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MINIREVIEWS

Probiotics as a complementary therapeutic approach in nonalcoholic fatty liver disease

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is currently recognized as one of the most common causes of chronic liver disease. It involves a spectrum of conditions that include pure steatosis without inflammation, steatohepatitis, fibrosis and cirrhosis. The key factor in the pathophysiology of NAFLD is insulin resistance that determines lipid accumulation in the hepatocytes and, thus, oxidative stress, which is followed by inflammatory response. However, NAFLD pathogenesis is still largely unknown and has been extensively investigated. Although life style modification with the aim of losing weight has been advocated to treat this disorder, its effectiveness is limited; additionally, there is no specific pharmacologic treatment until nowadays. Recent evidence suggests that the gut microbiota may play a role in the development of insulin resistance, hepatic steatosis, necroinflammation and fibrosis. Differences in gut microbiota between NAFLD patients and lean individuals as well as presence of small intestinal bacterial overgrowth in NAFLD subjects have been demonstrated. Furthermore, some data indicate that the immunoregulatory effects of probiotics may be beneficial in NAFLD treatment as they modulate the intestinal microbiota; improve epithelial barrier function and strengthen the intestinal wall decreasing its permeability; reduce bacterial translocation and endotoxemia; improve intestinal inflammation; and reduce oxidative and inflammatory liver damage. In this article, we review the clinical trials on the use of probiotics in the treatment of NAFLD and discuss the effects of these agents and their efficacy as an emerging therapeutic resource to treat NAFLD patients.

Key words: Fatty liver; Nonalcoholic fatty liver disease; Nonalcoholic steatohepatitis; Probiotic; Intestinal microbiota

Core tip: Nonalcoholic fatty liver disease (NAFLD) is an important cause of chronic liver disease. Its pathogenesis is largely unknown, and until nowadays

there is no effective treatment for this disorder. Recent evidence from animal and human studies suggests that gut microbiota may play a role in the development of NAFLD. Furthermore, some data indicate that probiotics may be beneficial in NAFLD treatment. In this context, we conducted a systematic review on the use of probiotics in the treatment of NAFLD and discuss the effects of these agents and their efficacy as an emerging therapeutic resource to treat NAFLD patients.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) has been recognized as the most common chronic liver disease in the western world[1]. NAFLD defines a spectrum of liver disosrders that can progress from simple steatosis (nonalcoholic fat liver) to nonalcoholic steatohepatitis (NASH) with or without hepatic fibrosis/cirrhosis. This condition is also a risk factor for hepatocellular carcinoma[2]. NAFLD is a multifactorial disease with a complex pathogenesis involving mechanisms not fully elucidated. It is well known that this condition is strongly associated with insulin resistance (IR), visceral obesity and dyslipidemia. NAFLD prevalence is increasing rapidly due to the current epidemics of obesity and type 2 diabetes[1,2]. There are several clinical trials[3-8] on pharmacologic treatment of NAFLD/NASH; however, no specific pharmacologic treatment has been established until nowadays[1].

Although NAFLD pathogenesis has not been fully elucidated, it has been proposed the "two-hit" theory to explain its development. The "first hit" involves hepatic lipid accumulation due to IR[9]; and, the "second hit" is characterized by oxidative stress followed by lipid peroxidation, secretion of proinflammatory cytokines [*e.g.*, tumor necrosis factor (TNF)-α] and adipokines, and mitochondrial dysfunction, which lead to the progression from simple steatosis to NASH[9,10]. Currently, some authors have considered NAFLD a pathogenetically "multiple-hit" disease[11].

Some evidence suggests that the gut-liver axis could be a point of attack to treat NAFLD[12-19]. Gut microbiota consists of about 10^4 microorganisms that live in a symbiotic relationship with the host, and is influenced by several factors such as diet, age, antibiotic therapy, hygienic habit and infection. These intestinal bacteria produce endotoxin that reach the liver and is phagocyted by the Kupffer cells. Therefore, the liver is constantly exposed to gut-derived lipopolysaccharide (LPS), lipopeptides, unmethylated DNA and double-stranded RNA, which may evoke intense inflammatory reaction[20] that contributes to the progression from steatosis to NASH.

Probiotics are live microbes able to modulate the intestinal microflora[20]. This article aimed to review the clinical trials on the use of probiotics in the treatment of NAFLD and to discuss the effects of these agents and their efficacy as an emerging therapeutic resource to treat NAFLD patients.

