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Current Experimental Perspectives on the Clinical Progression of Alcoholic Liver Disease

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Abstract

Chronic alcohol abuse is an important cause of morbidity and mortality throughout the world. Liver damage due to chronic alcohol intoxication initially leads to accumulation of lipids within the liver and with ongoing exposure this condition of steatosis may first progress to an inflammatory stage which leads the way for fibrogenesis and finally cirrhosis of the liver. While the earlier stages of the disease are considered reversible, cirrhotic destruction of the liver architecture beyond certain limits causes irreversible damage of the organ and often represents the basis for cancer development. This review will summarize current knowledge about the molecular mechanisms underlying the different stages of alcoholic liver disease (ALD). Recent observations have led to the identification of new molecular mechanisms and mediators of ALD. For example, plasminogen activator inhibitor 1 was shown to play a central role for steatosis, the anti-inflammatory adipokine, adiponectin profoundly regulates liver macrophage function and excessive hepatic deposition of iron is caused by chronic ethanol intoxication and increases the risk of hepatocellular carcinoma development.

Keywords

Steatosis; Steatohepatitis; Liver Fibrosis; HCC

CHRONIC INTOXICATION OF the liver with alcohol causes organ damage [reviewed in Albano (2008) and De Minicis and Brenner (2008)]. Several studies suggest that this damage progressively increases with amount of alcohol and duration of exposure but nevertheless whether an individual patient develops serious liver damage or not certainly depends on additional cofactors and predispositions [reviewed in Sorensen (1989)].

Alcoholic liver disease (ALD) ranks among the major causes of morbidity and mortality in the world (Grant et al., 1988b) and alcohol accounts greatly for premature mortality in Central and Eastern Europe (Rehm et al., 2007). Up to 12,000 deaths each year specifically due to alcohol induced liver disease have been reported in the United States (2004).

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Excess consumption of alcohol is generally widespread in western countries. Approximately 18 million adults in the United States currently suffer from alcohol abuse (Grant et al., 2004), and some of this population will develop ALD. ALD initiates in the development of alcoholic fatty liver, progresses with ongoing alcohol intoxication to alcoholic steatohepatitis (ASH), fibrosis, cirrhosis, and may finally lead to alcoholic hepatocellular carcinoma (HCC) (Diehl, 1997). ALD is the most important organ manifestation of chronic alcohol abuse. It is noteworthy that although 90–100% of chronic heavy alcohol consumers develop steatosis, only 10–35% develops ASH and even less (8–20%) develop cirrhosis (Yip and Burt, 2006). This may be explained at least partially by the influence of genetic factors. While the early stages of ALD-like steatosis and steatohepatitis are fully reversible kinds of damage, later stages, especially progressed fibrosis and cirrhosis are beyond a certain point considered irreversible and up to now the only long-term treatment of such progressed damage is liver transplantation. In this review we will provide an overview about the current knowledge of the main molecular and cellular mechanisms that mediate the different stages and progression of ALD.

STEATOSIS

Alcohol consumption, be it acute or chronic, leads to hepatic steatosis in at least 80% of heavy drinkers (Grant et al., 1988a). This kind of rapid lipid accumulation in the liver is one of the first characteristics of the onset of ALD (MacSween and Burt, 1986). However, this stage of alcohol-induced liver injury readily reverses when the alcohol consumption is ceased (abstinence) (Siegmund and Brenner, 2005). Steatosis (“the first hit”) is proposed to predispose the liver to progress to more severe pathologies when the liver is subsequently exposed to metabolic and⁄or other stressors (“second hit”) (Mantena et al., 2008; Yang et al., 1997).

Whereas steatosis was originally thought to be pathologically inert, more recent work indicates that it may play a critical role not only in the initiation but also in the progression of ALD (Day and James, 1998). Indeed, results of clinical studies indicate that patients with fatty liver are more likely to develop later stages of the disease [e.g., fibrosis and cirrhosis; Harrison and Diehl (2002)]. Due to an incomplete understanding of the mechanism(s) involved in the disease process, a universally accepted pharmacologic therapy to prevent or reverse ALD in humans is still lacking.

