Alteration of Intestinal Microflora Is Associated With Reduction in Abdominal Bloating and Pain in Patients With Irritable Bowel Syndrome

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OBJECTIVE: The influence of the gastrointestinal (GI) microflora in patients with irritable bowel syndrome (IBS) has not been clearly elucidated. This study was undertaken to see if patients with IBS have an imbalance in their normal colonic flora, as some bacterial taxa are more prone to gas production than others. We also wanted to study whether the flora could be altered by exogenous supplementation. In a previous study we have characterized the mucosa-associated lactobacilli in healthy individuals and found some strains with good colonizing ability. Upon colonization, they seemed to reduce gas formation.

METHODS: The study comprised 60 patients with IBS and a normal colonoscopy or barium enema. Patients fulfilling the Rome criteria, without a history of malabsorption, and with normal blood tests underwent a sigmoidoscopy with biopsy. They were randomized into two groups, one receiving 400 ml per day of a rose-hip drink containing $5 \times 10^7$ cfu/ml of *Lactobacillus plantarum* (DSM 9843) and 0.009 g/ml oat flour, and the other group receiving a plain rose-hip drink, comparable in color, texture, and taste. The administration lasted for 4 wk. The patients recorded their own GI function, starting 2 wk before the study and continuing throughout the study period. Twelve months after the end of the study all patients were asked to complete the same questionnaire regarding their symptomatology as at the start of the study.

RESULTS: All patients tolerated the products well. The patients receiving *Lb. plantarum* had these bacteria on rectal biopsies. There were no major changes of *Enterobacteriaceae* in either group, before or after the study, but the *Enterococci* increased in the placebo group and remained unchanged in the test group. Flatulence was rapidly and significantly reduced in the test group compared with the placebo group (number of days with abundant gas production, test group 6.5 before, 3.1 after vs 7.4 before and 5.6 after for the placebo group). Abdominal pain was reduced in both groups. At the 12-month follow-up, patients in the test group maintained a better overall GI function than control patients. There was no difference between the groups regarding bloating. Fifty-nine percent of the test group patients had a continuous intake of fermented products, whereas the corresponding figure for the control patients was 73%.

CONCLUSIONS: The results of the study indicate that the administration of *Lb. plantarum* with known probiotic properties decreased pain and flatulence in patients with IBS. The fiber content of the test solution was minimal and it is unlikely that the fiber content could have had any effect. This type of probiotic therapy warrants further studies in IBS patients. (Am J Gastroenterol 2000;95:1231–1238. © 2000 by Am. Coll. of Gastroenterology)

INTRODUCTION

Irritable bowel syndrome (IBS) is a common gastrointestinal (GI) disorder. Typical symptoms reported are abdominal pain, flatulence, and irregular bowel movements. Studies indicate that 10–20% of all adults have these symptoms (1), corresponding to about 25–50% of all patients who visit a gastroenterologist’s clinic (2).

Many factors are likely to give rise to symptoms like those of IBS, e.g., food intake, endocrine imbalance, disturbances in the intestinal bacterial flora, malabsorption, postoperative changes, and psychosomatic influences (3, 4). All these factors by themselves or in combination can exert an effect on the motor function of the GI tract.

Studies have indicated that IBS may be caused by changed motility in the colon, especially at food intake (5), and that patients with IBS report pain with a lesser degree of abdominal distension than do healthy persons. Furthermore, psychometric tests have indicated personality traits especially associated with IBS patients, and psychotherapy can sometimes be helpful (6). Patients with IBS and symptoms consisting mainly of constipation are sometimes helped by a dietary fiber supplement but dietary fiber supplements have not been successful in the treatment of IBS as measured by double-blind trials (7). There is no efficacious medical treatment for IBS, and many patients rely on
changes in their diet, such as intake of fiber supplements to stimulate intestinal movements, or avoidance of foods that tend to produce gas. The influence of the GI flora in patients with IBS has been reported in a few studies. Balsari et al. showed in a study of 20 patients with IBS that there was a great homogeneity in the fecal flora and that there was a decrease of coliforms, lactobacilli, and bifidobacteria in patients, compared with healthy individuals (8). In another study by Wyatt et al. no major differences in the fecal flora, compared with controls, were seen (9). They studied only two patients, however. In a recent report by King et al. it was found that colonic-gas production was greater in patients with IBS than in controls and that both symptoms and gas production were reduced by exclusion diet (10). This may indicate a role for gut bacteria in the symptomatology of IBS patients.

