

Roles of Gut Microbiota in Alcoholic Liver Disease

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Abstract: Alcoholic liver disease (ALD)—one of the most common liver diseases — involves a wide range of disorders, including asymptomatic hepatic steatosis, alcoholic hepatitis (AH), liver fibrosis, and cirrhosis. Alcohol consumption induces a weakened gut barrier and changes in the composition of the gut microbiota. The presence of CYP2E1 and its elevated levels in the gastrointestinal tract after alcohol exposure lead to elevated levels of ROS and acetaldehyde, inducing inflammation and oxidative damage in the gut. At the same time, the influx of harmful molecules such as the bacterial endotoxin LPS and peptidoglycans from gut dysbiosis can induce intestinal inflammation and oxidative damage, further compromising the intestinal mucosal barrier. In this process, various oxidative stress-mediated post-translational modifications (PTMs) play an important role in the integrity of the barrier, eg, the presence of acetaldehyde will result in the sustained phosphorylation of several paracellular proteins (occludin and zona occludens-1), which can lead to intestinal leakage. Eventually, persistent oxidative stress, LPS infiltration and hepatocyte damage through the enterohepatic circulation will lead to hepatic stellate cell activation and hepatic fibrosis. In addition, probiotics, prebiotics, synbiotics, fecal microbial transplantation (FMT), bioengineered bacteria, gut-restricted FXR agonists and others are promising therapeutic approaches that can alter gut microbiota composition to improve ALD. In the future, there will be new challenges to study the interactions between the genetics of individuals with ALD and their gut microbiome, to provide personalized interventions targeting the gut-liver axis, and to develop better techniques to measure microbial communities and metabolites in the body.

Keywords: alcoholic liver disease, gut dysbiosis, modulators

Introduction

With socioeconomic development, lifestyle changes, and increased social openness, alcohol abuse, alcohol dependence, and alcoholism have become one of the most serious public health problems worldwide. In 2016, it was reported that 32.5% of the world's population drinks alcohol, of which 1.5 billion are male drinkers and 900 million are female consumers.¹ However, alcohol consumption varies from one country to another.² In the same year, alcohol ranked seventh among risk factors for disability or death and was the leading risk factor for dangerous diseases in people aged 15–49 years.¹ Alcoholic liver disease (ALD) is the most widespread type of chronic liver disease globally. ALD can be classified as mild alcoholic liver disease, alcoholic hepatic steatosis, alcoholic hepatitis, alcoholic liver fibrosis, and alcoholic liver cirrhosis.³ In 2017, alcohol-related liver cirrhosis and cancer accounted for 1% of all deaths, and this is expected to increase in the future.^{1,4} The prevalence of ALD in Asia has increased significantly from 3.82% in 2000–2010 to 6.62% in 2011–2020, and ALD is expected to be the leading cause of chronic liver disease in Asia.⁵ China is currently the second largest alcohol consumer worldwide.^{6,7} Alcohol has also become the second leading cause of liver injury after viral hepatitis.⁸ A Chinese survey has shown that the drinking rate of adult residents in Liaoning Province is 35.30%, while the drinking rate, hazardous drinking rate, and harmful drinking rate of residents in Tongzhou District of Beijing were 45.29, 3.63, and 4.03% respectively.^{9,10}

The pathogenesis of ALD has not been fully elucidated. Current studies mainly evaluate the direct toxic effects of ethanol and its metabolites on the liver, oxidative stress, lipid metabolism, autophagy, genetics, gender, non-coding RNAs, and the gut microbiome. Early stages of ALD are not often accompanied by obvious symptoms. Traditional biochemical testing methods are less sensitive, and histopathological testing is invasive. Currently, no effective drugs are approved for the treatment of patients with ALD.¹¹ Although abstinence from alcohol is the basic treatment for all stages of ALD, patient compliance is poor. When the disease progresses to advanced stages, liver transplantation is the only effective treatment. End-stage liver disease not only causes physical and mental burdens to patients and their families but also causes great socioeconomic pressure.¹²

ALD has several unmet clinical needs and challenges, including noninvasive screening methods for disease diagnosis and prognostic assessment, development of therapeutic targets, and selection criteria for liver transplant patients. This review summarizes the relationship between gut dysbiosis and ALD to provide new strategies for the treatment of ALD.

