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Intestinal dysbiosis and probiotic use: its place in hepatic encephalopathy in cirrhosis

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Abstract

The gut microbiota, which plays an important role in health and disease processes, is affected by many disease processes, such as cirrhosis, and dysbiosis can lead to the development of numerous liver diseases, including complications of cirrhosis. In this disease group, the intestinal microbiota shifts towards dysbiosis for reasons such as endotoxemia, increased intestinal permeability, and decreased bile acid production. Although weak absorbable antibiotics and lactulose are among the treatment strategies in cirrhosis and its most common complication, hepatic encephalopathy (HE), this may not be the most appropriate treatment option for all patients, in view of its side-effects and high costs. Accordingly, it seems possible that probiotics could be used as an alternative treatment. The use of probiotics in these patient groups has a direct effect on the gut microbiota. Probiotics can also provide treatment with multiple effects through various mechanisms, such as lowering serum ammonia levels, reducing oxidative stress and reducing the intake of other toxins. This review was written to explain the intestinal dysbiosis associated with HE in cirrhotic patients, and the role of probiotics in treatment.

Keywords Cirrhosis, microbiota, probiotics, hepatic encephalopathy, intestinal dysbiosis

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Introduction

Cirrhosis is defined as the histological development of regenerative nodules surrounded by fibrous bands in response to chronic liver damage, leading to liver hypertension and end-stage liver disease [1]. Decompensation occurs in cirrhotic patients at an annual rate of 2-5%. Therefore, it is difficult to assess the true prevalence and incidence of cirrhosis in the general population, since many patients with cirrhosis are asymptomatic until decompensation occurs. It is estimated that the prevalence of chronic liver disease or cirrhosis in the world is 100 per 100,000 people (between 25 and 400); however, it has been reported to differ greatly by country and geographical region [2]. The main factor and interactive cofactors that cause the patient to develop cirrhosis can vary from patient to patient. The main causes can be listed as advanced age, male sex, chronic hepatitis B and C, obesity, metabolic syndrome, drugs, and alcohol. Since the etiology of cirrhosis cannot be determined precisely, it is characterized as cryptogenic [3] (Fig. 1).

Hepatic encephalopathy (HE), on the other hand, represents a spectrum of neuropsychiatric abnormalities in patients with impaired liver function after exclusion of other known brain diseases [4]. HE is generally classified as overt (OHE) and minimal (MHE). MHE refers to the group of patients without any clinically detectable neurological anomalies, but with abnormal neuropsychiatric or neurophysiological test results [5]. Oxidative stress, inflammation and neurosteroids have a synergistic role in the pathogenesis of HE. Via the creation of intestinal barrier dysfunction and systemic inflammation, intestinal flora and their byproducts—such as ammonia, indoles, oxindoles, and endotoxin—also play an important role in the pathogenesis of HE [6].

Differences between healthy individuals and patients with cirrhosis in terms of intestinal microbiota have been previously demonstrated [7]. Bajaj et al, one of the leading research groups in this area, introduced the concept of the cirrhosis/dysbiosis ratio (CDR), expressed as the "good vs. bad" taxon abundance ratio, to determine the degree of dysbiosis in cirrhosis and to make comparisons. They showed that the CDR was significantly lower in healthy controls than

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Conflict of Interest: None

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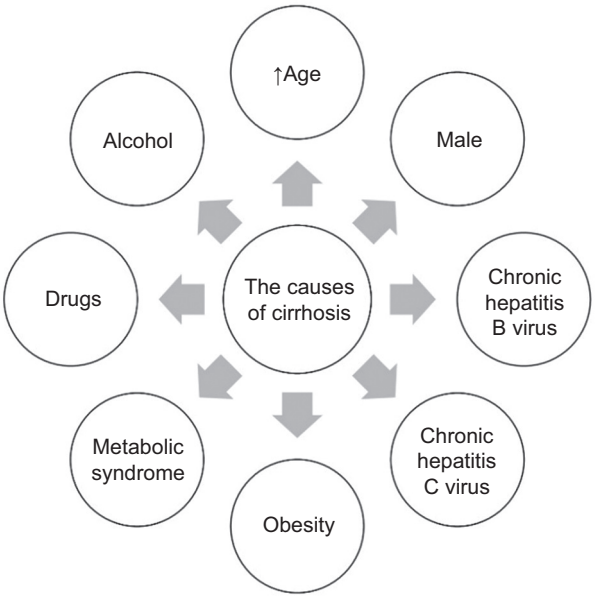

