Journal of Crohn's and Colitis (2011) 5, 115–121

available at www.sciencedirect.com

A randomised double-blind placebo-controlled trial with *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* subsp. *lactis* BB-12 for maintenance of remission in ulcerative colitis

Signe Wildt[a,*], Inge Nordgaard[a], Ulla Hansen[b], Elke Brockmann[c], Jüri J. Rumessen[d]

[a] *Dept. of Medical Gastroenterology, Hvidovre Hospital, University Hospital of Copenhagen, Denmark*
[b] *Dept. of Pathology, Hvidovre Hospital, University Hospital of Copenhagen, Denmark*
[c] *Chr. Hansen A/S, Hoersholm, Denmark*
[d] *Dept. of Gastroenterology F, Gentofte Hospital and University of Copenhagen, Denmark*

Received 17 September 2010; received in revised form 11 November 2010; accepted 11 November 2010

A correction notice has been published, see: https://doi.org/10.1093/ecco-jcc/jjac104

KEYWORDS
Ulcerative colitis;
Probiotics;
Maintenance of remission;
Lactobacteria

Abstract

Background and aims: To investigate the clinical effect of treatment with *Lactobacillus acidophilus* La-5 and *Bifidobacterium animalis* subsp. *lactis* BB-12 (Probio-Tec AB-25) to maintain remission in patients with ulcerative colitis.
Methods: Patients with left-sided ulcerative colitis in remission — including proctitis and at least one relapse within the last year were randomised (2:1) in a double-blind placebo-controlled study to Probio-Tec AB-25 or placebo for 52 weeks. The patients were evaluated clinically, endoscopically and histologically at entry and if relapsing. No other medication for ulcerative colitis than the study drug was allowed during the study. Primary endpoint was maintenance of clinical remission, secondary endpoints comparisons of days to relapse, and safety and tolerability of the study drug. The concentrations of the probiotic bacterial strains in stool were analysed in a subset of patients.
Results: Thirty-two patients were randomised. Twenty patients received Probio-Tec AB-25 and twelve patients received placebo. Five patients (25%) in the Probio-Tec AB-25 group and one patient (8%) in the placebo group maintained remission after 1 year of treatment ($p=0.37$). The median time to relapse was 125.5 days (range 11–391 days) in the probiotic group and 104 days (range 28–369 days) in the placebo group respectively, ($p=0.683$). Probio-Tec AB-25 was overall well tolerated.

* Corresponding author. Department of Medical Gastroenterology 439, Hvidovre Hospital, University of Copenhagen, Kettegaard Alle 30, 2650 Hvidovre, Denmark. Tel.: +45 36323096; fax: +45 36323777.
E-mail address: siw@dadlnet.dk (S. Wildt).

1873-9946/$ - see front matter
doi:10.1016/j.crohns.2010.11.004

Conclusions: In this small randomised placebo-controlled trial no significant clinical benefit of Probio-Tec AB-25 could be demonstrated in comparison with placebo for maintaining remission in patients with left-sided ulcerative colitis. A difference may be achieved in larger studies, but the clinical significance of this would be questionable. This study was registered in ClinicalTrial.gov (NCT00268164).

1. Introduction

Ulcerative colitis (UC) is a chronic inflammatory disorder of the colon with clinical manifestations of diarrhoea and bleeding. The disease is characterised by acute exacerbations separated by periods of remission. Maintenance therapy is important in UC since up to 76% of patients relapse within a year without treatment.[1] Aminosalisylates (5-ASA) and azathioprine (AZA) as maintenance treatment in patients with UC is well documented.[2–4] However treatment is not always effective, is not well tolerated by every patient, and may have potential serious side effects. Consequently alternative treatments are welcomed.

