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Effects of *lactobacillus casei* probiotic on mild to moderate ulcerative colitis: a placebo controlled study

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ABSTRACT

Background: The effects of probiotics on ulcerative colitis has still remained a contraversy. The aim of this study was to assess the effects of Lactobacillus casei strain ATCC PTA-3945 in treating ulcerative colitis. **Materials and Methods:** Thirty four patients with mild to moderately active ulcerative colitis randomly received a probiotic preparation of L. casei strain ATCC PTA-3945 (n = 17) or its placebo (n = 17) plus conventional medical therapy for their active disease. After a maximum of 2 months, remitted patients were again randomised to receive L. casei strain ATCC PTA-3945 or placebo, and also maintained on mesalazine or sulfasalazine tablets for a maximum of 6 months. **Results:** The numbers of patients achieving remission did not statistically differ between probiotic and placebo groups (82% vs. 76% at intention to treat analysis [P = 1.00], and 100% vs. 81.2% at per-protocol analysis [P = 0.23], respectively). The mean time to clinical remission were 25 days and 32 days in probiotic and placebo groups, respectively (P = 0.11). Relapse rates also did not significantly differ between probiotic and placebo groups (14.3% vs. 26.7% at ITT analysis [P = 0.65] and 16.7% vs. 33.3% [P = 0.64], respectively). Mean time to relapse were 96 days and 74 days in the probiotic and the placebo group, respectively (P = 0.51). **Conclusion:** The results of this preliminary study showed no significant effect by using L. casei strain ATCC PTA-3945 probiotic in the treatment of ulcerative colitis patients.

Key words: *Lactobacillus casei, probiotic, ulcerative colitis*

INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory bowel disease (IBD) affecting colon and rectum. It has been shown that environmental, genetic and immunologic factors play important roles in the pathogenesis of the disease.[1] One of the controversies in the pathogenesis of UC is the role of microbial flora in the intestine.[2,3] It seems that UC is the result of impaired interaction between colonic mucosa and environmental antigens such as microbial flora.[4-7] It has also been shown that increases in *Bacteroides vulgatus* or *Fusobacterium varium* and decreases in *Lactobacillus* and *Bifidobacteria* in colonic microflora have causative roles in the pathogenesis of the disease.[4,5,8,9]

Probiotics are living microorganisms supplemented to change the microflora of the intestine. Theoretically, probiotics can modify the bacterial flora of the gut, so that they can prevent the overgrowth of potentially pathogenic organisms and improve the integrity of intestinal mucosa.[10-12] Accordingly, they can be assumed as a potential treatment for UC, but data regarding their effects are not convincing.[3]

Lactobacilli are safe microorganisms commonly used in probiotic preparations. These organisms ameliorate disturbances in native microflora,[13] have anti-carcinogenic effects.[14] and lead to nonspecific activation of immune system.[15] So, we designed a study to evaluate the effects of a *Lactobacillus* preparation in patients with mild to moderately active UC.

MATERIALS AND METHODS

Aims and objectives

Our primary objective was to compare time to remission and rate of remission in patients with active ulcerative colitis treated with conventional medication of the disease in addition to a probiotic preparation of *Lactobacillus casei* strain ATCC PTA-3945, or its placebo. A secondary objective was to compare the rate and time to relapse after ulcerative colitis has remitted.

Patients

We conducted this randomized, double blind, placebo controlled study at 4 private practices of 4 gastroenterologists. The study was performed according to the Helsinki Declaration, and all the patients signed the written informed consent forms prior to their entery into the study. The patients could withdraw from the study at anytime they wished.

Thirty four patients between 15 and 65 years of age with ulcerative colitis were included. The patients either had newly been diagnosed or recently relapsed ulcerative colitis, based on clinical, endoscopic, and histological findings, and had a mild to moderately active ulcerative colitis according to Truelove and Witt's criteria,[16] and a Clinical Activity Index,[17] of ≥4 and ≤12. The exclusion criteria were; substantial cardiac, renal or hepatic diseases, severe immunocompromized patients, existing or intended pregnancy or breast feeding, regular treatment with non-steroidal anti-inflammatory drugs, intestinal major operation, steroids dependency, known intolerance to sulfur-free preparations of mesalazine, ulcerative colitis exacerbated by infectious colitis, toxic megacolon, use of antibiotics within 14 days prior to first visit for more than 1 week,

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use of corticosteroid injection within the last 30 days, use of immunosuppressive treatment within the last 90 days and use of mesalazine enema or corton enema within the last 14 days.

