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Fecal microbiota transplantation for treatment of non-alcoholic fatty liver disease: Mechanism, clinical evidence, and prospect

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Abstract

The population of non-alcoholic fatty liver disease (NAFLD) along with relevant advanced liver disease is projected to sustain growing, whereas currently no medications are approved for treatment. Fecal microbiota transplantation (FMT) is believed a novel and promising therapeutic approach based on the concept of the gut-liver axis in liver disease. There has been an increase in the number of pre-clinical and clinical studies evaluating FMT in NAFLD treatment, however, existing findings diverge on its effects. Herein, we briefly summarized the mechanism of FMT for NAFLD treatment, reviewed randomized controlled trials for evaluating its efficacy in NAFLD, and proposed prospect of future trials on FMT.

Key Words: Non-alcoholic fatty liver disease; Fecal microbiota transplantation; Randomized controlled trial; Mechanism; Efficacy.

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Core Tip: There are several reviews contributed to the role of gut microbiome in the pathophysiology, therapeutic potential and clinical trials for non-alcoholic fatty liver disease (NAFLD). However, no consensus is available in the literature about the clinical efficacy of fecal microbiota transplantation (FMT) on NAFLD. This is a first review to summary recent randomized controlled trials for evaluating the effects of FMT on blood lipid profile, liver function, histological changes and other parameters in patients with NAFLD, discussed its therapeutic mechanism, and proposed the obstacles and prospect of FMT in future trials.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD), recently proposed as metabolic dysfunction-associated fatty liver disease (MAFLD), is a chronic liver disease with a global prevalence of about 25% in adult population[1]. NAFLD has a bidirectional association with components of obesity and metabolic syndrome. In view of the ongoing obesity epidemic worldwide, the rise in metabolic syndrome, and other factors, the population of NAFLD along with advanced liver disease is projected to sustain growth[2]. Although patients with NAFLD at early stage have no self-conscious symptoms, these with advanced NAFLD are at markedly increased risk of adverse outcomes, such as increased overall and liver-specific mortality[3]. Due to its large population base, NAFLD is emerging as a rapidly increasing cause of liver-related mortality worldwide[4] and an important cause of end-stage liver disease[5], thus leading to a substantial health economic burden.

The pathophysiology of NAFLD is extremely complicated and involves multiple pathogenic pathways that are not completely elucidated. On the other hand, there is currently no approved therapy for NAFLD, although several drugs are reserved for patients with biopsy-proven non-alcoholic steatohepatitis (NASH). In search of potential novel therapeutic options, the manipulation of gut microbiome has attracted a lot of attention. Evidence from human observational studies and animal experiments have found the alterations in gut microbiome in NAFLD and indicated numerous pathophysiologic mechanisms relating the gut microbiome to NAFLD[6-9]. In brief, gut dysbiosis damages the intestinal barrier integrity, leading to increased gut permeability to bacterial products and hepatic exposure to harmful substances, thus triggering fat accumulation, hepatic inflammation and fibrosis[10-12].

Considering the close link between gut microbiome and NAFLD, many therapeutic approaches targeting gut microbiota, such as probiotic, prebiotic or fecal microbiota transplantation (FMT) have been tested for that whether they could provide the clinical benefit of alleviation of NAFLD. FMT is the latest mode of gut microbiota manipulation for the treatment of NAFLD[13], which seems to be more effective than other existing approaches of microbiota manipulation. FMT from a healthy donor to the host is aimed

to quickly re-establish a healthy gut microbial community, which can help treat gastrointestinal diseases. As evidenced by several animal-based studies, FMT could effectively improve the manifestations of NAFLD[14-16]. On the other hand, there has been an increase in the number of clinical trials evaluating the role of FMT in NAFLD treatment[17-19]. Herein, we briefly summarized the mechanism, randomized controlled trials (RCTs), and prospect of FMT in the treatment of NAFLD.

