











Faecal Microbiota Transfer – a new concept for treating cytomegalovirus colitis in children with ulcerative colitis

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Abstract

Introduction. Cytomegalovirus (CMV) infection in patients with inflammatory bowel disease (IBD) is reactivated by the use of immunosuppressive drugs. CMV infection may produce IBD flares refractory to standard therapy.

Objective. The aim of our study was to assess the efficacy and safety of faecal microbiota transplantation (FMT) for the treatment of CMV colitis in patients with ulcerative colitis (UC) flare.

Materials and method. A total of 8 children, with mild to severe UC, positive for CMV PCR in colonic biopsies, received 50–100 ml FMT by nasogastric tube on 5 consecutive days in each of 2 weeks. During the study, the subjects were treated with 5ASA and FMT. Immunosuppressant therapy was withdrawn, when CMV colitis was diagnosed by positive DNA PCR in colonic tissues. The clinical response was defined as a decrease of Paediatric UC Activity Index by ≥ 20 points.

Results. At the 6th week of the study, negative colonic CMV DNA PCR was measured after 10 infusions in 7/8 patients. For one boy, 20 infusions were administered to assess CMV elimination from colonic biopsies. A clinical response was observed in 3/8 patients, with clinical remission in 3/8 patients. Faecal calprotectin decreased significantly in 3 patients. CRP normalized in 2 patients after 6 weeks. No serious adverse effects were observed during and after infusions.

Conclusions. FMT seems to be an effective and safe treatment option for CMV colitis in children with UC. This is the first study to demonstrate the application of FMT as a new therapeutic option for CMV colitis.

Key words

Inflammatory bowel disease, faecal microbiota transplantation, cytomegalovirus infection

INTRODUCTION

Cytomegalovirus (CMV) is a DNA virus commonly found worldwide among all age groups. After primary infection, which is usually asymptomatic, the virus is retained in mononuclear cells of the host. In immunocompetent hosts, CMV remains in a latent form for the duration of the host's life. However, in immunocompromised individuals, CMV can be reactivated from the latent stage, leading to the production of new viral particles. CMV infection is diagnosed by PCR (polymerase chain reaction) or positive serologic tests. CMV disease, such as CMV colitis, may be diagnosed when the virus causes significant clinical symptoms. CMV reactivation is most commonly triggered by immunosuppressive drugs [1].

Patients with inflammatory bowel disease (IBD) are considered to be immunosuppressed, mainly due to the immunosuppressive treatment they receive, including steroids, immunomodulators and biologicals, as well as malnutrition. Although the data are limited, it seems that CMV reactivation is common in patients with severe IBD

flares, especially in steroid-resistant patients with colonic involvement, with a reported prevalence of 4. – 16.6% that can be as high as 25% in patients requiring colectomy for severe colitis [2–4]. Some authors have noted that patients with CMV have a higher mortality and colectomy rate [5].

Because of the deleterious impact of CMV infection on the course of IBD, the European Crohn's and Colitis Organisation (ECCO) recommends that patients with acute steroid-resistant colitis be screened for CMV infection, preferably by tissue PCR or immunohistochemistry. In patients with confirmed CMV disease, discontinuation of immunosuppressive drugs should be considered and antiviral therapy initiated [6]. The ECCO guidelines recommend treatment with intravenous ganciclovir and an eventual switch to oral valganciclovir as the first line of treatment. Although the data are not entirely consistent, the efficacy of ganciclovir was found to range between 75% – 100% in patients with ulcerative colitis (UC) [7]. Moreover, treatment with ganciclovir significantly reduced the colectomy rate, especially in those patients with high grade CMV infection [8]. Unfortunately, the adverse effects of ganciclovir are serious and include neutropenia, thrombocytopenia, rash, hypotension, nausea, vomiting and headache [9]. In the case of ganciclovir resistance or intolerance, the ECCO

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recommends foscarnet as an alternative. More recently, granulocyte/monocyte adsorptive apheresis (GMAA) was found to be effective for the treatment of UC flares-up with associated CMV reactivation [10, 11]. However, GMAA is not currently recommended by the American or European guidelines.

