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REVIEW

Alterations of the gut microbiome and metabolome in alcoholic liver disease

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Abstract

Alcohol consumption is one of the leading causes of liver diseases and liver-related death worldwide. The gut is a habitat for billions of microorganisms which promotes metabolism and digestion in their symbiotic relationship with the host. Alterations of gut microbiome by alcohol consumption are referred to bacterial overgrowth, release of bacteria-derived products, and/or changed microbiota equilibrium. Alcohol consumption also perturbs the function of gastrointestinal mucosa and elicits a pathophysiological condition. These adverse effects caused by alcohol may ultimately result in a broad change of gastrointestinal luminal metabolites such as bile acids, short chain fatty acids, and branched chain amino acids. Gut microbiota alterations, metabolic changes produced in a dysbiotic intestinal environment, and the host factors are all critical contributors to the development and progression of alcoholic liver disease. This review summarizes recent findings of how alcohol-induced alterations of gut microbiota and metabolome, and discusses the mechanistic link between gastrointestinal dyshomeostasis and alcoholic liver injury.

Key words: Alcoholic liver disease; Microbiome; Gut metabolome

Core tip: Excessive alcohol consumption causes alcoholic liver disease (ALD) with the mechanisms of pathogenesis largely unknown. Alterations of gut microbiota and metabolites are critical contributors to the development of ALD, which may lead to identification of therapeutic targets for ALD. This review summarizes recent findings of how alcohol-induced alterations of gut microbiota and metabolome, and discusses the mechanistic link between gastrointestinal dyshomeostasis and alcoholic liver injury.

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INTRODUCTION

Alcohol abuse is one of the leading causes of liver disease-related morbidity and mortality worldwide. Alcoholic liver disease (ALD) may progress from steatosis (fatty liver) to steatohepatitis, liver cirrhosis, and eventually hepatic carcinoma[1,2]. According to the National Institute on Alcoholic Abuse and Alcoholism, liver cirrhosis is the 12th leading cause of death in the United States, about 50% of which are alcohol related[3]. Even though enormous efforts have been made, the pathogenesis of ALD is still poorly understood, which makes the progress in finding proper treatments slow. In the last decade, the

role of the gastrointestinal tract (GI) in the pathogenesis and progression of ALD has drawn more and more attention. It is estimated that there are multiple times more microbial cells in the gut than the total number of cells in the human body[4]. The microbes contribute to a complex of biological processes such as digestion[5], synthesis of vitamins[6], and regulation of immunity[7]. Disruption of intestinal homeostasis and alterations in the gut microbiome and metabolome contribute to the pathogenesis of many disorders including ALD[4,8,9]. This review summarizes recent findings on how alcohol affects the composition of the gut microbiota and metabolites, and discusses the mechanistic link between GI dyshomeostasis and the pathogenesis of alcohol consumption-induced liver injury.

INTESTINAL MICROBIOME AND ALCOHOLIC LIVER DISEASE

Quantitative (bacterial overgrowth) and qualitative (dysbiosis) changes of the GI microbiome have long been associated with liver diseases including ALD[10]. Disturbed gut microbiota homeostasis results in dysfunction of the intestinal barrier and translocation of bacteria and/or bacterial products, which eventually contribute to the progression of ALD. Interventions focusing on gut bacteria and/or bacterial products in preventing ALD have drawn increasing attention in the last decade.

Intestinal bacterial overgrowth and translocation in the development of ALD

Alcohol consumption is well known to elicit bacterial overgrowth along the GI tract[11]. The number of both aerobic and anaerobic bacteria cultures of jejunal aspirates from alcoholic patients was distinctly higher than that from the control patients[12]. Similar trends were observed in patients with alcoholic cirrhosis[13]. Bacterial overgrowth has also been documented in experimental models of ALD[14,15]. Overgrowth of bacteria affects ethanol metabolism. Experimental induction of bacteria overgrowth resulted in enhanced endogenous and/or exogenous ethanol metabolism and high concentrations of acetaldehyde in both the intestinal lumen and the portal blood[16-18]. Oral administration of metronidazole, an antibiotic drug, led to a higher level of intracolonic acetaldehyde by increasing aerobic bacteria and reducing anaerobic bacteria in the intestine[19]. On the other hand, intracolonic acetaldehyde accumulation was prevented by antibiotic ciprofloxacin, which decreased colonic microbiota and fecal alcohol dehydrogenase activity[20].