Figure 1 The causes of cirrhosis

in cirrhotic patients. At the same time, the stool microbiome profile changed as the severity of cirrhosis increased [8]. Poorly absorbed antibiotics and probiotics have been identified as potential therapeutic interventions for dysbiosis in patients with cirrhosis, and prebiotics such as oligosaccharides, and poorly digestible starch and dietary fiber, which are weak digestible dietary components that promote the growth of beneficial bacteria and suppression of harmful bacteria, were previously used for HE treatment [9]. Lactulose, a special sample synthetic disaccharide, has been shown to improve HE by reducing blood ammonia levels [10]. Probiotics, prebiotics, and synbiotics are functional food ingredients that modulate the gut microflora to improve host health and well-being [11]. Probiotics affect the intestinal flora in the intestinal system, causing a decrease in the pathogenesis of urease-producing bacteria and thus reducing the production of toxic substances, especially ammonia, derived from the intestinal system [9]. Probiotics have been shown to improve MHE and prevent OHE by reducing blood ammonia levels [9]. Probiotics can also reduce oxidative stress by showing antioxidant activity, thereby providing positive modulation of the gut microbiota [12]. Thus, probiotics can contribute to HE patients' health by modulating the intestinal microbiota [13]. The purpose of this review is to explain the possible effects of probiotics in cirrhosis and HE via modulating gut microbiota.

Intestinal dysbiosis in cirrhosis

Human intestinal microbiota play a vital role in maintaining intestinal homeostasis, essential for general health and wellbeing [14]. The human microbiome contains 10-100 trillion bacteria, including 500-1500 different bacterial species developed by humans [15], and can be considered as an organ of similar size to the liver [15]. The intestinal microflora differs significantly among individuals. Host genotype, age, health status, diet and exposure to antibiotics are critical parameters regulating the configuration of the intestinal microflora [16]. However, approximately 90% of the intestinal microbiota in adulthood consist of *Firmicutes* (mostly Gram-positive bacteria) and *Bacteroidetes* (mostly Gram-negative bacteria) [17,18]. The *Firmicutes* include more than 200 species, such as *Lactobacillus, Bacillus, Clostridium, Enterococcus, Ruminicoccus* and *Streptococci*, while the *Clostridium genus* represents 95% of *Firmicutes phyla*. *Bacteroidetes*, on the other hand, consist of dominant species such as *Bacteroides, Prevotella, and Saprospirae* [17,19]. Recent findings underline that changing intestinal microbiota should be seen as a contributing factor of some diseases [15].

Major complications in liver cirrhosis, such as HE, spontaneous bacterial peritonitis and esophageal variceal bleeding, are characterized by serious changes in the intestinal microflora [16]. Dysbiosis and dysfunctions in the intestinal epithelial barrier are supported by liver fibrogenesis with multiple physiopathological procedures. Reduced portal vein blood flow and intestinal vascular obstruction are characteristic anomalies of cirrhotic patients that may lead to increased intestinal permeability [16]. In one study, intestinal permeability was evaluated with the lactulose/mannitol test in 18 cirrhotic and 15 healthy individuals; the cirrhotic patients were found to have greater intestinal permeability [20]. In another study, a lactulose-L-rhamnose intestinal permeability test was performed on 35 patients with liver cirrhosis and 6 healthy individuals. The mean lactulose-L-rhamnose excretion rates were found to be 0.124 and 0.049 in patients with liver cirrhosis and in the control group, respectively, while intestinal permeability was also found to be high in cirrhosis [21]. Furthermore, decreased bile acid production and low intestinal motility are associated with impaired liver function and significant changes in intestinal bacterial communities [22]. Summary information about intestinal dysbiosis in cirrhosis is presented in Fig. 2.