LITERATURE RESEARCH

The systematic review was conducted in the PubMed and Medline databases using the following terms: "(NAFLD or NASH or "nonalcoholic steatohepatitis" or "nonalcoholic fatty liver disease" or "fatty liver") and (probiotic or prebiotic or synbiotic or microbiota)". From the 305 articles, we restricted the search to clinical trials performed in humans and written in English. Five clinical trials were initially considered for the present review article. Then, we also searched the reference lists of each selected study by hand, and at the end, we included a total of 8 clinical trials and 1 meta-analysis.

Thus, there were selected all controlled clinical trials in which probiotics or synbiotics were used to treat NAFLD or NASH diagnosed by imaging methods and/or histological evaluation, regardless of the age, sex and ethnic origin of the participants. The trials in which the hepatic function and/or the metabolic and inflammatory parameters were not evaluated before and after the use of the probiotic/synbiotic were excluded from this review.

CLINICAL EVIDENCE OF PROBIOTIC EFFICACY IN THE TREATMENT OF NAFLD IN HUMANS

Loguercio *et al*[12] provided the first evidence that treatment with probiotics could improve some parameters of liver damage and function in patients with different types of chronic liver diseases including 10 biopsy-proven NAFLD males. The patients were given a mixture containing different species of bacteria [*Lactobacillus acidophilus* (*L. acidophilus*), *Bifidobacterium bifidum* (*B. bifidum*), *Lactobacillus rhamnosus* (*L. rhamnosus*), *Lactobacillus plantarum* (*L. plantarum*), *Lactobacillus salivarius* (*L. salivarius*), *Lactobacillus bulgaricus* (*L. bulgaricus*), *Lactobacillus casei* (*L. casei*), *Bifidobacterium lactis* (*B. lactis*) and *Bifidobacterium breve* (*B. breve*)] associated with fructooligosaccharides (FOS), and vitamins (B6, B2, B9, B12, D3, C, K) and minerals (zinc and iron) supplementation during 2 mo, followed by a 1 month wash out period. Treatment was followed by a reduction in the serum levels of the aminotransferases, markers of oxidative stress (malondialdehyde and 4-hydroxynonenal) and TNF-α in the NAFLD patients.

Three years late, the same group published

another study in a larger population (22 biopsy-proven NAFLD patients) which received the probiotic VSL≠3, a mixture containing 450 billion bacteria of different species (*Streptococcus thermophilus, B. breve, B. longum, B. infantis, L. acidophilus, L. plantarum, L. casei* and *L. bulgaricus*) at a dose of 2 sachets twice daily during 3 mo. The results corroborate the findings of the previous study as the authors observed improvement in the serum levels of the lipid peroxidation markers malondialdehyde and 4-hydroxynonenal, and also S-nitrosothiols[13].

In 2008, another clinical trial using VSL≠3 in NAFLD patients was published. Four patients received one sachet of the probiotic compound daily during 4 mo. The outcome was chiefly evaluated by the investigation of liver fat by proton magnetic resonance spectroscopy (MRS), which was performed at baseline, after 4 mo of treatment, and at month 7; *i.e.*, after a 3-mo washout. A comprehensive metabolic panel, protime, glycosylated hemoglobin, TNF-α, interleukin (IL)-6, IL-1β and interferon-γ were also evaluated in blood, monthly. All subjects experienced a significant increase in steatosis, and 3 of the 4 patients presented a meaningful change with more than 3% increase in liver fat. After washout, 3 of the 4 participants presented values of liver fat similar to those observed at baseline. There were no significant changes in any of the blood parameters[14].

A double-blind, placebo-controlled pilot study, including 20 obese children (mean age 10.7 ± 2.1 years) with persisting hypertransaminasemia and ultrasonographic (US) bright liver who were non-compliant with lifestyle interventions, was conducted with the aim of evaluating the effects of an 8-wk probiotic treatment. Ten individuals received daily, 12 billion colony forming units (CFU) of *L. rhamnosus*, strain GG; and 10 children received placebo. Evaluation at baseline included: US hepatorenal ratio, standard liver function tests, oral glucose tolerance test, serum TNF-α and antipeptidoglycan-polysaccharide polymers antibodies, and the glucose hydrogen breath test. After the probiotic treatment, it was observed a significant decrease in alanine aminotransferase (ALT) and antipeptidoglycan-polysaccharide antibodies levels; however, there were no alterations in the body mass index (BMI) Z score and visceral fat. Additionally, TNF-α and US bright liver parameters remained stable at the end of the treatment[15].

