [104] and ribosomal defects [105,106], contributes to decreased functioning of the oxidative phosphorylation system resulting in accumulation of reduced respiratory carriers in complexes I and III following chronic ethanol consumption. These disturbances in structure and function of the electron transport chain have been proposed as being associated with increased production of mitochondrial free radicals and oxidative injury in steatotic liver [96,101]. The alterations provoked by ROS translate into modifications to the mitochondrial proteome, which not only includes reductions in 30 mitochondrial encoded polypeptides, but also decreases in several nuclear encoded proteins that compose the oxidative phosphorylation complexes [104]. Proteomic analyses have also shown alcohol-dependent modifications in levels of mitochondrial

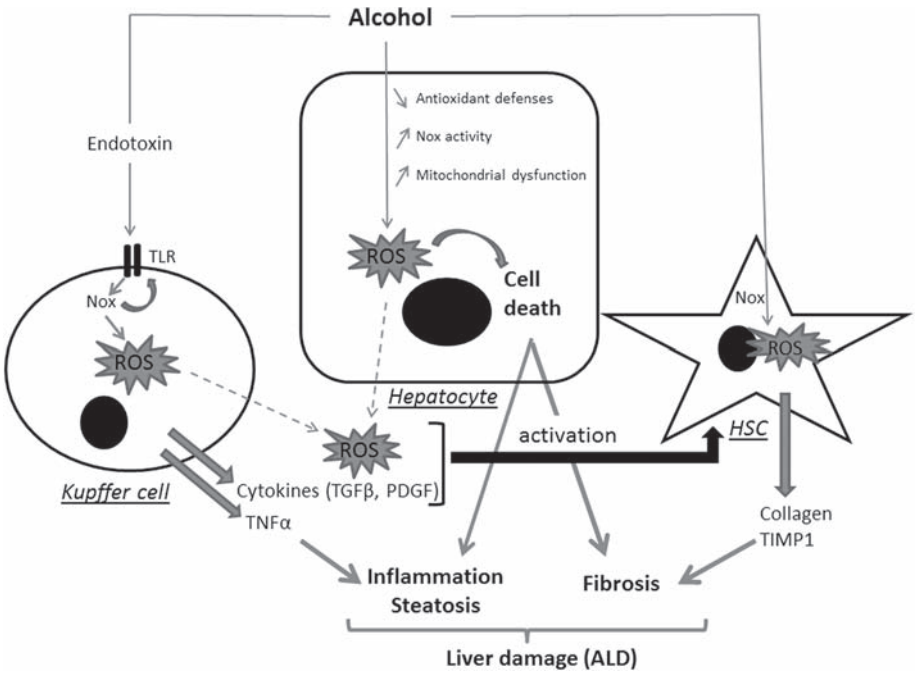

Figure 2. *Contribution of ethanol-induced oxidative stress to the progression of ALD.* Ethanol impairment of antioxidant defenses and mitochondrial structure and function may increase the production of ROS and cause hepatotoxicity, leading to fibrosis, inflammation and steatosis. ROS can also induce cellular responses which are strongly involved in Kupffer cell activation and which contribute to an increased inflammatory response leading to liver injury. Moreover, activated Kupffer cells release ROS and cytokines that are crucial for HSC activation and to induce the pro-fibrogenic pathway.

matrix enzymes, as well as post-translational changes [107,104].

A second hypothesis is related to P450 and other enzyme activities. Robin et al. [108] demonstrated that ethanol increased microsomal and mitochondrial CYP2E1 in cultured rat hepatocytes and in the liver of lean mice, suggesting an additional source of oxidants in alcohol-exposed mitochondria. Mitochondrial ROS generation has also been ascribed to several matrix enzymes, including α-ketoglutarate dehydrogenase [109] and α-glycerophosphate dehydrogenase, which are controlled by the NADH/NAD^+ ratio [110]. Whether these enzymes contribute to increased ROS production during conditions of alcohol-induced fatty liver disease remains to be defined, but it is expected that higher ROS levels in mitochondria during these pathologies will negatively affect mitochondrial and cellular functions by oxidative modification and alteration in redox-sensitive signaling pathways [111].

Finally, mitochondrial GSH depletion as a result of alcohol-mediated alterations in mitochondrial membrane dynamics underlies the susceptibility of hepatocytes from alcohol-fed models to tumor necrosis factor-alpha (TNFα) [112–114]. Hepatocyte signaling of TNFα through its membrane receptor, TNFR1, from complex I to complex II, is similar whether or not the hepatocytes are depleted in mitochondrial GSH, but hepatocellular susceptibility to TNFα occurs only if mitochondrial GSH is depleted. Thus, mitochondrial GSH appears to be a critical factor in the development of steatohepatitis through sensitization of hepatocytes to inflammatory cytokines [115].

