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Acetaldehyde adducts in alcoholic liver disease

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Chronic alcohol abuse causes liver disease that progresses from simple steatosis through stages of steatohepatitis, fibrosis, cirrhosis, and eventually hepatic failure. In addition, chronic alcoholic liver disease (ALD), with or without cirrhosis, increases risk for hepatocellular carcinoma (HCC). Acetaldehyde, a major toxic metabolite, is one of the principal culprits mediating fibrogenic and mutagenic effects of alcohol in the liver. Mechanistically, acetaldehyde promotes adduct formation, leading to functional impairments of key proteins, including enzymes, as well as DNA damage, which promotes mutagenesis. Why certain individuals who heavily abuse alcohol, develop HCC (7.2–15%) versus cirrhosis (15–20%) is not known, but genetics and co-existing viral infection are considered pathogenic factors. Moreover, adverse effects of acetaldehyde on the cardiovascular and hematologic systems leading to ischemia, heart failure, and coagulation disorders, can exacerbate hepatic injury and increase risk for liver failure. Herein, we review the role of acetaldehyde adducts in the pathogenesis of chronic ALD and HCC.

Introduction

Chronic alcohol abuse causes liver pathology that progresses from simple steatosis through stages of steatohepatitis, followed by fibrosis, then cirrhosis, and finally end-stage liver disease. In addition to liver degeneration, chronic alcohol abuse serves as a potent co-factor in the pathogenesis of hepatocellular carcinoma (HCC). Besides liver, chronic alcohol abuse causes permanent injury to the brain, leading to cognitive-motor impairments and neurodegeneration, the cardiovascular system, resulting in heart failure and enhanced atherosclerosis, and various other organs in which it serves as a co-factor in malignant transformation. However, the toll of chronic alcohol abuse is heaviest in liver and brain.

Alcoholic liver disease (ALD) accounts for 40% of deaths due to cirrhosis, and 28% of all liver disease related deaths in the US.[1] These figures correspond to 3.2–3.5% of all deaths, and 3.6% of all cancer deaths globally.[2,3] The risk of developing hepatocellular carcinoma (HCC) increases with dose of chronically consumed ethanol[1,4] and alcohol consumption in excess of 80 g/day is correlated with high rates (7.2–15%) of HCC, especially in the setting of cirrhosis.[1,3,5-7] HCC is the fifth most common malignancy, with an estimated half million new cases diagnosed worldwide each year. Prognosis is generally poor (median survival 1–2 months)[8] due to late discovery and limited effective therapeutic options. Only one-third of patients with HCC are deemed suitable for curative procedures, many of which have not yet been fully validated.[9] Given the role of alcohol abuse in the pathogenesis of both liver degeneration and HCC, along other major untreatable and irreversible diseases, including those that afflict the brain and cardiovascular system, it is imperative that we improve our understanding of how alcohol mediates its adverse effects.

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Alcohol Metabolism

Alcohol is detoxified and eliminated primarily in the liver via a series of oxidative metabolic reactions.[10,11] The three major steps are: (1) reversible oxidation of ethanol to acetaldehyde, which is toxic; (2) non-reversible metabolism of the toxic acetaldehyde to acetate; and (3) breakdown of acetate to water and carbon dioxide (**Fig. 1**). The first step in alcohol oxidative metabolism is effectuated by key enzymes, including alcohol dehydrogenase (ALD), cytochrome P450 2E1 (CYP2E1), and catalase. ADH is the main oxidizing enzyme; it has a high affinity for alcohol[12] and breaks down ethanol in the cytoplasm. CYP2E1 is utilized by a distinct pathway that is induced by chronic alcohol consumption, and results in acetaldehyde formation in peroxisomes. A third path of first-step ethanol metabolism is mediated by catalase oxidation of ethanol in microsomes.[13,14]