Gut Microbiome

The gut microbiota contains numerous bacteria, archaea, fungi, and viruses. The number of bacteria in the gut is similar to that of human cells.^{13,14} Although the human microbiome has a genome of over 3 million genes, it is far more complex than the human genome.^{13,14} *Thickobacterium* and *Bacillus* are the two most dominant bacterial phyla in the gut, accounting for almost 90% of all bacteria; the former includes over 200 different genera (eg, *Lactobacillus*, *Bacillus*, *Clostridium*, and *Enterococcus*).¹⁵ Approximately 85% of bacteria (such as *Lactobacillus* and *Bifidobacterium*) are commensal organisms, while the rest (such as *Clostridium* and *Clostridium*) may be pathogenic.¹⁶ Studies have confirmed that normal gut microbiota not only participates in the digestion, decomposition, synthesis, and absorption of substances in the intestinal lumen, provides nutritional support to intestinal mucosal cells, and maintains normal physiological functions of the body but also resists the colonization and growth of foreign bacteria, activates the intestinal immune system, and constitutes an intestinal mucosal barrier together with intact intestinal mucosal epithelial cells.

The various biological functions of the liver are related to the normal gut microbiota. Bile acids secreted by the liver can inhibit pathogenic bacteria in the gut and regulate the balance of gut microbiota. Additionally, gut microbiota metabolites can participate in the metabolism of fats, proteins, sugars, vitamins, and hormones by the liver through enterohepatic circulation. All imbalances or alterations in the taxonomic composition and/or function of the gut microbiota are referred to as “dysbiosis”.¹⁷ Currently, various studies have demonstrated that changes in the gut microbiota are associated with diabetes,¹⁸ Alzheimer’s disease,¹⁹ obesity,²⁰ nephropathy,²¹ autism,²² polycystic ovary syndrome,²³ amyotrophic lateral sclerosis,²⁴ childhood malnutrition,²⁵ premature aging,²⁶ tumors,²⁷ inflammatory bowel disease,²⁸ irritable bowel syndrome,²⁹ and celiac disease.³⁰ Recent studies have shown that liver diseases are closely associated with gut dysbiosis, including chronic viral infections, non-alcoholic fatty liver disease, ALD, and hepatocellular carcinoma.^{31,32} The liver receives most of the blood from the gut through the portal vein and is, therefore, most exposed to potential bacterial products or metabolites such as lipopolysaccharides, peptidoglycan, short-chain fatty acids, and bile acids.³³ Bacterial products or metabolites can activate Kupffer cells, neutrophils, hepatocytes, sinusoidal endothelial cells, and stellate cells, promoting the release of inflammatory mediators (tumor necrosis factor alpha (TNF- α) and interleukin 6 (IL-6)), leading to liver injury and disease.³³

The Effect of Alcohol on the Number and Composition of Gut Microbiota

Diets with much fat or sugar can affect the composition of the gut microbiota.³⁴ Excessive alcohol consumption can lead to a predominance of pathogenic bacteria. Mutlu et al³⁵ demonstrated that the ileum and colon of rats gavaged with alcohol daily for 10 weeks showed dysbiosis. It was found that alcohol promoted the growth of Gram-negative bacteria such as the *Aspergillus* phylum in the gut, thereby reducing the number of anaerobic bacteria such as *Bifidobacterium*.³⁶ Compared with healthy controls, alcoholics had more Gram-negative anaerobic and aerobic bacteria in their jejunal fluid.³⁷ In the ALD group, the incidence of small intestinal bacterial overgrowth was almost three times higher than that in non-alcoholic controls.³⁸ Alcoholic hepatitis patients have an elevated proportion of cytolytic-positive fecal enterococci, which correlates with the severity and mortality of liver disease.³⁹ In addition to bacteria, the role of gut fungi has attracted considerable attention. Compared with non-alcoholic controls, alcoholics had a lower abundance and diversity of fungal species.^{40,41} *Candida albicans* and its exotoxin candidin were found to exacerbate ethanol-induced ALD, which is associated with increased mortality.⁴² Table 1 summarizes the studies that observed changes in the gut microbiota of ALD patients.

