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Therapeutic potential of an anaerobic cultured human intestinal microbiota, ACHIM, for treatment of IBS

P. Benno [a, b, *], E. Norin [a], T. Midtvedt [a], P.M. Hellström [b]

[a] Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden
[b] Department of Medical Sciences, Uppsala University, Uppsala, Sweden

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ABSTRACT

By administering an anaerobic cultivated human intestinal microbiota (ACHIM) via upper gastrointestinal route using endoscopy we aimed to rectify intestinal dysbiosis and simultaneously achieve a treatment response in IBS patients. The study population fulfilled the Rome III IBS criteria and comprised 50 patients. During 10 days, patients recorded the irritable bowel syndrome symptom severity scale (IBS-SSS) along with the Bristol stool scale and number of stools/day. The enrolled patients were categorized as follows: 37 with diarrhea, 5 with constipation and 8 with mixed symptoms. The treatment response showed reduction in a majority of patients, 32 of which with 50-point reduction of IBS-SSS and 21 with a 100-point IBS-SSS reduction. The percentage improvement was 36 (23–49) and 28 (18–38) for women and men respectively. Short-chain fatty acids were not changed. We consider fecal microbiota transplantation in the form of ACHIM as an option for the future therapeutic armamentarium in IBS.
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Introduction

Irritable bowel syndrome (IBS) affects up to 11% in different communities [1]. The condition is often associated with low quality of life and may render the patients with considerable disabilities. The aetiology and underlying pathophysiology is assumed to be multifactorial. This is reflected by numerous treatment options; most often with suboptimal clinical results.

There is a growing body of evidences indicating that disturbances of the diversity of gut microbiota may play an important role in the development of IBS [2]. The basic concept for an underlying dysbiosis in IBS is that about 10% of the patients who once have had an intestinal infection develop post-infectious IBS [3]. In such cases, locally acting antibiotics such as rifaximin may have a positive, but often transient, therapeutic effect [4] whereas broad-spectrum antibiotics may increase the risk of developing IBS [5].

In line with experience from recurrent *Clostridum difficile* infection (CDI) as generally accepted condition of dysbiosis, where fecal microbiota transplantation is used with marked therapeutic results [6–8], We decided to study patients that developed IBS symptoms after an episode of traveller's diarrhea or a course of antibiotic treatment. In a phase 2A study, we employed an anaerobic cultivated human intestinal microbiota (ACHIM), instilled via the upper gastrointestinal route by use of an endoscope, in order to rectify a suspected intestinal dysbiosis and at the same time achieve a treatment response in patients with symptoms of IBS.

The primary end-point of this feasibility study was to asses IBS symptoms as evaluated by the number of patients moving from one category of IBS-SSS (severe, moderate, mild, remission) to another [9]. Secondary endpoints were changes of bowel movement frequency and fecal consistency as evaluated by the Bristol stool scale [10].

Material and methods

The study was conducted at Läkarhuset Hötorgscity, Stockholm, during 2016–2017. The research protocol was approved by the regional Ethics Board at Karolinska Institutet (Dnr 2016/1166-31/1). All participants provided written informed consent to participate in the study.

Study population

All study participants were referred from specialist colleagues in

* Corresponding author. Department of Medical Sciences, Uppsala University, Uppsala University Hospital, bldg. 40, SE-751 85, Uppsala, Sweden.
E-mail address: Peter.Benno@endoskopienheten.se (P. Benno).

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gastroenterology in the Stockholm catchment area to Läkarhuset. Since previous conventional treatments had been unsuccessful, these patients themselves requested alternative treatment with an intestinal microbiota. The study population fulfilled the IBS criteria defined by Rome III [11] and comprised fifty-two patients (25 males; 27 females).

During a period of **7** days, the patients recorded all bowel movements in a stool diary based on Bristol stool scale, number of stools per day, mean stool consistency on a 7 point scale in order to determine the IBS subtype, i.e. constipation predominant IBS (IBS-C), diarrhea-predominant IBS (IBS-D) or IBS with mixed bowel habits (IBS-M) [10]. Based on this system, patients enrolled for the study were categorized as follows: 37 IBS-D, 5 with IBS-C and 8 with IBS-M. Demographic details of the patients are shown in Table 1.