The second step, which is mainly carried out by mitochondrial aldehyde dehydrogenase (ALDH), is to metabolize acetaldehyde to acetate. In addition, acetaldehyde can be metabolized by CYP2E1 through an NADPH-dependent pathway (microsomal acetaldehyde-oxidizing system).[15] The resulting acetate is unstable and spontaneously breaks down to water and CO_2. When these oxidative mechanisms become overwhelmed, acetaldehyde accumulates and exerts its toxic effects. The electrophilic nature of acetaldehyde[12] enables it to bind and form adducts, i.e. covalent chemical addition products, with proteins, lipids, and DNA.[3,11,16-18] Adducts are broadly pathogenic because they impair functions of proteins and lipids, and promote DNA damage and mutation.[11]

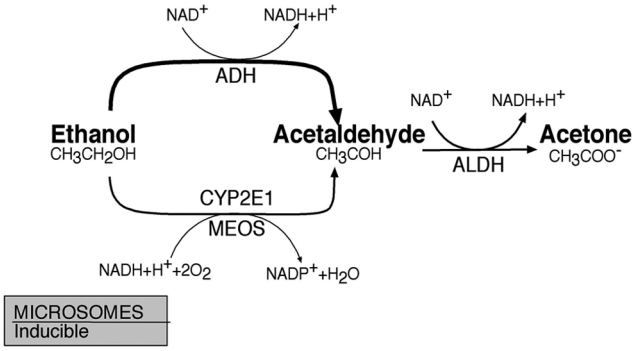

Figure 1. Metabolic pathways of alcohol. The main oxidizing enzyme is cytosolic alcohol dehydrogenase (ADH), which converts ethanol to acetaldehyde. Cytochrome P450 2E1 (CYP2E1), an enzyme of the microsomal ethanol oxidizing system (MEOS) that is inducible with chronic alcohol consumption also metabolizes alcohol. The conversion of acetaldehyde (a toxic metabolite) to acetate is by mitochondrial aldehyde dehydrogenase (ALDH). Both ADH and ALDH have polymorphisms that affect the rate at which acetaldehyde is generated and metabolized.

Alcohol Enzyme Polymorphisms

Propensity for acetaldehyde accumulation vis-à-vis ethanol consumption is governed in part by genetic factors. Polymorphisms in the ADH and ALDH genes can affect the rates of acetaldehyde generation and metabolism, and therefore govern proneness to acetaldehyde toxicity. In humans, there are at least 8 iso-enzymes of ADH and 4 iso-enzymes of ALDH. The most important ADH iso-enzymes are those encoded by the ADH1A, ADH1B and ADH1C genes.[3,19] In addition, various ADH1B and ADH1C alleles account for differences in the activity of ADH. For example, the ADH1B*2 allele encodes an enzyme that is 40 times more active than the one encoded by the ADH1B*1 allele. Rapid metabolism of alcohol to acetaldehyde, in individuals who express the ADH1B*2 allele, results in its toxic accumulation. The clinical manifestations of this effect include, flushing, sweating, tachycardia, nausea and vomiting after ethanol consumption. This ADH1B*2 allele is predominantly found in Asian populations. Although short-term responses to ethanol cause considerable discomfort, the adverse physiological response could serve as a deterrent for chronic alcohol misuse. The ADH1C*1 allele is another ADH iso-enzyme with increased (2.5-fold) activity, but the physiological effects associated with alcohol consumption are modest compared with those of ADH1B*2.[3] Nonetheless, individuals carrying at least one ADH1C*1 allele are at increased risk (3.6-fold) for developing alcohol-related HCC compared with those who are homozygous for ADH1C*2.[20] This suggests that genetic polymorphisms in ADH that result in increased generation of acetaldehyde also enhance ethanol's pro-carcinogenic effects.