Table I Studies That Assessed Changes in the Gut Microbiota in ALD Patients

Study	Participant (n)	Methodology	Overgrown Microbes and Depleted Microbes in the ALD Group
ALD without cirrhosis			
Bode et al ³⁷	Alcoholic patients (27) vs Healthy control (13)	Aerobic and anaerobic bacterial culture of jejunal juice aspirated in the fasting state	↑Gram-negative anaerobic bacteria, ↑Endospore-forming rods, ↑Coliform microorganisms
Kirpich et al ⁴³	Alcoholic patients (66) vs Healthy control (24)	Quantitative culturing of stool samples	↓ <i>Bifidobacteria</i> , ↓ <i>Enterococci</i> , ↓ <i>Lactobacilli</i>
Mutlu et al ⁴⁴	Alcoholics with and without ALD (47) vs Healthy control (18)	PCR and multi-tag pyrosequencing of sigmoid mucosa biopsies	↓ <i>Bacteroidetes</i> , ↑ <i>Proteobacteria</i>
Leclercq et al ⁴⁵	Alcohol dependent patients (60) vs Healthy control (15)	16S rRNA and qPCR of stool samples	↑ <i>Bifidobacterium</i> spp., ↑ <i>Lactobacillus</i> spp., ↓ <i>Holdemania</i> spp.
Chu et al ⁴²	Healthy control (11) vs patients with AUD (42) vs patients with AH (91)	ITS sequencing and qPCR	↑ <i>Candida</i> spp. positive <i>Candida albicans</i>
Duan et al ³⁹	Healthy controls (14) vs patients with AUD (43) vs AH (75)	16S rRNA and PCR of stool samples	↑ <i>Enterococcus faecalis</i>
Alcoholic liver cirrhosis (ALC)			
Chen et al ⁴⁶	ALC (12) vs Hepatitis B cirrhosis (24) vs Healthy control (24)	16S rRNA and PCR of stool samples	↑ <i>Prevotellaceae</i> , ↓ <i>Bacteroidetes</i> , ↑ <i>Proteobacteria</i> , ↑ <i>Fusobacteria</i> , ↓ <i>Lachnospiraceae</i>
Bajaj et al ⁴⁷	ALC (43) and non-ALC (170) vs Healthy control (25)	16s rRNA of stool samples	↑ <i>Enterobacteriaceae</i> , ↑ <i>Halomonadaceae</i> , ↓ <i>Lachnospiraceae</i> , ↓ <i>Ruminococcaceae</i> , ↓ <i>Clostridiales</i> XIV
Tuomisto et al ⁴⁸	ALC (13) vs Alcoholics without cirrhosis (15) vs Non-alcoholic control (14)	PCR of stool samples	↑Gram-negative <i>Bacteroides</i> spp., ↑gram-negative <i>Enterobacteriaceae</i> , ↑gram-negative <i>Enterobacter</i> spp.
Dubinkina et al ⁴⁹	ALC (27) vs ADS (72)	"Shotgun" metagenome analysis of stool samples	↑Two genera (<i>Klebsiella</i> , <i>Lactococcus</i>), ↑four species (<i>K. pneumoniae</i> , <i>Lactobacillus salivarius</i> , <i>Citrobacter koseri</i> , <i>Lactococcus lactis</i> subsp. <i>cremoris</i>)
Bajaj et al ⁵⁰	ALC with active alcohol misuse (37) vs abstinent cirrhotic patients (68) vs Healthy control (34)	16s rRNA of duodenal, ileal, and colonic mucosal and fecal microbiota	↑ <i>Proteobacteria</i> (<i>Enterobacteriaceae</i>), ↓ <i>Lachnospiraceae</i> , ↓ <i>Bacteroidaceae</i> , ↓ <i>Prevotellaceae</i>
Yang et al ⁴¹	Healthy controls (8) vs ALD (AH (6), ALC (4))	ITS sequencing	↑ <i>Candida</i> , ↓ <i>Epicoccum</i> , ↓unclassified fungi, ↓ <i>Galactomyces</i> , ↓ <i>Debaryomyces</i>
Zhong et al ⁵¹	AFL patients (21) vs ALC patients (17) vs healthy controls (27)	16S rRNA of fecal microbiota	↓ <i>Opitutales</i> , ↓ <i>Helotiales</i> , ↓ <i>Ophiostomatales</i> , ↑ <i>Proteobacteria</i> , ↓ <i>Ruminococcaceae</i> , ↑ <i>Fusobacteria</i> , ↓ <i>Faecalibacterium</i> , ↑ <i>Fusobacteriaceae</i> , ↓ <i>Lachnospira</i> , ↑ <i>Enterobacteriaceae</i> , ↓ <i>Agathobacter</i> , ↑ <i>Burkholderiaceae</i> , ↓ <i>Ruminococcus</i> , ↑ <i>Fusobacterium</i> , ↑ <i>Escherichia-Shigella</i>

Abbreviations: ALD, Alcoholic liver disease; PCR, polymerase chain reaction; AUD, alcohol use disorder; ITS, Internal Transcribed Spacer; AH, Alcoholic Hepatitis; ALC, Alcoholic liver cirrhosis; ADS, alcohol dependence syndrome; AFL, alcoholic fatty liver.

