Probiotics and Nonalcoholic Fatty liver Disease

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ABSTRACT

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide, both in adults and in children. NAFLD represents a spectrum of liver diseases that range from hepatic steatosis to steatohepatitis and cirrhosis. However, NAFLD is more prevalent in overweight and obese individuals. Evidences thus far suggest that hepatic triglyceride accumulation is not always derived from obesity; gut microbiota can also play a role in the development of insulin resistance, hepatic steatosis, necroinflammation and fibrosis. On the other hand, probiotics can strengthen the intestinal wall, reducing its permeability, bacterial translocation, and endotoxemia according to animal and human studies. They can also reduce oxidative and inflammatory liver damage, while improving the histological state in certain situations. This review article focuses on research that has been conducted on probiotics and NAFLD, highlighting their efficacy as a novel therapeutic option for the treatment of this condition.

KEYWORDS
Probiotics; Nonalcoholic fatty liver; cirrhosis

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INTRODUCTION

"Nonalcoholic fatty liver disease (NAFLD) is currently the most common cause of chronic liver disease, becoming a serious public health concern, as a result of the obesity epidemic, unhealthy dietary patterns, and sedentary lifestyles.[1,2] NAFLD represents a wide spectrum of conditions associated with the deposition of fat in the liver, that ranges from simple steatosis to nonalcoholic steatohepatitis (NASH) and cirrhosis.[3,4] The pathogenesis of NAFLD is unclear. Initial theories regarding its development have been based on the '2-hit hypothesis', where the "first hit" involves hepatic lipid accumulation, and insulin resistance is proposed to be the key contributing factor for steatosis development.[5] Oxidative stress followed by lipid peroxidation, as well as the action of proinflammatory cytokines (e.g. tumor necrosis factor [TNF]-α), adipokines and mitochondrial dysfunction initiate the second hit, which progresses from simple steatosis to nonalcoholic steatohepatitis (NASH).[6-10] In a recent review article, Dowman et al.

have referred to a further component, or a 'third hit' which also plays a role in NAFLD pathogenesis. This "third hit" is also caused by oxidative stress, which inhibits the replication of mature hepatocytes resulting in the over population of the hepatic oval cells.[11]

The existing treatment strategies for NAFLD have already focused on reducing metabolic risk factors, with lifestyle modification techniques such as weight loss through diet and exercise, as the key therapy,[12,13] as well as pharmacotherapy, and the use of hepato-protective agents such as anti-oxidants, and anti-inflammatory drugs, to protect the liver from further damage.[14-17] Many of these strategies have been shown effective in different pilot studies.[18] However, since there is sometimes no relationship between liver histology and serum levels of liver enzymes, the results of trials that use biochemical markers of liver injury as study end points need to be interpreted carefully, especially when there is no control group used in the study design.[19,20]

An endogenous factor contributing to NAFLD development is gut microbiota.[21] Recently, it has been reported that NAFLD might be linked to small intestinal bacterial overgrowth (SIBO), which induces liver injury by gut-derived lipopolysaccharides (LPS) and TNF- α production.[22] It has been suggested that gut microbiota increases the liver's exposure to endotoxins, thus promoting steatohepatitis.[22] Possible mechanisms as described by Solga and Diehl include "bacterial overgrowth, release of the LPS constituent of the gram-negative bacteria, and impaired intestinal barrier integrity, resulting in increased endotoxin absorption".[10] Thus, it seems that the manipulation of enteric flora may be a novel therapeutic strategy in the management of NAFLD. Probiotics can modulate the gut flora and influence the gut-liver axis. Available data indicates that probiotics may promote the intestinal mucosal barrier function and mucosal recovery during a pathological condition.[23] Because of the close anatomical and functional relationship between the gut and the liver, and the immunoregulatory effects of probiotics, the aim of this review article is to summarize the use of probiotics in NAFLD, specifically focusing on the probiotics' mechanisms of action, and highlighting their efficacy as a novel therapeutic option to treat this condition.

Gut-liver axis

The gut and the liver are closely associated, forming the gut-liver axis.[22] The interplay in this axis depends on two things: an intact intestine and a liver that is well-able to handle immunologic responses as well as the metabolism of endogenous and exogenous compounds.[22,24] The intestinal mucosa serves as a defense barrier that helps prevent the entrance and the systemic spread of bacteria and endotoxins, most of which are LPS from the cell walls of gram-negative bacteria.[25] However, under certain conditions, this intestinal barrier fails, resulting in bacterial and endotoxin invasion into the gastrointestinal (GI) tract, after which the pathogens reach systemic organs and tissues; this process is termed bacterial translocation.[26] There is also some evidence that a small degree of endotoxemia of gut origin is usually present in the portal circulation (which drains the GI tract), serving as the ideal route of transportation to the liver, for the intestinal bacteria and bacterial products such as LPS. But under normal conditions, this endotoxemia is rapidly cleared by the liver's reticuloendothelial system.[27-30] However, at times of liver disease, or with long-term exposure of the liver to LPS, which are hepatotoxins, a cascade of morphological and functional changes start in the liver, inducing an acute inflammatory response and a build-up of the polymorphonuclear cells.[31] Neutrophils then release reactive oxygen metabolites, proteases and other enzymes from the granules, with a worsening of liver damage.[31] Because of these factors, the modification of the gut microbiota to treat NAFLD, has produced growing interest in the potential for the use of probiotics as an effective dietary treatment.[32]