ALDH2 is encoded by two main alleles, ALDH2*1 and ALDH2*2. ALDH2*2 is an inactive enzyme, and when expressed, acetaldehyde accumulation can be 10- or 20-fold higher than when the ALDH2*1 active enzyme is expressed, rendering alcohol consumption virtually intolerable.[21] The ALDH2*2 enzyme is not found in Caucasians, but is homozygous in 10%, and heterozygous in 40% of Asians.[3] Low ALDH activity increases risk for aero-digestive cancers,[22] and among Japanese people, the phenotype increases the risk for malignancy in general.[23,24] Together, the findings indicate that specific genetic polymorphisms in the ADH and ALDH genes have important roles in increasing susceptibility to alcohol-related cancers among heavy drinkers, and that the adverse effects of ethanol are likely mediated through the toxic and mutagenic effects of acetaldehyde.

Roles of Alcohol and Acetaldehyde in Carcinogenesis

Experimental animal models have provided convincing evidence that alcohol is a mutagen,[3] and that acetaldehyde should be regarded as a carcinogen (**Table 1**).[10,11,25,26] The role of alcohol/acetaldehyde as a carcinogen is particularly relevant to the pathogenesis of upper aero-digestive tract cancers.[3,27] Mechanistically, alcohol mediates its mutagenic effects through: (1) formation of acetaldehyde adducts;[3,28] (2) increased oxidative stress;[29,30] (3) induction of Kupffer cells by gut-derived endotoxins and release of TNF-α;[31-33] (4) inhibition of DNA methylation; and (5) impairing retinoid metabolism, which is important in cell differentiation.[1] Iron acts either independently[34,35] or synergistically[21,34,36] to promote the toxic and mutagenic effects of acetaldehyde. It is noteworthy that similar mechanisms contribute to the pathogenesis of chronic alcohol-induced liver injury leading to fibrosis or cirrhosis.

Acetaldehyde exerts its mutagenic effects by interacting directly with DNA,[37] and causing lesions ranging from point mutations to more extensive chromosomal damage.[22] For example, acetaldehyde-induced point mutations in the hypoxanthine phosphoribosyltransferase gene (HPRT1) impair DNA synthesis, DNA repair mechanisms, particularly nucleotide excision and excision- repair processes that maintain stability and integrity of genomic DNA.[37] In addition, acetaldehyde causes DNA damage by inducing sister chromatid exchanges (SCE's).[16,22] Any one of these mutagenic effects can activate cancer-generating pathways, or contribute to liver injury leading to cirrhosis.

Acetaldehyde as an Indirect Carcinogen

(1) Acetaldehyde protein adducts. Acetaldehyde impairs cellular functions and gene expression by forming adducts with proteins and DNA.[11,16,38,39] Acetaldehyde produces protein adducts by interacting with the epsilon amino group of lysine, or the α amino group of N-terminal amino acids.[12] Stable acetaldehyde adducts alter the structure and function of proteins, including enzymes. For example, acetaldehyde adducts formed with O6 methylguanine methyltransferase,[22] impair DNA repair mechanisms, which could mediate carcinogenesis.[40] Other major proteins targeted for acetaldehyde adduct formation include, tubulin,[41] collagen,[42] ketosteroid reductase

Table 1. Mechanisms of tissue injury: Ethanol, acetaldehyde and lipid peroxidation adducts

