Fecal microbiota transplantation is an effective rescue therapy for refractory inflammatory bowel disease

Bota Cui1*, Pan Li1*, Lijuan Xu1, Zhaoyuan Peng1, Youquan Zhao2, Huiquan Wang3, Zhi He1, Ting Zhang1, Guozhong Ji1, Kaichun Wu4, Daiming Fan4, Faming Zhang1

1Medical Center for Digestive Diseases, the Second Affiliated Hospital of Nanjing Medical University, 121 Jiang Jiayuan, Nanjing 210011, China
2School of Precision Instrument and Optoelectronics Engineering, Tianjin University, 92 Weijin Road, Tianjin 300072, China
3Biomedical Engineering Department, School of Electronics and Information Engineering, Tianjing Polytechnic University, 399 Binshui west street, Tianjin 300378, China
4State Key Laboratory of Cancer Biology & Xijing Hospital of Digestive Diseases, the Fourth Military Medical University, Xi’an 710032, China

*These authors contributed equally to this work.
Correspondence: Faming Zhang
E-mail: fzhang@njmu.edu.cn
Received: March 27, 2015
Published online: April 13, 2015

Increasing evidence has indicated the potential role of fecal microbiota transplantation (FMT) for treatment of inflammatory bowel disease (IBD). However, the protocol of FMT preparation has not been standardized. Importantly, the literature of using FMT to treat IBD is very limited. Therefore, we aimed to establish standardized laboratory protocol and clinical work flow, including donor identification, purification of fecal microbiota, bank of frozen fecal microbiota, endoscopic infusion procedures, patient preparation and clinical evaluation system. A novel automatic system (GenFMTer) was designed and developed for preparation of fecal microbiota from feces. The results from our registered trials (NCT01790061, NCT01793831) demonstrated the rescue therapy role of FMT for refractory IBD. A recent study reported that single FMT through mid-gut might be an effective therapy for refractory Crohn’s disease.

To cite this article: Bota Cui, et al. Fecal microbiota transplantation is an effective rescue therapy for refractory inflammatory bowel disease. Inflamm Cell Signal 2015; 2: e757. doi: 10.14800/ics.757.

Introduction

Increasing studies have demonstrated the link between the dysbiosis and the pathogenesis of inflammatory bowel disease (IBD) [1, 2], which indicates that the reconstruction of intestinal microbiota should be propounded as a new strategy for IBD treatment [3, 4]. Fecal microbiota transplantation (FMT) has been proved to have fascinating efficacy in the treatment of recurrence Clostridium difficile infection (RCDI) by a randomized controlled trial [5], and is recommended for RCDI [6]. The more interesting trend is that FMT also has been used to treat IBD in several medical centers worldwide [7-9]. Two registered clinical trials for refractory Crohn’s disease (CD) and ulcerative colitis (UC) (NCT01790061, NCT01793831) are ongoing at our center.

Our recent report on FMT for CD showed that the rate of clinical improvement and remission at the first month was 86.7% (26/30) and 76.7% (23/30) respectively. These results indicated the promising application of FMT for IBD. However, the results varied among different case or case serials from different centers [10-14]. In Borody’s study [15], overall 91.9% (57/62) of UC patients had response to FMT, and 67.7% (42/62) got clinical remission based on their
24-years’ experience. Sha’s review [16] reported close to 90% patients with UC and overall 78.4% of patients with IBD achieved clinical remission after FMT. Another review reported that 45% (54/119) of patients with IBD achieved clinical remission, efficacy in UC and CD were 22% and 60.5%, respectively [17]. Conversely, Patrizia K [18] reported that no efficacy was shown in six patients with refractory UC by a single colonoscopic FMT. The variations might be related to the different criteria in different medical centers, including patient inclusion, evaluation methods, FMT lab protocol, delivering way and amount of fecal microbiota (not equals to fecal suspension). It is necessary to establish a standardized protocol to confirm the efficacy of FMT and use this therapy. Our recent study presented the efficacy of a standardized FMT procedure established in our medical center for the patients with refractory CD. Herein, we discussed the methodology and efficacy of FMT in detail.