In a double-blind clinical trial performed in 28 adults with biopsy-proven NAFLD, Aller *et al*[16] evaluated the effects of an acute treatment with a mixture of probiotics. The patients were randomized to receive *L. bulgaricus* and *S. thermophilus* (1 tablet daily containing 500 million CFU) during 3 mo or 1 placebo tablet (120 mg of starch). In the probiotic group, the serum levels of ALT, aspartate aminotransferase (AST) and gamma-glutamyl transpeptidade (GGT) decreased following treatment. In the placebo group, all liver biochemical parameters remained unchanged. The anthropometric parameters and cardiovascular risk factors remained unchanged in both groups.

In 2012, it was published the first and only study in which liver histology was assessed before and after synbiotic treatment of a group of 66 NASH patients. The subjects were randomly and equally separated into 2 groups receiving *B. longum* plus FOS associated with lifestyle modification *vs* lifestyle modification alone. The serum parameters were assessed 4 wk before the beginning of the dietary period, and at weeks 0 (randomization), 6, 12, 18, and 24. Liver biopsies were performed at entry and repeated after 24 wk of treatment. A significant reduction in the serum levels of TNF-α, C-reactive protein (CRP), AST, homeostasis model assessment of insulin resistance (HOMA-IR), and endotoxin was observed in the *B. longum* plus FOS and lifestyle modification group when compared to the individuals who underwent lifestyle modification alone. Likewise, steatosis and the NASH activity index showed a significant improvement[17].

Unlike the earlier results reported by Solga *et al*[14], a chinese group[18] demonstrated in a sample of 20 patients with histological-proven NASH, that probiotic treatment was superior in reducing liver fat, measured by MRS, when compared to the usual care. The subjects were randomized to receive Lepicol probiotic formula (*L. plantarum*, *L. deslbrueckii, L. acidophilus, L. rhamnosus* and *B. bifidum*) (n = 10) or usual care (n = 10) during 6 mo. The results showed a reduction in intrahepatic triglycerides (IHTG) from 22.6% ± 8.2% to 14.9% ± 7.0% in the probiotic group (P = 0.034) and no changes in the usual care group. Furthermore, in most patients (n = 6) of the probiotic group, the IHTG reduced by more than 30% from baseline, whereas the same reduction was observed in only 2 subjects of the usual care group (P = 0.170). The probiotic group also presented a higher reduction in the serum levels of AST (P = 0.008). However, there were no significant changes in BMI, waist circumference, and glucose and lipid serum levels.

Very recently, a novel randomized, double-blind, placebo-controlled clinical trial conducted as a pilot study in 52 NAFLD patients was published. The subjects were supplemented twice daily, for 28 wk, with either a synbiotic (n = 26) or a placebo capsule (n = 26) to evaluate the effects on hepatic fibrosis score (determined by transient elastography), and serum levels of liver enzymes and inflammatory markers. Both groups were advised to follow an energy-balanced diet and physical activity recommendations. At the end of the study, the synbiotic group presented improvement in all the following markers: AST, ALT, GGT, high-sensitivity CRP, TNF-α, total nuclear factor k-B and fibrosis score with significant differences, when compared to the placebo group[19].

According to a review article published in 2011 high-quality preclinical studies and few randomized controlled trials support the therapeutic use of probiotics in liver diseases[20]. The only meta-analysis[21] performed

Table 1 Summary of clinical trials using probiotics to treat nonalcoholic fatty liver disease patients