Probiotics

Probiotics were originally defined as "microorganisms causing growth of other microorganisms", and later on as "live microorganisms which when

consumed in adequate amounts, result in healthy benefits to the host".[33] A microorganism is considered to be probiotic when it meets the following conditions: it should be of human origin, non-pathogenic, should highly resist passing through the intestine, should have the ability to adhere to mucus while preventing the adherence of other pathogenic microorganisms, and should be beneficial to the immune system and human health in general.[34] Probiotics have been suggested as a treatment for the prevention of chronic liver damage, because they prevent bacterial translocation and epithelial invasion, and also inhibit bacterial mucosal adherence, and the production of antimicrobial peptides, while decreasing inflammation, and stimulation of host immunity.[35,36]

Probiotics and inflammation

Inflammation can develop as a result of both internal and external factors. The most important triggers for inflammation occur when microorganisms are present at locations where they do not belong. The aggressiveness of the pathogenic bacteria can weaken the barrier function of the mucosa and allow penetration of bacterial components into the body. "These components then end up in the liver, and the liver responds with inflammation".[37] Probiotics confer a health benefit to the host by different conceivable mechanisms such as decreasing the invasion of proinflammatory agents from the gut into the body. Bacterial phagocytosis and clearance most extensively occur in the liver and by macrophages called Kupffer cells.[38] According to Racanelli and Rehermann, these Kupffer cells, when exposed to LPS or other bacterial products such as lipopeptides, unmethylated DNA, and double stranded RNA, mediate an inflammatory response via pro-inflammatory cytokines, chemokines and reactive oxygen/nitrogen species, all of which impact liver tissue, causing injury.[38] As Su explains, these microbial products start proinflammatory actions by mediating through a specific class of receptors, termed Toll-like receptors (TLRs). TLRs detect signature molecules derived from pathogens and enable the host to regulate innate immune responses.[39] TLRs are expressed in many different hepatic cell types including Kupffer cells, hepatocytes, and hepatic stellate cells (HSCs). The extraordinarily powerful effects of TLRs on inflammation and their expression in the liver as well as the hepatic exposure to TLR ligands from the intestine, suggest that TLRs act as an important link between hepatic inflammation, injury, and fibrosis. The intestinal microbiota and TLRs represent a major link between inflammation and wound-healing responses in the liver. Among the many different TLRs, TLR4 has a prominent role in promoting inflammation and injury in conditions such as alcoholic liver disease and NASH.[39] TLR4 associates with CD14 on the cell surface to initiate LPS-induced signal transduction, notably, activation of nuclear factor κB (NFκB) and the subsequent production of proinflammatory cytokines, such as TNF and cyclooxygenase 2.[39,40] As explained by Baldwin, the activation of TLR4 by LPS triggers an essential intracellular inflammatory cascade, includes stress-activated and mitogen-activated protein kinases, c-Jun-N- terminal kinase, p38 and the NFκB pathway. Elimination of the inhibitor of NFκB kinase subunit β (IKK-β), allows NFκB to translocate to the nucleus, where it causes the expression of genes involved in certain inflammatory pathways, producing TNF-α and IL-1β.[41]

Probiotics have several anti-inflammatory effects that can contribute to their clinical benefits in NAFLD.[10] Based on explanations by various researchers, these effects include their competition with pathogenic bacteria for limited nutrients,[23] the modification of inflammatory pathways induced by intestinal bacterial overgrowth via alteration of cytokine signaling,[42] the amelioration of intestinal barrier function through modulation of cytoskeletal and tight junction proteins,[43,44] enhancement of the integrity of the intestinal epithelium by providing essential nutrients particularly in the form of medium-chain fatty acids that inhibit apoptosis, direct inhibition of the production of proinflammatory mediators such as TNF-α,[45] induction of anti-inflammatory responses in intestinal epithelial cell–leukocyte cocultures,[46] and stimulation of IgA release.[47]

The role of probiotics in insulin resistance

Insulin resistance seems to have a crucial role in the pathogenesis of NAFLD and NASH.[48] Besides the suggested role of insulin resistance in the development of steatosis, hepatic insulin resistance can promote hepatocyte injury and inflammation. Gut flora and gut derived endotoxemia are involved in the development of insulin resistance, especially through the LPS–TLR 4–monocyte differentiation antigen CD14 system.[45,49-52] Although this evidence has been largely derived from animal models, one study documented elevated plasma levels of LPS among patients with type 2 diabetes mellitus compared to matched controls.[53] Suppression or modification of SIBO, causing a reduction in proinflammatory cytokine production, leads to a fall in fasting insulin concentrations and decreased insulin resistance.[45,54] Administration of probiotics has been shown to lower blood glucose levels by an insulin-independent mechanism in a diabetic rat model.[55]