Because many patients present with symptoms of abdominal distension and increased flatulence, symptoms often seen in patients with a disturbed microbial flora in the gut, it would be of interest to see whether this group of patients has an imbalance of the normal colonic flora. Some bacterial groups are more prone to gas production than others and therefore fractions of the colonic flora, such as clostridia and Enterobacteriaceae, may contribute to the symptomatology (11–13). In previous studies we have characterized the mucosa-associated lactobacilli in healthy and diseased individuals (14, 15). The Lactobacillus spp. commonly found in healthy bowel mucosa was identified, and specific strains of Lactobacillus plantarum were defined by restriction endonuclease analysis of total chromosomal DNA (16). We have also shown that administration of one strain can induce colonization of these bacteria from jejunum to rectum in healthy volunteers (17). Simultaneously with the colonization by Lactobacillus plantarum there was a decrease in the colonic mucosa of bacterial groups with gas-producing ability, such as clostridia.

We therefore wanted to study, in a group of patients with IBS, whether administration of a rose-hip drink mixed with 5% (w/w) of an oatmeal soup fermented with Lb. plantarum DSM 9843 could be efficacious in relieving symptoms in patients with IBS, and especially symptoms relating to bloating.

MATERIALS AND METHODS

Study Design

This study was performed as a double-blind placebo-controlled trial. Patients were recruited via newspaper advertisement. They were examined by one of the investigators (S.N.). Patients fulfilling the Rome criteria (4), without a history of malabsorption, and with normal blood tests (Hb, C-reactive protein [CRP], thyroid-stimulating hormone [TSH], and T<sub>4</sub>) were included in the study. The patients should also have had a normal colonic examination within 6 months before study entry (colonoscopy or barium enema). Thereafter, 60 patients with diagnosed IBS were randomized into two groups, one receiving 400 ml/day of a rose-hip drink containing 5 × 10<sup>7</sup> cfu of Lactobacillus plantarum DSM 9843 (= strain 299v) (17) and 0.009 g/ml oat flour (test product), and the other group receiving plain rose-hip drink (placebo), comparable in color, texture, and taste. Administration lasted for 4 wk, and patients were not allowed to consume other products containing lactic acid bacteria during a period starting 2 wk before administration and ending on the final day of administration. Adverse events and medication were recorded throughout the study period.

One year after completion of the study all persons in the study were contacted and asked to report their present symptomatology with the same questionnaire as that previously used.

The study was approved by the Human Ethics Committee at Lund University.

Exclusion Criteria

Persons younger than 18 yr of age, pregnant, with previous abdominal surgery or diverticulitis, mental disorders (including all symptoms from severe disorder to anxiety and nervousness), and other organic intestinal diseases or severe systemic diseases were excluded. Persons were also excluded if they started or ended treatment with antibiotics, antiarrheaa agents, laxative agents, or spasmolytics during the period of study.

Recording of Symptoms

Patients started recording their GI function 2 wk before administration (weeks 1 + 2) and continued this registration during the treatment (weeks 3–6). Parameters studied each day were number of defecations, fecal consistency (very loose, rather loose, normal, rather hard, or very hard), and presence or absence of abundant gas. Once a week the patients recorded their overall GI function (perfect function–worst possible function), pain (no pain–worst possible pain), and flatulence (no flatulence–worst possible flatulence) during the week, on visual analog scales (VAS).

Microbiological Analysis

Fecal samples and rectal biopsies for microbiological analyses were taken before intake and at the end of administration and immediately transported to the laboratory for analysis. Rectal biopsies were taken at rectoscopy and washed three times in a solution (0.9% NaCl, 0.1% peptone, 0.1% Tween 80, 0.02% cysteine) before inoculation.

Viable counts were obtained from Rogosa agar (Oxoid) that was incubated at 37°C for 3 days for isolation of Lb. plantarum DSM 9843, from violet-red bile glucose agar (Oxoid) that was incubated at 37°C for 24 h (Enterobacteriaceae count), from perfringens agar base (Oxoid) + TSC selective supplement (Oxoid) that was incubated at 37°C for 3 days (sulfite-reducing clostridia count), and from Slunetz Bartley agar (Oxoid) that was incubated at 44°C for 2 days (Enterococci count). Colonies suspected to be Lb. planta-
rum DSM 9843 on the Rogosa agar (large, creamy, white-yellowish, and somewhat irregular) were counted. Representative colonies were picked, purified on Rogosa agar, and identified by Randomly Amplified Polymorphic DNA (RAPD) typing (18).

**Statistical Evaluation**
Statistical evaluation of changes within groups was carried out using the Wilcoxon signed-rank test. The Mann-Whitney U test, the unpaired Student’s t test, or Fischer’s exact test were used for comparison between groups.

**RESULTS**

**Tolerance of the Products**
All patients tolerated the products well, and no adverse events were reported during the period of intake.

**Efficacy Evaluation**
Five patients in the test group and three patients in the placebo group were excluded from the study because of failure to fill in the forms or failure to follow the dietary or medical restrictions. Thus, the efficacy evaluation included 25 persons in the test group (16 women and nine men; age range, 24–78 yr; mean age, 51 yr) and 27 persons in the placebo group (20 women and seven men; age range, 21–66 yr; mean age, 46 yr).

