

CASE REPORT

Fecal microbiota transplantation augmented by a sulfide-reducing diet for refractory ulcerative colitis: A case report with functional metagenomic analysis

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Abstract

Fecal microbiota transplantation (FMT) is effective for induction of remission in ulcerative colitis (UC). Diet has potential to augment the efficacy and durability of FMT by encouraging engraftment of transplanted microorganisms. A trial of FMT combined with a defined diet was undertaken as salvage therapy for a 71-year-old woman with active steroid-refractory extensive UC. A multidimensional sulfide-reducing diet (4-SURE diet) was commenced followed by single-donor FMT administered by colonoscopy and then enemas over 7 days. Dietary adherence, clinical evaluation, and stool samples for metagenomic profiling were undertaken at weeks 0, 4, 8, and 24. Colonoscopy was performed 8 weeks post-FMT. Shotgun metagenomic profiling of the donor fecal suspension was also performed. A rapid clinical response to FMT and 4-SURE diet was observed with normalization of stool frequency (≤ 2 motions/day) and resolution of rectal bleeding within 2 weeks. Dietary adherence was excellent. Colonoscopy at week 8 revealed no evidence of active colitis (Mayo endoscopic sub-score 0) with histology showing no evidence of acute or chronic lamina propria inflammatory cell infiltrate. Sustained clinical and endoscopic remission out to 24 weeks was observed. Metagenomic sequencing confirmed sustained engraftment of beneficial donor microbiota with increased alpha-diversity and capacity for short-chain fatty acid production, including *Faecalibacterium prauznitzii* and *Eubacterium hallii*. This case report supports the rationale of prescribed diet therapy to support engraftment of donor microbiota following FMT for UC. Further large trials with a diet-arm control group are needed to evaluate FMT augmented by a defined diet in UC.

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Introduction

Ulcerative colitis (UC) is a lifelong relapsing and remitting inflammatory disease of rising global prevalence.¹ The gut microbiota contribute to the pathogenesis of UC, however, current therapies for UC are “immunocentric,” solely targeting the immune response to the gut microbiota without altering the luminal environment. Fecal microbiota transplantation (FMT) involves transferring gut microbiota from a healthy individual to another with disease to restore diversity and function. Early data show FMT can be used for induction of remission in UC with rates of clinical and endoscopic remission comparable to available biologic therapies.²

Fermentable dietary substrate has a key role in shaping the composition and function of the gut microbiota, including modulation of potentially harmful microbial metabolites such as hydrogen sulfide (H₂S). Colonic fermentation of excess animal protein increases production of H₂S, whereas fermentation of dietary fibers suppresses and attenuates H₂S production via the gut microbiota preferentially fermenting complex carbohydrate over protein sources that would otherwise produce H₂S.^{3,4} Emerging observational data suggest modulation of fermentable fibers, total protein, and sulfur proteins, and sulfated food additives, strategies central to the 4-SURE diet (*FOUR* strategies to *Sulphide-REduction*), are efficacious in inducing clinical and endoscopic response in UC.⁵ Moreover, a single case report used combined FMT and 4-SURE diet as therapy and reported induction and maintenance of clinical and endoscopic remission out to 12 months.⁶

It is biologically plausible that a specific diet may augment the efficacy and durability of FMT by providing a favorable luminal milieu, encouraging engraftment of transplanted microorganisms and restoring luminal homeostasis.

Case Report

A 71-year-old woman with steroid-refractory extensive UC underwent microbial manipulation with FMT and 4-SURE diet, resulting in sustained clinical and endoscopic remission out to 24 weeks. Metagenomic sequencing confirmed sustained engraftment of beneficial donor microbiota.

Diagnosis. She was diagnosed with left-sided UC at the age of 54 years. Her disease was initially responsive to sulfasalazine therapy; however, over subsequent years she developed steroid-dependent disease complicated by osteopenia. Six months before FMT she was hospitalized for severe *Campylobacter jejuni* gastroenteritis. Thereafter, her disease became steroid-refractory,

with persistent bloody diarrhea (>6 bloody motions/day). Colonoscopy 4 months following hospitalization revealed severe extensive colitis characterized as Mayo endoscopic sub-score 3. Biopsies confirmed moderately active chronic colitis without features of cytomegalovirus infection, and both biopsies and serial stool cultures excluded persistent bacterial infection.

Escalation to immunomodulator or biologic therapy was precluded on account of concurrent medical comorbidities and patient reticence. Given salvage colectomy was the next most appropriate step in management, a trial of FMT combined with 4-SURE diet was undertaken. Usual therapy with sulfasalazine was continued. Ethics approval was granted (Central Adelaide Local Health Network HREC/18/CALHN/646) and written informed consent gained from the patient.