Recently great focus has been on studies of the bowel flora and the potential alteration of the flora with probiotic treatment. Probiotics are defined as living microorganisms that upon ingestion act with benefit on the host, by altering the microbiological balance of the bowel.[5] Randomised trials have indicated, that certain bacterial strains may be equivalent or superior to mesalazine or balsalazide given alone or as add-on for induction of remission in patients with UC.[6,7] Similarly, data from a few large-scale controlled clinical studies with mesalazine have suggested the equality of effectiveness of probiotics for maintenance of remission in UC.[7–9] Only one double-blind placebo-controlled study of the effect of probiotics in UC in remission have been reported, in which no difference in relapse rates between patients given *Lactobacillus salivarius, Bifidobacterium infantis* or placebo as add-on therapy to aminosalicylates were detected.[10] We report the results of a 1 year, prospective, randomised, double blind, and placebo-controlled trial using a combination of *Lactobacillus acidophilus* LA-5 and *Bifidobacterium animalis* subsp. *lactis* BB-12 (Probio-Tec AB-25) for maintenance of remission in patients with left sided UC.

2. Methods

2.1. Participants

From June 2004 to March 2006 patients with UC in remission were enrolled from two participating centers in Denmark. Inclusion criteria were age $\geq$18 years, an established diagnosis of UC, and left sided disease (endoscopic changes distally to the splenic flexure) — including proctitis. Patients were in remission for a minimum of 4 weeks during stable monotherapy with 5-ASA or no medication at all, and patients had a minimum of one relapse within the last year. Remission was defined by the presence of two out of three criteria: a simple clinical colitis activity index (SCCAI) score $\leq$4 [11,12], endoscopically grade 0–1 after Baron et al. [13], and/or histologically grade 0–1 after Truelove et al.[14].

The exclusion criteria were pregnancy (a positive urine HCG) or breast feeding, chronic liver or kidney disease, severe chronic disease of vascular or cardio-pulmonal etiology, malignancies, immunosuppressive disease or treatment, inflammatory bowel diseases besides UC, malabsorption syndromes, and former surgical procedures involving the gastrointestinal tract — with the exception of appendectomy. Treatment with azathioprine, 6-mercaptopurine, biological immunomodifiers, and treatment with steroids within 1 month of entry.

2.2. Study drug and randomisation

The probiotic used in this study was: Probio-Tec AB-25, a mixture of *L. acidophilus* strain LA-5 and *B. animalis* subsp. *lactis* strain BB-12 (Chr. Hansen A/S, Hoersholm Denmark). One capsule contained 1.25×10^{10} CFU (colony forming units) of each bacterium leading to a total delivery of 2.5×10^{10} CFU in each capsule. Placebo medication (Chr. Hansen A/S, Hoersholm, Denmark) was identical in appearance, size, and taste.

Eligible patients were randomised in blocks of 6 according to a table-generated randomisation list to receive either Probio-Tec AB-25 (two capsules three times daily, resulting in a total delivery of 1.5×10^{11} CFU daily) or to receive placebo (two capsules three times daily) in a 2:1 ratio. Compliance was evaluated by interview and by counts of the returned study drug. Subjects who had taken 80% or more of the trial medication were considered to be compliant.

2.3. Study design

The study was a randomised, double blind, placebo-controlled study exploring maintenance of remission in patients with UC. Study drug were administrated for 52 weeks. No other medications for UC were allowed during the study period. The primary endpoint was to assess the efficacy of the probiotic mixture Probio-Tec AB-25 compared to placebo to maintain remission in UC. Secondary endpoints were days to relapse, concentrations of the probiotic bacterial strains in stool and finally to assess the safety and tolerability of the probiotic mixture. i.e. registration of new symptoms related to the study drug.

At inclusion patients were asked to stop treatment with 5-ASA. Patients were evaluated clinically at weeks 0, 4, 16, 28, 40, 52 (±1 week). All patients kept a standardized diary throughout the study period with weekly records of SCCAI. Potential side effects and intake of concomitant medication were recorded. At visits to the clinic diaries were collected, SCCAI was performed and compliance was evaluated. At weeks 0, 16, 28, 40, 52 and at relapse blood tests were drawn

with measurement of haemoglobin, C-reactive protein (CRP), white blood cell count and albumin. Sigmoidoscopy was performed at enrolment and in case of relapse. At endoscopy biopsies were obtained in the rectum and in the sigmoid colon for histological evaluation. In case of relapse/treatment failure the participants were withdrawn from the study. Relapse was defined as an SCCAI score>4 and/or endoscopically changes grade 2–3.