Bacterial translocation is defined as the passage of viable bacteria from the GI tract to extraintestinal sites, such as the mesenteric lymph node, liver, kidney, and bloodstream. Experimental induction of small bowel bacterial overgrowth caused bacterial translocation in association with hepatic inflammation in rats[21]. The translocation of bacteria has been reported as early as 14 d after alcohol consumption in rats[22], while some studies did not show significant bacterial translocation after alcohol administration for 2 wk[23,24]. Moreover, Yan *et al*[14] reported that the bacterial translocation occurred prior to changes observed in the microbiome in a mouse model of continuous intragastric alcohol feeding for up to 3 wk. On the contrary, in a rat model of ALD combined with bacterial inoculation, rats chronically fed with alcohol presented markedly less bacterial translocation to the mesenteric lymph nodes and to the other organs examined compared to rats fed with an isocaloric liquid diet[25].

Bacterial products and gut permeability in the development of ALD

Bacteria, particularly the Gram-negative bacteria, produce endotoxins in the GI tract. Under physiological condition, endotoxin is excluded out of the body along with feces, and only trace amount of endotoxin can penetrate through the GI epithelium to the systemic circulation due to the gut barrier[26]. Alcohol consumption increases the serum endotoxin level, namely endotoxemia. The development of endotoxemia mainly results from bacterial overgrowth and/or increased gut permeability. Endotoxemia has been well documented in patients with ALD[26], and the blood endotoxin levels correlate well with tumor necrosis factor α (TNF-α) levels and the severity of ALD[27-29]. Elevated endotoxin in systemic circulation activates hepatic Kupffer cells *via* Toll-like receptor 4 to produce inflammatory cytokines and chemokines which, in turn, attract neutrophils and monocytes into the liver[30]. In addition to endotoxin, other bacterial products, such as bacterial DNA, peptidoglycan, and flagellin, could also translocate from the intestinal lumen to extraintestinal space and organs, and play a critical role in ALD progression. It was reported that bacterial DNA was elevated in the plasma of patients with alcoholic cirrhosis[31]. Bacterial DNA is recognized by TLR9 and sensitizes the liver to endotoxin-induced injury[32]. Alcohol exposure increased peptidoglycan levels and injected peptidoglycan deteriorated liver injury and inflammation in alcohol-fed mice[33,34].

Intestinal barrier dysfunction has been repeatedly reported in alcohol-induced endotoxemia and liver damage. Alcoholic patients showed increased gut permeability to a variety of macromolecules, such as polyethyleneglycol, lactulose/mannitol, or ^{51}CrEDTA[35-38]. In animal studies, gut permeability to macromolecules such horse radish peroxidase was also increased in association with alcohol-induced endotoxemia and liver damage[39-43]. Orally administrated lipopolysaccharide could be detected in the plasma of acute alcohol-intoxicated mice but not in the control mice[44]. Chronic alcohol exposure reduced the distribution of tight junction proteins, but did not significantly affect the intestinal histopathology[45]; and the gut leakiness only occurred in the ileum instead of in the duodenum or jejunum[45]. Taken together, intestinal barrier dysfunction enables bacteria and bacterial products to translocate from the intestinal lumen to the liver which, as a result, facilitates the development of ALD.