Numerous animal experiments have confirmed the importance of gut microbiota and their metabolites in ALD. Visapää et al⁵² used ciprofloxacin in a rat model of ALD and found that the concentration of acetaldehyde in the gut lumen and portal blood was greatly reduced, confirming the role of the gut microbiota in alcohol metabolism. Llopis

et al⁵³ used humanized mice and found that the gut microbiota modified the susceptibility of individuals to ALD. In another study, fecal microbes from alcohol-tolerant mice were transplanted into alcohol-sensitive mice, which showed tolerance to alcohol.⁵⁴

However, the mechanisms by which ethanol alters microbial composition are not known. During alcohol consumption, alcohol is rapidly absorbed by diffusion, mainly in the upper gastrointestinal tract. The effects of alcohol on the distal small intestine and colon arise mainly from the circulation balance between the lumen of the gastrointestinal tract and the vascular space. Little is known about how relatively small concentrations of ethanol in the large intestine cause profound changes in the fecal microbiota.⁵⁵ Recent studies have shown that ethanol is not directly metabolized by the gut microbiota and that ethanol-related changes in the gut microbiota are a side effect of elevated acetate levels in humans.⁵⁶

Mechanisms of Alcohol-Induced Intestinal Damage Through Oxidative Stress, Leading to Leaky Gut and Endotoxemia

Typically, intestinal monolayers form tightly connected barriers with various proteins, forming intestinal tight junctions (TJ), adherens junctions (AJ), and bridging particles.⁵⁷ This barrier keeps microorganisms in the intestinal lumen away from blood flow, while also allowing luminal nutrients to enter the portal vein, thus ensuring a useful, nontoxic blood supply to the recipient organ.⁵⁸ Disruption of the gut barrier is an important factor in the pathogenesis of ALD, and the main mechanisms are related to alcohol and its metabolite acetaldehyde, impaired small bowel motility,⁵⁹ changes in gastric acid secretion,⁶⁰ dysfunction of gut mucosal epithelial cells,⁶¹ and increased lipopolysaccharides from enterobacteria. This paragraph will place special emphasis on the mechanisms and effects of gut ecological dysbiosis and alcohol exposure on the intestinal barrier, particularly on increased intestinal permeability. Alcohol and acetaldehyde can cause mucus erosion and ulceration, alter the glycosylation of the protective mucus layer, and increase intestinal permeability.⁶² Alcohol can be absorbed in the duodenum and jejunum.⁶³ After entering the monolayer by simple diffusion from the mucus layer, ethanol is either metabolized in the barrier or continues to diffuse into the circulation for delivery to various body sites.⁶³ Importantly, ADH is more highly expressed and active in both the small and large intestine compared to ALDH, suggesting a greater accumulation of reactive acetaldehyde than acetate in the monolayer following alcohol metabolism.⁶⁴ In addition, the presence of CYP2E1 and its elevated levels in the gastrointestinal tract after alcohol exposure due to low levels of ALDH2 expression in the gut^{65–67} lead to elevated levels of ROS and acetaldehyde, inducing inflammation and oxidative damage in the gut and liver.^{68,69} Alcohol and acetaldehyde activate the expression of toll-like receptor 4 (TLR4) on gut mucosal cell membranes and protein kinase C activity, thereby inhibiting the expression of cell tight junction-related connexins such as occludin and zona occludens-1.^{70,71} Studies have reported that alcohol can reduce the secretion of regenerating insulin lectin (REG3) in gut epithelial cells, leading to the parasitization of harmful bacteria in the intestinal mucosa.^{72–74} At the same time, the resulting dysbiosis of the intestinal ecology alters intestinal metabolism and the influx of harmful molecules such as the bacterial endotoxin LPS and peptidoglycan can induce intestinal inflammation and oxidative damage, further compromising the intestinal mucosal barrier.⁵⁸ In this process, various oxidative stress-mediated post-translational modifications (PTMs) play an important role in the integrity of the barrier, eg, the presence of acetaldehyde will result in the sustained phosphorylation of several paracellular proteins.⁷⁵ Continued damage to the barrier can lead to leaky gut, which subsequently leads to a localized immune response in the gut, increased levels of harmful gut-derived compounds (eg, lipopolysaccharides (LPS), peptidoglycans, exosomes, etc.) entering the circulation, resulting in endotoxemia, and more.

