

Alcohol and hepatocyte-Kupffer cell interaction (Review)

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Abstract. Alcoholic liver disease accounts for 12,000 deaths per year in the United States and is the second leading indication for liver transplantation. It covers a spectrum of disease conditions ranging from steatosis and cirrhosis to hepatic malignancies. Epidemiological data clearly show a strong correlation between alcohol consumption and liver diseases. A large body of evidence has accumulated over the years in determining the molecular mediators of alcohol-induced liver injury. In this review, we provide an overview of such mediators, which include alcohol metabolites and reactive oxygen/nitrogen species, endotoxin via bacterial translocation from the gut and TNF- α , and highlight the role of the sympathetic nervous stimuli, norepinephrine and the α_{2A} -adrenergic receptors in contributing to the deleterious effect observed in alcohol-induced hepatic dysfunction.

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1. Introduction

... if the surfeit of delicacies, or the hereditary wine of my country dared to disturb my health or the equilibrium of my poetry, from you, dark monarch, giver of syrups and of poisons, regulator of salts, from you I hope for justice: I love life: Do not betray me! Work on!

—Pablo Neruda, *Oda al Hígado* (1)

Alcoholic liver disease (ALD) affects 1% of the North American population and accounted for over 12,000 deaths in 2001. It is the second most frequent indication for a liver transplant in the United States, accounting for 18% of all patients awaiting liver transplants (2-4). A recent study from Europe showed a 2.8% prevalence of advanced hepatic fibrosis in the general population, with alcohol consumption, singly or in combination with hepatitis C infection, being the predominant risk factor in 75% of those affected (5). ALD covers a spectrum of increasing hepatic dysfunction, ranging from steatosis to liver failure and malignant hepatic disease. The most prevalent types of ALD are fatty liver, alcoholic hepatitis and cirrhosis (6,7). As a major synthetic and metabolic organ, the liver plays an essential role in the body. It is a remarkably resilient organ and its parenchymal cells are capable of extraordinary regeneration.

Hepatic injury is primarily caused by infections and toxins, including alcohol, which contributes substantially to the burden of liver disease worldwide (4,8). Liver injury resulting from alcohol use is mediated through several processes, including the generation of harmful metabolites and oxygen species in the local milieu, alteration of intestinal permeability and increases in bacterial toxins and changes in the levels of endogenous mediators (9,10). The surest way of preventing ALD would be complete abstinence. Indeed, epidemiological data show a decrease in chronic liver disease by the end of the Prohibition in the US in 1914 (11). Alcohol, however, has also beneficial effects, and moderate alcohol consumption has been shown to improve digestion and cholesterol profile, and to lower the risk of ischemic heart disease (12,13). Epidemiological data has shown a clear correlation between excessive and prolonged alcohol consumption and liver disease (6,7). The molecular mechanisms underlying these interactions are still being elucidated.

The molecular mediators of alcohol-induced hepatic dysfunction have been elucidated over the years. It is widely

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thought that the autonomic nervous system is involved in alcohol-induced hepatic damage, and that the level of plasma norepinephrine (NE) is an independent prognostic factor in cirrhosis (14,15). Our recent study suggested that NE-induced inflammatory response is a potential cause of alcohol-induced hepatic dysfunction. In addition, a large body of literature indicates the role of alcohol metabolites, reactive oxygen/nitrogen species (RNS/ROS), bacterial translocation from the gut, TNF- α and hepatic stellate cell (HSC) activation as the combined cause of alcohol-induced hepatic damage. Therefore, a consensus has been emerging concerning the general pathway culminating in severe ALD involving a complex orchestration between Kupffer cells (KCs), HSC and hepatocytes (7,9,16). This study reviews these concepts and highlights the key role played by the noradrenergic stimuli NE and its receptor in the causation of alcohol-derived injury in the liver.