In 16 patients (50%) faecal samples were collected at weeks 0, 4 and 28 to determine the presence and concentration of bacteria present from the probiotic mixture. The stool samples were collected and stored immediately at −20 C. The microbiological procedures were performed at Chr. Hansen, Department of Molecular Strain Characterisation. The laboratory was blinded to the randomisation code.

2.4. Quantification of LA-5 and BB-12 in faeces by qPCR

BB-12 and LA-5 cell DNA-equivalents were quantified by a *B. animalis* subsp. *lactis* resp. LA-5 specific qPCR assay. DNA extraction and *B. animalis* subsp. *lactis* DNA quantification was performed as described in Taipale et al.[15] LA-5 cell DNA-equivalents were quantified using two *L. acidophilus* specific qPCR assays, named LA-5-5 resp. LA-5-10. Each of the two assays is specific to a subgroup of *L. acidophilus* strains and only LA-5 has been observed to be positive for both assays. The reaction conditions for the two assays were as follows: 50 μl 2× Probe Mastermix (EuroGentec, Belgium), 200 nM each primer, 150 nM probe and 1 μl of undiluted DNA in a total volume of 100 μl. Reactions were run on a ABI Prism 7500 with the following program: 1 cycle of 2 min at 50 °C followed by 10 min at 95 °C, 45 cycles of 15 s at 95 °C followed by 1 min at 59 °C. Oligonucleotides employed for assay LA-5-5 are LA-5-5-F (5′-TTACGCCAGTCCAAGGG TAG-3′), LA-5-5-R (5′-CAGAATGCCCGCAAGTTATC-3′) and LA-5-5-P (5′-FAM-TGCCGCA TTAGCAATTTTATAAATCCG-BHQ-3′) and for assay LA-5-10 resp. LA-5-10-F (5′-CCCTAGC TGGAAGACA-GATCC-3′), LA-5-10-R(TCCATTAGTTAAACCAAGCTGAA-3′) and LA-5-10-P (5′-JOE-GGTGAACATGTTCCCCGCACC-BHQ-3′).

2.5. Power calculations and statistical analysis

Assuming a 1 year remission rate of 70% with probiotic treatment and 30% with placebo treatment, using a 2:1 randomisation scheme, a cohort of 48 patients with UC were required to detect a difference at the 5% level of significance with a statistical power of 80%. Due to logistic problems and because of a high relapse-rate in the first evaluated 27 patients, a relapse-rate that indicated that no benefit towards the active treatment could be reached, we decided to cease enrollment after 32 patients. The results were analysed according to an intention to treat approach, and we also performed per protocol analysis. Data concerning baseline comparability, comparison of proportions in the two groups remaining in remission (primary endpoint), and comparisons of the number of days to relapse between the two groups (secondary endpoint) are presented as median and ranges. Non-parametric statistics were applied, using the Mann–Whitney test for quantitative data and Fischer's exact test for categorical data. Data concerning the detection of BB-12 and LA-5 in stool are presented as means (SD). The statistical software package SPSS 8 for Windows was used. All tests were two-tailed and p-values less than 0.05 were considered significant.

2.6. Ethics

The Regional Ethical Committees, The Danish Data Protection Agency and The Danish Medicines Agency approved the study. The study was registered in ClinicalTrial.gov (NCT00268164). Written and oral informed consent was acquired from all study participants.

3. Results

Thirty-two patients (10 males) with UC (23–68 years of age, median 37.5) participated in the study. Twenty patients were randomised to Probio-Tec AB-25 and twelve patients to placebo (Fig. 1). Clinical and demographic baseline characteristics in the two groups were similar, with the exception of the number of patients on medication, which was higher in the Probio-Tec AB-25 group than the placebo group (Table 1).

One patient was excluded from the study after 6.5 months due to pregnancy. She was included in the intention to treat analysis as a non-responder. Compliance was good and similar in the two groups (>80% of participating days, patients were taking all six capsules a day) except in one patient receiving Probio-Tec AB-25 who stopped taking the study drug for 21 days (47% of participating days) due to lack of efficacy and relapse.

Levels of serum albumin, CRP, haemoglobin, and white blood cell count were similar in the two treatment arms throughout the study (data not shown).