Probiotics as an option for anti-fibrotic therapy

Chronic liver injury is characterized by fibrosis development in the liver, since repeated and continuous damage to the liver cells causes HSC activation, which are recognized as the main matrix producing cells in liver fibrosis.[56] Through an increased portal delivery of endotoxins, intestinal bacteria seem to be able to produce fibrotic liver disease via the activation of Kuppfer cells, induction of TGF-β production and subsequent activation of HSCs. Besides being the main precursors of myofibroblasts, the HSCs are also the "predominant targets through which TLR4 ligands promote fibrogenesis".[37,57] HSCs may also have an important role in generating the liver inflammatory cascade associated with endotoxemia.[58,59] TLR4 is expressed, not only on Kupffer cells, but also on HSCs; while Kupffer cells contribute to fibrogenesis through the production of proinflammatory and profibrogenic mediators, HSCs are, as stated, the major source of the extracellular matrix deposited in the fibrotic process.[56] Activated HSCs are highly responsive to LPS through a TLR4-dependent pathway.[56,59] In HSCs, LPS induces IL-8 and CC-motif chemokine 2 production and activates the transcription factors NFκB and c-Jun (now known as transcription factor aP1) through TLR4, indicating that LPSs exert direct effects on HSCs during fibrogenesis.[59]

Probiotics in NAFLD in animal studies

Several animal models have provided evidence that probiotics may reduce NAFLD progression.[45,60-63] A study using a type of VSL#3 mixture of three types of bacteria (*Streptococcus*, *Bifidobacterium*, *Lactobacillus*) for 4 weeks in ob/ob mice showed a reduction in hepatic total fatty acid content and liver inflammation as well as an improvement in hepatic insulin resistance.[45] Another study suggested a direct decrease in pro-inflammatory cytokines via the down-regulation of NF-κB activity using probiotic treatments.[60] In a NAFLD animal model fed a high fat diet (HFD), VSL#3 supplementation reduced the expression of lipid peroxidation markers, TNF-α, iNOS and cyclooxygenase 2 as compared to the control group.[62] Furthermore, the administration of VSL#3 improved insulin resistance in liver and adipose tissues and counteracted the development of NASH and atherosclerosis in ApoE(−/−) genetically dyslipidemic mice.[64] Recently, it was shown that oral Bifidobacterium supplementation in mice with HFD induced-NASH attenuated hepatic fat accumulation without improvement of intestinal permeability. Histological aspects of the liver showed microvesicular steatosis with basically normal hepatic lobules in the Bifidobacterium group compared to the high-fat fed mice, where the structure of the hepatic lobule was destroyed and was associated with micro and macrovesicular steatosis.[65] Only one study assessed the effects of probiotics in liver fibrosis.[63] In a methionine choline-deficient (MCD) diet induced mouse model of NASH, Velayudham et al showed that the administration of VSL#3 reduced the progression of liver fibrosis by diminishing the accumulation of collagen and α-smooth muscle actin, but without significant attenuation of steatosis or inflammation. A decrease in the expression of procollagen I α1 and matrix metalloproteinases (MMPs) was observed in mice fed MCD + VSL#3 as compared to those fed

the MCD diet alone.[63] Velayudham et al. demonstrated that VSL#3 administration inhibited fibrosis by triggering the production of pseudoreceptor Bambi. Thus, in the presence of VSL#3, high levels of Bambi could prevent TGF- β-induced signals to release, and control the unrestricted activation of HSCs due to ongoing inflammation.[63] This study suggested that VSL#3 may improve fibrosis. Several strains of lactobacillus have demonstrated a protective effect on NAFLD,[66-68] whereas a recent meta-analysis pointed out the association of certain species (L. fermentum and L. ingluviei) with weight gain.[69]

Probiotics in NAFLD in human studies

Preliminary data showed that both VSL#3 and a synbiotic (combination of pro/prebiotics) given to NAFLD patients for two to three months, improved liver enzyme levels, TNF-α and oxidative stress markers.[70] More recently, two randomized double blind placebo controlled studies, showed a significant decrease in liver aminotransferases with probiotic administration in children[71] and in adults.[72] Malaguarnera et al. also demonstrated that oral administration of B. longum with fructo-oligosaccharides, combined with lifestyle modification, ameliorated the serum profile of aspartate aminotransferase, low-density lipoprotein cholesterol, TNF-α and endotoxins, while improving insulin resistance, steatosis and the NASH activity index.[73] These promising results are strongly indicative of a great benefit to use probiotics in the treatment of NAFLD. Nonetheless, as stated in a Cochrane meta-analysis, larger randomized studies are still needed.[74]

CONCLUSION

Given the evidences described above regarding the possible fundamental role of gut-derived microbial factors in the development and/or progression of NAFLD, a logical proposition is that the modification of intestinal microbiota may have a beneficial effect on this pathological condition. Complications of liver disease could potentially be reduced by altering the microbiota either quantitatively or qualitatively. Probiotics are safe, inexpensive and have no known adverse effects with long-term use, and supplementation with probiotics in the management of NAFLD/NASH seems to be a practical therapeutic strategy. Since different probiotic strains may have different effects, further understanding of the diverse functions of the various strains as well as their effects on different diseases is required.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this work.

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