Interaction Among Gut Dysbiosis, Intestinal Barrier Dysfunction, and ALD

Alcohol may act as the initiator of liver damage. After the impairment of the intestinal mucosal barrier, translocation of harmful components such as bacteria, bacterial DNA, bacterial peptidoglycan, bacterial flagellin, and endotoxin further contributes to the development and progression of ALD.

Intrinsic immune cells of the liver, Kupffer cell, activation through specific receptors such as toll-like receptors (TLRs) mediated by LPS and oxidative stress driven by metabolism of the ethanol by hepatic CYP2E1 and from activated NOXs, increases large amounts of the pro-inflammatory effect of cytokines such as IL-1, IL-6, TNF α ,

leukotrienes, platelet-activating factor, oxygen radicals, nitric oxide, nitrous oxide, etc., causing multi-organ failure and secondary liver injury, cirrhosis, hepatocellular carcinoma, or liver failure.^{52,76–78} Eventually, sustained oxidative stress, LPS infiltration, and hepatocyte damage will lead to hepatic stellate cell activation, resulting in hepatic fibrosis and sustained liver injury.^{79–81} The pathophysiology of ALD is shown in Figure 1.

Intervention of the Gut Microbiota

Recently, there has been a surge in research on whether patients with ALD can be treated with probiotics, prebiotics, synbiotics, fecal microbial transplantation (FMT), bioengineered bacteria, gut-restricted FXR agonists and others by modulating gut microbiota via different mechanisms (Figure 1, Table 2).

Probiotics, Prebiotics, and Synbiotics

The World Health Organization defines probiotics as “living microorganisms that are beneficial to the health of the host”.⁶² *Lactobacillus* and *Bifidobacterium* can fight pathogenic bacteria by promoting the growth of the intestinal epithelium and modulating the host immune system.^{93–95} In 1994, it was found that feeding *Lactobacillus* strains that survived in the gastrointestinal tract reduced endotoxemia and liver damage in a rat model of ALD.⁹⁶ *Lactobacillus rhamnosus* (LGG) was the first probiotic to be tested in a rodent model of ALD and was effective in leaky gut and liver inflammation.^{97,98} *Muciniphila* can also reduce ethanol-induced liver injury.⁹⁹ While probiotics are safe in healthy individuals, caution is needed in certain patients, including premature infants, the elderly, and patients with low immune function, short bowel syndrome, central venous catheters, or heart diseases.¹⁰⁰ Clinical trials have shown that probiotics are associated with bacteremia, endocarditis, gastrointestinal toxicity, and the transfer of antibiotic resistance in the gastrointestinal flora.¹⁰⁰

