Pathogenesis of Alcoholic Liver Disease: Newer Mechanisms of Injury

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Abstract. The understanding of how alcohol damages the liver has expanded substantially over the last decade. In particular, the genetics of alcoholism, the genesis of fatty liver, the role of oxidant stress, interactions between endotoxin and the Kupffer cell, and the factors that control activation of the hepatic stellate cell (HSC) have been the focus of a great deal of research. Genetic mechanisms for increasing the risk of alcoholism include alterations in alcohol metabolizing enzymes as well as neurobiological differences between individuals. The development of fatty liver may involve both redox forces, oxidative stress, and alterations in peroxisome proliferator activated receptor function. Oxidative stress is now known to involve both microsomal and mitochondrial systems. Recent studies implicate stimulation of Kupffer cells by portal vein endotoxin as a cause of release of cytokines and chemokines, hepatocyte hyper-metabolism, and activation of HSC. These actions appear to be in part gender-dependent and may explain the susceptibility of women to alcoholic liver disease. Activation of HSC underlies liver fibrosis and cirrhosis of all types; control of this activation might permit control of the progression of fibrosis. These advances suggest a number of new approaches as therapy for alcoholic liver injury. (Keio J Med 48 (4): 184-188, December 1999)

Key words: alcoholic liver disease, alcohol metabolism, Kupffer cells, hepatic stellate cells, oxidative stress

Introduction

Alcoholic liver disease (ALD) continues to be a major cause of cirrhosis and death around the world. While the ultimate control of alcoholic liver disease will require the prevention of alcohol abuse, better understanding of the hepatotoxicity of alcohol may lead to treatments of fatty liver and alcoholic hepatitis, prevention or delay of occurrence of cirrhosis, or modulation of the interactions between alcohol consumption and HCV infection. Furthermore, insights gained from studying alcoholic liver injury may extrapolate to non-alcoholic fatty liver and steatohepatitis.

Advances in understanding alcohol toxicity that have occurred in the last decade can be grouped under the topics of genetics of alcoholism, causes of fatty liver, the roles of oxidant stress and protein adducts, the importance of endotoxin, control of hepatic stellate cell (HSC) activation, and the risks of alcoholic liver disease in women. These will be discussed in turn.

Genetic Predisposition to Alcoholism

The genetic predisposition to alcoholism has been proven by a number of classical genetic studies, including twin, adoption, and high-risk familial clustering studies. The strongest genetic associations identified to date are those between risk of alcoholism and genes encoding alcohol metabolizing enzymes. In particular, individuals having the genes encoding high activity alcohol dehydrogenase (ADH) (β2 ADH encoded by ADH2*2) or the dominant negative allele for aldehyde dehydrogenase ALDH2 (ALDH2*2) are at reduced risk of alcohol abuse in that population.4 The effect of these vari-
ants can best be explained by either increased rates of formation (high activity ADH) or decreased rates of clearance of acetaldehyde (ALDH2 deficiency), which can cause aversive reactions to drinking.

Two new polymorphisms in ADH and ALDH2 genes have recently been reported. An A/C substitution at −75 bp in the promoter of ADH4 (encoding π ADH) was found to affect the expression of transfected reporter plasmids. A mutation in the promoter of the ALDH2 promoter was simultaneously reported by Harada et al. and the author's laboratory. This A/G variant occurs at about −360 bp from the start site and is adjacent to a site bound by transcription factors belonging to the steroid receptor family. The A allele is less active than the G allele in reporter gene transfection assays. Of great interest, Harada's group showed that the A allele was also less common in a group of alcoholics with active ALDH2. These variants were found in all ethnic groups examined. It will be very interesting to see if the observations on the association of the A allele with protection from alcoholism can be extended to Caucasians and Africans.

The National Institute on Alcohol Abuse and Alcoholism has initiated a genomewide search for other genes underlying the observations that alcoholism is both familial and heritable. In the first 10 years of funding of the Consortium on Genetics of Alcoholism, screening and diagnostic tools were created and validated, families with multi-generational alcoholism were identified, and the patients' phenotypes were established. DNA has been banked for a subset of families and the first pass of screening for microsatellite repeats has been completed for over 250 families. A number of potential loci associated with alcoholism have been identified which are currently being studied with more closely spaced markers.

As far as genetic risk of alcoholic liver disease, the largest existing study is the U.S. VA Twin Panel Study. This study reported in 1981 that there was a substantially higher concordance for cirrhosis in monozygotic twins than in dizygotic twins, indicating the presence of a genetic component to the risk. This database was re-analyzed in 1994. The analysis again supported the notion that concordance for cirrhosis was higher in the monozygotic twins, but found that most of the genetic liability for cirrhosis was the result of shared risk for alcoholism. Thus, the idea that there is a genetic risk of alcoholic liver injury has yet to find support in large population studies. However, case control studies have indicated the possibility of increased genetic risk for alcoholic liver disease through inheritance of ALDH2*2, and polymorphisms of the tumor necrosis factor-α (TNF-α) and interleukin-10 (IL-10) promoter, and glutathione S-transferase.