The IBS-SSS was used to assess the severity of IBS symptoms. The system incorporated intensity of pain, days with pain during a ten days period, bloating, bowel dysfunction and quality of life. Mild, moderate and severe cases are indicated by the scores 75–175, 176–300, and 301–500, respectively [9].

The study inclusion criteria were: age over 18 years, a history of traveler's diarrhea or a course of antibiotic treatment precipitating IBS symptoms and IBS-SSS of moderate-severe degree, i.e, exceeding 175 IBS-SSS points. The exclusion criteria were: celiac disease, inflammatory bowel disease, microscopic colitis, bile salt malabsorption, diabetes mellitus and short bowel syndrome. All patients were devoid of *Salmonella, Shigella, Yersinia, Campylobacter* and parasites (cysts and ova).

Donor feces

The microbiota transplant that was used originates from fresh fecal matter collected 1996 from a healthy donor of Scandinavian descent on ordinary Western diet and with minimal exposure to antibiotics. The fecal sample was quickly taken care of and processed/cultivated. The donor had been screened using a questionnaire addressing risk factors for potentially transmissible diseases. The feces were investigated for relevant pathogens, e.g. it was investigated for absence of hepatitis A, B and C, cytomegalovirus, Epstein–Barr virus, human immunodeficiency virus, rotavirus and calicivirus. Furthermore, the feces was screened for absence of Salmonella, Shigella, Campylobacter, Yersinia and *Clostridium difficile* [12]. Over the years, the fecal microbiota has been re-cultivated under strict anaerobic conditions at 7-day intervals. A quality control of the ACHIM has been carried out regularly.

Intervention

Under fasting conditions ACHIM was given via the upper gastrointestinal route. Using lidocaine spray (Xylocain, 10 mg/ml; AstraZeneca, Södertälje, Sweden) for surface anesthesia of the pharynx, the esophagus was intubated with a standard gastroscope (Olympus GIF-Q 180, Shinjuku-ku, Tokyo, Japan). The instrument was introduced into the descending part of the duodenum for delivery of the microbiota. With the aid of a flush tube, two vials of 30 mL containing at least 10^9 bacteria/mL was given and the catheter thereafter flushed with saline solution. The procedure was carried out on two occasions with 1-week interval. After the instillation to the patient using the endoscope, the patient was allowed to recover for 15 min before returning home.

Patient assessment and follow up

The patients rated the IBS-SSS during a 10 days period before the first treatment and then at four weeks, after the second instillation. The mean value for daily stool consistency (Bristol stool scale) and bowel movement frequency were estimated during one week.

Fecal sampling and analysis of short chain fatty acids

The subjects delivered a fecal sample before and four weeks after instillation of the microbiota. The fecal samples were collected in closed plastic vials and frozen at −20 °C within 5 min after collection. For analysis, sample were thawed at room temperature and homogenized manually.

Short chain fatty acids (SCFAs) in the fecal samples were analyzed after homogenization by addition of distilled water containing of 2-ethylbutyric acid (3 mmol/L; internal standard) and sulphuric acid (0.5 mmol/L). A 2-mL sample of the homogenate was vacuum-distilled according to the method of Zijlstra et al., modified by Hoverstad and Bjørneklett [13]. Analysis was done using gas-liquid chromatography and quantified with an internal standardization and the results are expressed as mmol/kg wet weight. The SCFAs acetic acid, propionic acid and butyric acid were analyzed in a subgroup of 11 paired samples.

Statistical analysis

All participants were evaluated according to the "before – after" concept without any placebo control. Data are presented as mean ± SEM or 95% confidence interval. Statistical differences between the observation times (before – after) were calculated using paired or unpaired Wilcoxon *t*-test, whereas multiple observation time points were evaluated with the Friedman test.

Table 1
Demographic characteristics of patients evaluated in the study.