Intestinal dysbiosis in the progression of ALD

Alcohol consumption not only results in quantitative changes of the intestinal microbiota, but also leads to enteric dysbiosis. Enteric dysbiosis refers to an imbalance in the intestinal bacterial composition that participates in the normal activities of the GI tract. Clinical studies have shown that patients with alcoholic cirrhosis had a lower proportion of *Bacteroidetes* and higher ones of *Proteobacteria* in the colon as compared to alcoholic patients without liver cirrhosis[46]. In another study, patients with alcoholic liver cirrhosis showed higher amounts of *Prevotellaceae* in the feces compared to cirrhotic patients with hepatitis B or healthy controls[47]. Animal studies also demonstrated that alcohol consumption for 10 wk altered colonic mucosa-associated microbiota composition in rats[48]. The abundance of *Bacteroidetes* and *Verrucomicrobia* were elevated in the cecum of mice intragastrically fed alcohol for 3 wk, while *Firmicutes* bacteria (including *Lactobacillus*, *Pediococcus*, *Leuconostoc*, and *Lactococcus*) were predominant in the control mice[14]. A recent animal study showed that chronic alcohol feeding for 8 wk caused a decline in the abundance of both *Bacteroidetes* and *Firmicutes* phyla, with a proportional increase in the Gram-negative *Proteobacteria* and Gram-positive *Actinobacteria* phyla in mice feces[15].

The interactions between alcohol-induced liver injury and alterations in the amount/proportion of certain bacteria phylum remain largely unknown. The *Proteobacteria* phylum includes Gram-negative bacteria, most of which are regarded as pathogenic species. Alcohol exposure-induced *Proteobacteria* expansion in the GI tract strongly indicates a link between alcohol-induced alterations of gut microbiota and the elevated plasma endotoxin level as well as hepatic inflammation. Studies have described opportunistic infections of *Corynebacterium*, a member of the *Actinobacteria* phylum, in individuals with ALD[49,50]. As mentioned above, intestinal bacteria like *Escherichia coli* metabolize alcohol and increase luminal acetaldehyde levels through alcohol dehydrogenase-dependent[51] or catalase-dependent pathway[52]. Acetaldehyde is known to disrupt the intestinal barrier through disassembling tight junction proteins[53-56], which implicates another mechanism of how microbiota participate in the development of ALD. The relevance of the gut microbiota changes for ALD progression still requires further investigation.

Intervention for ALD via modulating intestinal microbiome

Efforts on exploring therapeutic strategies for treating ALD have been made for decades, and one of the major attempts was to ameliorate alcohol-induced endotoxemia. Indeed, animal studies demonstrated that abrogating endotoxin signal cascade in the liver by administration of antibiotics[57] or neutralization of circulating endotoxin[58], led to attenuation of alcohol-induced cytokine production and liver damage. Dietary supplementation of milk osteopontin prevented alcohol-induced liver injury through blocking enteric Gram-negative bacterial translocation and the endotoxin-mediated effects in the liver[59].

The effects of probiotics and prebiotics in modulating alcohol-induced liver injury in both patients with ALD and experimental models have been widely studied and the related references are summarized in Table 1. The first report was the study by Nanji *et al*[60], which showed that *Lactobacillus GG* treatment reduced endotoxemia and the severity of ALD. Treatment with *Lactobacillus GG* attenuated alcohol-induced intestinal barrier stress, gut leakiness, and liver injury in rats[40,61-64] and mice[65,66]. A short-term therapy with *Bifidobacterium bifidum* and *Lactobacillus plantarum 8PA3* to alcoholic patients lowered plasma alanine aminotransferase and aspartate aminotransferase levels, restored the gut microbiota, and improved ALD compared to patients treated with standard therapy (abstinence plus vitamins) alone[67]. Another human study showed that *Lactobacillus casei* Shirota administration for 4 wk restored neutrophil phagocytic capacity in alcoholic cirrhotic patients[68]. Notably, the beneficial effects of probiotics were achieved not only by live bacteria, but also by heat-inactivated bacteria[63,69] or bacteria culture supernatant[70].

Short-chain fructooligosaccharides and other prebiotics are used to stimulate the growth and activity of probiotics such as *Lactobacillii* and *Bifidobacteria*. Dietary supplementation of oats prevented alcohol-altered colonic musoca-associated microbiota composition in rats[48]. It was shown that administration of prebiotic (fructooligosaccharides) to alcohol-fed mice reduced bacterial overgrowth and ameliorated alcoholic steatohepatitis through partially restoring the host antimicrobioal protein Reg3g[14].