**Microbiological Evaluation**
*Lb. plantarum* DSM 9843 was found in fecal samples from 84% of the participants in the test group (the detection limit was about $10^5$ bacteria [cfu]/g of feces). The median amount was $1 \times 10^7$ cfu/g with values ranging from $1 \times 10^5$–$8 \times 10^7$ cfu/g of feces. *Lb. plantarum* DSM 9843 was also found in rectal biopsies from 32% of the patients in the treatment group (the detection limit was $10^3$ cfu/g of mucosa). The median was $8 \times 10^4$ cfu/g of mucosa, with values ranging from $2 \times 10^3$–$4 \times 10^5$ cfu/g of mucosa (Table 1). There were no changes in the *Enterobacteriaceae* count in either group at the end of the study period compared with before the start. The same holds true for the sulfite-reducing clostridia, although there was a small increase in the placebo group in the rectal mucosa. During the study period the *Enterococci* count remained the same in the test group, whereas there was a small increase in the placebo group at the end of intake (Table 1).

**Flatulence**
Flatulence rapidly and significantly decreased in the test group, but at the end of the study period a significant decrease was also seen in the placebo group (Fig. 1). The decrease was significantly more pronounced in the test group than in the placebo group ($p < 0.05$ using the Mann-Whitney U test) during the last 2 wk of treatment (weeks 5 + 6). In the test group, 44% of the patients reported a reduction in flatulence of at least 50%, compared with 18% of the patients in the placebo group (data not shown).
The number of days with abundant gas production was significantly lower in the test group than in the placebo group during the last 2 wk of treatment (Table 2, Fig. 1).

**Abdominal Pain**

Abdominal pain was reduced in both groups, even though the reduction was more rapid and more pronounced in the test group (Fig. 2). During the last 2 wk of treatment (weeks 5–6), 36% of the patients in the test group reported a pain decrease of more than 1.5 (VAS) compared to before intake (weeks 1–2). The same reduction was seen in 18% of the patients in the placebo group (data not shown). Despite a clear trend, the pain decrease in the test group was not significantly more pronounced than in the placebo group during the last 2 wk of treatment ($p = 0.30$ using the Mann-Whitney U test).

**Defecation**

Significant improvements in the function of defecation were obtained on the VAS in the test group (Fig. 3). The patients in the placebo group also reported improvement, although not to an extent that was fully statistically significant ($p = 0.06$).

**FREQUENCY.** During the two reference weeks very few patients had an abnormal defecation frequency, i.e., 4 days without defecation or $\geq 1$ days with more than three defecations. In the test group, five patients had 4 days without defecation during this period. Three of these had a reduced number of days without defecation during the last 2 wk of treatment ($p = 0.30$ using the Mann-Whitney U test).

**CONSISTENCY.** Only a few patients in each group had $\geq 1$ days of very loose or very hard stools during the two reference weeks.
Abdominal pain reported once a week on a VAS (evaluated from 0 to 10 [no pain–worst possible pain]). The mean degree of symptoms is reported ± SEM. Significant changes are noted as *p < 0.05, **p < 0.01, and ***p < 0.001. Comparison with weeks 1 and 2; comparison with weeks 1 + 2/2; comparison with wk 1. No differences were seen between the test group and the placebo group during weeks 1 + 2/2 using the Mann Whitney U test and Student’s t test. □ = treatment arm (n = 25); □ = placebo arm (n = 27).

Defecation function reported once a week on a VAS (evaluated from 0 to 10 [perfect function–worst possible function]). The mean degree of symptoms is reported ± SEM. Statistically significant changes are noted as *p < 0.05, **p < 0.01, and ***p < 0.001. Comparison with weeks 1 and 2; comparison with weeks 1 + 2/2; comparison with wk 1; comparison with wk 2. No difference was seen between the test group and the placebo group during weeks 1 + 2/2 using the Mann Whitney U test and Student’s t test. □ = treatment arm (n = 25); □ = placebo arm (n = 27).
In both groups almost all of the patients with very loose stools improved during the two last weeks of treatment (six of nine in the test group and seven of seven in the placebo group; data not shown). Similarly, when the number of days with rather loose to very loose stools were summed up for each person, a significant decrease was seen in both groups during the last 2 wk of treatment (Table 3). This coincided with an increased number of days with normal stool consistency in both groups.

No effects were seen in either group on those with rather hard to very hard stool.

**Overall GI Function**

Overall GI function reported on the VAS was significantly improved in the test group, but not in the placebo group (Fig. 4). The difference in the rate of improvement in the test group during the last 2 wk of administration compared with the placebo group did not reach statistical significance, however ($p = 0.06$ Mann-Whitney $U$ test). During the last 2 wk of treatment (weeks 5 + 6), 44% of the patients in the test group reported a pain decrease of more than 1.0 on the VAS, compared with before intake (weeks 1 + 2). The same reduction was seen in 26% of the patients in the placebo group (data not shown).