Currently, it is well established that the microbiota is a key factor in maintaining the health-inflammation balance in the gut. An incompletely defined imbalance of the gut microbiota, called dysbiosis, may lead to the exacerbation of IBD. Dysbiosis is also responsible for reinfection of gut pathogens, such as *Clostridium difficile* and, probably, CMV. Recently, the most promising therapeutic option for *Clostridium difficile* infection (CDI), both in patients with and without IBD, is faecal microbiota transplantation (FMT). There are some similarities between CDI and CMV reinfection in patients with IBD. First, both pathogens may be present in the gut without any clinical symptoms; the exact factors that reactivate them to trigger IBD are still unknown. Second, immunosuppressive treatment favours the 2 infections. Third, both infections are more common in UC patients. Beside the fact that FMT is effective in treating of CDI, FMT has also been shown to induce remission in adults with UC in randomized trials [12]. Taking these facts together, the efficacy of FMT for the treatment of UC and for the treatment of coexisting gut infections, it seems reasonable to apply FMT in active colitis with CMV reinfection. To-date, such a study has not been performed in adults and children with UC.

OBJECTIVES

The aim of the study was to assess the efficacy and safety of FMT in the eradication of colonic CMV infection in patients with UC flare.

MATERIALS AND METHOD

Study design and study population. This is the report of a pilot, single-centre, open, prospective trial conducted in the Department of Paediatric Gastroenterology and Nutrition at the Medical University of Warsaw, Poland. The participants began to be recruited in September 2014, and the study is still ongoing. Children with steroid-dependent or resistant UC flare, whose doctors decided to perform colonoscopies or sigmoidoscopies, were screened for the presence of CMV DNA in colon tissues. Patients with positive CMV DNA PCR results in colonic biopsies were invited to participate in the study. The patients were at different UC stages and were on different therapy schemes. The diagnosis of UC was based on clinical signs and symptoms, as well as on endoscopic, histological and radiological results according to the revised Porto criteria [13]. The severity of UC, as well as the subjects' clinical response to therapy, were evaluated using the Paediatric UC Activity Index (PUCAI). Additionally, the Mayo endoscopic score was used to evaluate UC severity. The following laboratory markers were also assessed: CRP, albumin level and faecal calprotectin (FCA). The diagnosis of CMV colitis was based on positive CMV DNA results by PCR (Gene Proof, CMV PCR kit, Czech Republic) in colonic tissues taken during colonoscopy or sigmoidoscopy.

Donor selection and stool preparation. Donors were screened for HIV, HAV Ab (IgM and IgG), HBV (HBsAg, anti-HBc), HCV (Ig G anti HCV), EBV (IgM and IgG), CMV (IgM and IgG) and *Treponema pallidum*, and underwent stool testing for norovirus, rotavirus, *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, *Escherichia coli*, *Borrelia*, *Clostridium difficile* toxins, pathogenic flora, ova and parasites. The donors were generally healthy, ate a regular diet and had no antibiotic treatments during the 3 months prior to donating material. The donors were not related to the patients.

The stool samples were collected by the donors at home. Immediately after collection, the samples were placed in sterile tubes and frozen at -20°C before being forwarded to the Department of Microbiology Laboratory. The stool samples were thawed 2 hours before the transplantation. For children, approximately 30–60 g were mixed with 30–50 ml sterile saline to prepare a homogeneous suspension. Next, the suspension was filtered several times through sterile filters under biological safety cabinet class 2 conditions. The residue on the filter was mixed with 30–50 ml of sterile saline and slowly filtered under the same conditions. The final volume of approximately 50–100 ml was placed in a sterile syringe ready for use. The transfer was given to the recipient 4 hours after preparation.

Study intervention. At the study entry, demographic data, medical history and physical examination were obtained. Previous and current treatment was recorded. PUCAI, Mayo endoscopic score and laboratory markers were assessed. The study subjects stopped taking immunomodulators and biologics, but were maintained on 5-ASA agents. The subjects did not receive any antibiotics or bowel lavage before transplantation. Every day during the whole induction FMT course, the study subject took a proton pump inhibitor at a dose 1 mg/kg and the anti-vomiting drug ondansetron 4–8 mg orally in the morning on the day of FMT administration. Each patient received 1 x 100 ml or 1 x 50 ml FMT for patients with body weight ≥ 40 kg and < 40 kg, respectively, via nasogastric tube (NG), for 5 days in each of 2 weeks (in total, 10 FMT procedures in 12 days). FMT was administered in 10-min sessions. All the patients were monitored for 1 h and were allowed to drink 1 h and eat 2 h after FMT administration. The clinical response was defined as a decrease of the PUCAI score by ≥ 20 points, and the clinical remission defined as PUCAI < 10 points. The outcome measures were assessed at 2 and 6 weeks of the study.

Ethical considerations. The study was approved by the Clinical Research Ethics Committee of the Medical University of Warsaw, Poland. All the parents and children aged ≥ 16 years signed the informed consent before participation in the study. All the patients and donors were informed of the benefits and potential risks of standardized FMT and laboratory screening.