Steatosis owing to alcohol has historically been considered to be the direct result of the adverse effects of alcohol metabolism. The concept of toxicity of alcohol metabolism is based on the rationale that chemical breakdown of ethanol leads to formation of acetaldehyde, an electrophilic metabolite, and that expression of “leaky” metabolizing enzymes (i.e., CYP2E1) is induced. Furthermore, the metabolism of alcohol directly causes a shift in the cellular NADH (oxidized forms of nicotinamide adenine dinucleotide phosphate) pools to a more reduced state. The net effect of this redox shift is that fatty acid synthesis and esterification are elevated, coupled with a simultaneous decrease in mitochondrial β-oxidation of fatty acids. Finally, these biochemical changes cause lipids to accumulate in the hepatocytes. However, whereas alcohol metabolism is indeed likely to contribute to alcohol-induced steatosis, this process of hepatotoxicity alone may not be

sufficient to explain all effects of alcohol uptake. For example, a number of drugs and genetically altered mice decrease the steatotic response to alcohol without any apparent effect on alcohol metabolism, per se [e.g., Arteel (2003) and Kono et al. (2001a,b)]. These results suggest that alcohol metabolism is not the only cause of alcoholic fatty liver.

A possible alternative or additional mechanism by which alcohol may cause steatosis is via induction of cytokines such as tumor necrosis factor (TNF)-α. For example, TNF-α increases free fatty acid release from adipocytes in the periphery of the liver (Hardardottir et al., 1992), it increases lipo-genesis in hepatocytes (Feingold and Grunfeld, 1987), and it inhibits β-oxidation of fatty acids (Nachiappan et al., 1994) (see Fig. 1). Cytokines that are induced by alcohol may also impair transport and secretion of triglycerides as very low density lipoprotein (VLDL) (Navasa et al., 1998). Indeed, fat accumulation caused by chronic alcohol ingestion was almost completely blocked in mice lacking TNF-α receptor 1 (TNFR1) (Yin et al., 1999). However, the specific mechanism(s) by which cytokines may mediate these effects have not been completely determined and need to be clarified by future studies.

Development of steatosis by chronic alcohol consumption has also been linked to 2 signaling pathways that lead to increased lipogenesis in the liver: Adenosine monophosphate kinase (AMPK) inhibition and sterol regulatory element binding protein (SREBP)-1 activation. AMPK is able to inhibit lipogenesis and it activates fatty acid oxidation. In a chronic alcohol feeding model (mice fed the Lieber-DeCarli diet) AMPK activity is inhibited and SREBP-1 is activated (You et al., 2002, 2004) leading to induced expression of several lipogenic genes and triglyceride accumulation in the liver which contributes to enhanced lipid synthesis and development of fatty liver. In line with this SREBP-1 knock out mice are protected against ethanol-induced steatosis (You et al., 2004). Another study further demonstrated that over expression of adiponectin, a known activator of AMPK, results in a dramatic reduction of SREBP-1c expression in the mouse liver (Shklyaev et al., 2003). These studies establish a direct link between AMPK action, SREBP synthesis, and hepatic fat deposition.

There have been recent findings that broaden our understanding of the mechanisms of ALD, which include the potential role of plasminogen activator inhibitor (PAI)-1 in the initiation and progression of ALD [reviewed in Arteel (2008)]. PAI-1 is normally only expressed in adipocytes and endothelial cells; however, PAI-1 can be induced to high levels in multiple cell types under conditions of injury and/or inflammation (Fearns and Loskutoff, 1997). One of the many known strong inducers of PAI-1 is the pro-fibrogenic cytokine transforming growth factor (TGF)-β (see also below). PAI-1 is the major inhibitor of both tissue-type plasminogen activator and urokinase-type plasminogen activator (uPA). It therefore plays a major regulatory role in inhibition of fibrinolysis by blocking the activation of plasminogen. PAI-1 has been shown to potentially mediate fibrin accumulation in hepatic inflammation [e.g., Beier et al. (2009)]. It was recently shown that acute ethanol rapidly and robustly induced hepatic PAI-1 expression, and that steatosis under these conditions was prevented by genetic (PAI-1 −/−) mice) or pharmacologic inhibition of PAI-1 expression (Bergheim et al., 2006). As mentioned above, ethanol also inhibits the synthesis and excretion of VLDL lipids which can lead to fat accumulation. PAI-1 has been shown to blunt the upregulation of VLDL synthesis in response to alcohol mediated by uPA and subsequent Hepatocyte Growth

Factor (HGF) signaling (Bergheim et al., 2006). Similar protection against ethanol-induced steatosis was observed in TNFR1−/− mice in the same study, a strain in which the induction of PAI-1 expression caused by ethanol was also significantly blunted. Steatosis owing to chronic enteral alcohol exposure was also blunted by preventing the induction of PAI-1 expression (Bergheim et al., 2006). These data support the hypothesis that PAI-1 plays a critical role in alcohol-induced steatosis, analogous to previous findings in experimental non-alcoholic fatty liver disease (NAFLD) (Barck et al., 2004).

Taken together, there are multiple proposed mechanisms by which alcohol causes steatosis. These mechanisms are not mutually exclusive and likely to work in tandem to cause initiation and progression of ALD.