Apart from the above, dysbiosis in the intestinal microbiota changes the circumference of the colonic lumen, as well as its pH. This causes increased ammonia production from the intestinal microbiota and increased ammonia transition from the colon lumen to the blood [14]. Studies have shown differences in gut microbiota between healthy individuals and patients with cirrhosis [7,23,24]. The prevalence of potentially pathogenic bacteria, such as *Enterobacteriaceae* and *Streptococcaceae*, may affect the prognosis, together with the reduction of beneficial populations such as *Lachnospiraceae* in patients with cirrhosis [23]. However, the microflora imbalance is also associated with cirrhosis severity [25]. The main causes of this imbalance are thought to be hypochlorhydria, decreased IgA secretion and intestinal motility, and malnutrition [26]. The change in intestinal microbiota in patients with cirrhosis is summarized in Fig. 3 on the basis of phyla and family [7,23,27-32].

Treatment of cirrhosis and HE

The traditional treatment for HE is antibiotics or non-absorbed polysaccharides [33]. Among the preferred non-

absorbable disaccharides are lactulose (β-galactosidofructose) and lactitol (β-galactosidosorbitol) [34]. Fecal *Bifidobacteria* and *Lactobacilli*, thought to have health-promoting properties, can be increased by the addition of lactulose to the diet. Ten grams of lactulose per day increases fecal bifidobacterial numbers [35], and lactulose can be considered a prebiotic [35, 36]. With its prebiotic potential, lactulose promotes the growth of probiotic bacteria, such as *Bifidobacterium* species, known to have health-promoting effects [37]. However, in a systematic review it was shown that there is insufficient evidence to support or refute the use of non-absorbable disaccharides for HE, and antibiotics are superior to non-absorbable disaccharides in the treatment of HE. Moreover, this difference was not clinically significant. In this review, it was concluded that the available evidence is not sufficient to support or reject the use of non-absorbable disaccharides for HE treatment [38]. In a more recent systematic review, the efficacy and safety of non-absorbable disaccharides were evaluated for the prevention and treatment of HE in patients with cirrhosis, but insufficient evidence was found to recommend their use in clinical practice. However, they have been reported to show a significant beneficial treatment effect on both MHE and OHE [39].

Antibiotics are preferred for patients who respond poorly to disaccharides or do not show diarrhea or acidification in the stool. In the case of chronic antibiotic use, carefully conducted kidney, neurological and/or autological monitoring is required [40]. The most commonly used antibiotics are neomycin and rifaximin. These antibiotics reduce ammonia obtained from the activity of organisms containing colonic urease, and from mediator metabolism in the intestine [41]. Neomycin causes a decrease in mucosal glutaminase activity, thereby reducing the ability of the mucosa to consume glutamine and produce ammonia [42]. Rifaximin can be used as a first-line antibiotic in the treatment of HE, especially in patients intolerant to neomycin or renal dysfunction [41].

The use of lactulose or antibiotics for the prevention of HE is not a routine practice [43]. It has been stated that synbiotic (probiotics and fermented fiber) treatment is associated with a significant reduction in endotoxemia, and this treatment may be an alternative to lactose in the treatment of HE in patients with cirrhosis [44]. It is stated that treatment with probiotics, synbiotics or fermented fiber may play an alternative role in the treatment of MHE, especially in patients with cirrhosis [33, 43-45].

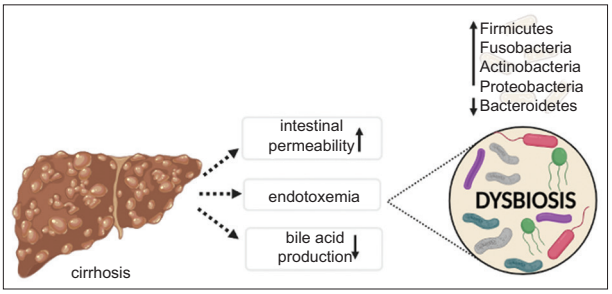

Figure 2 Intestinal dysbiosis in cirrhosis (Created by BioRender.com)

The role of probiotics in treatment

Probiotics are defined as living microorganisms that affect the host beneficially by improving the intestinal microbial balance [46]. The balance between species in the microbiota community is called eubiosis, while a disruption of eubiosis, which causes infectious and non-communicable diseases, is called dysbiosis [47]. Probiotics, on the other hand, can reverse intestinal flora dysbiosis. Probiotics can inhibit the colonization of pathogenic bacteria in the intestine, helping the host establish a healthy intestinal mucosa protective layer [48]. For example, short-chain fatty acids (SCFAs), whose production is increased by probiotics [49], can activate specific G-protein-coupled receptors (e.g., GPR41/43) expressed on enteroendocrine L-cells, thereby enhancing the energy metabolism of different intestinal peptides such as glucagon-like peptide -1 and -2, and are involved in the regulation of intestinal barrier function.