There are few reports addressing the impact of probiotic and/or prebiotic supplementation on gut microbiome during the development of ALD. To date the most comprehensive study employed 16S ribosome RNA sequencing to characterize gut microbiome changes in mice feces after chronic alcohol exposure and *Lactobacillus GG* supplementation[15]. *Lactobacillus GG* not only reduced bacterial overgrowth in alcohol-fed mice, but also prevented the alcohol-induced expansion of the *Proteobacteria* and *Actinobacteria* phyla.

INTESTINAL METABOLOME AND ALD

Research into alterations in gut metabolome in ALD is unfortunately not as advanced as that for alterations in gut microbiota. To the best of our knowledge, our group, for the first time, applied mass spectrometry-based high throughput technology for characterization of the metabolic alterations of the GI tract contents in a rat model of chronic alcohol consumption. First of all, we conducted a comprehensive metabolite profiling using a high performance liquid chromatography time-of-flight mass spectrometry (HPLC-TOF MS). Secondly, since the HPLC-TOF MS-based profiling approach may not be able to detect or generate accurate data of short chain amino acids (SCFAs) and branched chain

Table 1 Summary of references related to the protective effects of probiotic/prebiotic against alcoholic liver disease

Probiotic/prebiotic	Subjects	Duration of treatment	Outcome	Ref.	Year
Probiotics					
Lactobacillus rhamnosus GG	Male Wistar rats	1 mo	Probiotic feeding reduced alcohol-induced endotoxemia and liver injury	Nanji *et al*[60]	1994
A mixture containing 450 billion bacteria (VSL #3)	Alcoholic cirrhosis patients	3 mo	Treatment of probiotic lowered plasma levels of cytokines and oxidative stress parameters	Loguercio *et al*[103]	2005
L. casei Shirota	Alcoholic cirrhosis patients	4 wk	Probiotic supplementation restored neutrophil phagocytic capacity	Stadlbauer *et al*[68]	2008
Heat-killed *L. brevis* SBC8803	C57BL/6N mice	35 d	*L. brevis* SBC8803 ameliorated alcohol-induced liver injury and fatty liver	Segawa *et al*[69]	2008
Bifidobacterium bifidum and *L. plantarum* 8PA3	Male Russian adults	5 d	Patients treated with probiotics had significantly lower ALT and AST activity, and restored gut microbiota compared to patients treated with standard therapy alone	Kirpich *et al*[67]	2008
L. rhamnosus GG	Male Sprague-Dawley rats	10 wk	*L.* GG reduced alcohol-induced gut leakiness and blunted alcohol-induced oxidative stress and inflammation both in the intestine and liver	Forsyth *et al*[40]	2009
L. rhamnosus GG	Male C57BL/6N mice	Last 2 wk of the 8-wk feeding	*L.* GG supplementation reduced alcohol-induced endotoxemia and hepatic steatosis	Wang *et al*[65,66]	2011, 2013
L.paracasei	Male Fischer 344 rats	10 wk	*L. paracasei* altered the fatty acid composition of the plasma and liver	Komatsuzaki *et al*[61]	2012
L. rhamnosus GG culture supernatant	Male C57BL/6N mice	5 d	Bacteria-free *L.* GG culture supernatant ameliorated acute alcohol-induced gut leakiness and liver injury	Wang *et al*[70]	2012
Combined *Bifidobacterium*, *Lactobacillus*, *Enterococcus* and *Bacillus cereus* tablets	Male Sprague-Dawley rats	Up to 8 wk	Probiotic administration reduced plasma elevated-endotoxin levels caused by alcohol and altered gut microbiota	Zhang *et al*[62]	2012
Live or heat-killed VSL #3	Male rats	Up to 12 h	VSL #3 administration reduced plasma endotoxin level and cytokine production caused by acute alcohol exposure	Chang *et al*[63]	2013
Heat-killed *L. casei* MYL01	HepG2 cells	20 h	*L. casei* MYL01 modulated proinflammatory cytokine production	Chiu *et al*[104]	2014
Escherichia coli Nissle 1917 secreting pyrroloquinoline quinone	Male Foster rats	10 wk	Probiotic treatment ameliorated alcohol-induced oxidative damage and hyperlipidemia in rats	Singh *et al*[64]	2014
Prebiotics					
L. rhamnosus GG or oats	Male Sprague-Dawley rats	10 wk	Supplementation of *L. rhamnosus* GG or oats prevented alcohol-altered colonic musoca-associated microbiota composition in rats	Mutlu *et al*[48]	2009
Fructooligosaccharides	Male C57BL/6J mice	3 wk	Administration of fructooligosaccharides to alcohol-fed mice reduced bacterial overgrowth and ameliorated alcoholic steatohepatitis through partially restoring the host antimicrobial protein Reg3g	Yan *et al*[14]	2011

