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Intestinal microbiota in the Treatment of Metabolically Associated Fatty Liver Disease

Treatment strategy of MAFLD

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Abstract

Metabolically associated fatty liver disease (MAFLD) is a common cause of chronic liver disease, the hepatic manifestation of metabolic syndrome. Despite the increasing incidence of MAFLD, no effective treatment is available. Recent research indicates a link between the intestinal microbiota and liver diseases such as MAFLD. The composition and characteristics of the intestinal microbiota and therapeutic perspectives of MAFLD are reviewed in the current study. An imbalance in the intestinal microbiota increases intestinal permeability and exposure of the liver to adipokines. Furthermore, we focused on reviewing the latest "gut-liver axis" targeted therapy.

Key Words: Intestinal microbiota, metabolically associated fatty liver disease (MAFLD), Gut-liver axis

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Core Tip:

Recent animal studies and Clinical researches have placed the gut microbiota as a potentially important player in the pathogenesis of metabolically associated fatty liver disease (MAFLD). It is logical to target the gut microbiota to develop new strategies for MAFLD therapy. In addition, we assessed the therapeutic potential of intestinal microbiota manipulation for treating MAFLD and discussed the specific doses and duration of use can provide more help for clinicians in choosing treatment options.

Introduction

MAFLD is a spectrum of liver disorders, including metabolically associated steatohepatitis (MASH), and cirrhosis^[1]. MAFLD is the most common liver disease worldwide, affecting over half a billion people^[2]. This disease can progress from fibrosis to cirrhosis and can be complicated by hepatocellular cancer.^[3]

Obesity and insulin resistance are two of the many factors that can promote the progression of MAFLD and MASH^[4]. The most common manifestation of MAFLD is excessive fat accumulation in hepatocytes^[5]. The underlying mechanisms leading to MAFLD are still unknown^[6]. Defective lipolysis has been recognized as an important mechanism underlying human MAFLD pathogenesis^[7]. Primary and secondary changes in the bile acid pool have also been implicated in MAFLD pathogenesis^[8]. Recently, a study reported that *H. pylori* (HP) eradication may prevent metabolic syndrome including MAFLD^[9]. However, this study found that HP eradication did not affect metabolic indices at all. It was suggested that intestinal microbiota might regulate insulin resistance in MAFLD patients. A potential role for the gut microbiota in the pathogenesis of MAFLD has been found in recent animal studies^[10].

¹ In this review, we summarized the role of intestinal microbiota in the treatment of MAFLD. Furthermore, we discussed the specific doses and duration of use of intestinal microbiota manipulation for treating MAFLD and discussed the therapeutic potential.

Intestinal microbiota

Microbial communities are widely distributed in the gut, lungs, skin, and other epithelial surfaces^[11]. Approximately a thousand different species of bacteria live in the human gut^[12]. The human gut microbiota includes bacteria, viruses, and fungi^[13]. Bacteroidetes and Firmicutes are the dominant groups of bacteria among the several other groups of bacteria found in the

normal intestinal microflora^[14]. The non-bacterial intestinal microorganisms are also important for human health as bacteria^[15]. Future research should focus more on non-bacterial gut microbes and human diseases.

Bacteria in the gastrointestinal tract produce vitamins, absorb ions, protect the host from pathogens, induce histological development, and enhance immune function, among other benefits^[16]. The absence of beneficial microorganisms promoting appropriate immune development results in inflammatory responses underlying various human immune diseases^[17]. For example, inflammatory bowel disease (IBD) pathogenesis is associated with disrupted intestinal barrier function, gut microbiome imbalance, and subsequent dysregulated mucosal immune responses to gut commensal bacteria^[18, 19]. Their significance for human health has been demonstrated by several studies. The composition and distribution of gut microbiota change under different conditions due to their variation. Dysbiosis of the intestinal microbiota may impact human health and disease^[20]. Patients with MAFLD and MASH do not only show compositional changes in the gut microbiota (Table 1.)^[21], but also have a higher prevalence small intestinal bacterial overgrowth^[22, 23].