3.1. Primary endpoint

Five patients (25%) in the Probio-Tec AB-25 group and one patient (8%) in the placebo group maintained remission after 1 year of treatment (p=0.37) (Table 2).

3.2. Secondary endpoints

The median time to relapse was 125.5 days (range 11–391 days) in the probiotic group and 104 days (range 28–369 days) in the placebo group respectively, p=0.683 (Table 2).

Per protocol analysis of primary and secondary endpoints also revealed no significant differences.

The overall safety and tolerance of Probio-Tec AB-25 and placebo was good. In no case were side effects the cause of withdrawal. Gastrointestinal symptoms were reported equally in both treatment groups and a relation between Probio-Tec AB-25 and gastrointestinal side effects could not be established (Table 3).

Sixteen patients delivered a faecal sample for microbiology at study entry and at week 4. Six were taking placebo and 11 were taking probiotics. Only 4 of these 16 patients were in

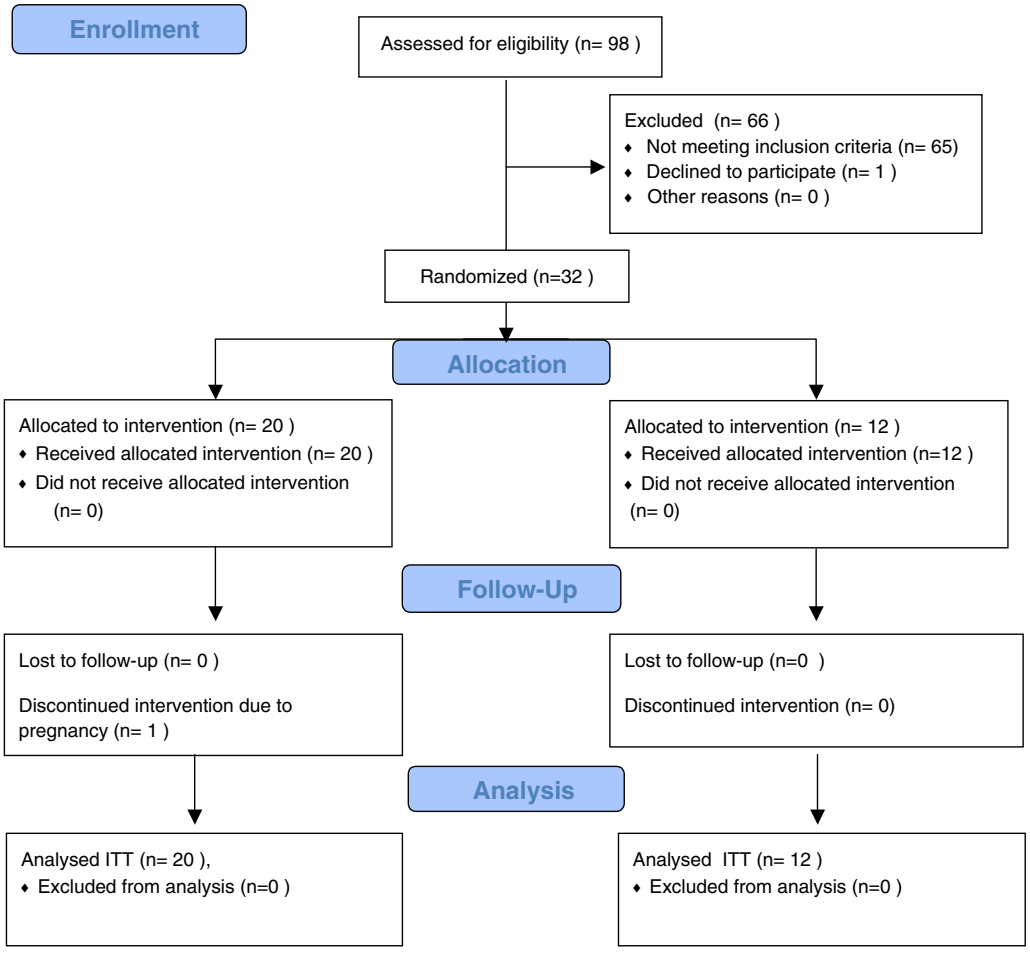

Figure 1 Study flow chart. Disposition of subjects through the study.

remission at week 28 and delivered a third faecal sample. At entry to the study no patients in the probiotic group had BB-12 or LA-5 in their faecal samples. At week 4 and week 28, BB-12 or LA-5 was detected in all patients receiving probiotics. Three patients in the placebo group had at study entry either BB-12, LA-5 or both bacteria detected in their faecal samples. At week 4 and week 28 BB-12 and LA-5 were identified in faecal samples from 2 patients (Table 4).