**Genesis of Fatty Liver**

Fatty liver has long been recognized as the earliest response of the liver to chronic alcohol consumption. This was attributed to the effect of increased levels of cytosolic and mitochondrial NADH, which inhibits oxidation of fatty acids and stimulates their synthesis. Although this hypothesis has been dominant in the field, it was challenged as early as the mid 1960's by studies in which anti-oxidants prevented development of fatty liver in animals receiving large single doses of alcohol or chronic feeding of alcohol in the diet. The mechanisms by which alcohol imposes oxidative stress are discussed in the next section. We recently addressed this question by generating cell lines expressing rat class I ADH. These cells lack other forms of ADH and ALDH, as well as cytochrome P4502E1. The cell lines express high levels of ADH and metabolize alcohol present in the culture medium. Moreover, the rate of NADH production in these cells is sufficient to increase the lactate/pyruvate ratio in the medium from about 9 to 45. This degree of redox stress is at least as great as that occurring in the liver during alcohol metabolism. Coincident with this, the HeLa cells accumulate large amounts of triglyceride and free fatty acids. This was associated with inhibition of fatty acid oxidation and an increased rate of fatty acid synthesis.

To determine the mechanism underlying this effect, various inhibitors were tested. As expected, the ADH inhibitor 4-methylpyrazole blocked the accumulation of fatty acids and triacylglycerols. Addition of tocopherol to the medium did not prevent fat accumulation, arguing that oxidative stress was not involved. On the other hand, methylene blue, which non-enzymatically accepts electrons from NADH, reduced the lactate/pyruvate ratio and attenuated the accumulation of fat in the cells. These results argue that a high NADH/NAD⁺ ratio alone is sufficient to initiate the accumulation of fat and the development of fatty liver.

An interesting development in our understanding of lipid metabolism may be relevant to the development of alcoholic fatty liver. The peroxisome proliferator activated receptor (PPAR) is now recognized to be a fatty acid receptor. This receptor forms heterodimers with retinoid X receptor, binds consensus response elements (PuGGTCAnPuGGTCA), and activates gene transcription. Many genes involved in fatty acid binding (fatty acid binding protein), fatty acid oxidation (peroxisomal fatty acyl CoA oxidase, mitochondrial medium chain fatty acyl CoA dehydrogenase, microsomal lauryl hydroxylase), and lipoprotein synthesis (apo CIII and Al) appear to be regulated by PPAR. It would be expected that high levels of fatty acids in the livers of individuals who drink alcohol would activate these genes, but some of them (e.g., fatty acyl-CoA oxidase)
are not induced. PPAR or fatty acyl-CoA oxidase knockout mice develop steatohepatitis similar to that seen with alcoholics. PPAR-α mRNA was also reported to be decreased in the liver of rats fed ethanol chronically. Finally, the author’s laboratory has observed that alcohol metabolism by hepatoma cells impairs the function of transfected PPAR-α (unpublished observations, Galli and Crabb). Further work will be required to understand the role of this interesting receptor in the development of fatty liver.

Roles of Oxidant Stress and Adduct Formation

One problematic finding is that with prolonged alcohol feeding of baboons, the redox shift in the liver becomes less severe, but the fat accumulation persists. This suggests that after several weeks of alcohol use, the redox state is no longer the primary cause of fatty liver. The best candidate for the perpetuation of fatty liver at this stage is oxidative stress. There are two primary ways by which chronic alcohol use can induce oxidative stress: induction of CYP2E1 and reductive pressure on the mitochondrial electron transfer system. Induction of CYP2E1 is well-established to occur with chronic alcohol use; in fact, cells expressing CYP2E1 are known to undergo oxidative damage and apoptosis that can be prevented with free radical scavengers. CYP2E1 is leaky in that electrons transferred to it from CYP450 reductase can be transferred to molecular oxygen in the absence of substrate. The preferential expression of CYP2E1 in the central zone of the liver is consistent with the prominence of alcohol-induced injury to this part of the liver. More recently, several groups have shown that ethanol metabolism is capable of causing acute oxidative stress (as demonstrated by dichlorofluorescein fluorescence) in perfused liver and isolated hepatocytes. This oxidative stress apparently can be handled in most individuals by antioxidant defense mechanisms. The most prominent defense in the mitochondrion is the presence of glutathione and glutathione peroxidase. It is noteworthy that mitochondrial glutathione is preferentially depleted in the alcohol-fed baboon or rat. The existence of oxidative stress has been demonstrated in humans by the finding that ethanol use increases the urinary excretion of 8-epi prostaglandin-F2α and the breath excretion of ethane (both products of lipid peroxidation and decomposition).

Protein adducts continue to be recognized as a potential mechanism for ethanol toxicity. Adducts are formed between a number of liver proteins and acetaldehyde, hydroxyethyl radical, and peroxidative aldehydes like 4-hydroxynonenal. The proteins identified to date include tubulin, Δ4-3-ketosteroid 5β-reductase (37 kD protein), CYP2E1, and various membrane proteins in hepatocytes and Jun N-terminal kinase in stellate cells. Among the most provocative studies are those that showed increased sensitivity of the liver to ethanol in animals immunized with protein-acetaldehyde adducts, then fed ethanol. The immunized, alcohol-fed animals developed hepatic inflammation and fibrosis, while animals that were only immunized or only alcohol-fed did not.