For the maintenance phase of the trial, patients with remmitted ulcerative colitis who had not been in our trial in the active phase of their disease were also included into the study. These patients had to be in remission for less than 3 months. The exclusion criteria were also applied for these patients. A colonoscopy was done and biopsies were taken to confirm remission in these patients prior to study entry.

Study design and medication

This was a randomized, multicenter, double blind, placebo controlled clinical trial to find out whether probiotic supplementation with *L. casei* strain ATCC PTA-3945 might be effective in inducing remission of active ulcerative colitis or preventing relapses over a 6 month period. A summery of the study design is shown in Figure 1.

Patients were randomized to receive either 1 capsule of *L. casei* strain ATCC PTA-3945 preparation (n = 17) or its placebo (n = 17) twice a day taken after lunch and dinner. Patients also received conventional medical treatment for active ulcerative colitis according to the severity and extension of their disease [Table 1]. The probiotic preparation was supplied as hard gelatin capsules containing 5×10^5 live active cells of *L. casei* strain ATCC PTA-3945 per capsule. Placeboes were indistinguishable from the *L. casei* preparation.

Randomizations were done using a random number table with odd numbers for probiotic and even numbers for placebo; both in the trial entery and in the begginning of the maintenance phase of the study. In the trial entery, randomization was stratified according to the use of mesalazine or sulfasalazine, and to the clinical severity of the disease (mild or moderate).

Patients not in remission after a maximum of 8 weeks were excluded from the further trial, as would any patient who deteriorated clinically. Remission of ulcerative colitis was when a patient did not have more than 3 well formed stools per day and was without visible blood in the stools and any clinical symptoms of ulcerative colitis and had a Clinical Activity Index of <4.

Relapse was defined as an increase in bowel frequency with blood for at least 1 week. A colonoscopy was performed and biopsies were taken to confirm relapse. Patients were asked to contact their gastroenterologists immidiately if symptoms of a relapse occurred. At the time of screening, a questionnaire about the patients' demographic information and the details of their