Initiation of the alcohol-induced liver disease

Steatosis. One of the best known biological effects of high ethanol consumption is the production of fatty liver, or steatosis [116]. The mechanisms underlying alcohol-induced hepatic steatosis involve the disturbance of several signaling pathways. Original hypotheses regarding the mechanism for this effect included redox shifts generated by the oxidation of ethanol by alcohol and aldehyde dehydrogenases, oxidative stress and mobilization of peripheral triglyceride [117]. Although the supplementation with antioxidants ameliorates steatosis in the enteral alcohol feeding model [118], the actual contribution of oxidative mechanisms is still incompletely clarified [119].

The source of triglycerides in the livers of alcohol-fed animals may be stored adipose lipid, derived from dietary fat, or *de novo* synthesized in the liver. In addition, ethanol consumption has been shown to cause activation of sterol regulatory element-binding protein-1 (SREBP-1), which activates genes involved in lipid biosynthesis. Conversely, ethanol consumption down-regulates the PPARα transcription factor, which regulates enzymes involved in fatty acid oxidation, and simultaneously prevents the import of fatty acids into the mitochondrion for oxidation [120]. Recently, Cederbaum's group showed that oxidative stress induced by ethanol (via induction of CYP2E1) upregulates Nrf2 activity, which in turn regulates ethanol induction of CYP2A5. Interestingly, results obtained from primary hepatocytes, mice gavaged with binge ethanol or fed with chronic ethanol, showed that this Nrf2-regulated ethanol induction of CYP2A5 protects against ethanol-induced steatosis [121,122]. In addition, Nrf2 activation

prevented alcohol-induced accumulation of serum triglycerides and hepatic free fatty acids by decreasing genes involved in lipogenesis, such as gene coding for SREBP-1 [123]. On the other hand, Supakul and Liangpunsaku [124] suggest a possible role of ceramide and acid sphingomyelinase (ASmase) as the key elements in ethanol-induced hepatic steatosis. Work from Crabb's group has shown that ethanol leads to "metabolic remodeling" of the liver resulting in hepatic steatosis. This process involves the inhibition of activated protein kinase (AMP) leading to a decrease in fatty acid oxidation and an increase in fatty acid synthesis [125]. Activation of PP2A through the generation of ceramide by activation of ASmase is likely the key mechanism in the inhibitory effect of ethanol on AMP phosphorylation [126].

Hepatic steatosis has long been considered rather harmless. However, it is now being recognized as a condition leading to steatohepatitis, fibrosis, and ultimately cirrhosis [127,128]. The reasons why some individuals progress from fatty liver to the more advanced stages of liver disease are not clear. The current concept is the "two-hit" hypothesis in which the first hit is steatosis. This is followed by a second hit in the form of cytokine production, mitochondrial dysfunction and oxidative stress. These hits are widely believed to be major contributors to alcohol-induced liver injury and may compound the initial steatosis. Thus ethanol-induced hepatic lipid accumulation may not only initiate but also enhance the progression of ALD [120].

Endoplasmic reticulum stress in ALD. Endoplasmic reticulum (ER) stress, a condition under which unfolded/misfolded protein accumulates in the ER, also contributes to alcoholic disorders of liver. Potential mechanisms that trigger the alcoholic ER stress response are related to alcohol metabolism, which include toxic acetaldehyde and oxidative stress. [129,130]. The unfolded protein response (UPR) appears to be activated in several liver diseases, which are associated with steatosis, raising the possibility that ER stress-dependent alteration in lipid homeostasis is a mechanism that underlies the steatosis [131]. Indeed, several distinct enzymatic lipogenic pathways, including the fatty acid elongation machinery, cholesterol biosynthesis and complex lipid biosynthesis, are compartmentalized in the ER. In addition, the ER stress response is correlated with elevated transcripts of lipogenic enzymes such as fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC). Recent studies using genetic or dietary models of fatty liver have demonstrated a key interconnectedness between hepatic steatosis and ER stress, as well as the physiological role of the UPR sensors in lipid homeostasis [132–134]. Moreover, the recent identification of Hsp70, Hsp90 and protein disulfide isomerase (PDI), which are involved in protein folding, as *in vivo* targets for modification by the lipid peroxidation product 4-HNE, suggests that impairment of their activity could contribute to the initiating events of ER stress and subsequent hepatic lipid accumulation [41].

Hence, restoration of ER homeostasis prior to ER stress-induced cell death may provide a therapeutic rationale in the ALD. Future studies should elucidate how ER stress impacts lipid assembly and trafficking from the ER and how chronic physiological ER stress leads to fatty liver [131].

Oxidative stress in alcohol-induced liver inflammation

The innate immune response is the first line of defense against invading pathogens, acting on a few highly conserved antigens present on microorganisms, including lipopolysaccharide (LPS). Endotoxemia and endotoxin-mediated alteration of liver cell function play a crucial role in the pathogenesis of ALD. Evidence suggests that a possible mechanism involved in alcohol-induced endotoxemia is disruption of intestinal barrier function and an increase in permeability to endotoxins and bacteria [135]. As depicted in Figure 2, alcohol consumption appears to increase the translocation of gut-derived endotoxins to the portal circulation where they stimulate intrahepatic Kupffer cells, which constitute the primary effector cells of the innate immune response within the liver [136]. Oxidative stress-induced cellular responses are strongly involved in innate immune cell activation. Indeed, some researchers have demonstrated that LPS derived from the intestine, as well as endotoxin, could stimulate NADPH oxidase leading to ROS generation in Kupffer cells [137,138].