Single-donor FMT was prepared and administered as per a previously described protocol.⁶ Following a polyethylene glycol bowel preparation, 200 mL of thawed fecal suspension was instilled into the patient's caecum via colonoscopy. Two further 100 mL aliquots of the same fecal suspension were administered by enema over the following 7 days.

Prior to FMT, habitual diet was assessed using a 7-day weighed food diary. A research dietitian provided individualized 4-SURE dietary advice with a 1-week set meal plan and recipes to be followed for 8 weeks.⁵ The 4-SURE diet was commenced first, followed by FMT. Adherence was assessed at weeks 4, 8, and 24 using a 3-day weighed food diary and self-reported dietary adherence checklists (≥76–100% always adherent).⁵

Stool samples were collected prior to FMT (week 0), and at weeks 4, 8, and 24 for metagenomic profiling and calprotectin. Clinical evaluation and blood testing including C-reactive protein were also undertaken at these time-points. Colonoscopy was performed 8 weeks post-FMT. Shotgun metagenomic profiling of the donor fecal suspension was performed as per established methods at Microba Life Sciences.

Outcome

Clinical. The patient reported a rapid clinical response to FMT and 4-SURE diet, with normalization of stool frequency (≤2 motions/day) and resolution of rectal bleeding within 2 weeks. Corticosteroid taper and cessation were achieved within 6 weeks. She remained in steroid-free clinical remission for 24 weeks following FMT and 4-SURE diet. Concordant with clinical improvement, biomarkers of inflammation normalized by 8 weeks following FMT (Fig. 1). Colonoscopy at week 8 revealed no evidence of active colitis (Mayo endoscopic sub-score 0) with histology showing no evidence of acute or chronic lamina

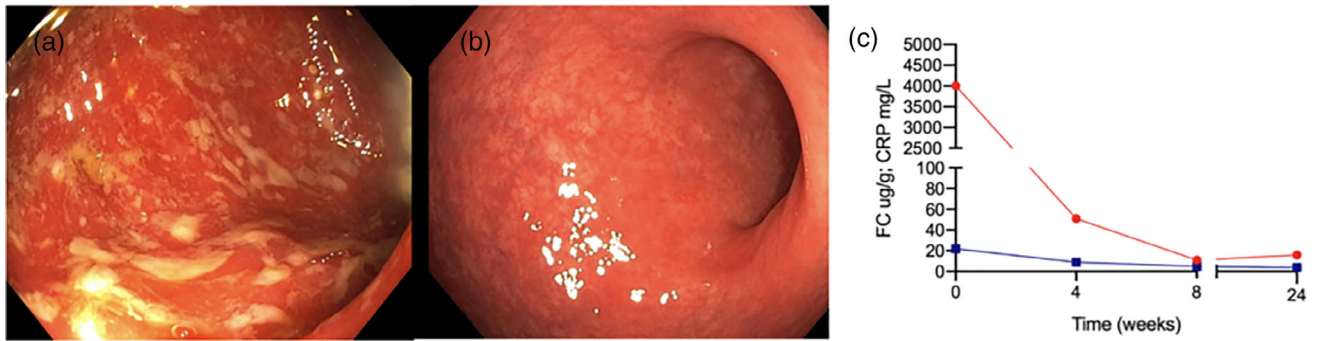


Figure 1 (a) Week 0 (day of fecal microbiota transplantation [FMT] and diet) showing ulceration, loss of vascular pattern, and erythema (endoscopic Mayo 3). (b). Week 8 post-FMT and diet showing normalization of colonic mucosal appearances with no visible evidence of inflammation (Mayo 0). (c) Graph of fecal calprotectin (FC) and C-reactive protein (CRP) over time course from commencement of FMT and dietary therapy (week 0) until week 24.

propria inflammatory cell infiltrate (Fig. 1). Durable remission and persistent normalization of inflammatory biomarkers were evident 24 weeks post-FMT and diet therapy (Fig. 1).

Diet. The 4-SURE diet was well tolerated with excellent self-reported adherence sustained to 24 weeks. This included maintaining a protein intake of 1.0–1.2 g/kg (75–90 g/day), increasing fermentable fibers to 25–30 g/day, and avoidance of specified food additives. Reduction of sulfur amino acids was not achieved. Food-related quality-of-life score improved from 61 to 116 (poorer 29, greater 145).