4. Discussion

In this randomised controlled trial of the probiotic Probio-Tec AB-25 we could not demonstrate an effect on maintenance of remission in patients with UC. Likewise no significant clinical benefit concerning number of days until relapse could be demonstrated.

Probio-Tec AB-25 consists of BB-12 and LA-5, bacteria shown to survive the acidic environments of the stomach and bile exposure,[16] and detectable in the stools several days after ingestion.[17,18] The bacteria can adhere to the mucus and epithelium of the intestine,[19] can stimulate the non-specific immune system,[20,21] and increase IgA production.[18] The bacteria were well tolerated and without serious side effects. The optimal doses of Probio-Tec AB-25 and of probiotics in general are unknown. We expected a daily dosage of 1.5×10^{11} CFU to demonstrate a potential clinical effect. However, trials have reported positive and safe results with dosages of probiotics as high as 3×10^{12} CFU,[22] and a dose–effect relationship is possible.[23]

All patients in the probiotic group excreted Probio-Tec AB-25 in stool samples, indicating good compliance. In four patients receiving placebo the probiotic bacteria were detected in different concentrations both before and after ingestion of placebo, indicating that either did the patients consume a probiotic product, (as no restriction on eating fermented products or yoghurts was given) or that bacteria present naturally in these patients were giving false positive results. However, all patients in the placebo group except one relapsed and any potential benefit of fermented or probiotic products did not influence the study results in a positive direction.

Although the proportion of patients remaining in remission after 12 months of treatment were numerically higher in the Probio-Tec AB-25 group than the placebo group, the majority of patients relapsed within a year (75% in the probiotic group and 92% in the placebo group). This relapse rate was significantly higher than anticipated in the study design, and could be due to the fact that patients before inclusion were stopped on 5-ASAs as we wanted to evaluate the true effect of the study drug alone and not as add-on therapy. If the patients were depending on the 5-ASAs for

Table 1 Baseline characteristics.

	Probio-Tec AB-25, n=20	Placebo, n=12	P value
Sex; F/M n	14/6	8/4	0.844
Age	40.5 (23–68)	35.5 (25–67)	0.192
SCCAI	0.5 (0–2)	0.5 (0–1)	0.212
Baron; grade	0 (0–1)	0 (0–1)	0.784
Truelove; grade	1 (1–2)	1 (1–2)	0.601
Disease duration; months	51.5 (3–288)	33.5 (2–194)	0.654
Disease location from anal valve; cm	20 (5–70)	22.5 (5–60)	0.953
Month since last relapse	4 (2–9)	5 (2–11)	0.279
Number of relapses the last year	2 (1–5)	1 (1–3)	0.317
Current smoker; n	4	3	0.710
Former prednisolon>40 mg; n	5	1	0.239
Former Azathioprine; n	1	0	0.439
Therapy at inclusion; n			
- None	1	5	0.018 *
- 5-ASA orally	12	4	
- 5-ASA rectally	5	2	
- 5-ASA orally and rectally	2	0	
- Salazopyrine	0	1	

Data are presented as median and range, or as sited in the table.
* p=0.018 (no medication versus medication — Fischer's exact test).

maintaining remission it could explain the high relapse rate seen in both groups. Secondly, many of the patients participating in the study had more than one relapse within the last year, proposing a more aggressive disease course in these patients despite the restriction to the left colon. In any case, the high relapse rate was the reason to cease enrollment after 32 patients. Even if all 48 patients had been included we would not have been able to detect any significant difference between groups with the relapse rates demonstrated in the study.