Compound	Injury Effect/Disease	Mechanism
Ethanol	Steatosis	Triglyceride accumulation
	Oxidative stress[29,30]	ER Stress, Insulin resistance, Mitochondrial dysfunction, CYP2E1 induction
	Cirrhosis	Acetaldehyde generation
	Carcinogenesis[10,11,25,26]	DNA damage
Acetaldehyde	Protein/Enzyme dysfunction	Protein,[11,12,16,22,38,39] DNA[16,22,37] and hybrid adducts[18,43,58]
	Oxidative stress	Lipid peroxidation adducts,[18,22,56] Pro-inflammatory cytokine activation, Glutathione scavenger function inhibited, ROS production increased[37,52]
	Fibrogenesis/Cirrhosis	Adducts in HSCs and myofibroblasts[11,64,65]
	Carcinogenesis	Mutagenesis by binding with DNA[37]
	Atherosclerosis	Oxidation of LDL[79,80,81,82,83]
	Cardiomyopathy	Impairs contractile function of cardiomyocytes[86,88,90]
	Erythrocyte macrocytosis	Acetaldehyde-modified erythrocyte membrane protein[107]
	Anemia and iron overload in liver	Immune mediated attack on erythrocytes[107]
	Impairs coagulation function	Inactivates clotting factors, e.g thrombin, fibrinogen, Factors II, VII, X, Xa, XIIIa[110,111,112]
Lipid Adducts (MDA; 4-HNE)	Cell death	Oxidative/ER Stress[11]
	Hepatic fibrosis/cirrhosis	Hepatic stellate cell activation with induction of collagen 1 synthesis and inhibition of pro-collagen negative feedback loop[77, 78]
	Carcinogenesis	Mutagenesis, including inhibition of oncosuppressor genes, e.g. p53[22]
	Atherosclerosis	Auto-immune response to modified proteins[82,83] Oxidation of lipoproteins forming plaques[80,81]

Abbreviations: ER stress, endoplasmic reticulum stress; CYP2E1, cytochrome P450 2E1; ROS, reactive oxygen species; HSCs, hepatic stellate cells; LDL, low-density lipoproteins; MDA, malondialdehyde; 4-HNE, 4-hydroxynonenal.

(catalyzes reduction of key intermediates in bile acid biosynthesis), CYP2E1,[43] and coagulation factors 7 and 9.[44] Protein adducts impair catalytic activity,[45] and consequently, functional impairment of CYP2E1 (NADPH-dependent) could lead to further acetaldehyde accumulation. Acetaldehyde binding to glutathione inhibits hydrogen peroxide scavenger function, thereby aggravating oxidative stress and lipid peroxidation.[46] Lipid peroxidation is probably the most important mediator of alcohol-induced cirrhosis and carcinogenesis.[31,47]

(2) **Acetaldehyde DNA adducts.** Acetaldehyde generates DNA adducts, the most prevalent of which is N2-ethyldeoxyguanosine (N^2-Et-dG).[22,37] N^2-Et-dG is detectable in livers of alcohol-exposed mice, leukocytes of human alcohol abusers,[16,22,48] and in humans with an ALDH2 genotype.[49,50] Due to its stability, N^2-Et-dG is detectable in alcohol-associated head and neck cancers,[51] and therefore could potentially serve as a marker of alcohol mis-use. 1,N(2)-propano-2'-deoxyguanosine (PdG), another acetaldehyde-DNA adduct, is distinguished by its genotoxic and mutagenic effects, and capacity to generate secondary lesions such as DNA-protein and DNA inter-strand cross-links[16] which impair DNA replication, thereby promoting cell death. Acetaldehyde-DNA adducts also promote carcinogenesis by triggering replication errors and mutations in oncogenes or onco-suppressor genes.[22]

(3) **Lipid peroxidation adducts.** Alcohol's mutagenic effects can be mediated by induction of CYP2E1,[22,52] which results in increased generation of reactive oxygen species (ROS)[37,52] leading to oxidative stress and cell death.[11] ROS-generated radicals,

including superoxide anion and hydroxyethyl radical (HER), are highly reactive and form adducts with lipids, proteins and DNA. Hydrogen peroxide, also generated through CYP2E1 enzymatic activity, can react with metal ions such as iron, to produce hydroxyl radicals.[37,53] Since chronic alcohol abuse causes iron to accumulate in liver, increases in CYP2E1 activity and H_2O_2 production can independently or synergistically exacerbate alcohol-induced liver injury and possibly liver cancer via increased hydroxyl radical formation,[21,36] and attendant DNA strand breakage, as well as a broad range of adverse biological responses.[37,54]

