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Review

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Microbiome manipulation with faecal microbiome transplantation as a therapeutic strategy in *Clostridium difficile* infection

B.H. MULLISH, J.R. MARCHESI, M.R. THURSZ and H.R.T. WILLIAMS

From the Section of Hepatology and Gastroenterology, Faculty of Medicine, St Mary's Hospital, Imperial College London, London, UK

Address correspondence to Dr B.H. Mullish Section of Hepatology and Gastroenterology, Faculty of Medicine, St Mary's Hospital Campus, Imperial College London, 10th Floor, QEQM Building, South Wharf Road, Paddington, London, UK. email: b.mullish@imperial.ac.uk

Summary

Faecal microbiome transplantation (FMT) has generated huge recent interest as it presents a potential solution to a significant clinical problem—the increasing incidence of *Clostridium difficile* infection (CDI). In the short term, however, there remain many practical questions regarding its use, including the optimal selection of donors, material preparation and the mechanics of delivery. In the longer term, enhanced understanding of the mechanisms of action of FMT may potentiate novel therapies, such as targeted manipulation of the microbiome in CDI and beyond.

Introduction

The concept of 'colonization resistance'—the ability of the healthy gut microbiome to inhibit colonization and overgrowth by invading microorganisms—has been recognized for over 40 years.[1] It is similarly well established that perturbation of the gut microbiome, or 'dysbiosis' (as may occur in response to antibiotics, along with other triggers) disrupts colonization resistance, with *Clostridium difficile* infection (CDI)-associated diarrhoea being the archetypal clinical manifestation.

Limitations of current antibiotic treatments for CDI have driven the search for novel treatments, with one option being faecal microbiome transplantation (FMT), i.e. generation of a liquidized bacterial suspension from the faeces of healthy donors, and delivery of this into the gastrointestinal (GI) tract of affected patients. Assessment of FMT in the setting of CDI has demonstrated that this is a viable treatment option.

The recognition that dysregulation of the gut microbiome is characteristic not just of CDI but a wide variety of human diseases[2] raises the possibility that manipulation of the composition or function of the gut microbiome could develop beyond CDI to be used more broadly as a therapeutic strategy.

CDI: a global problem

CDI ranges in clinical severity from mild diarrhoea to the life-threatening states of pseudomembranous colitis and toxic megacolon. Although the increasing impact of CDI over the past 15 years has been felt globally (with antibiotic use being the predominant risk factor), the burden has been greatest in Europe and North America.[3] One major factor contributing to this has been the arrival of newer, more virulent and increasingly antibiotic-resistant strains,

such as NAP1/ribotype 027. Although CDI acquisition still occurs most commonly in healthcare facilities, there has been increasing recognition of community-associated CDI, even amongst conventionally low-risk groups such as children.[4]

Standard therapy for CDI involves metronidazole for mild disease and vancomycin for severe or recurrent CDI (with pulsed/tapered regimens typically being used in recurrent disease[5]). Worryingly, however, the response to metronidazole has declined from ~90 to 70% over the past decade.[6] A further serious concern has been the increasing recognition of recurrent CDI. Recurrence occurs in ~20% of patients treated initially with either metronidazole or vancomycin[7]; the risk of further recurrence increases to 40% after a first recurrence, rising to 60–70% after more than two recurrences.[8] The presence of just three clinical criteria (age >65 years, severe disease and continued use of antibiotics after treating the initial CDI episode) are predictive of an almost 90% relapse rate.[9]

A number of different approaches have been proposed to address this problem, including intravenous immunoglobulin, probiotics, toxin binding and new antibiotics. An example of the latter is fidaxomicin, a macrocyclic antibiotic of narrow spectrum that is now approved for the treatment of CDI in Europe and North America following the outcomes of two randomized controlled trials. However, studies to date have not investigated the efficacy of fidaxomicin in cases of recurrent CDI, and alternative therapeutic strategies have been proposed.

Faecal microbiome transplantation

Efficacy

The recognition of CDI as a condition representing the loss of colonization resistance through antibiotic-associated gut dysbiosis prompted the hypothesis that reconstitution of the normal gut microbiota with FMT could be an effective therapeutic strategy. Many different techniques for the provision of FMT have been described, all with similar principles: collection of stool from a healthy donor (who has undergone screening for transmissible infections and has not recently used antibiotics); homogenization of stool (often in a domestic blender) and filtration of large particulate matter; and administration of the slurry into either the upper GI tract (via nasogastric or nasoduodenal tube), or the lower GI tract (via enema or colonoscopy).