RESULTS

Thirty steroid-dependent or steroid-resistant patients with UC flare were screened for the presence of CMV DNA in colon tissues. Of those, 16 patients were negative for CMV DNA PCR in colonic biopsies; 2 patients were excluded because of having severe colitis (PUCAI 65 points). Of 14

patients with positive CMV DNA PCR results in colonic biopsies, 4 patients did not agree to participate in the study. Finally, 8 patients with UC (4 boys), aged 2–17 years, were enrolled in this study. The baseline characteristics of the study participants are presented in Table 1. All the participants presented symptoms of UC exacerbation (mean PUCAI 46 points) and were positive for CMV DNA PCR in colonic biopsies. In all patients, FMT was administered by NG tube. After a 2-week course of FMT, almost all patients (7/8) became negative for CMV tissue DNA PCR. In the only patient (subject 4 in Table 1) who did not respond to the 2-week FMT course, it was decided to repeat the whole FMT course. As a result, CMV was eradicated after the second FMT course. In total, the patient received 20 FMT doses of 100 ml within 6 weeks, with a 2-week interval between courses. At the 6th week of the study, a clinical response was observed in 3/8 patients and clinical remission in 3/8 patients. In one patient (number 4), it was noticed that the PUCAI score only slightly decreased after 2 courses of FMT. An improvement in the Mayo endoscopic score was seen in 6/8 subjects. After the FMT course, a marked improvement was observed in laboratory markers: 2 normalized their CRP (patient number 5,8); a significant reduction of FCA was also found in 3 patients (patient numbers 2,5,8). Detailed changes in PUCAI score, as well as in laboratory markers, are shown in Table 2. The 2-week FMT course was very safe. Only mild, self-limiting adverse effects were observed: mild abdominal pain in 5 patients after the first dose of FMT. Additionally, 4 patients presented with nausea. The FMT procedures were tolerated in all the patients except for 2 episodes of vomiting within 2 h after infusion.

DISCUSSION

There is growing evidence that indicates the potential therapeutic role of FMT in patients with IBD. In

this group of patients, most FMT studies were focused on the treatment of IBD and the treatment of CDI in IBD [14–17]. CMV is another infectious agent which may play a significant role in the natural course of IBD, especially in UC. It has not been established whether the CMV is an innocent bystander or is a deleterious infectious trigger factor, which leads to the progression of UC. However, CMV infection is a marker of poor prognosis in IBD.

In this prospective study, it was found that FMT was highly effective in the eradication of colitis CMV in patients with UC flare. CMV eradication was achieved in all the study participants. It is difficult or even impossible to compare the results of the current study with any other trial, because to-date no such survey has been carried out. As CDI and CMV infection may have a similar impact on the course of IBD, we may refer to studies that assessed the use of FMT in CDI in patients with IBD. Unfortunately, to-date, very few papers have been published using FMT in the treatment of CDI in patients with IBD. In a study by Hourigan et al., all 5 patients (aged 10–17 years, 4 with CD and 1 with UC) with recurrent CDI who received 1 dose of FMT, were clear of CDI symptoms and CDI toxin negative at 12–20 weeks after FMT administration [14]. In a study by Russel et al., 2/3 children with CDI and IBD were cleared of CDI after FMT administration [15]. In adult studies assessing the efficacy of FMT in the treatment of CDI in patients with IBD, the response rates were 75% (15/20) [15], 75% (30/40)¹⁶ and 86% (31/36) after a single FMT administration [18]. The results of

Table 1. Baseline characteristics of patients

Subject No.	Age (years)	Gender	Disease duration (months)	UC Paris classification	Present medical treatment	GCS resistance/dependence	Disease severity (PUCAI)
1	15.5	F	54	E4S1	GCS, AZA, 5-ASA	dep	50
2	9	F	12	E3S1	GCS, AZA, 5-ASA, CycA,	res	50
3	1.5	F	2	E4S1	GCS, AZA, 5-ASA	dep	45
4	16.5	M	18	E3S1	GCS, AZA, 5-ASA	res	30
5	16	M	12	E4S1	GCS, AZA, 5-ASA, IFX	res	60
6	3.5	M	24	E4S1	GCS, AZA, ASA, CycA, IFX	res	35
7	15	F	12	E4S1	GCS, AZA, 5-ASA	dep	15
8	15	M	22	E4S1	GCS, AZA, ASA, CycA, IFX	res	45

GCS – glucocorticosteroids; AZA – azathioprine; 5-ASA – aminosalicylates; CycA – cyclosporine A; Tacro – tacrolimus; IFX – infliximab; ADA – adalimumab; res – resistance; dep – dependence; UC – ulcerative colitis; PUCAI – Paediatric UC Activity Index