ROS promotes formation of lipid peroxidation products, including malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), both of which are detectable in association with intense CYP2E1 immunoreactivity in oral squamous cell carcinoma or leukoplakia in alcoholics.[55] MDA and 4-HNE can react with DNA bases to form exocyclic DNA adducts. In this regard, MDA reacts with deoxy-guanosine residues, while 4-HNE reacts with deoxyadenosine and deoxycytidine,[22,37] yielding the adducts, 1,N6-ethenodeoxyadenosine (εdA) and 3,N4-ethenodeoxycytidine (εdC), which have important roles in the pathogenesis of chronic alcohol-related liver injury.[18,56] In addition, both εdA and εdC are highly mutagenic, and can cause mutations in the p53 gene.[22] Specifically, these adducts induce G:C to T:A transversions on codon 249, and 30–40% of HCCs contain a mutation of p53.[57]

(4) **Hybrid adducts.** Various types of aldehydes generated within cells can cross-react to form hybrid adducts. For example, MAA hybrid adducts are composed of different combinations of MDA-acetaldehyde-protein adducts. The clinical significance of this phenomenon is that hybrid adducts can act synergistically and potentiate carcinogenic potential of individual adducts.[18,58] In addition, hybrid adducts may mediate stabilization of protein adducts,[43] thereby perpetuating their genotoxic effects.

Adducts Promote Liver Disease

Adducts accumulate in perivenous regions (Zone 3) of rat[59,60] and human[56,61] livers, overlapping with the distribution of fatty change (steatosis), i.e. the earliest lesion in alcohol-induced liver injury,[62] and associated with increased serum aminotransferase levels.[63] Acetaldehyde protein adducts are detectable in alcohol-related disease-associated inflammation and fibrosis.[11,64] Ultrastructural and immunohistochemical staining methods revealed that acetaldehyde adducts are detectable in hepatic stellate cells (HSC) in the context of steatofibrosis or cirrhosis, and in myofibroblasts in zones with bridging fibrosis.[65] Liver injury is likely mediated by binding of acetaldehyde to lysine residues and secondary interference with lysine-dependent enzymes such as calmodulin, and also tubulin.[11] Acetaldehyde adduct formation with 5% or less of the available α-tubulin pools impairs microtubular function and causes derangement of the hepatocyte cytoarchitecture, as has already been described in ALD.[11] Similarly, accumulation of acetaldehyde and MDA adducts on areas of collagen deposits contributes to the formation of scar tissue, and subsequent hepatic fibrosis or cirrhosis.[11]

Aldehyde-protein adducts and hydroxyl radicals can cause liver injury by stimulating intense immune responses directed against the modified proteins, as demonstrated by antibodies detected in sera of chronic alcohol-exposed experimental animals[66] and humans.[11] Sera of heavy drinkers may contain high titers of IgM, IgG and IgA antibodies to acetaldehyde adducts.[67-69] Associated "auto-immune" attacks on hepatocytes cause necrosis,[70] and with continued rounds of inflammation, necrosis, oxidative stress, ROS generation, and further adduct formation, fibrosis ensues.[71] An assay for detecting IgA antibodies to acetaldehyde-protein adducts has been developed and can be used to monitor liver injury associated with moderate to heavy drinking.[69,72]

Chronic alcohol abuse leads to progressive liver disease through stages of simple steatosis, to steatohepatitis, fibrosis, and finally cirrhosis through activation of hepatic stellate cells (HSCs). Stellate cells are activated by oxidative stress and lipid peroxidation.[73] MAA adduct treatment of cultured hepatic endothelial cells stimulates secretion of cytokines and chemokines, including tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-2 (MIP-2).[74] In addition, MAA adducts activate HSCs by stimulating secretion of fibronectin, which leads to increased extracellular matrix deposition with attendant fibrosis, and eventually cirrhosis.[75] Mechanistically, adducts generated by alcohol metabolism and lipid peroxidation increase collagen mRNA and connective tissue protein expression.[43,76] Specifically, acetaldehyde induces collagen 1 synthesis in HSCs by activating the AP-1 transcription factor.[77] Protein kinase C phosphorylates p70S6k, which promotes protein synthesis and collagen deposition. Acetaldehyde also inhibits the negative feedback loop of procollagen by binding to the carboxyl-terminal propeptide,[78] further promoting deposition of fibrous matrix. Conversely, adduct scavengers such as chlormethiazole, abolish acetaldehyde-mediated fibrogenesis.[43] In essence, acetaldehyde adducts contribute to injury, degeneration, scarring and carcinogenesis, and therefore play key roles in the pathogenesis of various stages of alcohol-related liver disease.[3]