At present, FMT to treat CDI has been described for over 500 patients in the literature, with efficacy rates of >90%. The time from receiving FMT until response is variable; a median time to resolution of 1 day was reported in a cohort receiving colonoscopic FMT.[10] Although uncontrolled case series of FMT for CDI have been reported for over a decade, the first randomized controlled trial comparing FMT to standard therapy was published only recently.[11] Patients with recurrent CDI were randomized to one of three treatment arms: vancomycin 500 mg four times daily for 4 or 5 days followed by bowel lavage and then FMT; standard vancomycin therapy (500 mg four times daily for 14 days); or standard vancomycin therapy with bowel lavage. The primary outcome was resolution of diarrhoea without relapse at 10 weeks. FMT consisted of at least 50 g of fresh stool from donors unrelated to the recipient that was blended with 500 ml of normal saline and filtered before immediate administration via a nasoduodenal tube. Trial participants who failed to respond in their initial treatment arm were offered FMT 'off protocol'. The trial was stopped early (after randomizing 42 patients) following an interim analysis that demonstrated significantly improved outcomes from FMT compared with other treatment arms, with cure rates of 89% in the FMT group (94% after two infusions), 31% in the standard vancomycin group and 23% in the vancomycin-bowel lavage group.

Safety and acceptability

A major concern regarding FMT has been the potential for transmission of infectious diseases from donor to recipient, although no such cases have been reported. As such, donor risk assessment through clinical, social and travel assessment—along with blood and stool screening for transmissible diseases—has been recommended[12] and widely instigated (Table 1).

FMT appears to be well tolerated with few significant side effects. In the trial of van Nood *et al.*,[11] the most common side effects included diarrhoea, cramping and belching, consistent with other studies. Symptoms tended to resolve quickly without specific intervention. Aspiration was not observed when 500 ml of solution was infused over ~20 min in these patients.[11] No significant adverse sequelae have been reported in FMT recipients over longer term follow-up.[10]

Poor acceptability of FMT to patients has been a concern, but this is not borne out in practice. Many patients with recurrent CDI (and other conditions for which FMT has been proposed as therapy[13]) actively seek out FMT providers, often via online forums. Furthermore, those who received FMT generally found the procedure acceptable: 97% of patients who had undergone FMT for recurrent CDI reported willingness to undergo further FMT if required, with 53% stating that they

Table 1 Screening protocol for transmissible diseases for potential donors to an FMT programme[a]

Body fluid	Assays
Blood	• Hepatitis A serology. • Hepatitis B serology and hepatitis B DNA. • Hepatitis C serology and hepatitis C RNA. • HIV-1 and -2 and HTLV-1 and -2 serology. • Syphilis serology. • Cytomegalovirus and Epstein-Barr Virus serology. • *Entamoeba histolytica* serology. • *Strongyloides stercoralis* serology.
Stool	• Microscopy, culture and sensitivity for enteric bacteria (at least three samples over three separate days). • Analysis for ova, cysts and parasites (at least three samples over three separate days). • Acid-fast staining for *Cyclospora, Isospora* and *Cryptosporidium* • *C. difficile* toxin and PCR • Analysis for Rotavirus and Adenovirus. • *Helicobacter pylori* faecal antigen.

[a]This is the screening protocol currently used in the programme at Imperial College London; assays are repeated 6 monthly. In addition, donors complete detailed questionnaires regarding their medical, family and medication history when being considered as donors, and questionnaires regarding recent symptoms suggestive of GI disease or infection (as well as recent travel) when donating stool for processing into FMT.

would choose FMT as first-line treatment before antibiotics.[10]

Practical aspects of FMT administration

Recruitment and screening of stool donors can be a difficult and expensive process. Therefore, the ability to use pre-screened 'universal donors'—who have provided stool that can be processed into FMT in advance, then frozen prior to thawing on the day of use—is attractive. A standardized protocol for frozen FMT preparation (using glycerol as a cryopreservative) has been recently reported,[14] with efficacy against recurrent CDI of at least 90% from colonoscopic FMT using both fresh and frozen stool.

The mechanics of preparing the solution have varied between centres. Typically, 50–60 g of stool is homogenized with 250–300 ml of diluent. Saline, water and even milk have all been used successfully as diluents. Most centres administer large-volume bowel lavage prior to FMT, often regardless of the route of administration (to remove residual clostridial organisms and any antibiotic remnants). Recipients typically stop antibiotics anywhere between 1 and 3 days prior to the transplant, although this has not been compared in a trial setting to continuing antibiotics up to or even after the procedure. It has become conventional practice to administer loperamide prior to colonoscopic administration (to aid retention), and a proton pump inhibitor prior to upper GI administration (to minimize gastric acidity). Whether upper or lower GI administration of FMT is more efficacious has been recently addressed in an open-label randomized controlled trial[15]: there was no significant difference in outcome between administration colonoscopically or via a nasogastric tube.