Gut-liver axis

Intestinal barriers are comprised of biofilms, mucus layers, and epithelial cells^[24]. The intestinal barrier is characterized by physiological and immunological protection and maintains the digestive and absorptive functions of the intestine while restricting pathogen and toxic metabolite invasion into the circulation^[25]. The impaired intestinal barrier allows bacteria and their products, including pathogen-associated molecular patterns (PAMPs) into the circulatory system^[26]. In addition to the intestinal epithelial barrier (IEB), some researchers have discovered that the gut-vascular barrier (GVB) prevents the systemic dissemination of bacteria, bacterial antigens, and other luminal contents through the intestinal epithelial barrier^[27]. When the

microbiota is dysbiotic, it affects the GVB, leading to an increase in intestinal blood vessel permeability when consumed with a high-fat diet (HFD).^[28]. GVB-related research reveals how the gut-liver axis can be regulated to prevent MAFLD, as GVB impairment is a precursor to the disease^[28].

The portal vein provides the liver with 70% of its blood supply from the intestine^[29]. By combining with the Toll-like receptor-4 (TLR4), intestinal bacterial endotoxins participate in the oxidative stress response and promote the progression of liver disease^[30]. Thus, the intestinal microbiota influences MAFLD through the gut-liver axis.

Dysbiosis of the intestinal microbiota and MAFLD

There is an imbalance of microbial populations within the intestinal ecosystem when there is a loss of fragile equilibrium among these entities.^[31]. Multiple studies have shown that intestinal microflora imbalance is closely related to MAFLD^[32, 33]. The role of intestinal microbiota in occurrence of MAFLD is as followed (Figure 1) ^[34, 35]: (1) molecular dysbiosis causes the liver to produce more intestinal ethanol, which damages tight junctions and causes gut permeability problems.; (2) inflammation and fibrosis of the liver can be induced by PAMPs like endotoxin that bind to specific TLRs.; (3) dimethylamine and trimethylamine are formed by gut microbiota hydrolyzing choline. Choline deficiency results from increased choline metabolism, which prevents the expulsion of very low-density lipoprotein (VLDL) from the liver and leads to the accumulation of triglycerides.; (4) an altered gut microbiota might inhibit the secretion of fasting-induced adipocyte factor (FIAF, also known as angiopoietin-related protein 4, ANGPTL4), a specific inhibitor of endothelial lipoprotein lipase (LPL), which releases triglycerides from VLDL particles into the liver. Triglyceride storage in the liver is increased as a result of lipid β -oxidation inhibition; (5) inhibiting the action of adenosine monophosphate activated protein kinase (AMPK), excessive short-chain fatty acids (SCFAs) in the liver stimulate free fatty acids

(FFAs) synthesis and gluconeogenesis. Next, a summary of the role of intestinal microbiota dysbiosis in the occurrence of MAFLD will be provided.

Animal model studies

The intestinal microbiota is known to influence obesity and MAFLD. High sugar and fat diets are sufficient to induce obesity and insulin resistance in germ-free (GF) mice^[36]. It was observed that fecal microbiota can directly induce MAFLD in mice. This indicates a direct link between gut microbes and MAFLD development.^[37] HFD-fed C57BL/6J mice showed obesity-associated MAFLD^[38]. Both diets with lower carbohydrate or fat content stabilized weight and reduced adiposity when fed to diet-induced obese animals^[38].

Furthermore, MAFLD should be linked to bacteria or microbe-derived products^[39]. Endotoxin in the systemic circulation of individuals with MAFLD correlates with the severity of steatohepatitis in animal studies^[39, 40]. All fatty/fatty rats developed steatohepatitis after endotoxin treatment, with histologic evidence of focal hepatocyte necrosis, hepatic inflammation, and increased serum aspartate aminotransferase (AST) and aspartate aminotransferase (ALT)^[40]. In the same study, the obese/obese group's serum AST and ALT were ten times higher after endotoxin treatment than their lean littermates^[40]. The intestinal microbiota and their harmful metabolites (ethanol, endotoxin) may cause liver injuries. Intestinal microbes and their metabolites may play an important role in the advancement of MAFLD, but more research is needed to identify them.

Clinical research

Like the animal model studies mentioned above, MAFLD patients have endotoxin in their systemic circulation, correlating with the severity of steatohepatitis in clinical research^[41, 42]. Endotoxin levels were significantly higher in MAFLD patients than in controls^[42]. In addition to endotoxin, several studies have compared the intestine bacterial composition of healthy individuals and MAFLD patients. Using healthy controls, MAFLD and

MASH patients as comparison subjects, Mouzaki et al. characterized the composition of the intestinal microbiota in these three groups. They found that those with MASH had a lower number of Bacteroidetes compared to subjects with simple steatosis or healthy subjects, while the intestinal microbiota did not differ between MAFLD subjects and controls^[43]. There has been recent evidence that the composition of bacteria in stool varies with an individual's stage of fibrosis in MAFLD patients based on the sequencing technology^[44, 45]. Therefore, gastrointestinal microbiota dysbiosis plays an important role in MAFLD development and development into MASH.