Acetaldehyde Effects on the Cardiovascular System: Contribution to Liver Injury

While moderate consumption of alcohol has cardio-protective effects, excessive amounts worsen atherosclerosis and increase cardiovascular risk. Atherosclerotic plaques form in response to intimal accumulation of oxidized low-density lipoproteins (LDL) in foam cells, which promote oxidative stress and inflammation.[79] Since aldehyde adducts can mediate oxidation of lipids, including LDL, they potentially have an important role in the pathogenesis of atherosclerosis. Malondialdehyde (MDA), one of the oxidative products of unsaturated fatty acids and a component of atherosclerotic plaques, can also modify LDL. Moreover, both MDA and acetaldehyde can react with various proteins, including apolipoprotein B-100, a component of oxidized LDL, and generate additional adducts that cause endothelial cells to produce and release of pro-inflammatory cytokines and adhesion

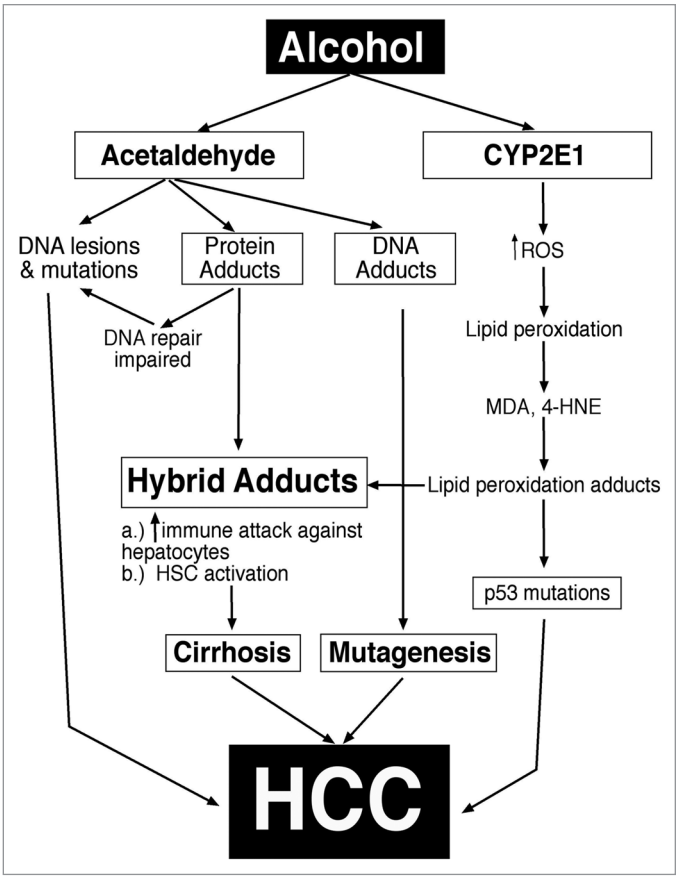

Figure 2. Proposed schematic of alcohol-mediated pathways resulting in hepatocellular carcinoma (HCC). The metabolism of alcohol generates acetaldehyde which promotes carcinogenesis pathways. Acetaldehyde (AA) directly causes DNA lesions, resulting in mutations. Indirectly AA forms covalent bonds with proteins, impairing DNA repair mechanisms, and increasing carcinogenesis potential. AA also binds to DNA, forming DNA adducts (N2-Et-dG and PdG), which are mutagenic. Chronic alcohol consumption results in CYP2E1 induction, generating reactive oxygen species (ROS), thereby increasing lipid peroxidation, which generates MDA and 4-HNE. These lipid peroxidation products cause p53 mutations and form adducts with DNA. AA-protein adducts combine with DNA adducts to form hybrid adducts, with resultant synergistic activation of auto-immune attacks against hepatocytes, ultimately resulting in cirrhosis, which is strongly associated with HCC development.