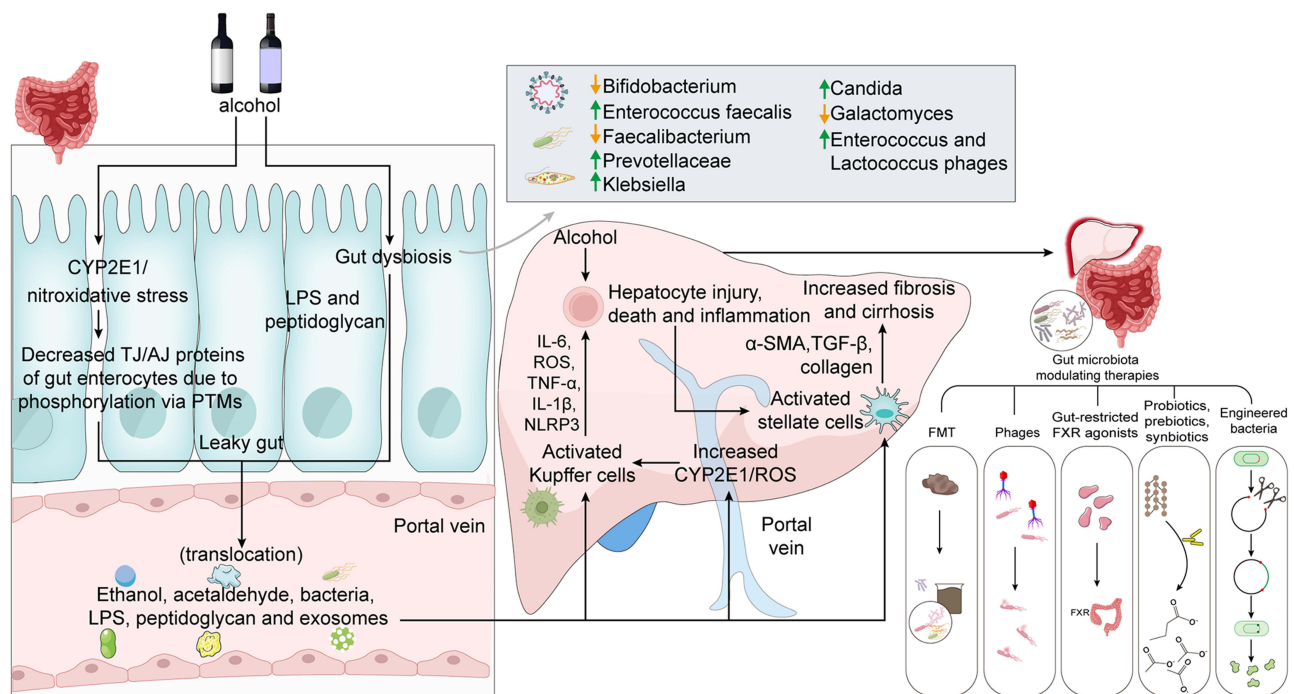


Figure 1 Pathogenesis and treatment of alcoholic liver disease. Alcohol consumption induces a weakened gut barrier and changes in the composition of the gut microbiota. The elevated CYP2E1 levels in the gastrointestinal tract after alcohol exposure lead to elevated levels of ROS and acetaldehyde, inducing intestinal inflammation and oxidative damage in the gut. The influx of harmful molecules such as the bacterial endotoxin LPS and peptidoglycan from gut dysbiosis can also induce inflammation and oxidative damage, further compromising the intestinal mucosal barrier. In this process, various oxidative stress-mediated post-translational modifications (PTMs) play an important role in the integrity of the barrier; eg, the presence of acetaldehyde will result in the sustained phosphorylation of several paracellular proteins (occludin and zona occludens-1), which can lead to intestinal leakage. Eventually, persistent oxidative stress, LPS infiltration and hepatocyte damage through the enterohepatic circulation will lead to hepatic stellate cell activation, hepatic fibrosis and hepatic cirrhosis.

Table 2 Clinical Trials Targeting the Intestinal Microbiome in ALD Disease

Type of Intervention	Cohort	Allocation Model	Intervention Details	Main Effects	Reference
Probiotics	66 patients with ALD (32 received probiotics with standard therapy, 34 received standard therapy only)	RCT	5 days probiotics of <i>Bifidobacterium bifidum</i> and <i>Lactobacillus plantarum</i> 8PA3	ALD patients had significantly lower AST and ALT levels after probiotic treatment	Kirpich et al ⁴³
	20 ALC patients were treated with probiotics and compared with 36 HCV-positive patients	Clinical trial	Probiotic VSL#3	VSL #3 resulted in lower levels of MDA and 4-HNE, improved cytokine levels (TNF- α , IL-6, and IL-10), and improved liver function in patients with ALC	Loguercio et al ⁸²
	89 patients with AH (44 received probiotics and 45 received the placebo)	RCT	The probiotics group received <i>L. rhamnosus</i> R0011/L and <i>helveticus</i> R0052 at 120 mg/day for 7 days	Probiotics ameliorated the Child-Pugh scores, declined the levels of ALT and γ -GGT, and changed the gut microbial composition	Gupta et al ⁸³
	12 patients with ALC vs 13 healthy controls vs 8 cirrhotic patients	Clinical trial	Patients with ALC received <i>Lactobacillus casei</i> Shirota	Probiotics restored neutrophil phagocytic capacity in ALC	Stadlbauer et al ⁸⁴
Prebiotics	50 AUD patients including early ALD	RCT	Prebiotic (inulin) versus placebo for 17 days	Prebiotics did not alleviate liver damage	Amadiou et al ⁸⁵
Synbiotics	10 patients with ALC and 10 patients with NASH received synbiotics	Clinical trial	Synbiotic treatment	Synbiotic improved ALT and GGT levels in both groups	Loguercio et al ⁸⁶
Antibiotics	50 patients with ALD	Clinical trial	24 patients received paromomycin sulfate 1 g three times daily over 3–4 weeks	Paromomycin did not improve ALD	Bode et al ⁸⁷
	13 patients with ALC and ascites	Clinical trial	4 weeks of treatment with rifaximin	Rifaximin increased the glomerular filtration rate and natriuresis while reducing levels of IL-6 and TNF- α	Kalambokis et al ⁸⁸
FMT	26 patients with SAH (8 patients received FMT and 18 were matched historical controls)	Clinical trial	Daily nasoduodenal infusion of 30 grams of donor stool for 7 days	Indices of liver disease severity improved significantly after FMT compared with the control group	Philips et al ⁸⁹
	51 patients with SAH (16 receiving FMT, others got current therapies only)	Clinical trial	Daily nasoduodenal infusion of 30 g of donor stool for 7 days in the FMT group	FMT improved survival beyond current therapies	Philips et al ⁹⁰
	1 patient with SAH	Case report	Daily nasoduodenal infusion of 30 g of donor stool for 7 days	FMT modulated microbiota beneficially, and improved clinical outcomes	Philips et al ⁹¹
	33 patients with SAH-ACLF (13 in the FMT arm and 20 in the SOC)	Clinical trial	30 grams of stool homogenized with 100 mL of normal saline administered a single time via the nasojejunal tube. SOC: Nutritional supplementation, supportive management.	FMT is safe, improves survival, and leads to improvement in clinical severity scores	Sharma et al ⁹²