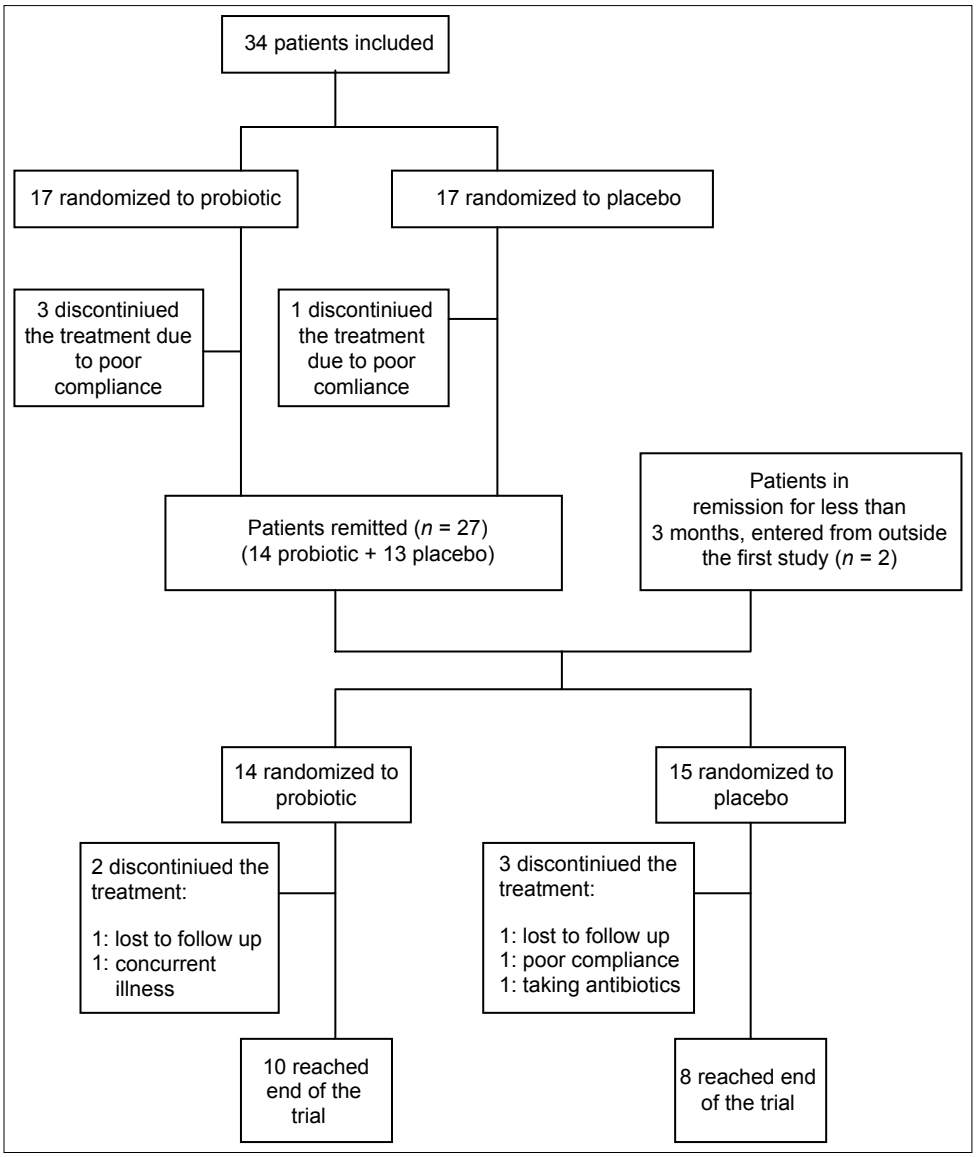

Figure 1: The study design

Table 1: Medications beside Probiotic/Placebo in the active phase of ulcerative colitis

Disease severity	*Disease extent*	*Medication*
Mild	Proctitis	Mesalazine suppositories[a]
	Distal	Sulfasalazine[b] or mesalazine[c] tablets
	Left-sided	Sulfasalazine[b] or mesalazine[c] tablets
	Pancolitis	Sulfasalazine[b] or mesalazine[c] tablets
Moderate	Proctitis	Sulfasalazine[b] or mesalazine[c] tablets+mesalazine suppositories[a]+prednisolone[d] tablets
	Distal	Sulfasalazine[b] or mesalazine[c] tablets+prednisolone[d]
	Left-sided	Sulfasalazine[b] or mesalazine[c] tablets+prednisolone[d]
	Pancolitis	Sulfasalazine[b] or mesalazine[c] tablets+prednisolone[d]

[a]Concurrent use of mesalazine tablets were allowed in patients with pretrial usage; [b]2-3 g/day; [c]2.4-3.6 g/day; [d]20-60 mg/day

ulcerative colitis symptoms was completed and medication in the preceeding weeks were recorded. The following indices were measured at trial entry, at remission, and at relapse: Haemoglobin, white cell count, platelet count, liver biochemichal tests, C-reactive protein, albumin, and erythrocyte sedimentation rate. Criteria for study termination were; patient lost to follow up visits, poor compliance, taking antibiotics for more than 10 consecutive days, disease deterioration requiring other forms of treatment in the active phase of the disease, pregnancy, concurrent illness, adverse events severe enough to make discontinuation of treatment advisable, and personal reasons not associated with the trial. Treatment could also be discontinued at any time following a patient's request. Poor compliance was if a patient discontinued the treatment for at least 3 consecutive days or if they had assumed less than 80% of the prescribed dosages during any of the intervals between follow-up visits. At the beginning of the maintenance phase of the study, patients were randomized to receive *L. casei* strain ATCC PTA-3945 or placebo and the randomization was stratified according to the use of mesalazine or sulfasalazine. The dose of prednisolone was tapered by 5 mg every 2-3 weeks untill completely stopped. Patients with mild proctitis, received mesalazine or sulfasalazine tablets after remission. In patients with moderate proctitis, mesalazine suppositories were stopped after remission. The rest of the drugs and their doses were kept unchanged.

Follow-up

In the active phase of the trial, patients had monthly visits with their gastroenterologist. In addition, they were phoned weekly to be asked about their ulcerative colitis symptoms, to see if they were taking their medication properly, if they have taken any other drugs beside their study medication, and if they have experienced any new adverse effects.

In the maintenance phase of the trial, patients had visits with their gastroenterologists every two months. In addition, they were phoned every two weeks to be asked similar as above.