In previously randomised, double blinded, controlled studies of probiotics for maintenance of UC in remission *E. coli* Nissle 1917 was compared to mesalazine in a low dose (1.2–1.5 g/day), and no difference between groups followed for 3–12 months were found.[7,8,24] However only one of these studies (including 327 patients) [8] seemed adequately powered to assess equivalency.[25] In a large-scale open label study Zocco et al. found no difference between *Lactobacillus GG*, mesalazine 2.4 g/day or both.[9] In a small open label trial, Ishikawa et al. found improved outcome in patients treated with a mixture of *Bifidobacterium breve*, *Bifidobacterium bifidus* and *L. acidophilus* in fermented milk as add-on therapy to usual medication. Taken together, these trials support the concept that some probiotics are as effective as standard therapy for maintaining remission in UC. However, very large patient study groups are necessary to prove equivalence or superiority for any medication with expected only modest clinical efficacy in head-to-head or add-on studies. Apart from the large study by Kruis et al.[8] neither superiority nor inferiority of probiotics has been shown in these studies. So, the question still remains whether probiotics have, or do not have, relevant biological effects on UC in remission. The most reasonable way to provide answers to these questions is to perform placebo-controlled trials and preferably avoiding add-on therapy. If indeed relevant and beneficial biological effects are present, their further clinical relevance should eventually be tested in well-designed non-inferiority studies. Placebo-controlled studies of probiotics for maintenance of remission in UC as the present study are very scarce, however. A small trial by Cui et al. compared the effect of placebo and bifidobacteria on indices of mucosal inflammation in UC in remission, and a beneficial effect of bifidobacteria was suggested. [26] The study of Shanahan et al. (not published in

Table 2 Primary and secondary endpoints.

	Probio-Tec AB-25, n=20	Placebo, n=12	P value
Remission after 52 weeks; no (%)	5 (25%)	1 (8%)	0.37
Days to relapse; median and range	125 (11–391)	104 (28–369)	0.68

Table 3 Adverse events.

	Probio-Tec AB-25 (n=20)	Placebo (n=12)
Flatulence, abdominal bloating and pain	4	2
Changes in faecal consistency	6	4
Muscolosceletal; (Arthralgia, sacroilitis)	2	0
Various; (Tiredness, incontinence, stress, oral blisters, eye dryness)	6	2
Headache, dizziness	2	2
Influenza, gastroenteritis, cystitis and pneumonia	3	2
Serious adverse events	0	0

Several patients presented with more than one adverse event.

Table 4 Quantification levels of LA-5 and BB-12.

	Probiotics (no. of positive samples and mean values (SD) of quantification levels)	Placebo (no. of positive samples and mean values of quantification levels)
Week 0		
La-5	0/11	2/6 5.74E+06 (3.73E+05)
La-5″10″	0/11	2/6 5.60E+06 (6.39E+05)
BB12	0/11	2/6 1.30E+07 (9.80E+05)
Week 4		
La-5	10/11 5.57E+06 (7.30E+05)	2/6 3.12E+06 (1.98E+05)
La-5″10″	10/11 5.46E+06 (4.02E+05)	2/6 2.96E+06 (3.68E+05)
BB12	11/11 1.60E+07 (1.72E+02)	1/6 9.49E+05 (5.98E+04)
Week 28		
La-5	2/2 9.05E+05 (6.26E+04)	0/2
La-5″10″	2/2 8.48E+05 (7.99E+04)	0/2
BB12	2/2 8.46E+06 (1.03E+06)	0/2

The quantified levels of LA-5 (*Lactobacillus acidophilus* strain LA-5) and BB-12 (*Bifidobacterium animalis* subsp. *lactis* strain BB-12) in faecal samples of 11 patients receiving Probiotics and 6 patients receiving placebo. Detection/quantification limits for LA-5 and BB-12 are 4.5E+04 and BB-12 1.4E+05 respectively.

full), reported no difference in relapse rates after 12 months between 157 patients randomised to either placebo, *L. salivarius* or *B. infantis* as add-on to aminosalicylates. However, only one third of these patients had left-sided colitis, and no power calculations are available.[10]