STEATOHEPATITIS

With continued alcohol intake, steatosis is accompanied by inflammation and further cytokine production and progresses to steatohepatitis. Kupffer cells, the resident macrophages in the liver, are critical to the onset of ethanol-induced liver injury. Activation of macrophages by endotoxin/lipopolysaccharide (LPS), a component of the cell wall of gram-negative bacteria, via the toll-like receptor 4 (TLR-4) leads to the production of a variety of inflammatory mediators such as TNF-α and reactive oxygen species (ROS). In addition to its role in steatosis, abnormal production of TNF-α is thought to be a critical component in the development of inflammation in the liver due to chronic ethanol exposure (Thurman, 1998; Tilg and Diehl, 2000). Despite the likely role of Kupffer cells and TNF-α in the aetiology of ALD, our understanding of the mechanisms for increased TNF-α production by Kupffer cells after chronic ethanol exposure is incomplete. Increased TNF-α production is probably both due to increased exposure to LPS resulting from increased intestinal permeability in response to ethanol (Fukui et al., 1991) as well as a chronic ethanol-induced sensitization of Kupffer cells to activation by LPS. The latter results from changes in the regulation of TLR-4 signal transduction in response to chronic ethanol and culminates in the dysregulation of transcriptional and post-transcriptional control of TNF-α expression.

While direct exposure of macrophages in culture can mimic some of the effects of ethanol (Kishore et al., 2001; Shi et al., 2002), other data suggest that there are multiple hepatic and extrahepatic responses to ethanol that serve to modulate Kupffer cells sensitivity to LPS. TLR-4 signaling can be modulated by a number of pro- and anti-inflammatory factors, including ROS, complement factors, cytokines as well as adipokines.

here is a growing appreciation of the specific role of ROS in the modulation/regulation of a number of signal transduction cascades (Finkel, 2003; Thannickal and Fanburg, 2000). ROS contribute to cellular responses to a variety of hormones, neurotransmitters, and cytokines (Finkel, 2003; Thannickal and Fanburg, 2000). Evidence also indicates that ROS contribute to LPS-stimulated signaling pathways both in cells of the innate immune system (monocytes/macrophages, neutrophils, etc.) as well as non-immune cells (Iles and Forman, 2002; Nagy, 2004). Chronic ethanol exposure causes oxidative stress in the liver and enhances the formation of free radicals; it is widely accepted that ROS play a critical role in

the development of alcoholic liver injury (Arteel, 2003; Hoek and Pastorino, 2002; Wheeler et al., 2001). ROS are produced during ethanol exposure along with the ethanol metabolism by hepatocytes (Arteel, 2003). Kupffer cells are also an important source of ROS during ethanol exposure (Wheeler et al., 2001) and in response to LPS (Spolarics, 1998). NADPH (reduced forms of nicotinamide adenine dinucleotide phosphate) oxidase-dependent production of ROS is implicated in ethanol-induced liver injury as p47phox −/−) mice which are deficient in this regulatory subunit of NADPH oxidase are resistant to chronic ethanol-induced injury (Kono et al., 2000). Chronic ethanol feeding increases the LPS-stimulated production of ROS by primary cultures of rat Kupffer cells; this increase is primarily due to an increase in NADPH oxidase activation after chronic ethanol feeding (Thakur et al., 2006a). Recently, Thakur and colleagues (2006b) have specifically identified NADPH oxidase-derived ROS as an important contributor to increased TLR-4-mediated signal transduction and TNF-α expression in primary cultures of rat Kupffer cells, particularly after chronic ethanol exposure.

Recent studies have further identified a role for adiponectin, a potent anti-inflammatory adipokine released by adipose tissue, in mediating increased TLR-4 signal transduction in Kupffer cells after chronic ethanol exposure. Adiponectin has broad effects on innate and adaptive immunity, with important anti-inflammatory activity. It both inhibits the growth of myelomonocytic progenitor cells and decreases the activation of mature macrophages (Wulster-Radcliffe et al., 2004; Yokota et al., 2000). Adiponectin also suppresses leukocyte recruitment by suppressing expression of endothelial adhesion molecules (Ouedraogo et al., 2007). Adiponectin suppresses phagocytic activity as well as LPS-stimulated cytokine production in macrophages (Thakur et al., 2006a; Wulster-Radcliffe et al., 2004; Yokota et al., 2000). In particular, LPS-stimulated nuclear factor κB and extracellular signal-regulated kinase 1/2 activation are sensitive to the inhibitory effects of adiponectin (Thakur et al., 2006a; Wulster-Radcliffe et al., 2004; Yamaguchi et al., 2005). Chronic ethanol feeding decreases serum adiponectin in both mice and rats (Thakur et al., 2006a; Xu et al., 2003; You et al., 2005) while in vivo treatment of mice with adiponectin prevents ethanol-induced liver injury by decreasing steatosis and serum alanine amino-transferase as well as hepatic expression of TNF-α (Xu et al., 2003). Further, treatment with adiponectin normalizes the increased sensitivity to LPS in primary cultures of Kupffer cells isolated from ethanol-fed rats (Thakur et al., 2006a). Importantly, primary cultures of Kupffer cells from ethanol-fed rats exhibited increased sensitivity to the anti-inflammatory effects of adiponectin (Thakur et al., 2006a) implying that use of this adipokine may give rise to future treatment strategies of ALD.