Targeting the gut-liver axis to treat MAFLD

The impaired gut barrier may allow endotoxin and other microbial metabolites to enter the portal vein, increasing pro-inflammatory cytokine production and aggravating MAFLD^[46]. In addition to using FXR agonists to treat MAFLD, there is growing research indicating that gut-liver axis may be a new approach.

Antibiotics application to MAFLD treatment

Antibiotics are used to reduce the quantity of intestinal microbiota to reduce the effects of the microbiome and their metabolites on host health. Non-absorbable antibiotics such as rifaximin are currently used to treat minimal and overt hepatic encephalopathy^[47]. Moreover, the combined use of antibiotics (neomycin and polymyxin B) reduced total hepatic triglycerides^[48]. Rifaximin can reduce circulating endotoxins and serum transaminases, resulting in therapeutic effects in MAFLD^[46, 49]. However, one clinical trial presented the opposite results. Cobbold et al. reported that rifaximin showed little therapeutic effects against hepatic lipid content^[50]. However, the inconsistency may be due to the small sample size, the relatively low treatment dose, the short duration of the study, and the broad-spectrum activity of rifaximin affecting both the harmful and the beneficial bacteria.

Furthermore, antibiotics can induce mutations resulting in antibiotic resistance^[51]. Antibiotics appear to alleviate MAFLD (Table 2), but their clinical use is still questionable. To have a beneficial effect on metabolic health and inflammation, future therapies targeting the intestinal microbiota need to be more nuanced.

Probiotics application to MAFLD treatment

Probiotics are "living microorganisms that confer a health benefit to the host when administered in adequate amounts"^[52]. Probiotics appear to alter intestinal microflora and may exert their effects by various mechanisms^[53]. Although most probiotics are derived from bacteria, fungi such as *Saccharomyces boulardii*, originally isolated to combat cholera, have also been proven to be effective probiotics^[54].

Gut microbiota modification using probiotics has shown beneficial effects on MAFLD mice^[55]. Promising results have been observed in adults and children with MAFLD responding to probiotic treatment^[56]. VSL#3 is a mixture of probiotic bacteria, including Lactobacilli, that has been used in many experimental and human MAFLD treatment studies. VSL#3 contains 450 billion bacteria per sachet from eight different bacterial species (Bifidobacterium longum, Bifidobacterium infantis, Bifidobacterium breve, Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus plantarum, Lactobacillus casei, and Streptococcus thermophilus)^[56]. A cornerstone paper published in 2003 reported that VSL#3 treatment significantly reduced hepatic inflammation, ALT levels, and hepatic oleic acid levels in a genetically obese obese/obese mice MAFLD model^[57]. Recent studies indicated that the multi-strain probiotic VSL#3 was effective in improving weight loss and liver fibrosis in obese children with MAFLD^[58]. It was demonstrated in a double-blind clinical trial that Lactobacillus GG significantly decreased serum ALT in obese children suffering from MAFLD (normalization in 80% of the cases)^[59]. Solga et al. studied the effect of VSL #3 on four MAFLD adult subjects in an

open pilot study over four months^[60]. All four subjects had a significant increase in liver fat and no significant differences in biochemical or clinical parameters^[60]. Researchers have identified a small sample size as an important limitation^[60]. Another study evaluated the effects of a different probiotic using a randomized, double-blind clinical trial^[61]. This study evaluated the effects of *Lactobacillus bulgarius* and *Streptococcus thermophilus* (1 tablet/day) in 28 MAFLD patients over three months^[61]. ALT, AST, and gamma-glutamyl transferase levels decreased^[61]. VSL#3 or Lactobacilli combined with a prebiotic and vitamin mixture (Bio-Flora) were well tolerated, improved conventional liver function tests, and reduced lipid peroxidation and TNF- α markers levels^[62]. A two-month Bio-Flora supplementation lowered liver enzyme levels in 10 biopsied adults with MASH^[62]. Both ALT and gamma-glutamyltransferase levels improved significantly one month after washout. The treatment also reduced oxidative stress markers [malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE)]^[62]. Probiotics may help to improve disease symptoms and limit the damage by promoting the reinforcement and repair of the epithelial barrier. Le Barz et al. reported that the probiotics strain Lb102 significantly upregulated the gene expression of two important tight junction proteins, ZO-1 and occludin^[63]. Probiotic *Clostridium butyricum*, known in Asia as MIYAIRI 588, prevented fatty degeneration from progressing to liver cancer in rats with MAFLD^[64, 65]. These studies demonstrated the therapeutic role of probiotics in MAFLD treatment (Table 2). Furthermore, the probiotic affected treatment of other diseases^[66]. The National Health Commission of China recommended using probiotics to maintain intestinal balance and prevent secondary bacterial infections in patients with severe COVID-19^[67]. However, there are still some unanswered questions regarding the role of probiotics against MAFLD. For example, it is unclear how probiotics change the composition of intestinal microflora. In populations from different regions, there is a great deal of variation in the composition of the gut microbiota. Therefore, the beneficial