fatty acids (BCAAs) due to their volatile properties, a gas chromatography mass spectrometry (GC-MS) was used to quantitatively measure specific metabolic panels of SCFAs and BCAAs. The methods were described in more detail elsewhere[71,72]. Thirdly, a targeted quantitative metabolomics approach for a panel of 20-30 bile acids using ultraperformance liquid chromatography-triple-quadrupole mass spectrometry was utilized[73]. Alcohol consumption markedly altered bile acids[73], increased fatty acids and steroids, decreased carnitines, amino acids, branched chain amino acids, and all short chain fatty acids except for acetic acid[71] in the GI luminal contents of rats after 8-wk of alcohol exposure. Bile acids, SCFAs, and BCAAs were the top three categories among the significantly changed metabolites by alcohol consumption. Therefore, they were quantitatively measured in our study and the results will be discussed in more detail below.

Global profiling of metabolites in the GI tract

Chronic alcohol consumption resulted in a global metabolite alteration including amino acids, fatty acids, steroids, lipids, carnitine, SCFAs, BCAAs[71], and bile acids[73] along the GI tract of rats. Almost all amino acids detected were decreased in GI contents of alcohol-fed rats compared to the control. Notably, high abundances of alanine, arginine, glutamic acid, proline, and threoine were observed in all the intestinal segments (from duodenum to rectum) and they were dramatically decreased after alcohol exposure. Amino acids derived from dietary protein may serve as substrates for luminal conversion by the gut microbiota which, in turn, regulate the host homeostasis. For example, one constituent of the gut microbiome, *Lactobacillus reuteri*, is able to convert L-histidine into histamine, which is an immune-regulatory signal suppressing TNF-α production[74]. Intestinal bacteria also involve in converting glutamate to γ-amino butyric acid *via* gluta-

mate decarboxylase[75]. Taken together, it is possible that the reduced abundance of amino acids in alcohol-fed rats was resulted from a perturbed gut microbial-host co-metabolism under the enteric dysbiosis condition.

The levels of steroids and steroid derivatives were significantly increased after alcohol consumption in the stomach, duodenum, jejunum, and ileum. Carnitines and metabolites involved in lipid metabolism were decreased in alcohol-fed rats. Most of the fatty acids detected were at higher levels including 17-HDoHE and 19,20-DiH-DPA, the two metabolic products from docosahexaenoic acid (DHA), and DHA itself. The elevation of DHA and DHA metabolites in the intestinal lumen, especially the large intestine, indicates a disrupted absorption of this nutrient induced by alcohol exposure.

Bile acids

Alcohol consumption significantly perturbed all 21 bile acids detected along the GI tract with the ileum showed the most significant alteration[73]. The concentration of unconjugated bile acids in control rats was low in duodenum (0.04 nmol/mg wet weight), whereas it was increased in the alcohol group (1.30 nmol/mg wet weight). Taurine-conjugated bile acids are the most abundant bile acids in the small intestine and the liver of control rats[73,76,77]. Alcohol consumption led to lower levels of taurine-conjugated bile acids in the duodenum and ileum (0.15 and 0.02 nmol/mg wet weight) compared to control rats (2.39 and 5.66 nmol/mg wet weight, respectively), which made unconjugated bile acids accounted for the largest proportion of the total bile acids in the entire GI tract. Meanwhile, the amount and proportion of taurine-conjugated bile acids were decreased both in the liver and blood[73].