Taken together, these data suggest that Kupffer cells are very responsive to the balance of pro- and anti-inflammatory signals and that changes in the local milieu of these inflammatory modulators during ethanol exposure is likely to contribute to the ethanol-induced sensitization of Kupffer cells. In particular, decreases in the anti-inflammatory adipokine, adiponectin, may play an important role in mediating the effects of chronic ethanol on hepatic macrophage function.

FIBROSIS

The next step within the alcohol-induced wound healing response of the liver is massive deposition of extracellular matrix proteins, mainly fibrillar collagens (for review, see Siegmund et al., 2005). The primary event of this fibrogenic process is commonly recognized to be the cytokine-associated activation of hepatic stellate cells (HSC) (Friedman, 2000, 2008) and TGF-β1 is regarded as the key pro-fibrogenic mediator of such chronic liver disease (Breitkopf et al., 2006; Gressner et al., 2002) because it is one of the most potent activators of HSC.

Acetaldehyde, the major metabolic product of alcohol in the body, increases secretion of TGF-β1 and induces TGF-β type II receptor expression in HSC (Anania et al., 1996) and both ethanol and acetaldehyde induce α2 (I) collagen gene promoter activation and upregulated protein expression (Chen, 2002). In cultured human HSC, acetaldehyde upregulates α2 procollagen type I mRNA expression via different mechanisms when discriminating between early and late responses. The early response is TGF-β1 independent, whereas the late response is not (Svegliati-Baroni et al., 2005). In line with this is the finding that acetaldehyde-responsive elements within the collagen type I genes bind among others transcription factors of the TGF-β signaling cascade, namely,Smad3 and Smad4 (Greenwel et al., 2000). Acetaldehyde does not change Smad3 and Smad4 protein concentrations but it selectively induces phosphorylation of Smad3 but not Smad2 (Greenwel et al., 2000). In addition, cotreatment with acetaldehyde and TGF-β1 together leads to an additive upregulation of the α2(I) collagen gene (Greenwel et al., 2000) implying that there exist also parallel inductive pathways of the 2 substances.

Activity of TGF-β seems to be connected to progression of ALD. This assumption is supported by a study of Stickel and colleagues (2001) within which a significant positive correlation between the serum levels of the fibrosis marker procollagen-III-N-propeptide and TGF-β1 was reported in samples from 61 patients with ALD. Furthermore, we measured phosphorylated Smad2 as marker for active TGF-β signaling in tissue sections from 68 patients with ALD [one representative example from this study which was part of our recently published work by Weng et al. (2009) is shown in Fig. 2] and found a significant correlation between TGF-βphospho-Smad2 and severity of liver disease (Weng et al., 2009). These results confirm an important role of TGF-β1 as mediator of alcohol-associated liver fibrogenesis.

Some effort has been made to identify possible genetic patterns within the TGF-β promoter which might prime a person for an increased risk of developing liver fibrosis. Because TGF-β1 polymorphisms comprising the C allele at position −509 and the T allele at codon of the TGF-β1 gene correlated with progression of liver cirrhosis in Chinese patients with chronic hepatits B virus (HBV) infection (Wang et al., 2008). TGF-β1 may be genetically linked to fibrosis/cirrhosis associated with HBV infection. However, the same connection was not identified for ALD. For example, no association between TGF-β1 codon 25 genotypes Arg/Pro or Pro/Pro and alcoholic liver cirrhosis was found in a study which enrolled 151 heavy drinkers without apparent ALD, 149 individuals with alcoholic cirrhosis, and 220 alcoholic cirrhotics who underwent liver transplantation (Osterreicher et al., 2008). In

another study, TGF-β1 single nucleotide polymorphisms at 3 locations (−509, C or T; +869, C or T, condon 10 and +915, C or G, codon 25) were tested in 165 alcoholics with advanced ALD and 185 healthy controls and no link was found between these TGF-β1 polymorphisms and the risk to develop advanced ALD (Oliver et al., 2005). So although there might very well exist genetic predispositions which underlie the fact that some individuals develop more severe ALD than others this most likely does not include polymorphisms of the TGF-β gene.