To confirm drug compliance, patients were asked to return unused medication at each visit.

Statistical methods

Kaplan-Meier graphs were used to compare the groups on remission and relapse time, and log-rank tests assessed the statistical significance of the difference, incorporating the stratification factor. $P < 0.05$ were considered statistically significant. Numbers of patients achieving and remaining in remission were compared with Fisher's exact test. All analyses were undertaken with SPSS for Windows, release 11.

RESULTS

Baseline characteristics

Seventeen patients were randomized to receive probiotic and 17 to treatment with placebo. There was not a significant difference between the two groups in factors such as age, sex, disease duration and extent, smoking, medication taken, and clinical activity index. The mean clinical activity index on the study entery was 6 in the probiotic group and 5 in the placebo group.

Remission

According to intention to treat (ITT) analysis, the numbers of patients achieving remission did not statistically differ between the two groups and were 14 (82%) in the probiotic group and 13 (76%) in the placebo group ($P = 1.000$). Per-protocol remission rates were 100% and 81.2%, respectively, which did not show significant difference between the two groups ($P = 0.23$). Mean time to clinical remission was 25 days in the probiotic group and 32 days in the placebo group ($P = 0.11$) [Figure 2].

Relapse

Number of patients relapsed were 2 (14.3%) in the probiotic group and 4 (26.7%) in the placebo group according to ITT analysis ($P = 0.65$). Also, per-protocol analysis showed no significant difference between the two groups (16.7% vs. 33.3%, respectively; $P = 0.64$). Mean time to relapse were 96 days and 74 days in the probiotic and the placebo group, respectively ($P = 0.51$) [Figure 3].

Follow up

Nine patients (5 in the probiotic group and 4 in the placebo group) discontinued the treatment: Mostly because of poor compliance (probiotic group 3, placebo group 2).

Seventeen patients reported 19 adverse events, from which 12 were remotely, probably, or definitely drug related [Table 2]. No serious adverse events were reported either in the group treated with probiotic or among the control patients.

DISCUSSION

The current medical treatment of UC mostly relys on conventional drugs, including aminosalicylates, corticosteroids and immunosupressive agents, but there is still a need for alternative therapies due to side effects of these medications, especially while being used for a long time.[18,19] Ulcerative colitis is a disease that occurs in human colon, where thousands of microorganisms reside. But it is not significantly found in germ-free animals.[20] There are about 400 different kinds of microorganisms in human intestine, mostly located in terminal ileum and colon.[21,22] These organisms produce cytotoxic compunds and sometimes have carcinogenic effects.[23-25] The interaction of these compunds at the apical surface epithelium of colon induces inflammation via activation of mucosal immune system.[20] Therefore, probiotics may be a potential treatment of UC by modifynig the bacterial flora of the gut, so that the overgrowth of potentially harmful organisms are prevented.