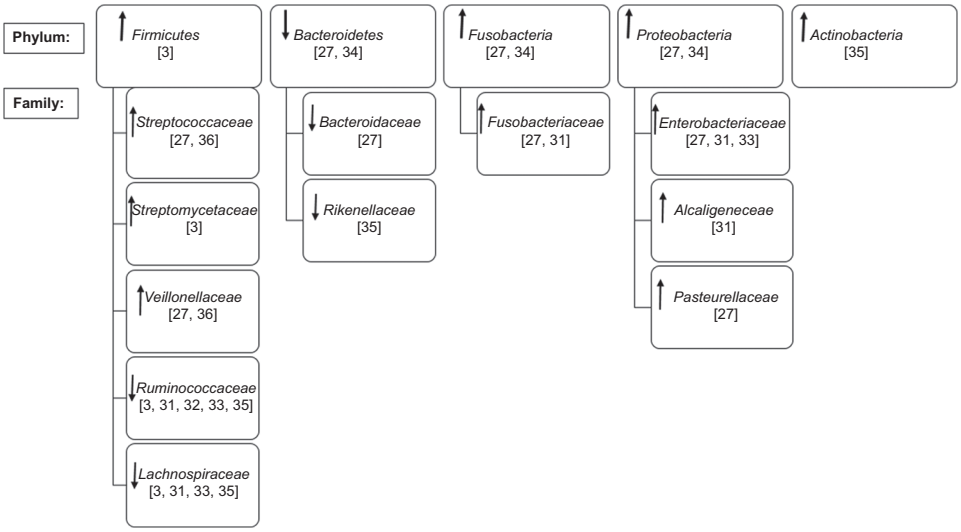

Figure 3 The change in intestinal microbiota in patients with cirrhosis

SCFAs can also modulate gene transcription through the inhibition of histone deacetylase activity [50]. However, there are many probiotic strains available and it is extremely important to know which strain has been shown to be effective in studies before using it in an intervention [51].

The beneficial effects of probiotic administration on different variables related to MHE have been confirmed in various studies [14,52-54]. Some studies on the possible health effects of probiotic use in HE are summarized in Table 1. Bajaj *et al* randomized cirrhotic patients with MHE to *Lactobacillus* GG (LGG) (n=14) or placebo (n=16). Among the patients followed for 8 weeks, only the LGG group showed a decrease in endotoxemia and tumor necrosis factor (TNF)-α, a change in the microbiome, while there was no difference in cognition [52]. Bajaj *et al* also demonstrated a significant amount of MHE reversal after treating cirrhotic patients with probiotic yogurt [53]. In another study, 67 patients with hepatitis B virus (HBV)-induced cirrhosis without OHE were randomized to receive probiotics (n=30) or no probiotics (n=37). In that study, the probiotic mixture containing *Clostridium*