Recently, new mechanisms of TGF-β1-mediated liver damage in ALD have been described. In ethanol-fed mice compared with pair-fed controls additional CCl_4 challenge induced enhanced fibrosis and reduced HSC apoptosis (Jeong et al., 2008). Interferon (IFN-γ) treatment improved liver fibrosis in pair-fed but not in ethanol-fed mice, and poly I:C activation of natural killer (NK) cell cytotoxicity against HSC was attenuated in ethanol-fed mice compared with pair-fed mice. The latter was due to reduced NK group 2 membrane D, TNF-related apoptosis-inducing ligand, and IFN-γ expression in NK cells from ethanol-fed mice. Furthermore, HSC isolated from ethanol-fed mice were resistant to IFN-γ-induced cell cycle arrest and apoptosis. This was based on production of oxidative stress and induction of suppressor of cytokine signaling protein expression leading to diminished acitivation of signal transducer and activator of transcriptor 1 (STAT1). Resistance of HSC from ethanol-fed mice to NK cell killing was overcome by a TGF-β1 neutralizing antibody. These data suggest that with chronic ethanol consumption the antifibrotic effects of NK cells/IFN-γ/STAT1 are attenuated via action of TGF-β1 (Jeong et al., 2008).

Taken together, TGF-β1 plays an important role in alcohol-associated liver fibrosis. However, TGF-β1 and its signaling obviously are not in charge of all adverse events occurring during alcohol-induced liver fibrogensis. Our recent study demonstrates that besides TGF-β1, interleukin (IL)-13 levels in liver tissue significantly correlate with fibrosis in patients with ALD (Weng et al., 2009). The results further suggest that TGF-β1 is not generally required as pro-fibrogenic cytokine in any kind of chronic liver disease. Other cytokines, e.g. IL-13, may play a critical role in this process as well.

Another factor that mediates progression of liver damage due to alcohol seems to be an imbalance of iron homeostasis. There are several indications that iron overload together with increased oxidative stress (H_2O_2) is an important risk factor for the development of severe ALD or even HCC. Patients with ALD frequently exhibit increased body iron stores, as reflected by elevated serum iron indices (transferrin saturation and ferritin) and hepatic iron concentration (Jeong et al., 2008; Oliver et al., 2005). Even mild to moderate alcohol consumption has recently been shown to increase the prevalence of iron overload (Ioannou et al., 2004). Moreover, increased hepatic iron content is associated with greater mortality from alcoholic cirrhosis, suggesting a pathogenic role of iron in ALD. Genetic hemochromatosis, a common iron overload disorder in the Caucasian population, in conjunction with excessive alcohol consumption exacerbates liver injury (Tavill and Qadri, 2004). It should be mentioned that iron per se is one of the most profibrogenic and genotoxic single factors and patients with hereditary hemochromatosis develop fibrosis in 50% and have a 200-fold increased risk for HCC (Niederau et al., 1985).

Despite these observations, the underlying mechanisms of iron accumulation observed in ALD are still poorly understood. Genome-wide microarray-based screening for candidate genes that could cause iron overload involved several genes not yet linked to iron metabolism (Hagist et al., 2009). Preliminary data from ALD patients and ethanol mice models suggest that hepatic iron uptake pathways are increased in the liver and potential mechanisms involve an increase of the transferrin receptor (TfR)1 and repression of the systemic iron hormone, hepcidin, that controls duodenal iron absorption and Reactive Oxygen Species (ROS)-mediated iron release via the iron exporter ferroportin (Harrison-Findik et al., 2006; Kohgo et al., 2005). Unexpectedly, TGF-β strongly induces hepcidin expression in cultured mouse hepatocytes (K. Breitkopf and S. Dooley, unpublished observation) and in line with this Wang and colleagues (2005) showed that hepatocyte-specific loss of Smad4 in mouse liver is associated with dramatically decreased expression of hepcidin which results in iron overload in multiple organs and premature death. These results imply that in terms of iron homeostasis high levels of TGF-β in the liver would be rather beneficial which is somehow contradictory to its known profibrogenic actions. However, the role of TGF-β in this context is controversially discussed and surely needs further research.