Functional microbiome analysis. A rapid change in the patient's microbiota was observed following FMT, with a shift toward the donor's microbiota profile (Appendix S1). Overall, the patient's microbiota remained stable in terms of most abundant organisms out to 24 weeks post-FMT, illustrating stable engraftment of the donor's microbiota profile. Moreover, an increase in alpha-diversity was observed over the follow-up period associated with the 4-SURE diet, with a steady increase in the patient's Shannon Diversity Index from 2.86 prior to FMT treatment, to 2.83 at 4 weeks, 3.19 at 8 weeks, and then 3.38 at 24 weeks, approximating the donor's Shannon Diversity Index of 3.32 at the time of stool donation.

At a species level, it was possible to categorize changes in the microbiota post-FMT to better conceptualize shifts in the ecosystem (Appendices S1 and S2). Engrafted species (Group C) were those not detectable in the patient at baseline and engrafted following FMT. These organisms may be drivers of the therapeutic effect of FMT or benefactors of the therapy. This included avid producers of short-chain fatty acids (SCFAs) such as *Faecalibacterium prausnitzii* and *Eubacterium hallii*. Suppressed species (Group D) were those present in the patient at baseline but contracted or became undetectable following FMT. Some of these organisms may be drivers of luminal inflammation or benefactors of an oxygen-rich inflamed microenvironment, including species with toxigenic potential such as enterotoxigenic-positive strains of *Bacterioides fragilis*.

The metabolic potential of organisms based on functional genes was extrapolated from metagenomic data. An increase in the relative abundance of organisms with capacity to biosynthesize

SCFAs was observed in the patient following FMT, sustained over 24 weeks (Appendix S2). Concurrently, loss of capacity for both *Bacteroides fragilis* toxin production and histamine production was evident. Sulfate-reducing capacity was diminished over the 24-week study period, consistent with preferential carbohydrate fermentation and suppression of protein fermentation.

Discussion

This is the first report demonstrating durable efficacy and sustained remission associated with microbial engraftment of induction FMT augmented by a diet designed to modulate colonic fermentation and sulfide metabolism in a patient with refractory UC. Mechanistic insights provided by functional microbiome analysis illustrate an exciting synergistic combined approach to microbial manipulation in UC.

Higher rates of FMT efficacy are thought to be related to a greater biodiversity and abundance of microbes as presumed with pooled donor FMT compared with autologous FMT.⁷ Oxygen-sensitive viable anaerobes such as *F. prausnitzii* are also more likely to be preserved through anaerobic preparation.⁷ As dietary substrate also directly influences microbial biodiversity and abundance, it is possible the diet of FMT recipients could affect engraftment and efficacy of FMT. The 4-SURE diet is hypothesized to facilitate donor organism engraftment and microbial diversity and modulate excess H₂S production at the colonic-luminal interface by reducing availability of dietary protein for reduction by sulfate-reducing bacteria and concurrently increasing the variety of slow and readily fermentable carbohydrates for degradation by SCFA-producing organisms.⁵ This “switch” to preferential carbohydrate fermentation increases the bioavailability of SCFAs as an energy source for colonocytes.⁸ The sustained increase in SCFA-producing organisms and sulfate-reducing capacity observed is plausibly related to bioavailability of 4-SURE dietary substrate and suppression of protein fermentation and yields insights of the possible mechanisms of efficacy of diet and FMT in UC.

Beyond a single pilot study, longevity of efficacy of FMT following an induction regimen is limited. Sood *et al.* reported significantly higher rates of endoscopic and histological remission using 8-weekly FMT via colonoscopy following a rigorous

multi-session FMT induction, with no difference in steroid-free remission between FMT and placebo groups at 48 weeks.^{6,9} Only one other FMT study in UC has provided functional metagenomic data, similarly showing an increase in alpha-diversity following FMT in those who achieved remission, as well as enrichment in SCFA-producing organisms including *E. hallii*.¹⁰ Interestingly, in contrast to the findings of this case report, *B. fragilis* was associated with a favorable donor microbial profile. However, *B. fragilis* has also been described as a part of a UC microbial signature, and enterotoxigenic *B. fragilis* strains are associated with UC flares.

Collectively, the possibility of microbial manipulation using FMT and a defined diet to achieve durable remission in UC holds promise and warrants further study in high quality randomized-controlled trials using a control diet-arm.

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Patient consent

Written informed consent was gained from the patient.

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Supporting information

Additional supporting information may be found in the online version of this article at the publisher's website:

Appendix S1. Supporting information.

Appendix S2. Supporting information.