2. Sympathetic nervous system and alcoholic liver disease

The autonomic nervous system influences many functions mediated by the neurotransmitter released and its interaction with the receptors. The primary responses of the autonomic nervous system are mediated via the cholinergic and adrenergic pathways. The adrenergic nervous system has NE as the major neurotransmitter in axon terminals of postganglionic fibers. The direct measure of sympathetic nervous activity is to measure the plasma concentration of NE. Norepinephrine from the synaptic cleft enters into the plasma, where its concentration reflects the activity of the sympathetic nervous system. A close association exists between plasma NE levels in venous blood in the forearm and sympathetic nervous activity in muscles during exercise, mental stress, hypoglycemia and in patients with liver disease (17,18). A large body of evidence has accumulated over the past several decades that confirms the importance of sympathetic nervous activity in cardiovascular and homeostatic alterations, and in the metabolic syndrome present in advanced liver disease (19,20). In addition, excessive consumption of alcohol may induce transient changes in the sympathetic nervous system. Autonomic dysfunction has been implicated in alcoholics. Even though there is increasing evidence of parasympathetic dysfunction, sympathetic defects may also be present in alcoholics (21). There has been increasing attention given to the potential role of the adrenergic system in ethanol consumption. Significantly elevated NE levels in active drinkers compared to 3-month abstinent alcoholics and non-drinking controls have been reported. In addition, NE concentrations have been shown to decline during the early withdrawal phase from days 1 to 14 of abstinence (22,23).

High levels of catecholamines in the portal venous plasma of patients with cirrhosis were first reported by Shaldon *et al* (24). Subsequently, at the beginning of the 1980s, studies with isotope derivatives and high-pressure liquid chromatography showed increased circulating levels of NE and epinephrine in patients with cirrhosis and portal hypertension (25-28). In fact, a positive relation exists between circulating NE and epinephrine and the progression of the disease, and thus the level of plasma NE became an independent prognostic factor of cirrhosis (14,15). This increased circulating NE in cirrhotic patients is caused by the sympathetic nervous activity of a

number of organs, such as the liver, the prehepatic splanchnic areas, the heart and the kidney.

Increased levels of NE have been reported in both humans and in animal studies following alcohol consumption. Ireland *et al* reported elevated blood pressure with increased epinephrine levels and a later rise in NE in young human males following acute ethanol intake (29). Kovacs *et al* showed elevated plasma epinephrine and NE levels in mice following intraperitoneal injection of 1.75 g/kg ethanol or ingestion of 5% ethanol in drinking water for 24 h (30). Using dopamine β hydroxylase (DBH) knockout mice, a mouse strain that has the DBH gene deleted and thus no NE in the brain, adrenal glands or circulation (31), Weinshenker *et al* showed decreased preference for and consumption of ethanol, and increased ethanol-induced hypothermia and sedation in these animals, concluding that NE is a critical component of the response to ethanol (32). It has been postulated that under stressful conditions, 30% or more of plasma NE originates in the adrenal medulla (33,34). Patterson-Buckendahl *et al* demonstrated that adrenomedullary gene expression from enzymes of the catecholamine synthetic pathway, tyrosine hydroxylase (TH), DBH and phenylethanolamine-N-methyl transferase (PNMT) was increased in rats after 7 weeks of consumption of 6% wt/vol ethanol in drinking water (35). In a similar study of a 1-week administration of a liquid diet containing 5% wt/vol ethanol in rats, ethanol potentiated a normal increase in the immobilization-induced increase in adrenomedullary TH, DBH and PNMT mRNA expression (36). These studies clearly demonstrated that NE levels are elevated in the plasma following chronic consumption of alcohol, and this increase is in part due to the up-regulation of the various mediators of the catecholamine synthesis pathway.