Characteristic	Females	Males	Total
Number	25	25	50
Age (years)	43.5 (36.5–50.5)	38.3 (31.5–45.1)	41.1 (36.3–45.9)
Duration of disease (years)	9.5 (6.7–12.2)	13.5 (7.3–19.6)	11.5 (8.2–14.8)
Cause of disease			
Antibiotics	12	12	24
Post-infectious	9	10	19
Inconclusive	4	3	7
IBS subtypes			
Diarrhea	16	21	
Constipation	4	1	
Mixed	5	3	

Values are mean and 95% confidence interval.

Results

Totally 52 patients were assigned to receive ACHIM, fifty of which were followed-up at 4 weeks. There were two drop-outs at the four-week control for reasons unrelated to the study (Table 1).

Table 2 shows results of the primary endpoint with symptom reduction at week 4. A standard 50-point IBS-SSS symptom reduction was achieved in 32 individuals (64%) and an extended 100-point symptom relief of IBS-SSS was achieved in 21 individuals (42%). The percentage improvement is 36 (23–49) and 28 (18–38) for women and men respectively. The remission rate at week 4 was independent of the severity of IBS at inclusion to the study. Also, there was no difference in remission rate between women and men (Fig. 1) (see Table 3).

The Bristol stool scale could not reveal any change in the stool consistency, nor the number of bowel movements before and after intervention. Nor could a subanalysis reveal any differences in these two parameters in the patients who went into remission. Analysis of the fecal output of SCFAs was not different from baseline to follow-up.

There were no safety issues with ACHIM. No serious adverse events (SAEs) were noted. There were no adverse events (AEs) and no adverse drug reactions (ADRs) documented, a majority of the patients reported a sensation of undefined gastrointestinal unrest within the first two days of treatment; a feeling that slowly subsided over the next few days. No AEs related to the cardiovascular, respiratory or central nervous systems were reported.

Discussion

There is no clear definition of dysbiosis but it signifies a microbial imbalance of the gut microbiota. However, the development of a *Clostridium difficile* infection with multiplying organisms producing toxins can be considered as a model for an acute, antibiotic-induced intestinal dysbiosis. Long experience shows that application of an intestinal microbiota in the form of a fecal microbiota is an effective treatment with disappearance of the *Clostridium difficile* cytotoxin and healing rates up to 90%. Dysbiosis may also be suspected in patients with IBS that develop a significant change in bowel habits, be it constipation or diarrhea, after a course of antibiotic treatment or an infectious diarrhea. We therefore decided to make an attempt to restore a suspected dysbiosis in patients with defined IBS by using microbiota from a healthy donor and thereby recreate a balanced flora and attenuate intestinal symptoms. In order to obtain a high safety margin for our treatment we used feces from one single donor, with little previous exposure to antibiotics and a stable socio-hygienic background. The donor had no previous history of any gastrointestinal, pulmonary or genitourinary infections. In addition to the safety and tolerability aspects of our treatment we also sought to describe some advantageous effects with ACHIM.

All the patients included in the study fulfilled the criteria for IBS as caused by suspected gastrointestinal dysbiosis initiated in conjunction with a course of antibiotics and/or infectious diarrhoea. A clinically significant improvement of symptoms was achieved in a majority of the patients after treatment with ACHIM. There was no unexpected AEs and no SAEs. These early findings with ACHIM are interesting in view of the fact that all the participants in the study had previously been seeking medical health care without favourable results remaining as moderate-severe IBS patients.

The IBS-SSS is a solid tool for assessing the severity of symptoms [9]. Reduction of the IBS-SSS by 50 points has been considered to be a significant clinical improvement, but a sharper goal of 100 point reduction of the IBS-SSS has been suggested. These treatment goals were reached in a majority of our patients. This high requirement for treatment response has previously been reported by several groups.