MECHANISM OF FMT FOR NAFLD

Structure and function of gut-liver axis

The functioning of gut-liver axis is established on the interaction between the gut, along with its microbiota, and the liver, through the portal vein. The gut microbiota is a complex community consisting of over 100 trillion microbes, prevalently bacteria[20]. The interface between the microbiome and intestinal epithelial cells is the gut mucus barrier, physically separating the microbiota from the epithelial lining, thus providing protection against an exaggerated inflammatory response[21] (Figure 1). Thus, the mucus barrier serves as a first line of defense. Underneath the mucus layer is the intestinal barrier, which is formed of a monolayer of epithelial cells. Adjacent epithelial cells are sealed together by tight junctions[22], and they function as a physical shield preventing bacteria from crossing the gut into the portal circulation. In addition, some mucosal immune cells patrol the epithelium, and cooperate to protect against strikes arising from the microbiota and infectious agents[23]. In case the epithelium is breached, there is another barrier, the gut vascular barrier that prevents bacteria from entering the portal circulation and disseminating systemically[24].

Due to the multiple barriers as mentioned above, the most of bacteria cannot directly interact with the host, but through the mediation by the bacterial products and metabolites. With the aid of this indirect mediation, the gut microbiota participate in nutrient digestion and absorption, host metabolism, and mucosal and systemic immunity[25]. For instance, gut microbiota breaks down dietary fibers into short-chain fatty acids (SCFAs), which could provide energy support for the host cells. More

notably, they also have been shown to regulate lipid metabolism, protect intestinal mucosal barrier, control the differentiation of several immune cells, and participate in the microbicidal activity of macrophages[26-28]. In addition, gut microbiota transform the primary bile acids into secondary bile acids, which act as the natural agonists of intestinal farnesoid X receptor (FXR). FXR engagement can regulate its downstream defense genes to enhance epithelial barrier properties[29], reduce lipogenesis in the liver[30], and improve insulin sensitivity[31]. In summary, the homeostasis constructed by the balance of gut microbiome and the intact physiological barriers is critical for controlling the reciprocal interaction between the gut and the liver to maintain health.

Disruption patterns of gut-liver axis in NAFLD

Current data indicate that altered gut microbiome, along with bacterial components, impair the intestinal barrier and vascular barrier, and facilitate the influx of bacterial products into portal vein in NAFLD[11,29]. Noticeably, the liver is especially vulnerable to the insults from these bacterial products, which consequently aggravate the hepatic metabolic abnormalities and inflammation (Figure 1).

Several clinical studies have shown that patients with NAFLD have the remarkable gut dysbiosis, generally characterized by the over-growth of bacteria and changes in microbiota composition[32]. For instance, recent research has found an increased abundance of *Escherichia coli* and *Bacteriodes vulgatus* in patients with NAFLD[9]. Abnormalities in intestinal microbiota have been established a link with the reduced thickness of the mucous layer, as well as the increased intestinal permeability, in patients with NAFLD[33,34]. The damage of intestinal barriers loses control of the passage of bacterial components and metabolites, which can reach the liver *via* the portal vein. Some of these components are agonists of Toll-like receptor (TLR) signal pathway, which result in enhanced hepatic expression of inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), thus promoting inflammation and fibrosis[32,35]. The altered gut microbiome is also likely contribute to decreased production of SCFAs, which promotes lipogenesis and lipid deposition in NAFLD[36]. In

addition, gut dysbiosis reduces bioavailability of choline and increases portal influx of trimethylamine, which also contribute to hepatic steatosis in NAFLD[37]. Disruptive bile acid pool is another consequence of microbiota abnormalities, and it suppresses FXR-mediated signalling in the intestine and the liver, leading to increased lipid accumulation, oxidative stress and inflammation[38,39].