Table 1 The role of probiotics in the treatment of hepatic encephalopathy

Study design	Intervention	Result	Ref.
Randomized controlled trial	For MHE treatment in patients with cirrhosis of the liver caused by the hepatitis B virus, the participants were divided into 2 groups: those who received probiotics (*Clostridium butyricum* combined with *Bifidobacterium infantis*; n=30; dose 1500 mg t.i.d.] and a control group (n=37), and they were followed for 3 months.	The dominant bacteria (*Clostridium cluster I* and *Bifidobacterium*) were significantly enriched in the probiotic-treated group, while *Enterococcus* and *Enterobacteriaceae* were decreased. Probiotic treatment also resulted in a significant reduction in venous ammonia, and significant improvement in the intestinal mucosal barrier and in patients' cognition	[14]
Randomized controlled trial	Nonalcoholic MHE cirrhotic patients were divided into 2 groups, consuming probiotic yogurt (n=17) or controls (n=8), and were followed for 60 days.	While MHE was reversed in 71% of the group consuming probiotic yogurt, this rate was 0% in the other group. In addition, while it was not seen in anyone in the group receiving probiotic supplements, 25% of those in the group not receiving treatment developed OHE. The treatment-receiving group also showed significant improvement in the NCT-A, BDT, and numeral symbol test compared to those who did not receive treatment	[53]
Randomized controlled trial	Patients with cirrhosis who survived an episode of HE in the previous month were divided into 2 groups as those who received daily probiotic (VSL#3) supplementation (n=66) and the control group (n=64), and were followed for 6 months.	There was a tendency for a decrease in the development of immediate HE in patients receiving probiotics (34.8% in the probiotic group versus 51.6% in the placebo group). Child-Turcotte-Pugh and end-stage liver disease scores were significantly improved in the probiotic-treated group, and the risk of hospitalization was significantly reduced	[56]
Randomized controlled trial	Cirrhotic patients with MHE were divided into probiotic (VSL#3) supplementation (n=42) and control (n=39) groups, and they were followed for 3 months.	There were significant improvements inarterial ammonia levels, psychometric tests, critical flicker frequency, SIBO, and delayed OCTT in the probiotic supplement group, and improvement in SIBO and OCTT was associated with reversal of MHE	[58]
Double-blind placebo-controlled study	Patients with cirrhosis of the liver and at least one major complication of cirrhosis were divided into 2 groups, those taking probiotic (*Lactobacillus acidophilus, Lactobacillus bulgaricus, Bifidobacterium lactis,* and *Streptococcus thermophiles*) supplements (n=18) and a placebo group (n=18), and followed for 6 months.	After the first month of treatment, it was determined that probiotic supplementation achieved a significant reduction in ammonia levels in patients with ammonia levels above normal at the beginning. Apart from this, no other clinical or laboratory effects were reported	[59]
Randomized controlled trial	Cirrhotic patients without OHE lactulose (n=35; dose 30-60 ml/day], probiotics (n=35; dose 1 capsule t.i.d.: each capsule contained *Streptococcus faecalis* 60 million, *Clostridium butyricum* 4 million, *Bacillus mesentricus* 2 million, lactic acid bacillus 100 million) and lactulose + probiotics (n=35), and they were followed for 1 month.	Significant improvement in abnormal psychometry tests and venous ammonia levels was seen after treatment in all 3 treatment groups. It was concluded that lactulose, probiotic or combination therapy would be equally effective in the treatment of MHE	[45]
Animal trial	Rats were divided into probiotics—*Lactobacillus plantarum UBLP40* (107 CFU/day, 14 days) and *Bacillus clausii UBBC07* (107 CFU/day, 14 days) combination—and standard drug-lactulose (2.5 mL/kg in 3 divided doses, 14 days) groups.	Probiotic treatment achieved improvement in liver function parameters (reduction in AST, ALT, ALP, and ammonia levels) and oxidative stress parameters in rats. Combination therapy has been reported to be effective in the preclinical phase of acute hepatic encephalopathy	[54]

AST, aspartate transaminase; ALT, alanine transaminase; ALP, alkaline phosphatase; OCTT, orocecal transit time, HE, hepatic encephalopathy; MHE, minimal HE; OHE, overt HE; SIBO, small intestinal bacterial overgrowth; NCT-A, number association test-A; BDT, block design test

butyricum (*C. butyricum*, CGMCC0313-1) combined with *Bifidobacterium infantis* (*B. infantis*, CGMCC0313-2) was administered to patients 1500 mg t.i.d. for 3 months. The investigators found that probiotic treatment containing *C. butyricum* and *B. infantis* contributed to improvement in the intestinal mucosal barrier, improvement in cognitive function, and a decrease in ammonia levels [14]. In a study of rats by Shahgond *et al*, groups receiving a combination of probiotics *Lactobacillus plantarum* UBLP40 (107 CFU/day, 14 days) and *Bacillus clausii* UBBC07 (107 CFU/day, 14 days), or standard drug-lactulose (2.5 mL/kg in 3 divided doses, 14 days) were created. Not only did the probiotic treatment improve liver function tests compared to the other group, but also an improvement in oxidative stress parameters was observed. Combination therapy has been reported to be effective in the preclinical acute phase of HE [54].