In conclusion, the present prospective randomised, double-blind placebo-controlled study of Probio-Tec AB-25 for maintenance of remission in UC uniformly addressed left-sided disease, and we avoided add-on therapy with concomitant mesalazine. With the doses and bacterial strains used, fewer relapses and longer remission periods were seen in the probiotic group, but the differences were not statistically significant. However, the study had a small placebo group, limited statistical power, and a relapse rate higher than expected, thus from this study it cannot be excluded that Probio-Tec AB-25 is better than placebo to maintain remission in UC but the 1 year remission rate must be much less than the anticipated 70%. A difference may be achieved in larger studies, but whether this will be of clinical significance is questionable. Other bacterial strains or doses may be more effective. Also, the effectiveness of probiotics may differ with the extent of colitis. We therefore recommend, that future randomised clinical trials of probiotics in maintenance of remission in UC should be stratified for extent and severity of colitis, and designed on basis of placebo-controlled studies of other probiotics, prebiotics or synbiotics. Finally, if probiotics should be considered a safe and effective replacement or adjuvant for aminosalicylates/mesalazine, the effects of prolonged (more than 1 year) therapy need to be addressed.

Study funding

The study drugs used in this study was provided by Chr. Hansen A/S, Hoersholm Denmark. The study was supported by grants from Chr. Hansen A/S, Hoersholm, Denmark and by grants received from P. Carl Petersens Foundation and The Danish Crohn Colitis Organisation.

The study sponsors had no involvement in the study design, collection, analysis and interpretation of data, in writing of the manuscript or decision to submit the manuscript. However one of the authors (EB) is employed at the laboratory at Chr. Hansen A/S.

Statement of authorship

SW: Participated in the design of the study, carried out the study and data analyses, performed the statistical analyses and drafted the manuscript.

IN, JR: Conceived of the study and participated in its design and coordination and helped to draft the manuscript.

UH, EB: Carried out samples analyses and interpretation of data and helped to draft the manuscript.

All authors read and approved the final manuscript.

Acknowledgements

The authors thank Morten Danielsen for providing the strain specific detection method for LA-5. The assistance of the laboratory technicians Karin Schlichter and Masoumeh Taremi is gratefully acknowledged.

References

1. d'Hans G, Sandborn WJ, Feagan BG, Geboes K, Hanauer SB, Irvine EJ, Léman M, Marteau P, Rutgeerts P, Schölmerich J, Sutherland LR. Clinical trials of medical therapy in adults with ulcerative colitis. *Gastroenterology* 2007;**132**:763—86 Ref Type: Abstract.
2. Sutherland L, Macdonald JK. Oral 5—aminosalicylic acid for maintenance of remission in ulcerative colitis. *Cochrane Database Syst Rev* 2006:CD000544.