Using novel in vitro and in vivo models (Mueller, 2000; Rost et al., 2007), we have recently demonstrated that H_2O_2 alone increases TfR1 via posttranscriptional and translation mechanisms ultimately leading to cellular accumulation of iron (Andriopoulos et al., 2007; Mueller et al., 2001). The data show that chronic exposure of cells to per se nontoxic levels of H_2O_2 lead to accumulation of iron via distinct regulatory mechanisms promoting Fenton chemistry. We suggest that increased oxidative stress in form of H_2O_2 is an important regulatory factor that causes continuous iron accumulation and may support ALD progression.

LIVER CANCER

Worldwide, HCC is one of the most common malignant tumors ranking the seventh most prevalent in men and the ninth in women. In developed countries, alcohol abuse is the major cause of liver endstage disease and HCC. The consumption of more than 80 g of ethanol per day for more than 10 years increases the risk for HCC by approximately 5-fold (Morgan et al., 2004). The risk for HCC in decompensated alcohol-induced cirrhosis is about 1–2% per year (Morgan et al., 2004). The International Agency for Research on Cancer (IARC Working Group, 1988) has classified ethanol as a human (group1) carcinogen as it induces HCC (among other tumors) in animals and increases the risk for developing HCC in man.

Although pathogenesis of HCC seems to require several factors (Seitz and Stickel, 2007) including the presence of cirrhosis, oxidative stress, altered methyl transfer resulting in DNA hypomethylation, and a decrease in retinoic acid, the precise mechanisms by which alcohol causes HCC at a molecular level are poorly understood. In addition, comorbidities such as viral hepatitis, diabetes mellitus, and obesity are conditions known to aggravate the development of HCC in patients with ALD. Furthermore, as described above, hereditary hemochromatosis represents a comorbidity which increases the risk for HCC by 200-fold (Niederau et al., 1985).

It is widely recognized that ROS such as superoxide anion and hydrogen peroxide play an important role in alcohol-induced liver injury and in hepatocarcinogenesis (Albano, 2006; Seitz and Stickel, 2007). Chronic ethanol consumption results in the generation of ROS via multiple pathways leading to lipid peroxidation (LPO) and LPO byproducts such as 4-hydroxy-2-nonenal (4-HNE) and malondialdehyde (MDA). These DNA-reactive aldehydes in turn form mutagenic exo-cyclic DNA adducts including 1N-6-ethenodeoxyadenosine (εdA) and 3N-4-ethenodeoxycytidine (Chapman et al., 1982; Frank et al., 2004; Wang et al., 2009).

Several enzyme systems are capable of producing ROS including the mitochondrial respiratory chain, the cytosolic enzymes xanthine oxidase and aldehyde oxidase as well as the microsomal cytochrome P450-dependent mono-oxygenases (Albano, 2006). One member of the latter system, cytochrome P450 2E1 (CYP2E1) is involved in the major pathway by which ethanol generates oxidative stress. Expression of CYP2E1 has been shown to correlate well with the generation of hydroxyethyl radicals and with LPO products such as 4-HNE and MDA (Whitfield et al., 2001). CYP2E1 is induced by chronic alcohol consumption within a week even at a relatively low ethanol dose (40 g/d), but the degree of CYP2E1 induction shows high variations between individuals (Oneta et al., 2002). Inhibition of CYP2E1 by chlormethiazole, a specific CYP2E1 inhibitor, improved ALD as shown in the Tsukamoto-French rat model (Tavill and Qadri, 2004). An increase of oxidative DNA adducts and of mutagenic apurinic and apyrimidinic DNA sites has been found in chronically ethanol-treated wild-type mice but not in mice that lack functional CYP2E1 (Bradford et al., 2005) further stressing the importance of CYP2E1 in the generation of DNA damage following ethanol ingestion. Furthermore, it has been shown that increased levels of Cyp2E1 potentiate pro-apoptotic effects of TGF-β resulting in increased cell death of hepatocytes (Zhuge and Cederbaum, 2006). Recently, we have been able to detect etheno-DNA adducts such as εdA in the livers of patients with ALD (Frank et al., 2004; Gebhardt et al., 1997). Our data support the notion that ethanol-mediated induction of hepatic CYP2E1 leading *interalia* to highly miscoding LPO-derived DNA lesions may play a central role in HCC development in ALD patients.

In summary, HCC is increasingly encountered in developed countries mostly due to ALD. Although the molecular mechanisms that ultimately lead to HCC by alcohol are poorly understood, generation of ROS and oxidative DNA damage play pivotal roles (Fig. 3). Induction of CYP2E1, formation of mutagenic etheno DNA adducts and accumulation of hepatocellular iron seem to be key events in ALD and may eventually offer novel therapeutic approaches to prevent disease progression and carcinogenesis.

Because of these multiple mechanisms by which alcohol may damage the liver, it is unlikely that one therapy will be sufficient to treat ALD. With better understanding of the mechanisms and risk factors that mediate the initiation and progression of ALD, rational targeted therapy can be developed to treat or prevent ALD.