FMT for remoulding gut-liver crosstalk to treat NAFLD

Given these attacks induced by gut dysbiosis promote liver steatosis, inflammation and fibrosis, manipulation of gut microbiota is exploring as a therapeutic target for NAFLD. Probiotics, prebiotics and symbiotic have been explored for treating the patients with NAFLD[40-42]. However, it does not come to a clear conclusion substantially that whether these agents generate significant clinical improvement in NAFLD patients. Moreover, data on adverse effects links to the use of probiotics[43]. These issues have paved the road for FMT application in NAFLD treatment, which is expected to yield better clinical efficacy and less side effect.

The fecal material for FMT contains distal gut microbiota from a healthy donor and is processed into an odorless and tasteless preparation. In clinical practice, the approaches for FMT include oral administration of microflora liquid or capsule, transplanting to middle digestive tract by endoscopic biopsy hole, and threading through colonic pathway to lower digestive tract[44]. FMT is used to replenish a healthy gut microbial environment and restore physiological colonization, leading to the recover of microbial diversity and abundance[12] (Figure 1). The repair of gut dysbiosis can elevate intestinal permeability through rebuilding the physiological barriers (*i.e.*, mucus barrier, epithelial barrier)[17], and suspending the uncontrolled influx of bacterial products to the liver. The restoration of intestinal structure and function can improve lipid metabolism, decrease insulin resistance, suppress inflammatory response, and consequently attenuate the symptoms of NAFLD (*i.e.*, reduction of fat content, liver steatosis, serum transaminases levels and inflammatory infiltrates)[16,45,46]. Finally, FMT has been demonstrated well tolerated and safe over the long-term use[47,48].

EVIDENCE FROM RCTS ON FMT IN NAFLD TREATMENT

To review RCTs for NAFLD treatment using FMT, references indexed in databases (PubMed, EMBASE, the Cochrane Library, and Web of Science) were searched with the combination of following keywords: 'non-alcoholic fatty liver disease/NAFLD/fatty liver/non-alcoholic steatohepatitis/metabolic dysfunction-associated fatty liver disease/MAFLD/steatohepatitis/Liver steatosis/hepatic steatosis', and 'fecal microbiota transplantation/ fecal microbiota transplant/fecal microbiome transplantation/fecal microbiome transplant/FMT'. The eligibility criteria was determined based on PICOS principle (population, interventions, comparisons outcomes, study designs). The inclusion criteria were as follows: (1) Study design was RCT; (2) participants were diagnosed with NAFLD by either liver histology or noninvasive imaging; and (3) study results included one of the following outcomes: serum cholesterol, triglycerides, alanine aminotransferase (ALT), aspartate aminotransferase (AST), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL-C), homeostatic model assessment of insulin resistance (HOMA-IR), body mass index (BMI), or assessment of histological change by any of the following modalities: liver biopsy, MRI or elastography. Specifically, liver biopsy outcomes included determination of NAFLD activity score (NAS), necro inflammation score, fibrosis score, or steatosis score, MRI outcomes included estimated fibrosis stage, and elastography outcomes included fat attenuation degree or fibrosis stage. The exclusion criteria were as follows: (1) Participants were under 18 years; (2) participants had severe diseases except NAFLD; and (3) study reported incomplete data. As a result, a total of 2778 references were initially identified, and finally, 3 published RCTs undergone full-text assessment were included in this review. The detail of study selection was recorded in Figure 2.

Evidences from Included RCTs

The three RCTs were conducted between 2013 and 2021, comprised of 117 adult participants with NAFLD. Two trials[17,18] were double-blind while one[19] was open-label. Regarding FMT intervention, two studies performed allogenic FMT through duodenal infusion, while one adopted colonic infusion. Among the outcomes, serum levels of cholesterol, triglycerides, HDL-C and LDL-C were reported in all trials, while only one trial[18] reported the histological change (NAS score, necro inflammation score, fibrosis score, steatosis score) in patients between baseline and endpoint. In all trials, adverse effects of FMT were not reported. Details of the general characteristics of all included RCTs were given in Table 1.