Some patients were scheduled for a return visit *ad hoc*. Among those revisiting at week 24, three had a recurrence of their IBS symptoms, whereas of those revisiting at week 52, only one had slightly worsened symptoms. The remaining nine were continually improved. In this open study there is a non-negligible risk of high placebo response. Nevertheless, the persistent long-term symptom reduction seen in many patients indicates a true therapeutic effect of the intervention. Interestingly, no differences were seen on feces consistency or bowel emptying rate even in those patients who went into complete remission suggesting that the evaluation of the severity of IBS cannot be determined by one single item but requires a more composite evaluation such as IBS-SSS [9]. Moreover, we found no differences in the elaboration of the SCFA pattern before and after the ACHIM treatment. In a similar manner, the spectrum of SCFAs pattern may not be sensitive enough to verify changes of the whole bacterial genome. Furthermore, the gut microbiota has previously shown to be capable of producing similar end-metabolic products irrespective of the dominating flora, why SCFA does not seem to represent a reliable marker of a dysbiosis, nor its rectification after treatment.

In order to evaluate possible mechanisms underlying some of the symptoms and findings in IBS, such as abdominal pain, constipation, diarrhea, altered intestinal motility and abdominal distension, it is relevant to mention that all these conditions may be influenced by variations in the host-microbe interactions of the gut. Such interactions are largely dependent on food intake. Especially certain carbohydrates are fermented by the microbiota and transformed to SCFAs [13] and gases. However, an increased gas production alone is unlikely to the cause of all symptoms. Importantly, in our study the patients were told not to change their dietary habits. However, it is important to underline that the examined number of paired fecal samples were limited and SCFA excreted in feces does not reflect the processes going on in proximal parts of colon.

Our intention with the study was to examine the tolerability for an inoculated exogenous microbiota both in terms of the exposure of the ACHIM matter *per se*, but also the mere fact that each individual's indigenous intestinal microflora would be exposed to an exogenous microbiota. Our investigation showed that the exogenous ACHIM had only negligible disturbing effects on bowel function. In a few subjects borborygmia and feelings of gut peristalsis were perceived over the first two-three days after treatment, but no cases of persistent diarrhea or constipation were reported. The vast majority of subjects had no unpleasant feelings with the treatment whatsoever. We found no specific IBS subtype or background traits in those individuals who experienced borborygmus. The reported sensations most likely stand for an increased bacterial metabolic activity that might act to repress a suspected dysbiosis.

In terms of clinical effects of the ACHIM treatment, we observed a significant effect at group level. However, since the underlying

Table 2
Improvement of evaluable patients in the study with reduction of IBS-SSS >50 steps, or the sharper requirement of >100 steps, and percent improvement in for each gender and the total group.

Characteristics	Women	Men	Total
	25	25	50
Patients improved ≥50 points	18	14	32
Patients improved ≥100 points	13	8	21
Treatment response, improvement (%)	36 (23–49)	28 (18–38)	32 (24–40)

Values are number of patients and mean with 95% confidence interval.

Table 3
Improvement rate at 4 weeks according to categorization of IBS at inclusion.

Number of patients in category severe IBS at inclusion migrating to another category							
No response		Moderate		Mild		Remission	
Men	Women	Men	Women	Man	Women	Man	Women
7	4	3	8	5	4	0	2

Number of patients in category moderate IBS at inclusion migrating to another category					
No response		Mild		Remission	
Men	Women	Men	Women	Man	Women
5	4	5	1	0	2

Categories were severe IBS: IBS-SSS >300, moderate IBS: IBS-SSS 175–300, mild IBS: IBS-SSS 75–175, remission: IBS-SSS <75.

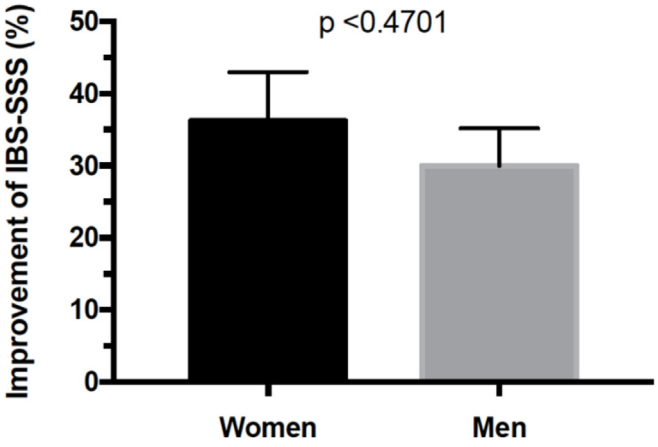

Fig. 1. Caption: The percentage improvement of IBS-SSS in women and men. Values are mean ± SEM.