3. Direct effect of ethanol on Kupffer cell activation by norepinephrine

KCs are the resident macrophages of the liver. They ensure maximal liver function by removing bacteria and phagocytosing foreign materials. The importance of KCs in causing alcohol-induced injury in the liver was illustrated by Adachi *et al* (37). They demonstrated that twice weekly treatment of male rats with gadolinium chloride (GdCl₃), a selective Kupffer cell toxicant, to inactivate KCs, prevented injury in a rat model of ethanol-induced injury (37). KCs are quiescent in the absence of stimulatory agents. When activated, KCs release several inflammatory cytokines. These include TNF- α , IL-1, IL-6 and TGF- β . Clinical studies have shown an increased production of TNF- α by circulating monocytes in patients with ALD. This has been corroborated by animal studies in chronically alcohol-fed animals (38,39). KCs also produce significant amounts of ROS and other inflammatory mediators when activated. Direct production of ROS by KCs is catalyzed by NADPH oxidase. Cytokines secreted by KCs, most significantly TNF- α , contribute to increased ROS production. In addition, Kupffer cell facilitation of neutrophil infiltration contributes to ongoing oxidative stress through the generation of ROS via the myeloperoxidase pathway.

NE acts in the liver to activate KCs. Using a cecal ligation and puncture (CLP) model of rodent polymicrobial sepsis, we previously demonstrated that the up-regulation of

pro-inflammatory cytokines, such as TNF- α , is caused by the increased release of NE from the gut during sepsis. This is evidenced by our studies (40) and others (41), which show that peripheral sympathetic activity increases during sepsis, resulting in the elevation of plasma levels of NE. Intraportal infusion of NE *in vivo* increased TNF- α release and was inhibited by co-infusion with yohimbine, a non-specific antagonist of the α_2 -adrenergic receptor. Cellular levels of TNF- α in KCs were also significantly increased following intraportal NE infusion, and were inhibited by co-infusion with yohimbine (42). KCs isolated from rats during early sepsis exhibited a marked increase in the mRNA expression of the α_{2A} -adrenergic receptor subtype (43,44). Furthermore, Zhou *et al* showed increased TH gene expression and protein levels in the intestine of septic rats, suggesting that the increased NE levels are due to an increase in NE biosynthesis from the gut (45). These studies in sepsis showed that NE released from the gut during sepsis enters the liver through the portal vein and binds to α_{2A} -adrenergic receptors on KCs, facilitating the increased production of TNF- α .

Although previous studies have reported that the adrenal medulla is the major contributor for ethanol-induced NE release, it is possible that the release of NE by the intestine as evidenced in sepsis also contributes to alcoholic liver injury via the gut-liver axis. Nevertheless, *in vitro* experiments from our laboratory with rat KCs treated with ethanol for 7 days, i.e., chronic condition, showed a 120% increase in α_{2A} -adrenergic receptor mRNA expression. This was reflected by a 98% increase in TNF- α mRNA expression in the cultured KCs, and is supported by the attenuation of the Kupffer cell response to the portal vein infusion of NE by the specific α_{2A} -adrenergic receptor inhibitor BRL44408 maleate (43). Paradoxically, NE also acts on KCs via the β -adrenergic receptor to depress their phagocytic and immune functions (46-48). Ethanol exposure leads to increased circulating NE and the activation of KC β -receptors. This results in the depression of KC immune function via the activation of adenylate cyclase. This particularly affects the phagocytosis and cytotoxic actions of these macrophages (48,49). Recently, Parlesak *et al* showed that ethanol significantly depressed the amount of ROS released by LPS-stimulated monocytes (50).