Bile acid metabolism is dependent on the biological activities of the gut microbiota and the host, and both bacterial and hepatic enzymes further modify bile acids during enterohepatic circulation[78,79]. Perturbed gut microbiome may result in a disturbance of bile acid metabolism and reabsorption, leading to altered bile acid profiles in the blood, liver, kidney, and heart[80]. Indeed, inhibiting intestinal microbiota with ampicillin increased the expression of the apical sodium-dependent bile acid transporter (ASBT/Slc10a2) in the brush-border membrane of the ileum, which in turn increased bile acid transport into portal blood[81]. Germ-free mice and rats have a higher proportion of taurine-conjugated bile acids in their livers and intestines[79,82], demonstrating a close association between gut microbiota and bile acid composition. It has been reported that the ratio of glycine-conjugated to taurine-conjugated bile acids is dependent on the hepatic taurine concentration[83]. In our study, we found that the hepatic bile salt taurine to glycine ratio was 30:1 in control rats, while the ratio was 1:1 in alcohol-treated rats. The majority of taurine is usually degraded by the gut microbiota to inorganic sulfate[84]. For this reason, an overgrowth of gut microbiota caused by alcohol exposure would be expected to decrease taurine bioavailability, which provides an explanation for alcohol-induced decrease in taurine-conjugated bile acids in our study. In addition, another investigation suggests that the reduction of taurine in the liver in alcohol-fed mice may be due to the formation and excretion of N-acetyltaurine, a novel metabolite synthesized from taurine and acetate[85].

SCFAs and BCAAs

Acetic acid, propionic acid, and butyric acid are the most predominant SCFAs within the intestine[86]. Our study revealed that the distal intestine (ileum to rectum, especially cecum) processed the majority of SCFAs, within which acetic acid, propionic acid, and butyric acid were predominant (85% in ileum, 94% in cecum, 97% in colon, and 93% in rectum)[71]. Alcohol consumption dramatically reduced all 9 SCFAs detected in the distal intestine except for acetic acid. SCFAs are mainly produced by microbial fermentation of indigestible dietary fibers in the gut[87]. The alteration of SCFAs in alcohol-fed rats may be a result from alcohol-perturbed gut microbiota. The elevated acetic acid levels after alcohol consumption may presumably be due to the oxidation of ethanol to acetaldehyde and subsequent oxidation to acetic acid[88]. Since bacterial aldehyde dehydrogenase activity is limited[18], gut microbiota may not be the major player for the elevated luminal acetic acid level. On the other hand, SCFAs may influence the gut microbiota through stimulating *Bifidibacteria* growth and inhibiting Gram-negative facultative and anaerobic bacteria[89]. SCFAs are known as energy sources to regulate the homeostasis of the intestine and other organs[86]. In a recent study, SCFAs were approved to be beneficial against alcohol-induced intestinal barrier dysfunction through activating AMP-activated protein kinase in Caco-2 cells[90].

BCAAs are essential nutrients obtained from food, as they cannot be synthesized *de novo* by mammals[91]. Gut microbiota, however, are capable to produce BCAAs efficiently[92]. BCAA supplementation has been widely used to improve energy metabolism[93,94], insulin resistance[95-97], and severity of liver disease[98]. Our study reported that all three BCAAs, valine, leucine, and isoleucine, in the GI lumen were predominant in the small intestine (duodenum, jejunum, and ileum) and to a lesser extent in the cecum in rats[71]. Alcohol consumption led to significantly lower levels of all three BCAAs in the GI contents[71]. Previous findings have shown that chronic alcohol consumption increased incorporation of leucine into hepatic proteins[99] and accelerated the absorption of leucine from the small intestine[100], which may explain the dramatic reduction of BCAAs in the gut lumen observed in our study. Notably, a low ratio of plasma BCAAs to aromatic amino acids is a hallmark of liver cirrhosis. Indeed, elevated leucine and isoleucine levels were reported in the plasma of non-alcholic steatotic and non-alcoholic steatohepatitic patients compared to healthy controls[101], which indicate the homeostasis of BCAAs may be involved in the pathogenesis of liver diseases. Moreover, branched chain SCFAs, 2-methylpropanoic acid, 2-methylbutyric acid, and 3-methylbutyric acid are derived from the catabolism of BCAAs[102]. The decreased enteric BCAA levels may