**Methodology of FMT**

The defined standardized FMT protocol involves the standardization of donor identification, purification of fecal microbiota, bank of frozen fecal microbiota, delivering of microbiota and a clinical work flow including patient preparation and clinical evaluation.

**Donor identification**

The criteria of donor screening was modified based on Brandt’s report [19], and was used to minimize the risk of infection diseases or specific changes of intestinal microbiota. The age of donor in our study was ranged from 8 to 15 year-old. The first reason for the age criteria is safety consideration. Children have less risk of sexual transmitted diseases than adults in China because they generally have no sexual life in this age period. The second consideration is from the literature evidence. The donor selection for the concept of FMT in Traditional Chinese Medicine was recommended to choose children aged around 12 year-old. Once the donor was defined, drinking or eating must be followed in accordance with the instruction of donor’s food preparation at least 3 days before feces collection. Twenty gram of Forlax (macrogol 4000) was given orally at the night before defecation.

**Fecal microbiota purification**

In our reported protocol [20], fresh feces were collected and added with 0.9% saline, mixed with blender thoroughly, and filtered with a special micro-strainer, then centrifuged the suspension and removed the supernatant, washed the precipitation with saline, and repeated the steps of centrifugation and washing for 4 times. The last precipitation was suspended with 3-fold volumes of saline and got the final production. The compositions of fecal microbiota before and after purification were detected by metagenomics analysis in previous study [20], which showed the similar microbiota composition between purified fecal microbiota and original feces. The results indicated the purification procedure should not affect the composition of microbiota.

However, we never used the reported protocol again. Recently, an automatic microbiota purification system named GenFMTer (FMT medical, Nanjing, China) was developed by our team and cooperators (Figure 1) [21]. This automatic system normalized the procedure of microbiota purification, excluded the bias of manual operation, kept the quality and quantity of microbiota production, and diminished the uncomfortableness of operators. The total spent time of whole procedure based on GenFMTer from feces collection to the last step before delivering the final product into patients’ gut was controlled within 60 minutes. The time of processes within GenFMTer was less than 15 minutes. This system and protocol should be helpful to reduce the exposure

**Figure 1. Infusion laboratory enriched fecal microbiota during endoscopy and the automatic system of fecal microbiota purification.** (A), (B) The image during infusion showed no observable particles under magnified endoscopic view with the fecal microbiota suspension indicated the effect of purification for fecal microbiota. (C) The automatic machine GenFMTer and the control system (FMT medical, Nanjing, China) used for fecal microbiota purification.
Bank of frozen fecal microbiota

The freshly purified fecal microbiota was used to transplant directly or was stored at -80°C in fecal microbiota bank for later use. Before infusion, the frozen microbiota was thawed at room temperature and centrifuged to remove the preservation solution, 3-fold volumes of saline was added for suspending the microbiota. This bank was the first non-profit bank in China and named as fmtBank. The procedures at fmtBank was standardized and was open to all hospitals for providing rescue therapy for refractory intestinal bacterial infection with the cooperation of the Second Affiliated Hospital of Nanjing Medical University (Nanjing, China) and Xijing Hospital of the Fourth Military University (Xi’an, China).

Delivering way

The suspension of fecal microbiota was delivered into the mid-gut of patients through the channel of gastroscope in our previous study [20]. Because of the colon or para-anus lesions the suspension might not be delivered into deep colon or hold with enough time in patients with IBD. The delivering way through mid-gut at least for IBD should be better than that through colon or enema. In our clinical practice, it was also infused through stoma in some patients if necessary according to their intestinal conditions. The delivering ways also were reported by nasogastric tube, oral microbiota capsule in other studies [22, 23]. The efficacy of different infusion methods had been discussed in the treatment of CDI [19, 23], and delivered by colonoscopy was recommended. However, in IBD, there was no evidence to indicate significant difference of FMT efficacy among various infusion methods.

