Linking Pathogenic Mechanisms of Alcoholic Liver Disease With Clinical Phenotypes





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Alcoholic liver disease (ALD) develops in approximately 20% of alcoholic patients, with a higher prevalence in females. ALD progression is marked by fatty liver and hepatocyte necrosis, as well as apoptosis, inflammation, regenerating nodules, fibrosis, and cirrhosis.[1] ALD develops via a complex process involving parenchymal and nonparenchymal cells, as well as recruitment of other cell types to the liver in response to damage and inflammation. Hepatocytes are damaged by ethanol, via generation of reactive oxygen species and induction of endoplasmic reticulum stress and mitochondrial dysfunction. Hepatocyte cell death via apoptosis and necrosis are markers of ethanol-induced liver injury. We review the mechanisms by which alcohol injures hepatocytes and the response of hepatic sinusoidal cells to alcohol-induced injury. We also discuss how recent insights into the pathogenesis of ALD will affect the treatment and management of patients.

Keywords: Alcoholic Hepatitis; Alcoholic Liver Disease; Hepatic Stellate Cell.

Ethanol leads to hepatocyte stress and injury through effects on hepatocytes and intestinal epithelia. Toxicity is mediated through ethanol as well as ethanol metabolism and its metabolites. What are the molecular mechanisms of these processes, and how do they affect disease development and phenotype?

Oxidative Stress

Ethanol is metabolized through 2 major oxidative and 2 minor nonoxidative pathways. Ethanol is oxidized by alcohol dehydrogenase and cytosolic aldehyde dehydrogenase 1 and mitochondrial aldehyde dehydrogenase 2,[1] which converts nicotinamide adenine dinucleotide$^+$ into reduced nicotinamide adenine dinucleotide. Ethanol also is oxidized by cytochrome P450 family 2, subfamily E, polypeptide 1 (CYP2E1) and catalase, which generates reactive oxygen species (ROS) and leads to cellular damage.[2] Although the liver metabolizes the most ethanol, other tissues also can metabolize alcohol.[3] For example, chronic ethanol intake induces CYP2E1 expression in Kupffer cells.[4,5] In addition to oxidative metabolism, 2 nonoxidative pathways can metabolize small amounts of ethanol.[3] However, the effects of these pathways on cell functions are not clear.[6]

Ethanol induces oxidative stress via multiple pathways (reviewed by Dey and Cederbaum[7]). In hepatocytes, increased oxidative stress directly damages mitochondria to induce cell death or sensitize hepatocytes to cell death in response to inflammatory cytokines. Increased oxidative stress in Kupffer cells also increases their sensitivity to lipopolysaccharide (LPS).[5] Livers from reduced nicotinamide adenine dinucleotide phosphate oxidase and inducible nitric oxide synthase knockout mice have decreased, whereas Cu,Zn-superoxide dismutase knockout mice have increased, oxidative and nitrate-induced stress and injury after chronic ethanol feeding.[8–10]

Strategies to down-regulate oxidative stress pathways therefore might prevent or reduce the development of ALD. Unfortunately, clinical trials in patients with ALD focusing on antioxidant therapies generally have not been successful. Rather than excluding a role for this approach in therapy, the results highlight the need to increase our understanding of oxidative stress and antioxidants, to guide appropriate selection of targets, as well as proper doses for human beings. Other antioxidants that have received attention

Abbreviations used in this paper: **ALD, alcoholic liver disease; CYP2E1, cytochrome P450 family 2, subfamily E, polypeptide 1; DAMP, danger-associated molecular pattern; ER, endoplasmic reticulum; HIF, hypoxia inducible factor; HSC, hepatic stellate cell; IL, interleukin; LPS, lipopolysaccharide; NASH, nonalcoholic steatohepatitis; NK, natural killer; RIP, receptor-interacting protein kinase; ROS, reactive oxygen species; SIRT1, sirtuin 1; TLR, Toll-like receptor; TNF-α, tumor necrosis factor-α; UPR, unfolded protein response.**

Most current article

0016-5085/$36.00
http://dx.doi.org/10.1053/j.gastro.2016.02.035

either in preclinical and small clinical trials and warrant further study include s-adenosyl methionine, betaine, folate, and methionine adenosyltransferase I α.

Hypoxia

Alcohol consumption induces pericentral hypoxia owing to increased oxygen consumption during ethanol metabolism.[11,12] Prolonged hypoxia promotes ROS production, impairs mitochondrial fatty acid oxidation, and stimulates expression of lipid synthesis genes, contributing to mitochondrial damage and cell death. Liver can adapt to hypoxia by activating transcription of genes that include hypoxia inducible factors (HIFs). Expression of HIF1A and HIF2A increases in liver with binge and chronic ethanol feeding. Unfortunately, although HIF1A protects the liver from hypoxia, a number of events also ensue that exacerbate alcohol-induced liver injury.[13–15] Mice with hepatocyte-specific disruption of *Hif1b* (*Arnt*) are resistant to chronic plus binge alcohol-induced steatosis and liver injury.[16] Activation of HIFs during alcohol-induced hypoxia therefore have complex effects on liver injury in mice. The roles of HIFs in human ALD have not been investigated.