The most commonly used probiotics today are *Bifidobacteria*, lactic acid bacteria, *Propionibacteria*, yeast (*Saccharomyces boulardii*), and Gram-negative *Escherichia coli* strain *Nissle* 1917 [55]. VSL#3 is a probiotic that comes to the fore specifically in the treatment of cirrhosis, and consists of 8 strains: 4 *Lactobacillus* spp. (*L. paracasei DSM 24733, L. plantarum DSM 24730, L. acidophilus DSM 24735*, and *L. delbrueckii subspecies bulgaricus DSM 24734*), 3 *Bifidobacterium* spp. (*B. longum DSM 24736, B. infantis DSM 24737* and *B. breve DSM 24732*), and *Streptococcus thermophilus DSM 24731*] [56]. In a double-blind study of patients with cirrhosis in India, patients were randomized to probiotic (VSL#3) (n=66) or placebo (n=64) daily. After 6 months of treatment, patients with cirrhosis who received daily VSL#3 had a significantly lower risk of hospitalization for HE. Moreover, VSL#3 also significantly reduced the pattern of Child-Turcotte-Pugh and end-stage liver disease scores, but no improvement was observed in the placebo group [56]. Currently, VSL#3 is recommended for liver health, including MHE, in the Canadian Probiotic Guidelines [57].

The use of probiotics in patients with liver cirrhosis should aim to maintain the natural biological balance of the intestinal system and modulate the growth of other bacterial groups to maintain the natural biological balance of the intestinal system, stimulate host resistance to infection, reduce the negative relationship between portal hypertension and both local and systemic hemodynamic changes, and finally prevent and/or improve HE [60,61]. The mechanisms to explain the possible beneficial effects of probiotics in the treatment of HE are summarized in Fig. 4 [62].

First, the selected appropriate, non-pathogenic bacteria can directly reduce ammonia production and absorption by changing the composition of the intestinal flora. This can be achieved by changes in intestinal metabolism, such as pH, intestinal permeability, and the nutritional status of the intestinal epithelium. Urease is a critical enzyme in bacterial luminal metabolism, resulting in ammonia production and pH increase [62]. As a result of increased urease activity, ammonia production may increase via intestinal hydrolysis of urea and absorption of nitrogenous products [13,62]. Probiotics can alter this process by increasing competitive inhibition and luminal bacteria concentration with urease-producing bacteria [62]. Reduction in serum ammonia is an important therapeutic goal, since ammonia level is closely related to the severity of HE, as well as the function of other organs, and is an independent risk factor for mortality [63]. In a healthy individual, ammonia is detoxified by glutamine formation from glutamate in the liver and brain, whereas in HE hepatocellular insufficiency and/or portosystemic shunt weaken the liver's ability to detoxify ammonia, so that arterial ammonia levels increase [64]. The intestines of cirrhotic patients contain more urease-active bacteria than those of healthy individuals, and this leads to increased urea hydrolysis and absorption of nitrogen products directly from the intestine [62]. In particular, *Klebsiella* and *Proteus*, 2 types of urease-producing bacteria, play an important role in increased ammonia production and HE development [16]. Small bowel dysmotility, frequently seen in cirrhosis, worsens the existing problem [62]. In addition, it has been suggested that probiotics can increase intestinal epithelial viability by providing essential nutrients (such as butyrate) that inhibit apoptosis of lumen epithelial cells [65]. Therefore, there are several possible mechanisms by which probiotics can reduce the absorption of ammonia into the portal blood flow. A recent meta-analysis reported that probiotics can reduce serum ammonia levels and prevent the development of OHE in patients with liver cirrhosis [66].

Another effect of ammonia is associated with altering mitochondrial function and inducing oxidative stress [67]. In cirrhosis and HE, proinflammatory cytokines such as TNF-α, interleukin (IL)-1β, and IL-6 are modulated with ammonia and show synergistic effects [13]. In patients with cirrhosis, an increase in pro-oxidant markers, such as malondialdehyde, and a decrease in antioxidant factors, such as superoxide dismutase, can be seen together with oxidative stress [68]. Therefore,