Table 2. Laboratory, clinical and endoscopic data

Subject No.	CRP [mg/dl]			CPT [µg/g]			PUCAI			Mayo Endoscopic Subscore	
	before FMT	2 weeks after FMT	6 weeks after FMT	before FMT	2 weeks after FMT	6 weeks after FMT	before FMT	2 weeks after FMT	6 weeks after FMT	before FMT	6 weeks after FMT
1	2.2	1.1	2.1	>1800	>1800	>1800	50	20	45	1	2
2	0.7	<0.5	<0.5	925	330	127	50	15	10	2	1
3	0.6	0.7	0.8	1280	1154	>1800	45	25	15	3	2
4	0.5	0.5	0.6	285	882	100	30	*30	0	3	3
5	5.3	3.9	<0.5	>1800	>1800	826	60	40	30	3	1
6	1.8	1.0	0.9	>1800	133	>1800	35	20	0	3	1
7	<0.5	<0.5	<0.5	1501	>1800	>1800	15	10	10	2	1
8	2.1	0.7	<0.5	>1800	>1800	844	45	20	5	2	0

all of these studies are very promising, and we believe that FMT, through gut microbiota restoration, may be a very promising therapeutic option for infections that develop on the basis of gut dysbiosis. However, while there are several hypothetical similarities between CMV and CDI, they are 2 different pathogens, and we cannot extrapolate data on CDI to CMV.

One of the most important issues regarding FMT therapy is the number of doses. Although the use of 1 or 2 doses of FMT in the treatment of CDI is well established, it was decided to administer multiple doses of FMT to treat CMV colitis. The main reason for this decision was the fact that in previous studies it was shown that multiple or repeated FMT administrations were more effective in the treatment of patients with IBD [12, 19–23]. Based on these findings, when the CMV eradication was not achieved after the 2-week FMT course, it was decided to repeat the whole course, which was successful. Undoubtedly, this approach needs further studies to establish how many FMT infusions are needed for the eradication of CMV from colonic tissue.

Importantly, a decrease of UC severity was noticed in the marked (90%) group of patients. This decrease corresponded with an improvement in the Mayo endoscopic score found in almost all patients (except patient 1 and patient 4). However, 2 FMT courses (20 doses) were needed to achieve endoscopic response in patient 4. It is not known whether FMT therapy healed the IBD and, through the restoration of microbiota, resulted in CMV eradication, or whether FMT therapy directly eradicated CMV infection. However, even if FMT is not effective in the treatment of IBD itself, the eradication of CMV from colonic tissue allows the safe introduction of immunosuppressive agents to UC therapy. Hence, the FMT as a safe therapeutic proposal has a beneficial impact on UC prognosis and future treatment.

In the presented trial, all 8 patients had mild or moderate activity of UC with CMV colitis, they therefore had no indication for the antiviral therapy (negative CMV IgM antibodies). According to the ECCO guidelines, in patients with severe steroid resistant UC and CMV detected in colonic tissues, antiviral therapy, as well as discontinuation of immunosuppressive therapy, is recommended [5]. There are a few reasons why the decision to use ganciclovir was suspended in the patients with severe UC and CMV colitis. First, it is not easy to distinguish IBD flare symptoms from symptoms of CMV colitis. Second, a prompt (after 2 days) and marked clinical response was observed, measured as drop in PUCAI of between 10 – 25 points in the FMT therapy. Third, the decisions were made before the ECCO guidelines on opportunistic infections (2014) were published.

The main advantage of this study is the fact that, for the first time it shows the successful eradication of colonic CMV with FMT in UC patients. FMT may be a new therapeutic option in colonic CMV, especially in those UC patients with mild or moderate activity and negative serologic markers of CMV infection. The study also has some limitations. Qualitative CMV PCR assessment was used because the quantitative CMV assessment is not available in Poland. The quantitative CMV PCR test is recommended for CMV colitis diagnosis; however, it has only been used in a few published studies [24–26]. Another shortcoming of the survey is the small number of patients. However, in already published studies regarding the therapeutic effect of FMT in children with IBD samples, the sizes were also very small. In June

2013, Kunde et al. for the first time demonstrated the safety and tolerability of FMT in 10 children and adolescents with UC (mean age 7–21 years) [19]. Suskind et al. reported that a single dose of FMT was safe and effective in treating 7/9 teenagers with CD, but not in 4 with UC [20,21]. The authors of the presented study believe that they will confirm the results of this pilot study.

CONCLUSIONS

The results of this pilot study show that FMT is highly effective in eradicating colonic CMV in paediatric patients with UC flare.

Conflicts of interest

The authors are not aware of any conflict of interests associated with this study.

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