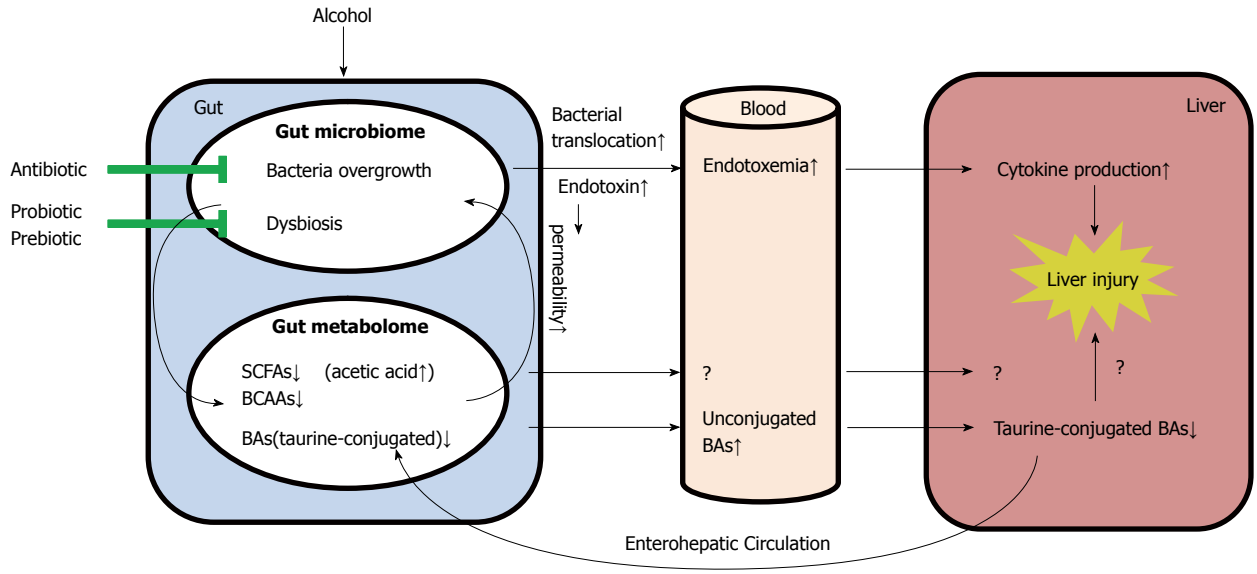

Figure 1 Schematic diagram of the impact of alcohol consumption on the gut microbiota and metabolome during the development and progression of alcoholic liver disease. BA: Bile acids; SCFA: Short chain fatty acid; BCAA: Branched chain amino acid.

further contribute to the decreased levels of branched chain SCFAs after alcohol consumption.

CONCLUSION

Alcoholic consumption is one of the leading causes of liver diseases and liver-related death worldwide. Of the major factors that contribute to the pathogenesis of ALD, the gut microbiota and metabolites have recently drawn more and more attention. Altered intestinal microbiota and gut-associated endotoxemia are recognized as pathophysiological factors in the development of ALD. Prebiotics and probiotics have been applied to prevent alcohol-induced disease development and progression. Taking the advantages of metabonomics approaches, detailed metabolic profiling provides novel information on alcohol-induced alterations in microbiota-host co-metabolism. The impact of alcohol consumption on the gut microbiome and metabolome during the development of ALD is summarized in Figure 1. Despite the recent progression in understanding the importance of the GI tract in the development of ALD, questions of how alcohol consumption results in gut microbiome and metabolome alterations and what are the consequences of such changes to the host have not been fully addressed. Future investigations on the cause-effect relationship between alterations of gut microbiome/metabolome and the liver pathophysiology will not only provide novel insights into the pathogenesis of ALD but also pave the way to the development of therapeutic interventions to cure ALD.

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