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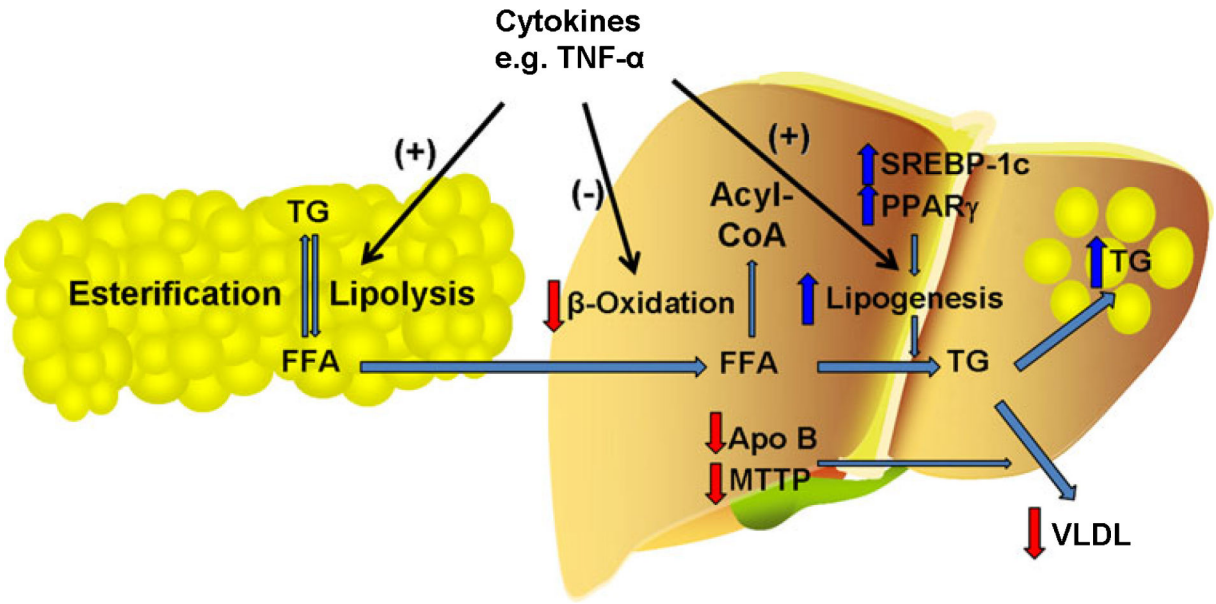

Fig. 1.

Schematic representation of the role of cytokines in the altered lipid metabolism caused by ethanol. It is proposed that alcohol alters, via cytokines (e.g., TNF-α), the balance between esterification and lipolysis in adipocytes of the periphery, which leads to an increased release of fatty acids. Furthermore,TNF-α may decrease the β-oxidation of fatty acids and increase lipogenesis in the liver, resulting in an intracellular lipid accumulation. TG: triglycerides, FFA: free fatty acids, MTTP: microsomal triglyceride transfer protein, VLDL: very low density lipoprotein, ApoB: apolipoprotein B, PPARγ: peroxisome proliferator activated receptor γ, SREBP-1c: sterol regulatory element binding protein, TNF-α, tumor necrosis factor.