Ref.	Sample size and underlying hepatic disorder	Design	Intervention	Duration	Results after treatment
Loguercio *et al*[12]	12 chronic HCV infection 10 alcoholic cirrhosis 10 NASH (biopsy-proven)	Prospective, single-center, nonrandomized, noncontrolled pilot study	*Lactobacillus acidophilus, Bifidobacterium bifidum, Lactobacillus. rhamnosus, Lactobacillus. plantarum, Lactobacillus. salivarius, Lactobacillus. bulgaricus, Lactobacillus. casei, Bifidobacterium. lactis* and *Bifidobacterium. breveassociated* plus FOS, vitamins and minerals	2 mo	NASH patients: decreased serum ALT, GGT, MDA, 4-HNE and TNF-α
Loguercio *et al*[13]	22 NAFLD (biopsy-proven) 20 alcoholic cirrhosis 20 chronic HCV infection 16 liver cirrhosis (without any other information)	Prospective, single-center, nonrandomized, noncontrolled	VSL#3 - 900 billion/2 × d	3 mo	NAFLD and alcoholic cirrhosis groups, improved MDA, 4-HNE. All groups improved S-NO plasma levels
Solga *et al*[14]		Open label pilot trial	VSL ≠3 - 450 billion/d	4 mo	All increase steatosis. No significant changes in metabolic panel, protime, glycosylated hemoglobin, TNF-α, IL-6 and 1β, and interferon-γ
Vajro *et al*[15]	20 NAFLD children (10/10) (US + increased aminotransferases)	Randomized, double-blind, placebo-controlled trial	*Lactobacillus rhamnosus* GG (12 billion CFU/d) *vs* placebo	8 wk	Improvement of ALT antipeptidoglycan-polysaccharide antibodies levels. No alterations in BMI z score, visceral fat, TNF-α levels and in US bright liver parameters
Aller *et al*[16]	28 (14/14) NAFLD (biopsy-proven)	Randomized, double-blind, placebo-controlled trial	*L. bulgaricus* and *Streptococcus thermophiles* (500 million CFU/d) *vs* placebo	3 mo	Improvement of AST, ALT and GGT levels No changes in anthropometric parameters and cardiovascular risk factors
Malaguarnera *et al*[17]	66 (34/32) NAFLD (biopsy-proven)	Randomized, double-blind, placebo-controlled trial	*Bifidobacterium longum* + FOS *vs* placebo	24 wk	Improvement of TNF-α, CRP, AST, HOMA-IR and endotoxin levels, steatosis, and the NASH activity index
Wong *et al*[18]	20 (10/10) NAFLD (biopsy-proven)	Randomized, double-blind	Lepicol probiotic formula *vs* nothing	6 mo	Improvement of AST levels and IHTG No differences in BMI, waist circumference, glucose and lipid levels
Eslamparast *et al*[19]	52 NAFLD (US, liver enzymes and fibroScan)	Randomized, double-blind, placebo-controlled trial	Synbiotic (200 million of 7 strain + FOS + probiotic cultures [magnesium stearate (source: mineral and vegetable) + vegetable capsule (hydroxypropyl methyl cellulose *vs* placebo	28 wk	Improvement of ALT, AST, GGT, CRP, TNF-α and total nuclear factor k-B levels, and fibrosis score

NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatites; HCV: Hepatitis C virus; ALT: Alanine aminotransferase; GGT: Gamma-glutamyl transpeptidase; MDA: Malondialdehyde; 4-HNE: 4-hydroxynonenal; TNF-α: Tumor necrosis factor; FOS: Fructooligosaccharides; VSL#3: Mixture of probiotics; S-NO, snitrosothiols; CFU: Colony-forming unit; BMI: Body mass index; US: Ultrasound; AST: Aspartate aminotransferases; CRP: C reactive protein; HOMA-IR: Homeostasis model assessment of insulin resistance; IHTG: Intrahepatic triglycerides; US: Ultrasound.

to investigate the effects of probiotics in NAFLD was recently published, and its authors concluded that probiotic therapy can reduce liver aminotransferases, total-cholesterol, TNF-α, and improve IR in this condition. The authors recommend modulation of the gut microbiota as new treatment for NAFLD. The summary of the clinical trial described above is presented in Table 1.

RESEARCH

Given the burden of NAFLD at present[22], the difficulty in maintaining lifestyle interventions, and the lack of effective treatment of this disorder, the prognosis of NASH is not optimistic[23]. The promising results of most clinical trials in which the use of probiotics were evaluated in humans, strongly suggest a benefit[12,13,15-19] of these agents in the treatment of NAFLD.

In 2001, the World Health Organization defined probiotic as a live commensal microorganism that, when consumed in adequate quantities, confers health benefit to the host. Lactobacilli and bifidobacteria are normal constituents of the human gastrointestinal microbiota[20], and they represent the main probiotics used in the clinical trials presented above[12,19]. Probiotic treatment aimed at modifying the colonic flora; and, modulate the enteric flora using probiotics are thought to produce benefit for several reasons: (1)

the intestinal bacterial flora favors the digestion and absorption of nutrients; (2) gut microbiota is related to overall immunity of the host[24,25]; and (3) microbiome may alter the synthesis of intestinal hormones such as glucagon-like peptide 1, and influences the host metabolism[24,26].