Dysregulation of Lipid Synthesis

Alcohol consumption dysregulates lipid synthesis and metabolism, resulting in steatosis.[17] Alcohol exposure inhibits hepatic activity of sirtuin 1 (SIRT1), leading to increased acetylation and stability, as well as increased transcriptional activity of sterol regulatory element-binding protein-1, which regulates lipogenesis.[18,19] Sterol regulatory element-binding protein-2, lipin 1,[20,21] and ceramide also regulate lipogenesis. SIRT1 regulates fatty acid synthesis and oxidation; ethanol-induced decreases in SIRT1 affect multiple processes to lead to steatosis.[22–24] Because SIRT1 regulates lipid metabolism, it could be targeted to prevent and/or treat steatosis associated with ALD.

Mitochondria, Endoplasmic Reticulum Stress, and Autophagy

Acute and chronic alcohol exposure alter liver mitochondria structure and function in animal models and human beings.[25] Alcohol exposure also damages mitochondrial DNA and ribosomes and decreases rates of mitochondrial respiration (state III) and oxygen consumption, leading to mitochondrial-mediated apoptosis.[11,26] However, liver can adapt to chronic alcohol-induced mitochondrial and metabolic stress by activating mitophagy,[27] mitochondrial fusion, or mitochondrial respiration, as well as mitochondrial biogenesis (via peroxisome proliferator-activated receptor γ, coactivator 1 α) in mice.[28] Therefore, mitochondrial plasticity allows for a balance between alcohol-induced mitochondrial damage and repair or biogenesis.

Alcohol metabolism can result in the formation of a variety of protein adducts, as well as impair proper protein folding in the endoplasmic reticulum (ER), resulting in accumulation of misfolded protein and ER stress.[29] Cells can adapt to ER stress by activating the unfolded protein response (UPR). The UPR attenuates ER stress and restores ER homeostasis by decreasing general protein translation and increasing protein folding capacity (by promoting expression of chaperone proteins). The UPR also promotes degradation of misfolded proteins by ER-associated protein degradation via the proteasome or by ER stress-mediated compensatory autophagy.[30,31] However, chronic alcohol consumption inhibits hepatic proteasome activity.[32] As a result, damaged or misfolded proteins accumulate in cells and form insoluble protein aggregates that are resistant to degradation by the proteasome and require removal by autophagy. In support of this model, autophagy was observed to be activated in mouse liver after acute or chronic alcohol exposure.[33,34] This process adds another layer of adaptive compensatory mechanisms for the regulation of proteostasis in response to ER stress and impaired proteasome function after alcohol exposure.

Autophagy is a catabolic process involved in maintaining normal liver physiology and the development of liver diseases.[35] Autophagy involves the formation of double-membrane autophagosomes that traffic and fuse with lysosomes to form autolysosomes, where autophagic cargo are degraded. Chronic alcohol impairs vesicular function and trafficking.[36–39] Cells can adapt to impaired vesicular function by promoting lysosome biogenesis and synthesis of early autophagosomes.[40] Therefore, autophagy could either decrease or increase, depending on the balance of impaired cellular trafficking and lysosomes, and the compensatory activation of de novo autophagosome synthesis and lysosome biogenesis after alcohol exposure. Although our knowledge in this area is far from complete, especially with regard to autophagy in human ALD, a number of newly developed and repurposed drugs have been shown to regulate the autophagy process and eventually may warrant evaluation for human ALD.

Interactions of Apoptosis, Necrosis, and Autophagy

Alcohol consumption leads to hepatocyte death via apoptosis, necrosis, or necroptosis (programmed necrosis). Ethanol induces apoptosis via the extrinsic (death-receptor regulated) or intrinsic (mitochondrial) pathways.[41–43] Necroptosis is similar in nature to necrosis but is initiated by death receptor activation and mediated by receptor-interacting protein kinase 1 (RIP1) and 3 (RIP3), and the downstream mixed lineage kinase domain-like protein.[44–46]

Although multiple pathways can contribute to hepatocyte cell death in the context of ethanol exposure, tumor necrosis factor (TNF)-α–induced cell death has been the most well studied. After TNF-α binds to its receptor (TNF receptor 1), it recruits downstream factors (Figure 1).[47] Formation of specific downstream effector complexes determines whether TNF-α induces hepatocyte survival, apoptosis, or necroptosis.[47] Recent reviews have summarized mechanisms that regulate the formation of these effector complexes.[48,49] RIP1 and RIP3 could have multiple