A viable treatment option

Consensus guidelines with regards to the role of FMT in CDI treatment have recently been published and broadly adopted[12] (Table 2). Some authorities argue that FMT should be considered early in the clinical course of CDI, and even as first-line therapy.[16] Experience of the use of FMT in severe CDI is more limited, but it appears effective in this setting.[17] Recent data suggest that FMT is of similar efficacy in immunocompromised patients, with no additional risk of infectious complications.[6]

FMT has been shown to be more cost effective than other treatment modalities for recurrent CDI.[18] Based on the available evidence, FMT is now recommended as treatment for recurrent CDI in professional guidelines both from the USA[5] and the UK, where FMT for recurrent CDI was recently advocated for use by NICE.[19]

Gut microbiome in human disease: from FMT to novel therapies

Recent research using molecular techniques (including sequencing of 16S rRNA genes and metabolic profiling platforms) has identified distinctive alterations in the composition and function of the

Table 2 Conventional indications and contraindications for use of FMT in the treatment of CDI (adapted from Bakken et al.[12,a])

Indications	Contraindications
• Recurrent or relapsing CDI: • At least three episodes of mild-to-moderate CDI and failure of a tapering vancomycin regimen, with or without an alternative antibiotic. • At least two episodes of severe CDI resulting in hospitalization and associated with significant morbidity. • Moderate CDI not responding to standard therapy (vancomycin) for at least a week. • Severe (and perhaps even fulminant) *C. difficile* colitis with no response to standard therapy after 48 h.	• Life-threatening food allergies, e.g. nut allergy. • Pregnancy or lactation. • Contraindication to preferred means of administration, e.g. oesophageal stricture limiting nasogastric tube insertion. • Patients with decompensated cirrhosis, uncontrolled HIV (CD4 count < 240 cells/mm^3), recent bone marrow transplant (within past 6 weeks), or other significant immunodeficiency. • Patients taking major immunosuppressive agents, including high dose corticosteroids (e.g. prednisolone ≥ 60 mg/day), calcineurin inhibitors, mTOR inhibitors, lymphocyte-depleting biologic agents, anti-TNF therapy, and recent use of chemotherapeutic anti-neoplastic agents (past 6 weeks).

[a]As described in the text, arguments have been made for using FMT earlier in the clinical course of CDI;[16] it may be that the concerns regarding the risk of FMT to treat CDI in immunosuppressed states have been overestimated.[6]

gut microbiome accompanying a wide range of human diseases.[2,20] Although many of these are primary GI/liver conditions (including inflammatory bowel disease, colorectal cancer and non-alcoholic fatty liver disease), many are not, including obesity, diabetes and even neurological conditions. Whether such dysregulation of the gut microbiome in disease states is causal or consequential remains largely unclear, although there is certainly now some evidence for the former: for instance, obese and lean phenotypes can be induced in germ-free mice by transfer of faecal microbiota from human twins discordant for obesity.[21] Similarly, men with metabolic syndrome who received FMT from lean male donors demonstrated an increase in gut microbial diversity and improvement in peripheral insulin sensitivity, when neither of these changes were seen when these individuals received FMT of their own processed stool.[22]

Such findings have clear implications for the screening of FMT donors; potential donors with any of the conditions in which gut microbiome dysregulation have been consistently linked are typically excluded. Additionally, given that FMT has demonstrated that sustainable alterations in the gut microbiome are achievable, the manipulation of the gut microbiome's structure (or modification of its functional activity) has been highlighted as a potential new mechanism of therapeutic intervention for a broader range of diseases.[23]

If 'microbiome therapeutics' are truly to represent a novel treatment modality, then the means by which such therapy may be optimally administered must be established. FMT has already been used as an experimental treatment for a number of conditions other than CDI, although the results to date have been highly variable.[24] Furthermore, FMT in itself clearly has significant drawbacks (not least its unpalatable nature), and other techniques for achieving manipulation of the microbiome merit exploration. One favoured idea is the administration of a 'defined microbiome ecosystem' of selectively cultured bacterial strains (ideally either as a drink or an oral capsule containing a mixture of lyophilized bacteria) that target specific dysregulated components of the gut microbiome.[25] An alternative strategy may be to design drugs that modulate microbial signalling or enzymatic activities and alter host metabolism.[23]

Conclusion

FMT is gaining widespread acceptance as a viable treatment option for CDI. Ongoing trials will help to clarify the uncertainties that still exist regarding the optimal means of administration. The recent identification of gut microbiome dysregulation as a feature of a broad range of diseases has raised the possibility that the success of FMT for CDI may be transferrable to other conditions, although the potential contribution of the microbiome to the pathogenesis of many of these diseases is much less well characterized than in CDI. Mechanisms of manipulating the gut microbiome in a more targeted way than FMT are clearly of great potential interest. The key next step is to understand the mechanism by which FMT exerts its efficacy in CDI, and further to explore the interaction between the gut microbiome and host metabolism in both health and disease states

(and the factors that influence this, including diet and antibiotic use). The true potential of 'microbiome therapeutics' may then begin to be realized.

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