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Gut-Liver Axis in Alcoholic Liver Disease

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Abstract

Alcoholic liver disease (ALD) has been amongst the leading causes of liver cirrhosis and liver-related death worldwide for decades. Early discoveries in alcoholic liver disease identified increased levels of bacterial endotoxin in the portal circulation suggesting a role for gut-derived "toxins" in ALD. Indeed, alcohol consumption can disrupt the intestinal epithelial barrier and result in increased gut permeability that is increasingly recognized as a major factor in ALD. Bacterial endotoxin, LPS, is a prototypic microbe-derived inflammatory signal that contributes to inflammation in ALD through activation of the Toll-like receptor 4 (TLR4). Recent studies also demonstrated that alcohol consumption is associated with alterations in the gut microbiome and the dysbalance of pathogenic and commensal organisms in the intestinal microbiome may contribute to the abnormal gut-liver axis in ALD. Indeed, bacterial decontamination improves ALD both in human and animal models. This short review summarizes recent findings and highlights emerging trends in the gut-liver axis relevant to ALD.

Keywords

gut permeability; inflammation; microbiome; lipopolysaccharide

Introduction to Gut-Liver Interactions

The anatomy of the liver provides its close interaction with the gut where nutrients and the microbiome contribute to the maintenance of a healthy metabolism and liver. Gut derived nutrients and other signals are delivered to the liver via the portal circulation that has several unique features. The slow blood flow in the liver sinusoids permits interactions between gut-derived substances and hepatocytes, other liver parenchymal cells and liver immune cells; this is further promoted by the fenestrated endothelium in the sinusoids[1]. The liver as the largest immune organ hosts the entire spectrum of immune cell repertoire and has a remarkable capacity to recruit and activate immune cells in response to gut-derived

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metabolic or pathogen-derived signals. The effects of gut microbiota in liver diseases has been a major interest in recent years[2]. A recent study places the liver in the center of the intersections between the host and the gut commensal microbiota[3]. Interestingly, bile acid produced by the liver can also modulate the microbiome as some bacteria utilize bile acids[4]. The interaction between the microbiome and the host liver is of particular interest in alcoholic liver disease where alcohol was shown to both change the composition of the microbiome and impair intestinal integrity and barrier function[5,6].

Alcoholic Liver Disease

Excessive alcohol use over a prolonged period of time often results in alcoholic liver disease. The spectrum of alcoholic liver disease includes steatosis, steatohepatitis, acute alcoholic steatohepatitis, alcoholic fibrosis and cirrhosis (Laennec's cirrhosis). Steatosis is and early steatohepatitis are reversible after cessation of alcohol use. Based on current understanding, multiple pathogenic factors are involved in the development of alcoholic liver disease. Alcohol and its metabolites induce reactive oxygen species and hepatocyte injury, though mitochondrial damage and ER stress[7–12]. There is early activation of chemokines, particularly MCP-1 that contributes to recruitment of macrophages and IL-8 that recruits neutrophil leukocytes in the liver[13,14]. Activation of Kupffer cells has been identified as a central element in the pathogenesis of ALD[15,16]. Previous studies demonstrated that KCs and recruited macrophages in the liver are activated by bacterial endotoxin (lipopolysaccharide-LPS) through Toll-like receptor 4 (TLR4) and that the level of LPS is increased in the portal as well as in the systemic circulation after excessive alcohol intake[17,18]. These observations suggest that gut-derived LPS is a central mediator of inflammation in alcoholic steatohepatitis.

Fibrosis is a dynamic and progressive process governed by stellate cell activation from inflammatory cytokines and gut-derived products[19]. Indeed, microbial components in the portal blood contribute to progression of fibrosis and the development of portal hypertension[19,20]. Advanced ALD predisposes to hepatocellular cancer where LPS-TLR4 interactions and stem cell Nanog expression seem to have mechanistic roles in animal models[21,22].