pathophysiology is unclear and probably multifactorial, results rather have to be considered individually in order to arrive at a robust assessment. In our hands, 32 of 50 subjects had a relevant symptom reduction and 21 of those had an even stronger response, making it likely that the introduction of an exogenous microbiota would be capable of rectifying the dysbiosis along with a normalization of the functional symptoms. Indeed, this was seen in four of our patients within the stipulated study time, and another at the *ad hoc* follow-up at 52 weeks. Hence, even if there is no placebo comparator, and only historical data from the previous three years are at hand, our data suggest efficacy of ACHIM in IBS.

Among 20 of our patients there was only a futile or passing effect of ACHIM treatment. Basically, an unsuccessful outcome might be due to four reasons: the amount of bacteria instilled at gastroscopy was too little, the administration route not optimal, or the matching of the flora between donor and recipient was too poor, and finally, an etiology of IBS that is a completely separate one from dysbiosis. We have taken extraordinary measures in order to minimize these erratic factors. The bacterium dose of ACHIM was doubled as previous experience has shown one vial containing 30×10^9 CFU of bacteria to be effective [8,14]. Furthermore, the administration of two vials of ACHIM was duplicated at a 1-week interval in order to ensure sufficient dosing of ACHIM. We chose the upper route of administration since this was considered to cover both the small intestine and the colon. This has previously been shown to be as effective the lower route for treatment of *Clostridium difficile.* [6]. As regards the matching of the donor and recipient microbiota, treatment of IBS is utterly challenging since there are no clues as to what bacterium or microflora that may be pathogenic. Likewise, in a recent study by Johnsen et al. [15] a success rate of 65% was found, which is similar to our present findings.

There are some shortcomings of our study. There is no control group and the follow-up time is short, which is due to the fact that the study was carried out as a first feasibility study in order to study the beneficial potential of ACHIM. However, this will be followed up by a placebo-controlled study with a 3-month follow-up time as the next strategic step in the therapeutic evaluation of ACHIM. Moreover, fecal samples could only be obtained from a limited number of patients why this could only give us a hint of possible changes in the gut functions with ACHIM.

We have previously coined the term *dysbiotic bowel syndrome* (DBS) as a new concept describing the effects and consequences of an unbalanced intestinal microbiota with related symptoms. As there is no clear signature microbiota that is representative of IBS we have to rely on a successful treatment response to fecal microbiota in order to identify a disrupted intestinal microbiota function.

Summary

As the onset of many cases of IBS are associated with a course of antibiotics or traveler's diarrhea a bacterial etiology is suspected for the symptoms that may fall within the definition of IBS, even though no real pathogens are disclosed upon repeated fecal analyses. The possible reason for this outcome is an outgrowth of one or many bacterial species that reduces the diversity of the microflora along with symptoms or IBS. It seems that the use of a fecal microbiota transplantation can be therapeutic in such cases either as an anaerobically cultivated flora, or possible any conventional fecal matter, prepared for transplantation. The efficacy of the fecal microbiota transplant in IBS may not be as effective as in *Clostridium difficile* infection. This might be due to the fact that in IBS patients the bacterial outgrowth may be so much more differentiated as compared to the single etiology in *Clostridium difficile* infection. Our finding is moving the frontier of IBS research forward towards a resolution of the syndrome, at least in some, if not all cases of IBS.

Practice Points

- An imbalance of the gut microbiota underlies many cases of irritable bowel syndrome where no pathogenic bacteria are disclosed
- Many IBS patients respond to treatment with fecal microbiota transplantation.
- Dysbiosis is supported as an underlying cause of IBS

Research Agenda

- Identification of super-donors for fecal microbiota transplantation
- Identification of the susceptible group of patients that should respond to fecal microbiota therapy
- Survey long-term effects of therapy where we today meet patients with total resolution of their symptoms, while others experience recrudescence of dysbiosis

Conflicts of interest

PB, EN & TM are applicants of a patent regarding the ACHIM culture. PMH has no conflict of interest to report.

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