A double-blind RCT conducted by Craven *et al*[17] included 21 patients to compare allogenic with autologous FMT. As a result, no significant changes was found in total triglycerides, cholesterol, HDL-C, LDL-C, HOMA-IR, weight, waist-to-hip ratio, BMI or hepatic proton density fat fraction in patients after FMT. However, patients experienced a significant increase in small intestinal permeability after FMT. The changes in fecal microbiota composition varied by individual in both groups after FMT. Moreover, a trend toward an increase in the fecal microbiota diversity was found in patients who had improvement in intestinal permeability, although changes in specific taxa were hardly discerned in allogenic transplant. It is noteworthy that the investigators only deployed FMT one time but evaluated its efficacy after 24 wk, and it was difficult to expect one session of FMT to achieve a long-lasting therapeutic effect, which could be one reason for the unsatisfactory results of most outcomes. In addition, this study was also limited by small sample size and lack of histological findings.

In another double-blind RCT conducted by Witjes *et al*[18], 21 participants with hepatic steatosis received either allogenic or autologous FMT. FMT administration was performed using duodenal infusion three times at 8-week intervals, with a duration of 24 wk. The results indicated a trend toward improvement in the necro-inflammation score, but finally no significant improvement in liver histology following allogenic FMT. Similarly, there was no significant improvement in biochemical parameters. However, significant changes in the expression of some hepatic genes associated with

inflammation and lipid metabolism were found in allogenic FMT group, compared with autologous FMT. The findings from fecal microbiota analysis suggested no significant changes in fecal microbiota diversity and composition between baseline and endpoint, in either groups.

Xue *et al*[19] carried out an open-label 4-wk RCT including 75 patients with NAFLD. Participators were randomly to receive allogenic FMT or oral probiotics. The fecal microbiota preparation was administered to patients by colonic infusion per day, for 3 d in total. The results showed no significant difference in blood lipid (*i.e.*, triglycerides, cholesterol, LDL-C, HDL-C) and liver function tests before and after treatment in either groups. However, significant decrease in liver fat attenuation degrees was found in FMT group, while significant increase was found in non-FMT group. Microbiota analysis indicated that certain bacterial contents had a trend toward healthy individuals after FMT. Furthermore, FMT had a greater impact on the microbial community structure in lean NAFLD than in obese NAFLD patients.

Pooled analysis of selected outcomes

The outcomes reported in more than two RCTs were selected for pooled analysis. The mean and/or standard deviation (SD) values of cholesterol, triglycerides, LDL-C, HDL-C, AST, ALT, BMI and HOMA-IR were extracted for assessment. Data were processed to obtain the changes between baseline and endpoint. The pooled effects were reported as weighted mean differences (WMD) with 95% confidence intervals (95%CI). Statistical analysis was performed using STATA 16.0 software (Stata Corp LLC, TX, United States) using a random-effects model. A *P*-value of < 0.05 was considered significant for the test for overall effect.

All three included RCTs[17-19] reported the changes of serum lipid profile (triglycerides, cholesterol, LDL-C and HDL-C) in patients between baseline and endpoint. The pooled results suggested that FMT failed to cause significant improvement of triglycerides (WMD -0.07, 95%CI -0.47 to 0.33, *P* = 0.74; Figure 3A), cholesterol (WMD -0.27, 95%CI -0.75 to 0.21, *P* = 0.27; Figure 3B), LCL-C (WMD 0.05,

95%CI -0.31 to 0.42, P = 0.78; Figure 3C), and HDL-C (WMD -0.05, 95%CI -0.22 to 0.15, P = 0.54; Figure 3D), as compared with control. There was no significant heterogeneity between studies. Two RCTs[18,19] reported the outcomes of ALT and AST, and pooled analysis suggested that FMT caused no significant reduction in ALT (WMD -0.51, 95%CI -22.57 to 21.55, P = 0.96; Figure 3E) and AST (WMD -2.78, 95%CI -10.26 to 4.71, P = 0.47; Figure 3F). Likewise, two RCTs[17,19] reported the outcomes of HOMA-IR and BMI, and pooled analysis found no improvement in them after FMT (HOMA-IR: WMD -1.09, 95%CI -2.43 to 0.25, P = 0.11, Figure 3G; BMI: WMD -0.64, 95%CI -2.73 to 1.44, P = 0.54, Figure 3H).