Gut barrier function – effects of alcohol

The integrity of the intestinal mucosa is determined by the function of several components: protective layer of defensins on the intraluminal surface of the intestinal epithelium; tight junction proteins between intestinal epithelial cells, and the gut immune cells in the intestinal wall[5]. Alcohol has both direct effects on these functions in the intestine and indirect effects by alcohol and/or its metabolites distributed via the blood stream[23]. Acute alcohol binge causes cellular damage and death of the intestinal epithelial cells when consumed at high concentrations[24]. In addition, increase in blood alcohol levels is associated with reduced expression of mRNA levels of important proteins involved in tight junctions between colon epithelial cells[25]. In Caco-2 intestinal epithelial cells, alcohol decreases the expression of the tight junction proteins, occludin and zona-occludens -1 (ZO-1).[25,26] It has been shown that microRNA-221 is involved in the downregulation of the tight junctions

proteins in the mouse model of alcohol-induced gut permeability[25]. Acetaldehyde, a highly toxic metabolite of alcohol, disrupts tight junction thereby contributing to the increased gut permeability caused by chronic alcohol use[27]. In Caco-2 cells, alcohol also increased the expression of the circadian clock proteins, CLOCK and PER2, via reactive oxygen species (ROS)-induces upregulation of CyP2E1 leading to intestinal hyperpermeability[28]. Further studies demonstrated that disruption of the circadian clock in mice resulted not only in increased intestinal permeability but also promoted alcohol-induced liver damage and inflammation[29]. Chronic exposure of Caco-2 cells to alcohol also increased the susceptibility of these epithelial cells to infections by bacterial pathogens[30]. In vivo, deficiency in intestinal mucin-2 ameliorated ALD in mice and this was linked to increased killing of commensal bacteria and prevention of bacterial overgrowth[31].

We recently found that acute alcohol binge drinking in healthy human volunteers resulted in a significant increase in serum endotoxin levels and this correlated with increased bacterial 16S rDNA increase suggesting gut microbial origin[32]. This is similar to the increased levels of serum endotoxin reported in patients with chronic alcohol consumption and liver disease[16,33]. In the animal model, we found that both acute alcohol binge and chronic alcohol administration increased serum endotoxin levels in mice[24]. The acute alcohol binge resulted in minimal inflammation in the proximal intestine while TNFa and NF-kB activation were robust after chronic alcohol feeding. The alcohol-induced intestinal inflammation correlated with reduced mRNA and protein levels of Reg3b and increased expression of microRNA-155 in the small intestine and further studies demonstrated that miR-155-deficien mice were protected from chronic alcohol-induced inflammation in the small intestine. Furthermore, there was no increase in serum endotoxin levels after chronic alcohol feeing in the miR-155-deficient mice suggesting that this microRNA-155 may have a role in alcohol-induced disruption of the gut integrity[24]. Another recently identified regulator of gut permeability is FoxO4 induced by alcohol[34]. It was found that alcohol significantly increase FoxO4 that can regulate intestinal permeability[34]. These recent studies imply the complexity of gut barrier function and highlight the multiple checkpoints where alcohol could interfere with normal function.

Gut microbiome and alcohol

Perhaps the majority of new information on gut-liver axis in recent years is related to understanding the role of the microbiome in human health and disease. The intestinal microbiota has a major role in shaping the host immune response and commensial bacteria shape the integriy of the gut mucosa[35]. A wide array of human diseases including obesity, insulin resistance and the related metabolic syndrome, and NASH, cancer, chronic inflammatory diseases and infections, and neuroinflammatory processes have been linked to change in the gut microbiome. In case of alcohol use, the microbiome is a very likely target given that in human alcohol consumption alcohol in the intestinal content has direct contact with components of the microbiome. In vitro studies demonstrated that alcohol has direct and selective effects on the growth of bacteria and intestinal overgrowth can produce ethanol that in turn may affect intestinal permeability[36].

Metagenomic analysis of alcohol-induced alteration in the intestinal microbiome revealed that alcohol feeding in mice decreases the bacterial diversity and shifts the phylum representation over time[37]. Compared to pair fed mice where the majority of bacteria were in the Bacteriodes and Firmicutes phylum, alcohol feeding dramatically increased the presence of Actinobacteria and increased the proportion of Firmicutes over altered Bacteriodes[37]. In another study in a mouse model of alcoholic liver disease, bacterial translocation was found before changes in the microbiome and the bacterial traslocation was associated with reduced expression of the bactericidal c-type lectins, Reg3b and Reg3g in the small intestine[38].

Chronic alcohol also alters the metabolic composition in the gastrointestinal content that changes the source of nutrition for microbes in the GI tract[39]. For example, alcohol feeding resulted in a decrease in all amino acids and branched chain amino acids in the gut[39]. These deteriorations indicate that chronic alcohol use directly and indirectly changes the composition of the gut microbiota.