Abbreviations: ALD, Alcoholic liver disease; ALC, Alcoholic liver cirrhosis; HCV, hepatitis virus C; RCT, randomized clinical trial; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; MDA, Malondialdehyde; 4-HNE, 4-Hydroxynonenal; TNF- α , tumor necrosis factor- α ; IL, Interleukin; AH, Alcoholic Hepatitis; γ -GGT, γ -Glutamyl Transferase; AUD, alcohol use disorder; NASH, Nonalcoholic Steatohepatitis; FMT, fecal microbial transplantation; ACLF, Acute-on-chronic failure; SOC, standard of care.

Prebiotics can promote the growth and activity of specific or minority microbiota in the host's gut.¹⁰¹ In alcohol-fed mice, pectin restored intestinal cupped cell function, increased the growth of anthropoid genera, and prevented liver injury.^{54,102}

Synbiotics, a combination of probiotics and prebiotics, are complex carbohydrates in the gastrointestinal tract that are not digested and metabolized by the pancreas and intestinal enzymes, and show advantages in the treatment of ALD.^{103–105}

Probiotics, prebiotics, and synbiotics may slow the progression of ALD; however, the dose, form, and regimen warrant further exploration. In addition, modulation of the gut microbiome may be transient, with recovery observed for only a few weeks to months. Nonetheless, longitudinal and long-term studies are still needed to determine better regimens.

FMT

Maintenance of normal gut flora is not the responsibility of one or two dominant gut bacteria.⁴³ FMT in healthy individuals may play a better role in ALD. FMT can be traced back to the Eastern Jin Dynasty when Ge Hong documented the use of fecal fluid to treat patients with food poisoning and diarrhea in “Post-Elbow Prescription”.¹⁰⁶ In 1958, Eiseman et al¹⁰⁷ completed effective treatment of FMT in patients with severe pseudomembranous colitis. The potential role of FMT in regulating the gut microbiota in ulcerative colitis and gastrointestinal and non-gastrointestinal diseases is strongly emphasized.^{108–111} Bajaj et al¹¹² used FMT and broad-spectrum antibiotics to treat recurrent hepatic encephalopathy. Xu et al¹¹³ treated a patient with cirrhosis and spontaneous bacterial peritonitis using FMT based on conventional therapy, which showed significant improvement in the general condition. A few studies have investigated the effect of FMT in ALD patients. Patients with severe AH who were not eligible for glucocorticoid therapy received FMT for 7 days and showed significant improvement in liver disease severity and survival.⁸⁹ It was reported that liver function, hepatic encephalopathy, and Model for End-Stage Liver Disease (MELD) scores of a 38-year-old patient with corticosteroid-refractory severe AH who consecutively received FMT for 1 week had improved.⁹¹ Washed microbiota transplantation (WMT) — a full process technique based on an intelligent fecal bacteria isolation system and strict quality control of the associated rinsing and transplantation routes — is a new stage in the development of FMT with greater safety. A prospective study showed that complete enteral nutrition combined with WMT improved the nutritional status and induced clinical remission in malnourished Crohn's disease patients.¹¹⁴ Currently, personalized and precise WMT that matches the patient and preserves autologous flora has been proposed.