Although these negative results might challenge the potential of FMT in the treatment of NAFLD, their reliability could be attenuated by several limitations. Firstly, only a very small number of RCTs was retrieved, as well as a very small sample size. Moreover, the available data of histological outcome which is the major prognostic index of NAFLD, was insufficient for pooled analysis. Finally, the management of FMT administration to patients varied among studies, which might influence on the efficacy of FMT. Therefore, future studies including standardized FMT sessions in more patients with robust outcome measures are still needed.

PROSPECT

The only recommended treatment for NAFLD is weight loss. However, it is hard to adhere to lifestyle modifications for achieving it, making the need of effective and safe medications more urgent. In past years, numerous pharmacological therapies have been investigated, such as medications targeting glucagon-like peptide-1 (GLP-1), peroxisome proliferator-activated receptors (PPARs) and intestinal microbiota. There is considerable evidence that the composition of gut microbial in NAFLD patients change significantly from control individuals[9,11,49], which contribute to obesity, insulin resistance and liver steatosis[12,16,50]. FMT is an emerging tool for manipulating intestinal microbiota, which is considered as a potential therapeutic approach for NAFLD. Although current outcomes on FMT therapy for NAFLD vary between RCTs and the

most are gloomy, aforementioned RCTs still demonstrate that patients might benefit from FMT due to improvement of small intestinal permeability, alleviation of hepatic necro-inflammation, and liver fat attenuation. Furthermore, the results from the three RCTs and pooled analysis are hardly considered as robust due to apparent limitations. Therefore, FMT is still expected to be promising in NAFLD treatment, meanwhile, there are a series of obstacles should be resolved.

Firstly, the alterations of gut microbiota could be variable by individual with NAFLD, or/and by the stage of NAFLD, and sometimes the results are contradictory[51,52]. Thus, the significant heterogeneity lying in gut microbiota makes the restoring of varied gut microbiome by a fixed FMT hardly to be achieved, and also hinders the transforming of the success of one FMT directly to another population. Moreover, the characteristics of donor feces, in particular fecal microbiota richness, diversity, and compatibility, have considerable influence on the efficacy of FMT, while the rigorous criteria for donor selection has not yet been determined. Furthermore, repetitive FMT should be required to maintain the improvement of gut microbiome, however, the management of FMT is still casual across trials. Although the safety of FMT is evidenced, long-term consequences of FMT are unknown as FMT still has rare risks. Therefore, the practice guidelines of FMT still need to be extensively investigated. Finally, FMT combined with other pharmacological therapies are worth considering in future trials since NAFLD involves multiple pathogenic factors.

CONCLUSION

In summary, our work summarized disruption patterns of gut-liver structure and function in NAFLD, and how FMT remoulades the homeostasis of gut-liver crosstalk to alleviate NAFLD. As FMT is suggested as a therapeutic of great potential, increasing clinical trials are carried out to evaluate its efficacy in NAFLD. We reviewed the published RCTs to analyze the evidence on the clinical efficacy of FMT in NAFLD patients. FMT failed to yield clinical benefit in blood lipid (*i.e.*, triglycerides, cholesterol, LDL-C, HDL-C), and liver function (*i.e.*, ALT, AST) parameters. By contrast, the

improvement of small intestinal permeability and alleviation of hepatic necro-inflammation in patients after FMT could be encouraging. Whereas, the reliability of above results is challenged by several limitations, especially small sample size and casual FMT administration scheme. It is believed that some obstacles still need to be resolved before fully inspiring the potential of FMT in the treatment of NAFLD. Future high-quality trials in more patients adopting more scientific management of FMT are essential to further validate the clinical benefit of FMT.

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