Microbial products, pattern recognition receptors, and the immune system

Pathogen-associated molecular patterns (PAMPs) are sensed by pattern recognition receptors including Toll-like receptors (TLRs), Nod-like receptors (NLRs), helicase receptors and others[40,41]. The microbiome contains a broad variety of PAMPs and owed to the intestinal barrier, these PAMPs don't reach the systemic circulation. The most studied gut-derived PAMP in the circulation is bacterial lipopolysaccharide (LPS) that is a component of Gram-negative bacterial wall. Many studies demonstrated that chronic alcohol consumption increases LPS levels in the portal as well as in the systemic circulation without entering the brain. This was found in human alcoholics with or without liver disease as well as in rats and mice[23,33]. In mice, a single acute alcohol gavage can increase serum LPS levels and administration of a 5% alcohol containing diet also increases serum LPS as early as in one week[42]. A recent study showed that acute alcohol binge in normal volunteers results in a rapid increase in serum LPS as well as in bacterial 16S DNA levels suggesting disruption of intestinal barrier function. In addition ot LPS and 16S bacterial DNA, peptidoglycan, a component of Gram-positive bacteria was also detected in human alcoholics[43]. It has also been shown that TLR4 or CD14 deficient mice that have disruption of the LPS receptor complex, are protected from alcoholic liver disease[17,18,44]. Consistent with this, sterilization of the gut also attenuated alcohol-induced liver disease in animal models[45].

Repeated engagement of TLR4/CD14 with LPS results in TLR tolerance in macrophages[46,47]. However, in the alcoholic liver environment with increase portal blood LPS, KCs and macrophages become sensitized to LPS[47] leading to increased TNFα production[48,49].

Activation of the TLR4 receptor complex by alcohol-induced LPS results in downstream activation of the NFκ-B pathway and induction of pro-inflammatory cytokines and chemokins. Amongst those are TNFα and IL-1β that have been shown to increase gut permeability thereby potentially amplifying the alcohol-induced initial gut leakiness and

liver disease process[50]. Locally in the intestinal mucosa, chronic alcohol results in increased expression of TNFα an IL-1β[24]. Recent reports described that IL-22 is a cytokine that regulates gut epithelia cells and immune functions. In a burn injury model, alcohol addition amplified reduction in IL-22 that correlated with increased gut permeability. In the same study, IL-22 administration prevented the increased gut permeability caused by the combined insult of alcohol plus burn injury[51]. In another study, IL-22 administration ameliorated alcoholic liver injury raising the question whether IL-22 acts directly on the liver or via the gut-liver axis in ALD[52]. IL-22 is produced by innate lymphoid cells (ILC) that reside in the bowel wall[5,53]. In the intestinal wall the interaction of immune cells is an important component in maintenance of the host and microbiome balance. Recent studies suggest that microbiota can dictate the crosstalk between macrophages and innate lymphoid cells (ILC)3 and this promotes intestinal homeostasis[54]. ILCs balance immunity, inflammation and tissue repair in the intestine, and may play a role in regulation of the gut-liver axis in ALD[55].

Clinical aspects of the impaired gut-liver axis in ALD

Excessive alcohol use in most cases is associated with alcohol dependence[56]. The role of intestinal permeability and inflammation has received recent attention in the biological and behavioral control of alcohol dependence[57]. A recent study found that intestinal permeability and LPS were increased in alcohol-dependent non-cirrhotic subject at hospitalization for detoxification compared to 3 weeks later after successful detoxification. Inflammatory cytokine increase was correlated with depression and alcohol craving suggesting that gut-brain axis may play a role in the pathogenesis of alcohol dependence[58].

Alcoholic hepatitis often arises in the setting of liver fibrosis raising the question whether the fibrotic liver was more susceptible to alcohol and/or gut-derived PAMPs induced by alcohol. It has been shown that in patient with portal hypertension the gut barrier function is impaired leading to increased gut “leakiness”[59]. Increased levels of LPS entering the liver has multiple biologic effects. First, LPS induces recruitment and activation of inflammatory cells and pro-inflammatory cytokine production. Second, LPS modulates hepatocyte functions and results in cholestasis[60]. Third, LPS and pro-inflammatory cytokines induce production of acute phase reactants by hepatocytes in the liver including serum amyloid A, LPS binding protein (LBP), fibrogen, C-reactive protein, Il0-6 and ceruloplasmin[61–63]. It has been proposed that normal hepatocytes have a role in “detoxification” of the portal blood including elimination of LPS[64,65]. Altered production of LBP, soluble CD14 and anti-LPS antibodies that all act by binding circulating LPS and modulate the biologically active form of LPS that leads to inflammation. The capacity of hepatocytes that are constantly exposed to alcohol is unlikely to be contact in the role of LPS detoxification, however, only a few studies have evaluated this question[66]. Indeed, levels of LBP and soluble CD14 were found to be elevated in advanced liver diseases[67,68]. Consistent with increased gut permeability, changes in gut bacterial populations and their translocation to the liver and ascites was found in alcoholic liver cirrhosis in various studies[69]. One of the potential clinical implications of the gut-liver axis in advanced ALD is promotion of bacterial infections often manifested as sub-acute bacterial peritonitis, hepatic encephalopathy, or severe systemic infections[70]. Selective gut decontamination with Rifaximin has improved

hepatic encephalopathy raising the possibility of beneficial effects of selective gut decontamination in advanced ALD[71].