effects of probiotics on MAFLD/MASH must be verified in ethnically diverse populations, and optimal formulations and dosages must be determined to develop commercialized probiotic products.

Prebiotics application to MAFLD treatment

Prebiotics, a selectively utilized substrate by host microorganisms, confer health benefits^[68]. Researchers have shown that prebiotics are effective in the treatment of MAFLD both in vitro and in vivo. Prebiotics are composed of oligosaccharides or short-chain polysaccharides. ⁴ The best-characterized prebiotics are fructosyl-oligosaccharides (FOS), including inulin (long-chain fructosyl-oligosaccharide), galactosyl-oligosaccharides (GOS), and other oligosaccharides present in milk, which are transformed by the gut microbiota into SCFAs and simultaneously promote proliferation of selected commensal bacteria in the colon^[69-72]. ¹ The de novo lipogenesis was threefold higher in patients with MAFLD, indicating that increased de novo lipogenesis is a defining characteristic of this disease^[66]. Animal studies show that prebiotic supplementation may improve MAFLD by inhibiting the fatty acid synthesis pathway^[73, 74]. ⁸ Rats fed with 10% oligofructose, a nondigestible but fermentable oligomer of β -D-fructose, showed significantly reduced fructose-induced hepatic triglyceride (TG) accumulation^[72, 75]. Prebiotics also modulate glucose homeostasis and lipid metabolism, thereby modulating MAFLD/MASH progression in clinical trials^[76]. Those suffering from obesity benefited from prebiotics by increasing Bifidobacteria growth and lowering plasma endotoxin levels^[77]. Lactobacillus, Bifidobacterium, and Gram-positive bacteria grow well on lactulose, while Gram-negative bacteria are inhibited by it^[78]. For six weeks, obese mice who were fed HFDs and given lactulose showed less inflammation and liver damage, which was correlated with lowered lipopolysaccharide levels^[79]. Additionally, chitin-glucan modulates gut microbiota, thereby limiting weight gain, glucose intolerance, triglyceride accumulation, and fasting hyperglycemia^[80].

In conclusion, prebiotics is a potential therapeutic tool against MAFLD (Table 2). However, studies showed that consuming prebiotics over 30 g/day could cause adverse gastrointestinal effects such as flatulence^[81]. Clinical trials that demonstrate high-quality results are necessary to generalize prebiotics' use for MAFLD given the limited research in this field.

Synbiotics application to MAFLD treatment

Gibson and Roberfroid coined the term "synbiotic" in 1995, defining it as "a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the GI tract, by selectively stimulating the growth or activating the metabolism of one or a limited number of health-promoting bacteria, thus improving host welfare"^[69]. Six months after therapy with Bifidobacterium and FOS, MASH patients had significantly lower serum ALT and AST levels than those who received placebo^[82]. A meta-analysis of 15 randomized controlled trials including 782 MAFLD patients revealed that synbiotics significantly reduced ¹ liver steatosis, ALT, AST, high-density lipoprotein, low-density lipoprotein, triglyceride and cholesterol levels, TNF- α expression, the degree of liver stiffness, and homeostasis model assessment-insulin resistance^[83]. Studies involving animals and clinical trials suggest that synbiotics could potentially treat MAFLD (Table 2).

FXR agonists application to MAFLD treatment

Liver enzymes ⁵ synthesize and conjugate bile acid (BA), a cholesterol derivative^[84]. ⁵ Micelles containing conjugated bile acids function to solubilize, digest, and promote the absorption of dietary lipids, cholesterol, and fat-soluble vitamins (A, D, E, and K) in the small intestine^[85].