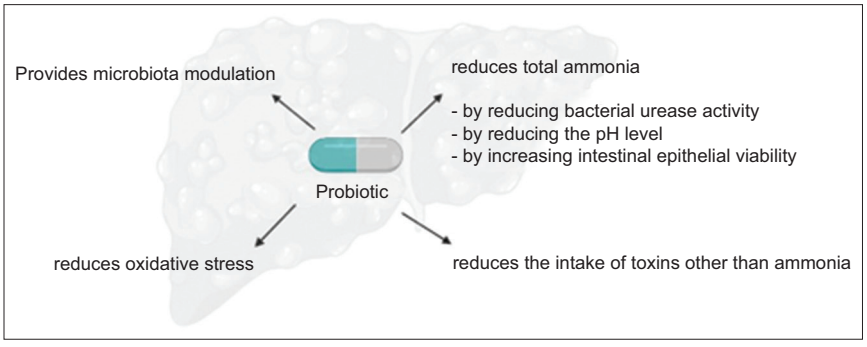

Figure 4 The beneficial effects of probiotics in the treatment of hepatic encephalopathy (Created by BioRender.com)

inflammatory signals derived from the gut negatively affect the hepatocyte itself, and treatment of the gut flora can limit or reverse this damage [62]. The use of antioxidants can be effective for the treatment of increased oxidative stress in liver diseases [69]. Probiotics can also be an alternative at this point. Probiotics can play a protective role against oxidative stress by reducing free radicals and increasing the number of antioxidant enzymes in the body [70]. *Bacillus* SC06 or SC08 was administered orally for 24 days in rats in which oxidative stress had been induced by diquat intraperitoneally. After probiotic supplementation, a decrease in *Bacillus*, especially SC06, alanine transaminase, aspartate transaminase, alkaline phosphatase and lactate dehydrogenase levels, a decrease in mitochondrial dysfunction, and alleviated damaged liver structure were observed. These findings suggest that probiotics can alleviate oxidative stress-induced liver damage [71].

Probiotics also can block the intake of toxins other than ammonia, which have not yet been identified. This effect is supported by research in patients with end-stage renal failure receiving hemodialysis therapy [62]. The mental state of these patients often changes because of intestinal toxins such as phenol, which is not removed by dialysis. In people with end-stage kidney disease, lactic acid bacteria have shown efficacy in altering the intestinal flora and thus reducing such toxins [72]. As noted above, part of the lactulose effect may be due to its action as a "prebiotic" that promotes the growth of the same lactic acid bacteria used in probiotics [62].

In a review that examined various studies evaluating the roles of probiotics, prebiotics, and synbiotics in MHE, it was stated that most of the evidence supports the use of probiotics for MHE. It has also been stated that the effect may be associated with modulating changes in the gut microbiota, with an increase in non-urease-producing bacteria such as lactobacilli and a decrease in urease producers such as *Escherichia coli* and *Staphylococcus aureus* [33]. *Clostridium cluster I* and *Bifidobacterium* were significantly enriched, while *Enterococcus* and *Enterobacteriaceae* were significantly decreased after 3 months of probiotic supplementation—*C. butyricum* (CGMCC0313-1) combined with *B. infantis* (CGMCC0313-2)—in patients with HBV-induced cirrhosis but without OHE [14]. In the literature, the type and amount of probiotics used in the studies varied, but the number of probiotics used was found to be $10^{6,7,8,9}$ [Q: Please explain the meaning of this] [14,73,74].

The main fermentation products of prebiotic metabolism are SCFAs, including acetate, propionate and butyrate. Butyrate in particular is considered a useful metabolite, associated with many biological functions in the gut. One of the important functions of butyrate is the epigenetic regulation of gene expression by inhibition of histone deacetylase [75]. Synbiotics, a combination of probiotics and prebiotics, have also been shown to be important in the treatment of MHE by reducing blood ammonia levels and increasing the non-urease-producing *Lactobacillus* in the intestinal flora [9,44]. In the results of a meta-analysis, treatment with probiotics and synbiotics has been shown to cause a significantly greater improvement in HE compared to placebo or lactulose [76].

Concluding remarks

HE is a serious and common complication of liver disease. Current treatment strategies, including lactulose and poorly absorbable antibiotics, may not be the most appropriate treatment for all patients with liver disease in view of their side effects and cost. Therefore, probiotics are seen as an alternative treatment for HE. The use of probiotics is associated with significant improvement, especially in MHE. Since probiotics are a suitable, safe, natural and well-tolerated treatment option for long-term use, probiotic therapy is considered to be ideal for HE. However, although it has been shown that probiotics can be an effective treatment in HE, more clinical studies are needed to better define factors such as the type and amount of strain to be administered and the duration of intervention. We believe that this would allow for more accurate recommendations to be made.

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