Ethanol potentiates the release of pro-inflammatory cytokines by up-regulating KC α_{2A} -adrenergic receptors, and their activation leads to the increased release of pro-inflammatory cytokines, in particular TNF- α . Thus, the interaction of ethanol and NE with KCs leads to several deleterious effects typified by the clinical effects observed in the chronic alcoholic. Ethanol potentiates cytokine-mediated injury by priming KCs to respond to NE by producing TNF- α , which mediates a variety of hepatotoxic effects by direct toxicity and the activation of HSCs. Ethanol also promotes LPS-associated hepatic damage by depressing the immune function of KCs. Ethanol consumption has been linked to endotoxemia and increased susceptibility to infections from microorganisms that would ordinarily have been cleared from the circulation by KCs. In addition, this suppression of KC cytotoxicity inhibits its tumoricidal activity and is implicated in the multifactorial development of hepatic malignancies (51,52).

KCs express α - and β -adrenergic receptors, however, and the relative activation of these receptor subtypes determines

the differential response of KCs to NE. However, a likely effect of chronic alcohol consumption is the up-regulation of α_{2A} -adrenergic receptors in KCs. This probably explains the contrasting effects of NE increase in the liver with moderate versus chronic or excessive alcohol intake. An intriguing new development has been the demonstration of endogenous NE secretion by macrophages and other immune and inflammatory cells (30,53,54). This requires further study, particularly as it raises the possibility of a self-perpetuating cycle of autocrine stimulation of KCs by endogenously-derived NE in the causation of hepatic injury.

4. Alcohol metabolites and ROS-associated alcohol-induced hepatic injury

Alcohol is primarily metabolized in the liver through two pathways: the alcohol dehydrogenase (ADH) pathway and the microsomal ethanol-oxidizing system (MEOS). After moderate intake, most of the alcohol is broken down by ADH, which converts ethanol to acetaldehyde with the reduction of nicotinamide adenine dinucleotide (NAD) to reduced NAD (NADH). Subsequently, the acetaldehyde is converted to acetate by a second enzyme, aldehyde dehydrogenase. The MEOS comes to play particularly after higher alcohol consumption. The main component of the MEOS is the enzyme cytochrome P450, which also converts alcohol to acetaldehyde. Acetaldehyde has been shown to cause the injury and death of hepatocytes. It forms adducts with proteins and DNA and therefore impairs microtubules, decreases protein secretion and causes protein retention and ballooning of the hepatocyte. Acetaldehyde exerts toxicity also with regard to other key cellular functions, particularly in the mitochondria, and it may promote the peroxidation of the cellular membranes (55-57).

Alcohol acting through its metabolites produces oxidative stress by enhancing the production of ROS/RNS and by inhibiting the protective antioxidant enzyme systems. Reactive species, primarily the superoxide radical $O_2^{\bullet-}$, the peroxide ($O_2^=$)-containing hydrogen peroxide radical (H_2O_2) and the hydroxyl radical ($\bullet OH$), cause hepatic damage by interacting with constituent cellular molecules, including lipids, in the cell membrane, cellular proteins and DNA. Lipid peroxidation of the bi-lipid plasma membrane of the cells generates more radicals and results in extensive cellular damage (10). Cysteine, methionine and histidine are amino acids that are particularly sensitive to attack and oxidation by the hydroxyl radical (58). This results in the inactivation of crucial enzymes, and conformational changes in protein structure. The results of these insults are permanent cell damage or death. Mutations that occur, when persistent, may lead to cell transformation and eventual malignancy.

5. Gut permeability and bacterial translocation in alcohol-induced hepatic damage

The permeability of the gut mucosa is increased following alcohol intake. This leads to a rise in the translocation of endotoxin from the gut into the circulation. Alcohol also alters the gut microflora, in particular when consumed chronically, with a shift towards increased gram-negative bacteria. This,

too, increases circulating bacterial endotoxin. Experiments in animals have revealed that the liver quickly removes intravenously administered endotoxin from the bloodstream (59,60). This would be the case in the portal circulation with relatively higher gut-derived endotoxin due to the first pass effect (61). Hepatic uptake and detoxification is essential for preventing systemic reactions to blood-borne LPS, a gram-negative bacterial endotoxin. The endotoxins released by these bacteria activate KCs by binding to TLR4 and its co-receptors, CD14 and MD-2 (62), leading to the release of inflammatory cytokines, in particular TNF- α .