Direct interaction of NADPH oxidase isozyme 4 (Nox4) with the Toll-like receptor 4 (TLR4) appears to be involved in LPS-mediated ROS production and NFκB activation in neutrophils [139]. Furthermore, NADPH oxidase induces TLR4 and TLR2 expression in human monocytic cells [140]. In chronically ethanol-fed rats, pretreatment with diphenyliodonium (an inhibitor of NADPH oxidase) or the antioxidant, dilinoleoylphosphotidylcholine, normalized ROS production, decreased LPS-induced ERK1/2 phosphorylation and inhibited the increased production of TNFα in Kupffer cells [138,141,142]. Moreover, the inhibition of NADPH oxidase prevented steatosis, upregulation in the gene expression of TLR2, 4, 6 and 9, and sensitization to respective ligand-induced liver injury [143]. These observations strongly suggest a cross-talk between oxidative stress and TLR pathways in ALD. Previous reports indicated that NADPH oxidase-deficient mice were resistant to alcohol-induced liver injury, further suggesting a crucial role for NADPH oxidase in inflammatory responses and liver injury [141]. Thus, ROS appear to play a key role in direct hepatocyte injury and also contribute to increased inflammatory responses, adding to the liver injury [144].

Oxidative stress in alcohol-induced liver fibrosis

Liver fibrosis is the scarring process that represents the liver's response to a variety of acute or chronic stimuli, including ethanol, drugs and toxins [145]. Hepatic fibrosis results from excessive accumulation of extracellular matrix proteins including fibrillar collagen along with insufficient remodeling [146,147] and is associated with numerous pathological and biochemical

modifications leading to structural and metabolic anomalies [148,149].

Various cell types are involved in the pathogenesis of hepatic fibrosis (Figure 2). Following liver injury, HSC lose their vitamin A content, acquire a myofibroblast-like phenotype, become proliferative, motile, pro-fibrogenic and show abundant rough ER [150]. This process can be enhanced by activated Kupffer cells, which release ROS and cytokines that are crucial for HSC activation [151]. Kupffer cells are a major source of two profibrogenic cytokines, TGFβ and platelet-derived growth factor (PDGF), which are considered to be key fibrogenic and proliferative, respectively, stimuli for HSC [152]. Moreover, the phagocytic activity of Kupffer cells generates large amounts of ROS, which could further activate HSC and enhance their fibrogenic potential [151,153].

ROS induce the expression of genes, such as pro-collagen Type I, monocyte chemoattractant protein 1 (MCP-1) and tissue inhibitor of metalloproteinase-1 (TIMP1), which are considered as critical fibrosis-associated genes (Figure 2). This ROS-dependent induction occurs via c-jun N-terminal kinase (JNK), activator protein-1 (AP1), and NFκB pathway activation [154]. The release of several known pro-fibrogenic mediators, including angiotensin II, PDGF, TGFβ, and leptin, results in ROS production by HSC and myofibroblasts [155]. The expression of NADPH oxidase by HSC [156,157] represents an additional mechanism for direct activation of HSC through the ROS generated by the NADPH oxidase complex, raising the possibility of a direct impact of ethanol on HSC. Indeed, ethanol has been shown to up-regulate the expression of Nox in many organs, including the lungs, the liver and the brain, as well as in mouse embryos [158–161], suggesting that Nox may be involved in ethanol-induced ROS generation. Interestingly, a CYP2E1-dependent generation of ROS has been found to be critical for increased collagen I protein synthesis in hepatocytes and HSC co-cultures [162]. This increase in collagen I represents a pro-fibrogenic response that can also occur as a result of a decrease in the antioxidant defenses associated with enhanced lipid peroxidation [163].

Taken together, these results demonstrate the involvement of various liver cell types in the contribution of ethanol-induced oxidative stress to the progression of ALD to liver fibrosis (Figure 2).

Conclusion

Experimental and clinical studies increasingly show that oxidative damage induced by ethanol contributes in many ways to the pathogenesis of alcohol hepatotoxicity. The whole of the data in the literature suggests a role of alcohol on antioxidant enzyme regulation but is not totally conclusive with regard to human pathology. Further studies should focus on developing novel antioxidant compounds with well-characterized pharmacokinetic and pharmacodynamic profiles. Indeed, mitochondrial GSH appears to be a critical factor in the development of steatohepatitis through sensitization of hepatocytes to inflammatory cytokines. Moreover, ROS seem to play a key role in direct hepatocyte injury and contribute to increased inflammatory responses, promoting liver injury. Finally it is remarkable to observe that various liver cell types are involved in the contribution of ethanol-induced oxidative stress to the progression of ALD to liver fibrosis.

Declaration of interest

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

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