The Role of Endotoxin and the Kupffer Cell

There is a growing appreciation that portal vein endotoxin and activation of the Kupffer cells are important in alcoholic liver injury. Recent studies show that alcohol feeding increases endotoxin levels in the portal vein, that chronic ethanol feeding induces the expression of the endotoxin binding protein and its receptor present on the Kupffer cell (CD14), and that inactivation of Kupffer cells by gadolinium chloride ameliorates the injury seen with chronic ethanol administration in the Tsukamoto-French model. The consequences of Kupffer cell activation are wide spread, as the cells release: TNF-α, which is a cause of hepatocyte apoptosis; IL-1 and IL-6 which elicit an acute phase response; eicosanoids, which are implicated in the increased rate of hepatocyte oxygen consumption during ethanol metabolism; reactive oxygen species; chemokines that stimulate migration of leukocytes into the liver; and platelet-derived growth factor (PDGF), a major stimulator of HSC proliferation (see below). It is also interesting to note that the mere presence of fatty liver (even without ethanol consumption) increases the sensitivity of the liver to the effects of endotoxin.

Pathways of HSC Activation

The final element in alcoholic liver injury is activation of the HSC and production of collagen in the hepatic interstitium. This fibrogenic process seems to require the activity of Kupffer cells and interaction of Kupffer cell-derived cytokines with the HSC. Currently, an area of great interest is the control of the earliest stage of HSC activation. One of the very earliest events is the activation of NF-κB in the HSC, but the signal responsible for this is as yet unknown. This might relate to changes in the matrix which they contact in the liver (for example, by modification by acetaldehyde), formation of protein adducts in the HSC (with acetaldehyde, 4-hydroxynonenal, malondialdehyde) or by oxidative stress originating in the hepatocytes or within the stellate cells themselves (HSC are known to express ADH and ALDH, but not CYP2E1). However, once NF-κB is activated, the cells can be stimulated to divide by PDGF, and stimulated to produce collagen by TGF-β and possibly IL-1. With time, this activation may
become irreversible, leading to progressive fibrosis and cirrhosis. The irreversible activation may be driven by the changes the cells make in the extracellular matrix, such as deposition of type I collagen, production of TGF-β by the HSC themselves (resulting in a positive feedback loop) and depletion of retinyl ester stores in the HSC. In addition, the HSC also make ICAM-1, MIP-2, PAF, SCF-1, and MCP-2, chemokines and adhesion molecules that may be important in the migration of leukocytes into the liver. All of these factors may lead to progressive liver damage even if the patient stops drinking.

Increased Risk of Alcoholic Liver Disease in Women

Given the recent new knowledge about the pathogenesis of alcoholic liver disease, it is worth revisiting the differences in responses to alcohol between men and women. Recent studies have shown that men and women have similar sized livers. This results in a larger liver mass/body mass ratio for women. When the rate of alcohol metabolism is normalized to liver mass, men and women have similar metabolic rates. However, blood alcohol levels after comparable doses of alcohol will usually be higher in women than in men because of the women’s lower body mass and lean body mass, resulting in a lower volume of distribution in the women. It has also been found that female rats are more susceptible to alcohol-induced liver injury in the Tsukamoto-French model. This may relate to more pronounced accumulation of fat in their livers, lower levels of fatty acid binding protein (and thus higher concentrations of unbound free fatty acids), increased plasma endotoxin, increased expression of the endotoxin receptor (CD14) in Kupffer cells and lipopolysaccharide binding protein, more pronounced central hypoxia, and more marked activation of NF-κB in the HSC. Thus, many mechanisms may conspire to make female alcoholics more prone to the development of alcoholic hepatitis and cirrhosis.

New Therapies for Alcoholic Liver Disease

Recent abstracts have indicated that S-adenosylmethionine may reduce mortality in cirrhosis due to its ability to protect against oxidative stress. Pentoxiphylline was also reported to improve outcomes in alcoholic hepatitis. This may be due to inhibition of activation of the HSC and reduction in production of inhibitors of metalloproteases. The full publication of these studies is eagerly awaited. Large, multi-center studies on the effectiveness of dilaunoil-phosphatidyl choline in hepatic fibrosis will be concluded soon. Propylthiouracil, which was reported in the 1980’s to improve alcoholic hepatitis, was recently reported to have additional effects. It can inhibit neutrophil myeloperoxidase, and may thereby reduce oxidative stress caused by hypochlorous acid made in the neutrophils. This discovery may lead to the development of other inhibitors that do not interfere with thyroxine production.

Future therapies may be suggested based on the mechanisms of injury discussed above. These include better antioxidants, inhibitors of CYP2E1, inhibitors of NF-κB activation or of eicosanoid production by Kupffer cells, and antagonists or antibodies against PDGF, TNF-α, and TGF-β. Of course, the best long term therapy will be the development of drugs or psychological therapies effective in reducing craving for alcohol and effective in treating alcoholism.

References