⁵ In addition to fat and cholesterol solubilization, BA has bacteriostatic properties inhibiting bacterial growth in the biliary tree^[86]. This signaling molecule functions as a link between the liver and intestine. Through a

feedback mechanism, BA receptors (also known as FXRs) are abundantly expressed in the liver and intestine^{[87][88]}.

The FXR agonists have been proven effective against MAFLD or MASH in many animal experiments and clinical trials (Table 3).

FXR agonists such as obeticholic acid which improve the liver's lipid and glucose metabolism and reduce liver inflammation and fibrosis in MAFLD^[89]. Furthermore, FXR agonists reduce pro-inflammatory cytokines expression in macrophages and hepatic inflammation in a mouse model of MAFLD^[88]. FXR agonists could also enhance the anti-inflammatory polarization of the macrophages *in vitro* and *in vivo*^[90]. The first study showing that obeticholic acid improves insulin sensitivity, suppresses hepatic inflammation, and reduces fibrosis has been published by Mudaliar et al in 2013^[91]. Younossi et al. recently reported the intermediate outcomes (after 18 months of treatment) of a phase III study evaluating the safety and efficacy of a daily dose of 10 or 25 mg of obeticholic acid in 931 patients (58% females) with F2/3 fibrosis (fibrosis evaluated by liver biopsy)^[92]. In MASH patients, obeticholic acid (at 25 mg dose) significantly improved liver fibrosis and several MASH disease activity indicators. Despite the encouraging results of this phase III trial, some questions persist (metabolic consequences, management of side effects including pruritus, and elevated LDL cholesterol in patients with elevated risk of cardiovascular disease). Consequently, obeticholic acid therapy should be studied in more detail in order to confirm its safety over the long term.

In addition to obeticholic acid, ursodeoxycholic acid (UDCA) is a frequently used FXR agonist. Bile acids such as UDCA, a naturally occurring hydrophilic bile acid, have been used for treating cholestatic liver disease for decades^[93]. Recently, UDCA has been considered a potential therapeutic agent for MAFLD. Patients with MASH showed a significant reduction in liver enzyme levels and steatosis with UDCA treatment in a small pilot trial^[94]. It is evident, however, that patients with MASH are ineffective when treated with UDCA^[95]. Therefore, the efficacy of UDCA for MAFLD/ MASH treatment

needs to be further confirmed.

Fecal microbiota transplantation (FMT) application to MAFLD treatment

A new approach to clinical treatment is FMT, in which fecal matter from a healthy donor is transplanted into the patient. Studies have shown that FMT can restore healthy microbiota and normalize blood lipid levels in patients with type 2 diabetes and ulcerative colitis^[96-100]. ¹Based on these reports, we can conclude that FMT is a potential therapeutic option for MAFLD and MASH (Table 3). A study involving mice with HFD-induced steatohepatitis showed that FMT was able to restore gut dysbiosis and increase cecal butyrate reduce endotoxin and inflammation factor generation, and ZO-1 concentrations in the small intestine^[100]. Researchers have found that after allogeneic FMT, patients with MAFLD experience a significant reduction in abnormal permeability of the small intestine.^[101] The efficacy and safety of FMT must be further assessed using high-quality clinical data. Furthermore, standardized protocols for sample preparation, archiving, formulations and dosages should be developed.

Conclusions

MAFLD is a common chronic liver disease progressing from simple steatosis to MASH and potentially to cirrhosis, a risk factor for liver cancer. It is now widely accepted that gut microbiota consisting of bacteria, archaea, fungi, viruses, and non-bacterial gut microorganisms, is closely related to chronic liver diseases. MAFLD is associated with disturbances in the gut-liver axis and intestinal microbiota, as evidenced by several recent studies. Therefore, it is logical to target the microbiota to alleviate MAFLD symptoms. Antibiotics, probiotics, prebiotics, synbiotics, and FXR agonists are safe and effective treatment options for MAFLD. Furthermore, FMT is a promising strategy for reversing the intestinal dysbiosis associated with MAFLD. For these agents to

be confirmed as effective in treating MAFLD, more well-designed and mechanism-based laboratory and clinical studies are required. It will also be important to examine the genomes of MAFLD patients to determine whether genetics can be a determinant of therapeutic response.

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