molecules that are critical mediators of atherosclerosis.[80,81] Correspondingly, atherosclerosis can be inhibited by treatment with recombinant antibodies to oxidized LDL[82] depleting B cells that produce autoantibodies to oxidized LDL[83] or administration of aldehyde scavengers.[84] It is noteworthy that MAA adducts are increased in human atherosclerotic vessels,[80] and like MDA, MAA's pro-inflammatory properties can exacerbate atherosclerosis.[80] Acetaldehyde toxicity and vascular degeneration can contribute to liver disease by causing chronic ischemic injury mediated by atherosclerosis of the aorta and hepatic artery.

Acetaldehyde quite likely has a pathogenic role in alcohol-associated cardiomyopathy.[85-89] In this regard, it is noteworthy that in experimental animal models, acetaldehyde accumulation in myocardium impairs contractile function,[86,88,90] disrupts cardiac excitation-contraction coupling[87,88,91] and promotes oxidative stress[85] with attendant increased lipid peroxidation.[87] Acetaldehyde inhibits protein metabolism and induces protein adducts, which together impair the integrity of actin and myosin filaments and depress cardiomyocyte contractile function. In addition, alcohol abuse leads to hypertension, cardiac arrhythmias, and non-ischemic cardiomyopathy.[92] Consequential reductions in cardiac output would lead to liver injury due to chronic passive congestion, and also exacerbate injurious effects of chronic ethanol exposure or acetaldehyde formation. On the other hand, ALDH2 is cardioprotective against ischemic heart disease[93] and acute cardiac toxicity[94] because it promotes rapid detoxification of acetaldehyde. Correspondingly, genetic polymorphisms that reduce ALDH2 enzymatic activity are associated with increased rates of diabetes mellitus among Chinese women with coronary artery disease (CAD), independent of alcohol intake,[95] while over-expression of ALDH2 in transgenic mice reduces alcohol-induced myocardial hypertrophy and impairments in insulin/Akt signaling.[96-98] Therefore, acetaldehyde can promote cardiovascular disease by several mechanisms including impairment of insulin signaling and cardiomyocyte function, and inducing atherosclerosis, which together could lead to liver injury mediated by ischemia or chronic passive congestion.

The relationship between cardiovascular disease and liver disease is particularly evident in regard to non-alcoholic fatty liver disease (NAFLD). Like alcoholic liver disease, NAFLD progresses through stages of increased inflammation with cell turnover and loss (non-alcoholic steatohepatitis; NASH), fibrosis, cirrhosis, and finally end-stage liver disease.[99] In addition, progression of NAFLD increases ones risk for developing HCC.[100] NAFLD is increasingly regarded as the hepatic component driving the increased risk of cardiovascular disease in individuals with diabetes mellitus, hyperlipoproteinaemia, or metabolic syndrome.[101-104] Moreover, obesity and excess dietary fat intake, which are largely responsible for the epidemic growth in NAFLD, promote systemic oxidative stress and lipid peroxidation,[105] thereby contributing to the increased risk of cardiovascular disease. Like ALD, progression of NAFLD is associated with increased formation of adducts, including acetaldehyde and MAA, and MAA adduct accumulation correlates with severity of NASH.[106] Therefore, NAFLD-mediated increases in acetaldehyde and hybrid adducts can contribute to progression of alcoholic liver disease via increased atherosclerosis.