3. Gisbert JP, Linares PM, McNicholl AG, Mate J, Gomollon F. Meta-analysis: the efficacy of azathioprine and mercaptopurine in ulcerative colitis. *Aliment Pharmacol Ther* 2009;**30**:126–37.
4. Timmer A, McDonald JW, Macdonald JK. Azathioprine and 6-mercaptopurine for maintenance of remission in ulcerative colitis. *Cochrane Database Syst Rev* 2007:CD000478.
5. Fuller R. Probiotics in human medicine. *Gut* 1991;**32**:439–42.
6. Tursi A, Brandimarte G, Giorgetti GM, Forti G, Modeo ME, Gigliobianco A. Low-dose balsalazide plus a high-potency probiotic preparation is more effective than balsalazide alone or mesalazine in the treatment of acute mild-to-moderate ulcerative colitis. *Med Sci Monit* 2004;**10**:I126–31.
7. Rembacken BJ, Snelling AM, Hawkey PM, Chalmers DM, Axon AT. Non-pathogenic *Escherichia coli* versus mesalazine for the treatment of ulcerative colitis: a randomised trial. *Lancet* 1999;**354**:635–9.
8. Kruis W, Fric P, Pokrotnieks J, et al. Maintaining remission of ulcerative colitis with the probiotic *Escherichia coli* Nissle 1917 is as effective as with standard mesalazine. *Gut* 2004;**53**: 1617–23.
9. Zocco MA, dal Verme LZ, Cremonini F, et al. Efficacy of Lactobacillus GG in maintaining remission of ulcerative colitis. *Aliment Pharmacol Ther* 2006;**23**:1567–74.
10. Shanahan F, Guarner F, Wright AV, Vilpponen-Salmala T, O'Donoghue D, Kiely B, Progid Investigators. A one year, randomised double-blind, placebo controlled trial of a Lactobacillus or a Bifidobacteria probiotic for maintenance of steroid-induced remission of ulcerative colitis. *Gastroenterology* 2006;**130** Ref Type: Abstract.
11. Jowett SL, Seal CJ, Phillips E, Gregory W, Barton JR, Welfare MR. Defining relapse of ulcerative colitis using a symptom-based activity index. *Scand J Gastroenterol* 2003;**38**:164–71.
12. Walmsley RS, Ayres RC, Pounder RE, Allan RN. A simple clinical colitis activity index. *Gut* 1998;**43**:29–32.
13. Baron JH, Connell AM, Lenard-Jones JE. Variation between observers in describing mucosal appearances in proctocolitis. *Br Med J* 1964;**1**:89–92.
14. Truelove SC, Richards WC. Biopsy studies in ulcerative colitis. *Br Med J* 1956;**1**:1315–8.
15. Taipale T, Pienihäkkinen K, Isolauri E, Nexmann-Larsen C, Brockmann E, Alanen P, Jokela J, Söderling E. *Bifidobacterium animalis* subsp. *lactis* BB-12 delivered with a novel pacifier may reduce the risk of respiratory infections in breastfed children. *Submitted Br J Nutr* 2010 Sep;**24**:1–7 (Epub ahead of print).
16. Hoier E. Saure-und gallentoleranz von lactobacillus acidophilus and bifidobakterien. *Lebenmittlindustrie Milchwirtschaft* 1992;**26**:769–72.
17. Alander M, Mättö J, Kneifel W, et al. Effect of galacto-oligosaccharide supplementation on human faecal microflora and on survival and persistence of Bifidobacterium lactis Bb-12 in the gastrointestinal tract. *Int Dairy J* 2001;**11**:817–25.
18. Fukushima Y, Kawata Y, Hara H, Terada A, Mitsuoka T. Effect of a probiotic formula on intestinal immunoglobulin A production in healthy children. *Int J Food Microbiol* 1998;**42**:39–44.
19. Ouwehand AC, Salminen S, Tölkkö S, Roberts P, Ovaska J, Salminen E. Resected human colonic tissue: new model for characterizing adhesion of lactic acid bacteria. *Clin Diagn Lab Immunol* 2002:184–6.
20. Hatcher GE, Lambrecht RS. Augmentation of macrophage phagocytic activity by cell-free extracts of selected lactic acid-producing bacteria. *J Dairy Sci* 1992;**76**:2485–92.
21. Miettinen M, Vuopio-Varkila J, Varkila K. Production of human tumor necrosis factor alpha, interleukin-6, and interleukin-10 is induced by lactic acid bacteria. *Infect Immun* 1996;**64**:5403–5.
22. Sood A, Midha V, Makharia GK, et al. The probiotic preparation, VSL#3 induces remission in patients with mild-to-moderately active ulcerative colitis. *Clin Gastroenterol Hepatol* 2009;**7**: 1202–9 1209.
23. Van Niel CW, Feudtner C, Garrison MM, Christakis DA. Lactobacillus therapy for acute infectious diarrhea in children: a meta-analysis. *Pediatrics* 2002;**109**:678–84.
24. Kruis W, Schutz E, Fric P, Fixa B, Judmaier G, Stolte M. Double-blind comparison of an oral *Escherichia coli* preparation and mesalazine in maintaining remission of ulcerative colitis. *Aliment Pharmacol Ther* 1997;**11**:853–8.
25. Fedorak RN, Dieleman LA. Probiotics in the treatment of human inflammatory bowel diseases: update 2008. *J Clin Gastroenterol* 2008;**42**(Suppl 2):S97–S103.
26. Cui HH, Chen CL, Wang JD, et al. Effects of probiotic on intestinal mucosa of patients with ulcerative colitis. *World J Gastroenterol* 2004;**10**:1521–5.