6. TNF- α and alcohol-induced hepatic damage

Clinical and animal studies have revealed TNF- α to be a key mediator of the hepatic damage that occurs in alcohol-induced liver injury (63,64). Its adverse effects on cells range from mitochondrial damage to oncotic necrosis and apoptosis. Activated KCs release TNF- α through a series of molecular steps involving sequential activation of transcription factor NF- κ B via the TIR domain-containing adaptor myeloid differentiation factor 88 (MyD88) and the phosphorylation of transcription factor inhibitor I κ B by IKK (I κ B kinase). The disinhibition of transcription factor NF- κ B by I κ B leads to the nuclear translocation of NF- κ B. NF- κ B initiates cytokine gene transcription leading to the up-regulation and release of TNF- α and other pro-inflammatory cytokines (62,65,66). TNF- α amplifies and prolongs the inflammatory response by activating cells to release pro-inflammatory cytokines, such as IL-1 and high mobility group B1 (HMGB1), as well as mediators, such as eicosanoids, nitric oxide and ROS. These promote further inflammation and tissue injury (67). Studies have shown that gut-derived NE itself induces the release of TNF- α from KCs and leads to hepatic dysfunction (43,44). However, as its action depends on its interplay with other cytokines and the general hepatic milieu (63,68), TNF- α does have physiologically beneficial effects, thus straightforward antagonism would not necessarily be an effective intervention.

7. Hepatic stellate cell activation in alcohol-induced hepatic damage

The interaction of KCs with other component cells of the liver, most notably HSCs, is another mechanism of alcoholic-induced hepatic damage. Physiologically, HSCs act as fat storage cells in the liver and are a reservoir for lipid soluble vitamins. Hepatic stellate cells, which are pericytes, are nominally differentiated cells capable of transforming to different lineages following liver damage. However, during chronic inflammation following continuous cytokine stimulation, these cells become activated and are transformed to myofibroblasts responsible for the hepatic scarring that culminates in liver cirrhosis. Activated HSCs proliferate and undergo phenotypic transdifferentiation to become myofibroblasts. This is believed to be the central pathogenetic event in the development of fibrosis. Cytokines secreted by KCs, particularly TGF- β and TNF- α , are central to this activation. HSC transformation to myofibroblasts can be summarized as a three step cascade: a pre-inflammatory phase that initiates HSC activation by discharge of mitogenic cytokines (TGF- α ,

IGF-1) from damaged and apoptotic hepatocytes, followed by the inflammatory phase, based on cytokines (platelet-derived growth factor, TGF- β , TNF- α) from activated KCs. The consecutive post-inflammatory phase is characterized by the secretion of fibrogenic cytokines from myofibroblasts and interacting matrix components potentially contributing to a perpetuation of the fibrogenic process even after cessation of the primary event (69,70).

8. Summary and conclusions

Alcohol abuse plays a significant role in the causation of liver disease worldwide. Hepatic damage resulting from alcohol consumption is manifested in a spectrum of clinical and histopathological changes. KCs play a central role in the initiation and propagation of hepatic dysfunction through their production of several intermediaries. Ethanol increases NE release and potentiates NE-mediated dysfunction by up-regulating the α_{2A} -adrenergic receptors, thereby promoting the release of pro-inflammatory cytokines by KCs. Ethanol also depresses KC phagocytosis and the clearance of endobacteria, and in association with its effect on increasing gut permeability, potentiates endotoxin-Kupffer cell-mediated damage. This, in addition to the production of ROS and the activation of HSCs by KCs, highlights its dynamic role in the pathogenesis of alcoholic liver disease. The modulation of the Kupffer cell response to ethanol presents an exciting opportunity to attenuate the deleterious response of the liver to ethanol consumption and reduce the enormous morbidity associated with it.

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