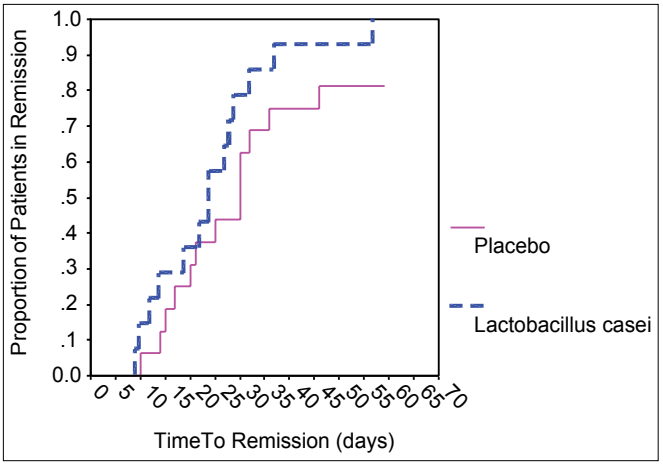

Figure 2: Proportion of patients remitting per day

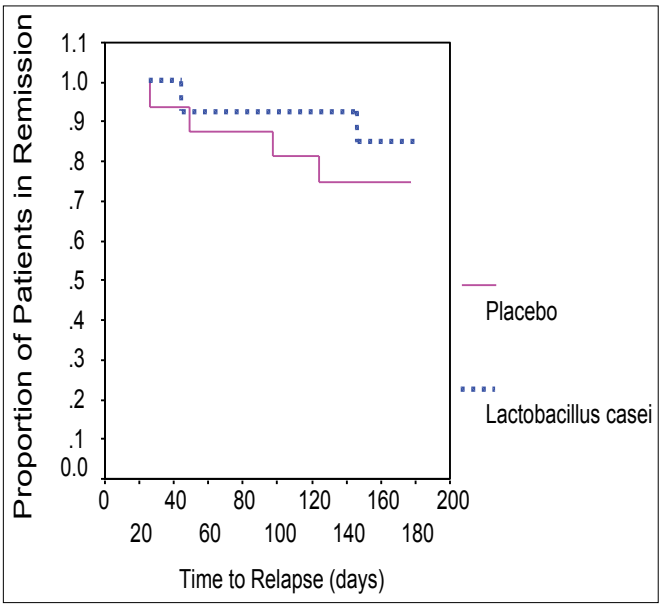

Figure 3: Proportion of patients maintaining remission per day

Table 2: Adverse events probably or definitely drug-related

Adverse event	*No of patients reporting adverse events*		*Drug's name*
	Probiotic group	*Placebo group*	
Acne	1	1	Mesalazine
Acne-Alopecia	1	1	Mesalazine-Prednisolone
Bloating	1	2	Probiotic/Placebo
Dyspepsia	0	1	Mesalazine
Hard stool	0	1	Probiotic/Placebo
Headache-Tenesmus	0	1	Sulfasalazine
Puffiness	0	1	Prednisolone
Rash	1	0	Mesalazine-Omeprazole

There are some studies assesseing the effects of probiotics on UC, some of which showed positive influence of probiotics,[26-28] and some others failed to show any significant difference between probiotics and control groups.[29-32] A randomized controlled trial of the effect of *Bifidobacteria*-fermented milk supplement in the treatment of ulcerative colitis showed success in maintaining remission.[33] A clinical trial on 327 patients with ulcerative colitis showed that *Escherichia coli* Nissle 1917 had efficacy and safety similar to the standard mesalazine.[30] The results of another study on 120 patients with ulcerative colitis suggest that treatment with a non-pathogenic *E. coli* has an equivalent effect to mesalazine in treatment of ulcerative colitis.[31] A systemic review of clinical trials comparing the effects of probiotics with conventional treatment of UC showed no significant difference between probiotics and anti-inflammatory drugs.[2] However, the latter study reported a significant difference between probiotics and placebo.[2]

According to the results of our study, the proportion of patients reaching remission and time to remission were not significantly different between the two groups. Also, relapse rates and the time to relapse did not show statistically significant difference between probiotic and its placebo. This was not unexpected since both treatment groups benefited from mesalazine/sulfasalazine and the anti-inflammatory properties of corticosteroides, which were likely to be more effective in resolving the active inflammation than probiotic. On the other hand, a difference of 22 days was observed in the period of time to relapse between the probiotic and placebo groups. This gives us the idea that maybe the small sample size had been the reason for this difference not to become significant.

The main limitations of our study were the small sample size and concurrent use of other treatments. Usage of other treatments were mostly because of ethical issues; as the efficacy of corticosteroides and mesalazine/sulfasalazine in induction and mesalazine/sulfasalazine in maintaining remission of ulcerative colitis have been proven, we could not deprive the patients from these medications. So, instead, we decided to see whether simultaneous usage of probiotic, which work with mechanisms of action different from corticosteroides and mesalazine/sulfasalazine, would increase the chance of remission and decrease relapse in ulcerative colitis patients. Regarding the study medication, the reason for not using mesalazine or corton enemas was that these products are not easily accessible for all the patients in Iran.

Another important issue influencing the effects of probiotics on ulcerative colotis is the type of probiotic used.[2] Depending on the type of probiotic, previous studies have shown that *Bifidobacteria* probiotcs were significantly more effective than *E. coli*.[2] But studies assessing the effects of *Lactobacillus* on UC are scarce.[2,29] Furthermore, the number of organisms in the probiotic preparations were almost low. It is possible that higher doses of the organism may show different results. Although the results of our study did not show a significant efficacy for *L. casei* strain ATCC PTA-3945 in ulcerative colitis, larger clinical trials with higher doses of the organism are needed to be held to further evaluate the efficacy of this prescription in ulcerative colitis.

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