Bioengineered Bacteria and Bacteriophages

Bioengineered bacteria that secrete beneficial metabolites is a new approach to precision medicine. *Lactobacillus* producing indole-3-acetic acid (IAA) reduced the severity of ALD.⁶¹ Precise editing of cytolytic fecal enterobacteria in fecal colonized sterile mice from patients with AH using phages reduced the severity of ethanol-induced liver in mice.³⁹ Bioengineered bacteria or phages have not been tested in clinical settings and large multicenter clinical trials are needed to determine their beneficial effects in humans.

Precision Medicine Approaches Targeting the Intestinal Microbiome

A recent study¹¹⁵ showed that blocking bile acid excretion into the intestine or silencing the bile acid receptor, farnesoid X receptor (FXR), promoted bacterial overgrowth in the small intestine and increased intestinal wall permeability and bacterial translocation, as well as systemic and local inflammation in the liver. Therapeutic targets should be devoted to the study of anti-lipopolysaccharide antibodies or TLR4 inhibitors to block liver damage from intestinal inflammatory factors in the future. Another new area of interest may be miR155 inhibitors. Intestinal permeability and endotoxin and inflammatory factor levels were alleviated in miR155-deficient ALD mice.¹¹⁶

Other Treatments for Modulation of the Gut Microbiota

Natural products and phytochemicals act through various pathways, such as modulating the intestinal microbiota, improving redox responses, and being anti-inflammatory. Supplementation with lychee pulp extract upregulated the

expression of intestinal tight junction proteins, antimicrobial proteins, and mucin in ALD mice and increased the relative abundance of *Lactobacillus* spp., *Acetobacter* spp., *Actinobacteria* phylum, and *Corynebacterium* spp. while decreasing serum endotoxin levels.¹¹⁷ Tang et al reported that oatmeal supplementation for 12 weeks maintained tight junctions and colonic mucosal integrity by preventing alcohol-induced leaky gut in rats.¹¹⁸ Natural products and related phytochemicals are ideal candidates against ALD, which warrants validation using clinical trials.

Modern studies have found that herbal medicines also exert therapeutic effects by adjusting the intestinal flora. Liu et al¹¹⁹ found that *Lycium barbarum* extract restored the growth of bifidobacteria and lactobacilli and adjusted the imbalance of the intestinal flora. Research has shown that the alcoholic extract of *Ocimum sanctum* can regulate the intestinal flora, protect the intestinal mucosa, and reduce the level of endotoxin leakage and the degree of alcoholic liver damage in rats.^{120,121}

Conclusions

Alcohol causes changes in the gut microbiota and weakens the gut barrier. Persistent oxidative stress, LPS infiltration and hepatocyte damage through the enterohepatic circulation will lead to hepatic stellate cell activation and hepatic fibrosis leading to the development and exacerbation of ALD. Probiotics, prebiotics, synbiotics, FMT, bioengineered bacteria, gut-restricted FXR agonists and others are promising therapeutic approaches that can alter gut microbiota composition to improve ALD. In the future, there will be new challenges to study the interactions between the genetics of individuals with ALD and their gut microbiome, to provide personalized interventions targeting the gut-liver axis, and to develop better techniques to measure microbial communities and metabolites in the body.

Main Concepts and Learning Points

- Alcohol causes changes in the gut microbiota and weakens the gut barrier.
- Persistent oxidative stress, LPS infiltration and hepatocyte damage through the enterohepatic circulation will lead to hepatic stellate cell activation and hepatic fibrosis leading to the development and exacerbation of ALD.
- Probiotics, prebiotics, synbiotics, FMT, bioengineered bacteria, gut-restricted FXR agonists and others can improve ALD.
- This will be a new challenge to study the interactions between the genetics of individuals with ALD and their gut microbiome, to provide personalized interventions targeting the gut-liver axis, and to develop better techniques to measure microbial communities and metabolites in the body.

Author Contributions

All authors made a significant contribution to the work reported in terms of the conception, study design, execution, acquisition of data, analysis and interpretation. They took part in drafting, revising or reviewing the article; gave final approval of the final manuscript to be published; agreed on the journal to which the article has been submitted; and agreed to be accountable for all aspects of the work.

Funding

This work was supported by Hainan Provincial Health Industry Research Project (22A200078), Hainan Provincial Postgraduate Innovation Research Project (Qhyb2022-133), the specific research fund of The Innovation Platform for Academicians of Hainan Province (YSPTZX202313), and Hainan Province Clinical Medical Center (No. 2021818).

Disclosure

The authors declare that they have no competing interests.

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