It is well known that the liver and the gut communicate through the portal venous system; therefore, intestinal-microorganism products may reach and affect the liver. Miele *et al*[27] demonstrated a high prevalence of small bowel bacterial overgrowth (SIBO) and increased gut permeability in NAFLD patients; furthermore, both findings were associated with steatosis severity. Disruption of the intercellular tight junctions was suggested by those authors as the mechanism of the increased intestinal permeability[27]. This increased intestinal permeability associated with the SIBO favors bacterial translocation, exposing the liver to gut-derived bacterial fractions and metabolites constantly[20]. Zhu *et al*[24] characterized the gut microbiomes in NASH subjects. According to their findings, there are increased abundance of alcohol-producing microbiota in these patients as well as elevated blood-ethanol concentration leading to increased oxidative stress and liver inflammation due to the alcohol metabolism.

Indeed, in addition to the increased production of ethanol, the intestinal bacterial microbiota also synthetizes LPS that promotes release of the pro-inflammatory cytokine TNF-α and IL-6 from the hepatic macrophages, which damage the liver, disrupt normal hepatocyte function, lead to mitochondrial oxidative stress, and reduce the clearance of toxins by the hepatocytes[17]. The only clinical trial in which serum endotoxin was measured demonstrated a decrease in their concentrations after the probiotic use[17]. Likewise, in 4 of the 6 clinical trials in NAFLD patients in which the serum markers of oxidative stress were evaluated, the authors observed a reduction in their levels after treatment with probiotics[12,13,17,19]. Serum concentrations of ALT and AST are well-recognized clinical markers of liver damage, and most clinical trials showed a decrease in at least one of his parameters, at the end of the probiotic use[12,13,15-19]. These findings suggest that colonization of the gastrointestinal tract by probiotics is followed by modification of gut flora with subsequent reduction in pro-inflammatory species and, then, improvement of the liver damage.

IR plays a central role in the development and progression of NAFLD. As a consequence of IR, subjects with NAFLD have decreased muscle glucose uptake, impairment in suppression of hepatic endogenous glucose production stimulated by insulin[28,29], and increased lipolytic effect in the adipose tissue resulting in high fatty free acids release[30]. All together these mechanisms can lead to hepatic steatosis. Additionally, the increase in the adipose tissue, especially the visceral fat, has been related to inflammation, decreased release of insulin-sensitizing and anti-inflammatory cytokines, and high expression of pro-inflammatory molecules[31]. Gut microbiota impacts on energy metabolism participating in the homeostatic control and insulin sensitivity[32]. Bäckhed *et al*[33] demonstrated in *germ-free* mice infected with the intestinal bacteria from conventionally raised mice, increase in body fat content even with low food intake (standard chow), IR, and glucose intolerance, within 14 d of the infection. Modulation of gut bacterial flora using probiotics did not result in better glycemic control in 2 clinical trial, evidenced by no changes in glycosylated hemoglobin[14] and glucose serum levels[18]. However, Malaguarnera *et al*[17], observed improvement in HOMA-IR after 24 wk of treatment with a synbiotic. In this situation, the FOS present in the synbiotic supplement may have helped to improve the glycemic control. The assays of intrahepatic fat content also presented different results: Solga *et al*[14] verified increased steatosis measured by MRS after probiotics; Varjo *et al*[15] did not observed any differences in bright liver parameters using US; and Malaguarnera *et al*[17], using histological evaluation, described a decrease in steatosis and NASH activity index at the end of probiotic treatment.

It has been described differences between distal gut microbiota of obese and lean humans. Obese people were shown to have lower Bacteroidetes and more Firmicutes in their distal gut compared to lean control. When obese individuals lose weight on either a fat- or a carbohydrate-restricted low-calorie diet, the prevalence of Bacteroidetes increases in their gut[34]. This finding suggests that microbiota plays a role in increasing the capacity of hosts to harvest energy from their diet. However, no differences in BMI, waist circumference and visceral fat were demonstrated in all clinical trials that evaluated the effect of probiotics in NAFLD patients[15,16,18].

The clinical trials discussed above have some limitations, which may be summarized as followed: (1) differences among the studies regarding the pharmaceutical formulations. In some studies, the mixture contained, in addition to the probiotic, other constituents such as oligoelements, vitamins and prebiotic, which may have influenced the results; (2) great variety in the probiotic doses, bacterial species and duration of treatment; (3) in the large majority, the response to probiotic use was not evaluated by liver biopsy; and (4) a small number of subjects in most studies. However, despite these limitations, the small number of clinical trials, and considering that probiotics are low cost, present good tolerability, and are safe, they should be considered a complementary therapeutic approach in NAFLD patients.

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