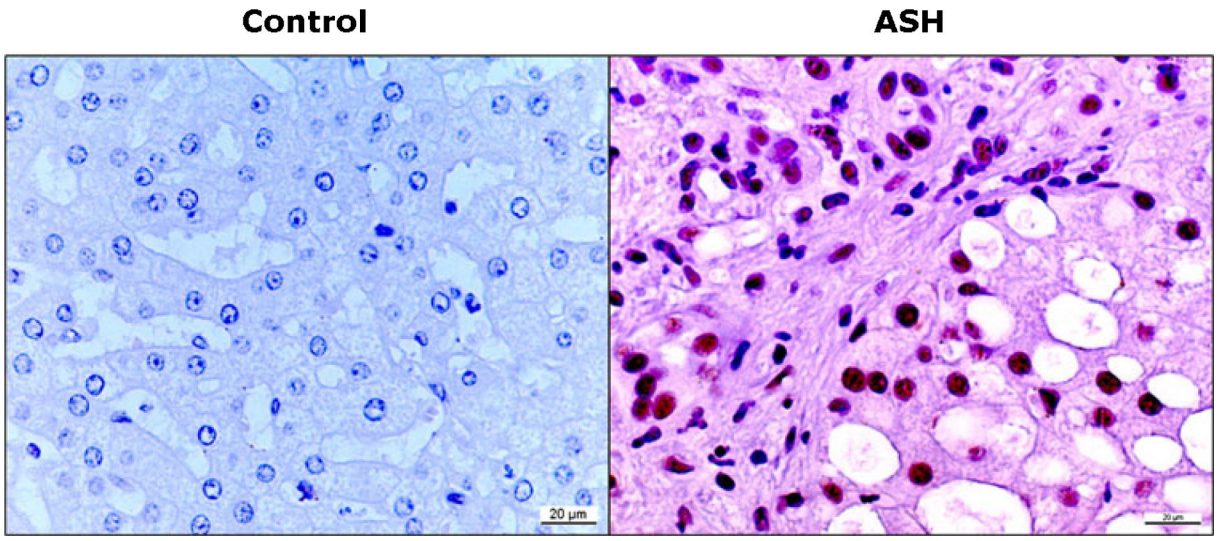

Fig. 2.

Transforming growth factor (TGF-β) signaling is highly activated in human liver samples from patients with alcoholic liver disease. The picture shows a representative immunostaining for phosphorylated Smad2 in liver tissue from a patient with alcoholic steatohepatitis (ASH; sample on the right). A high percentage of nuclei-stained positive for activated Smad2 (dark nuclei) imply highly active TGF-β signal transduction. A control liver tissue (sample on the left) was from a patient with gall stone and showed mainly p-Smad2-negative nuclei. Magnification: 400×.

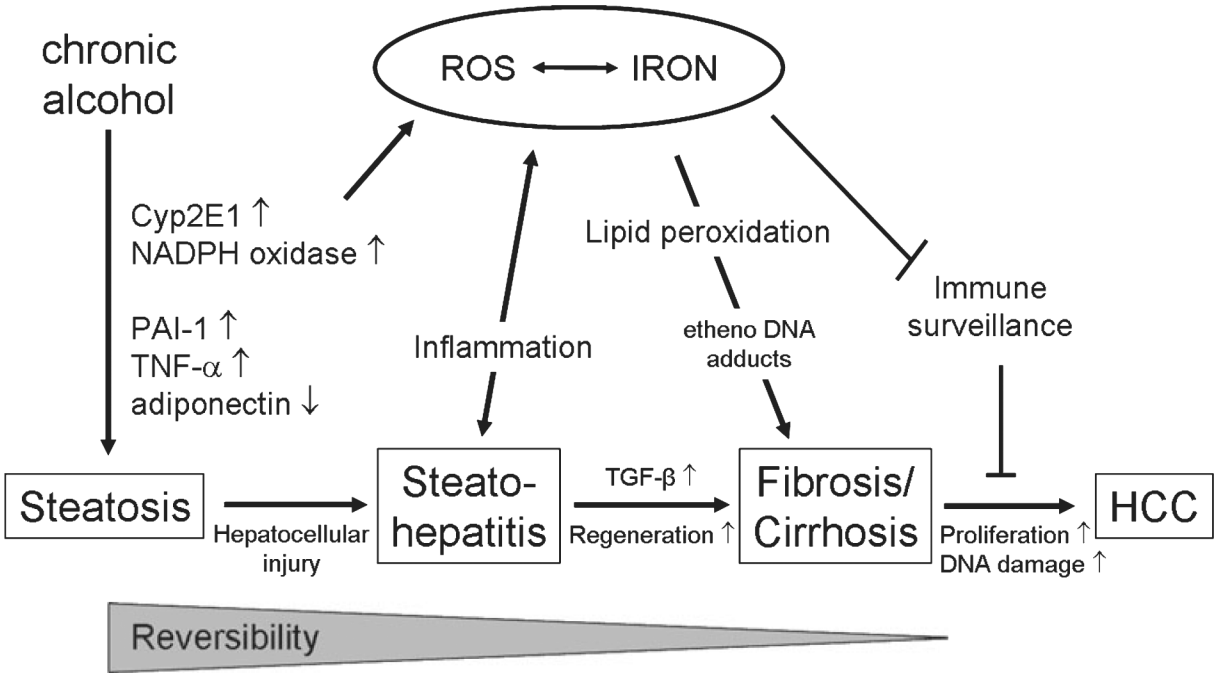

Fig. 3.

Schematic summary of the molecular mechanisms underlying the progression of alcoholic liver disease. CYP2E1: cytochrome P450 2E1, PAI-1: plasminogen activator inhibitor-1, TNF-α: tumor necrosis factor-α, ROS: reactive oxygen species, TGF-β: transforming growth factor-β, HCC: hepatocellular carcinoma; NADPH: reduced forms of nicotinamide adenine dinucleotide phosphate.