Clinical work flow

To evaluate the efficacy of FMT for CD, a clinical work flow including patients’ preparation and clinical evaluation was designed as supplementary figure 1 in our previous study [20]. All medicine or treatments of patients were stopped at one week before FMT. Mesalazine were given for avoiding the observation bias after FMT for maintenance therapy. The clinical symptoms, laboratory examination were evaluated at the scheduled time points. Sample of feces, urine and serum were collected simultaneously for fecal microbiota, metabolomics or proteomics analysis. Endoscopy, magnetic resonance imaging or computed tomography scan were performed for evaluation in partial patients at six months after FMT. The preparation before gastroscopy procedure for reducing the infusion time also should be highlighted here.

Results and Discussion

The FMT in this study showed a fast clinical response to the refractory CD in the first week after FMT, and the best efficacy of clinical improvement and remission was appeared at one month after the treatment. Four of seven patients who met the 15-months follow-up kept in remission, the longest remission until now had 27 months. Figure 2 showed the colonoscopic follow-up of a patient with refractory CD complicated with fistula, formation of intraperitoneal large inflammatory mass. (A) Severely inflamed sigmoid colon before FMT; (B) The improved sigmoid colon at 10 months after FMT; (C) The colonoscope passed through whole colon smoothly and showed the scar of previous ulcerative lesions in ascending colon.
after FMT, and then relapsed in short-terms. This phenomenon hints that these patients might benefit from repeated or sequential FMT therapies. To confirm this conception, a sequential therapy based on FMT was designed for the treatment of patients with steroids-dependent UC, which had obtained an encouraging result and will be published in another journal. It is unclear that if the efficacy of FMT was related to the degree of intestinal microbiota reconstruction. The compositions of fecal microbiota at different time points are being analyzed by 16sRNA sequencing by our group, and the correlation between clinical response and microbiota remodeling will be revealed in our coming reports.

FMT also showed surprising efficacy in the remission of extra-intestinal manifestation. Figure 3 (A) and (B) had shown the inflammation of reversal stoma (communication between abdominal skin and colon) was improved after FMT in a 25-year-old patient with CD after ileocolonic resection. And Figure 3 (C) and (D) showed the heavy tongue coating in a 22-year-old male with fistulizing CD (without fungus infection) was improved dramatically within 3 days after FMT. IBD related skin lesions may be caused by the autoinflammatory skin disorder \[24\] or the atypical mycobacteria infection \[25\] with immunocompromised. Our study reported 8 of 11 (72.7%) patients with skin lesions achieved remission within two weeks after FMT, which may be related to the regulation of immune homeostasis by reconstruction of intestinal microbiota.

The CD-related sustained abdominal pain of the patients was relieved by FMT (Figure 3). Abdominal pain in IBD is probably secondary to peripheral sensitization and inflammatory-induced visceral hypersensitivity \[26, 27\], which leading to misery, depression, and impacting patients’ daily life, and was recognized as a clinically challenge for a physician. Twenty-seven patients (including one case who was excluded for the complication of CDI in the report) who had sustained abdominal pain were treated with FMT, and 26 of them showed an immediately improvement and kept for more than one month (Figure 3). The CD-related pain remission induced by FMT implied a novel clue on the management of pain by remodeling gut microbiome.

Blood biochemistry examinations had revealed the
significant elevation of plasma hemoglobin, albumin, HDL, LDL and total cholesterol at 3 months after FMT. LDL were reported to decreased in activated IBD patients for the activation of lipoprotein-associated phospholipase A2 [28], an enzyme to hydrolyze oxidized phospholipids of LDL. The increasing level of plasma LDL indicated the remission of the IBD [28].

Conclusions

Overall, our results based on the protocol of standardized FMT through mid-gut showed an exciting efficacy for the patients with refractory CD, which inducing the clinical improvement or remission and the amelioration of extra-intestinal manifestation without severe adverse events. With the discussion of our results from UC, it should be concluded that FMT might be a novel, safe and efficient therapy for refractory IBD.

Conflicting interests

The authors have declared that no competing interests exist.

Acknowledgments

We thank our funding sources including public donated Intestine Initiative Foundation, Clinical Science and Technology Foundation of Jiangsu Province (BL2014097), the National Science and Technology Major Project (2012BAI06B03) and National Gastroenterology Research Project (2015BAI13B07).

References