Acetaldehyde Effects on Erythrocytes and Clotting Mechanisms

Chronic alcohol abuse has significant adverse effects on the hematopoietic system including erythrocyte and coagulation functions. Chronic alcohol abuse promotes red blood cell morphological changes such as erythrocyte macrocytosis.[72,107] This abnormality has been correlated with the presence of

autoantibodies to acetaldehyde-modified erythrocyte membrane proteins in peripheral blood[107] and bone marrow aspirates[108] Potential consequences of these immune-mediated attacks on erythrocytes are not completely known, but they may increase red blood cell destruction, and thereby promote both anemia and iron accumulation in liver. Excessive iron accumulation is a well-recognized mediator of liver injury and contributes to the pathogenesis of alcoholic liver disease. Moreover, acetaldehyde-bound to red blood cells can be distributed to various tissues and exert widespread toxic effects.[109] Finally, a broad range of hematologic disorders can adversely affect liver function. For example, hemolytic anemias and hemaglobinopathies can result in increased iron load in the liver, as well as promote cholelithiasis due to increased formation of bilirubin stones. Alcoholic and other forms of chronic liver disease impair function of coagulation factors, but with regard to alcohol abuse, acetaldehyde mediates these effects by inactivating thrombin, Factor Xa, fibrinogen, II, VII, and X.[110-112] In addition, acetaldehyde inhibits the transglutaminase activity of factor XIIIa,[112] and forms complexes with glycosaminoglycans to synergistically inhibit factors IX, IXa,[111] X, and Xa,[110] with consequential prolongation of clotting times.

Conclusions

The data strongly support the concept that chronic and excessive alcohol consumption contributes to and probably promotes progressive liver disease and HCC. These effects of alcohol are mainly mediated by acetaldehyde, which is generated during metabolism of alcohol, and accumulates consequential to genetic polymorphisms in alcohol metabolizing enzymes, increased oxidative stress, iron deposition in liver, and immune-mediated attacks on adducted proteins. Acetaldehyde-protein and DNA adducts promote oxidative stress, formation of lipid peroxidation products (MDA and 4-HNE), HSC activation with attendant inflammation and fibrosis, and mutagenesis, and they interact with other aldehyde adducts to form stable hybrids that promote substantially greater degrees of injury, including DNA damage (**Fig. 2**).

Based on these observations, we hypothesize that acetaldehyde accumulation → oxidative stress, inflammation, cell injury → HSC activation and cytokine activation → ROS generation →radical ion accumulation → lipid peroxidation, DNA and protein adduct formation → protein and enzyme malfunction, impaired gene expression, and DNA damage → mutagenesis and fibrogenesis. In light of the potency of hybrid adducts with respect to accelerating and intensifying injury, it could be that "second hits" that perturbate metabolism and promote additional aldehyde accumulations and adducts may play pivotal roles in governing the path toward progressive hepatic fibrosis and cirrhosis versus hepatocellular carcinoma. In this regard, other disease states such as non-alcoholic fatty liver disease (NAFLD) or chronic hepatitis C virus infection, and ischemia/hypoperfusion caused by heart failure or atherosclerosis result in increased accumulation of lipid peroxidation products and adduct formation.[113,114] When combined with chronic alcohol abuse, these co-factors increase risk for progression to cirrhosis and/or HCC.[115] Conditions that lead to increased iron deposition and consequently potentiate radical ion formation such as hemachromatosis,[35] or ADH and ALDH gene polymorphisms that impair efficient metabolism of alcohol to carbon dioxide and water, could serve as co-factors in promoting end-stage liver disease or HCC. Finally, tobacco smoke contains nitrosamines that promote adduct formation,[116] and since many alcohol abusers smoke or are exposed to smoke, they are at increased risk for generating adducts unrelated to acetaldehyde-associated adducts. Interactions between acetaldehyde and other adducts resulting in the formation of hybrid adducts may represent the pivotal factor governing long term consequences of chronic alcohol abuse with respect to the development of cirrhosis or HCC. Future investigations will need to focus on developing means to detect, characterize, and quantify hybrid adducts in liver, and identify approaches to limit their formation and accumulation in the context of chronic alcohol abuse.

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