Emerging therapeutic approaches that target the gut-liver axis

Given that alcohol disrupts the gut barrier function, it is attractive to explore therapeutic interventions that could prevent alcohol-induced gut “leakiness” and/or restore alcohol-induced defects. For example, it has been shown that alcohol-induced zinc deficiency contributes to the impaired gut barrier function[72]. More important, administration of zinc in mice with chronic alcohol feeding restored the alcohol-induced gut dysfunction[73].

Another approach is to modify the microbiome dysbalance associated with alcohol use. To this end, a recent study evaluated the effect of VSL3 in mice with chronic alcohol feeding and found improvement in gut permeability after VSL3 administration[74]. In a different study, administration of *Lactobacillus rhamnosus GG* in mice on chronic alcohol diet resulted in a decrease in fecal pH, attenuated serum endotoxin levels and attenuation of alcohol-induced liver damage (ALT and steatosis)[37]. These beneficial effects of *Lactobacillus rhamnosus GG* treatment during continued alcohol intake in mice was associate with improved gut permeability based on tight junction protein expression.[37]. Notably, *Lactobacillus rhamnosus GG* treatment also improved markers of intestinal barrier function and provided protection against non-alcoholic fatty liver induced by high fat diet in mice[75].

Fecal transplantation as a therapeutic approach demonstrated benefits in C. difficile infection[76]. Based on observation indicating altered composition of the intestinal microbiome in ALD, it is tempting to speculate that fecal transplantation may modulate the outcome or severity of ALD.

Dietary supplements have received interest in alcoholic liver disease as chronic alcohol use is often associated with deficient intake of nutrients and vitamins. Zinc deficiency is a well established consequence of chronic alcohol consumption[72]. Studies have shown that zinc deficiency augments alcohol-induced liver injury as well as the negative effects of alcohol on gut permeability[72]. It was shown that dietary zinc deficiency augmented alcohol-induced increases in serum endotoxin levels as well as most of the pathogenic features of alcohol-induced liver damage and inflammation[73]. Milk osteopontin, a component of milk was shown to ameliorate alcohol liver damage and serum endotoxin increase in a mouse model of alcoholic liver disease[77].

Studies in rat duodenum showed that administration of a 15% alcohol solution or red wine in the intraluminal surface of the duodenum increases duodenal permeability and this could be prevented by administration of melatonin[78]. Melatonin is produced in the gut enterochromaffin cells and it can act as a potent antioxidant (Stomlanski et al 2012).

New therapeutic approaches to target gut-derived inflammatory signals may consider anti-LPS antibody administration or TLR4 inhibition strategies. Another potential target could be inhibition of miR-155 based on the observation of attenuation of alcohol-induced gut permeability in miR-155 deficient mice[24].

Unanswered questions

Although the number of reports on the gut-liver axis in alcoholic liver disease has drastically increased in recent years, there are many remaining questions. Increase in gut permeability is not unique to alcoholic liver disease. In disease conditions such as Crohn's colitis or HIV infection serum LPS levels are elevated yet there is no liver disease. It appears that increased gut permeability is just one of potentially several factors that contributes to ALD. It is tempting to speculate that alcohol-induced effects on hepatocytes, whether it is induction apoptosis, ER stress, mitochondrial damage and/or modulation of inflammatory cell responses in the liver are fundamental elements in the process of ALD that provide an environment for gut-derived LPS (an/or other PAMPs) to result in the complex pathology of ALD. Nevertheless, most studies suggest that prevention of the alcohol-induced disruption of gut permeability and/or the entry of gut-derived inflammatory signals to the liver have proven beneficial effects on the development of alcoholic liver disease. In conclusion, the interactions between the gut microbiome, intestinal barrier and the liver appear to have a key role in the pathogenesis of alcoholic liver disease and further exploration of the gut-liver axis in ALD deserves attention.

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