**Follow-Up**

Forty-eight of the original 52 patients answered the questionnaire at the 12-month follow-up (22/25 belonged to the test group and 26/27 to the placebo group). In general, patients in the test group still had a significantly better function compared with study entry, whereas the patients in the placebo group did not exhibit any remaining improvement. The number of defecations was reduced in both groups. There were no major changes in gas bloating (Table 2). In the questionnaire questions had been added about any regular intake of fermented milk products or the test prod-

### Table 3. Mean Number of Days With Rather Loose to Very Loose Stools, Normal Stools, and Rather Hard to Very Hard Stools During Weeks 1 + 2 and 5 + 6

<table>
<thead>
<tr>
<th>Group</th>
<th>Weeks</th>
<th>No. of Days With Rather Loose to Very Loose Stools</th>
<th>No. of Days With Normal Stools</th>
<th>No. of Days With Rather Hard to Very Hard Stools</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test group (n = 25)</td>
<td>1 + 2</td>
<td>4.8 (0–13)</td>
<td>4.0 (0–9)</td>
<td>3.0 (0–10)</td>
</tr>
<tr>
<td></td>
<td>5 + 6</td>
<td>3.2 (0–10)*</td>
<td>6.2 (0–12)†</td>
<td>2.9 (0–11)</td>
</tr>
<tr>
<td>Placebo group (n = 27)</td>
<td>1 + 2</td>
<td>4.8 (0–14)</td>
<td>4.0 (0–14)</td>
<td>2.7 (0–8)</td>
</tr>
<tr>
<td></td>
<td>5 + 6</td>
<td>3.0 (0–14)‡</td>
<td>6.6 (0–14)†§</td>
<td>2.7 (0–13)</td>
</tr>
</tbody>
</table>

Figures within parentheses are minimum and maximum values. *$p = 0.02$ vs weeks 1 + 2 in the test group; †$p = 0.002$ vs weeks 1 + 2 within groups; and ‡$p = 0.04$ vs weeks 1 + 2 in the placebo group.

**Figure 4.** Overall GI function reported once a week on a VAS (evaluated from 0 to 10 [perfect function–worst possible function]). The mean degree of symptoms is reported ± SEM. Significance and comparisons as above. No significant difference was seen between the test group and the placebo group during weeks 1 + 2/2 using the Mann Whitney $U$ test and Student’s $t$ test. □ = treatment arm (n = 25); □ = placebo arm (n = 27).
uct, which is commercially available in the area. In the test group, nine of 22 persons (41%) did not have any regular intake of fermented products. In the placebo group, six of 22 patients (27%) had no regular intake of fermented products.

**DISCUSSION**

The results of this study indicate that administration of *Lactobacillus plantarum* DSM 9843 with known probiotic properties (17) decreases pain and flatulence in patients with IBS.

The etiology of IBS is largely unknown. Etiological considerations range from motility disorder or altered perception, a psychological disorder, a psychophysiological phenomenon, or even abnormal illness behavior (3–6). Many therapy modalities have been tried in controlled studies, e.g., fiber, dietary fiber supplementation, antispasmodic agents, antidepressants, and dopamine antagonists, to name just a few (19). Not a single study offers convincing evidence for an efficacious therapy for patients with IBS. The range of specific symptoms associated with IBS may indicate a multitude of etiological influences and some therefore advocate a multicomponent therapy (20).

In recent years, we have been interested in the mucosa-associated flora of the gut. We have investigated the flora in healthy persons and in patients with different GI symptomatology (14). In the course of this work we identified and isolated a few *Lactobacillus* strains of species commonly found in healthy intestinal mucosa and proved their colonization ability (17). During trials with administration of the *Lactobacillus plantarum* DSM9843 strain to healthy volunteers we saw a reduction in flatulence and in the amount of gas-producing bacteria in the feces, and an increased amount of carboxylic acids in the feces (21). The colonic flora of patients with irritable bowel syndrome has been reported in a few studies previously. Balsari *et al.* found in a group of 20 patients a decrease of coliforms, *Lactobacilli*, and, to some extent, *Bifidobacteria* (8). In another study of the microbial flora of two patients with food-related IBS, significant diet-related differences in bacterial viable counts were seen in both patients (9). That bacteria may play a role in the symptomatology of IBS has recently been shown in a study by King *et al.* (10). They found that colonic gas production, particularly of hydrogen, was greater in patients with IBS than in controls. They also showed that symptoms and gas production were reduced by exclusion diet. Reduction may be associated with alterations in the activity of hydrogen-producing bacteria.

We therefore wanted to perform this study and test the hypothesis that an increased colonization by *Lb. plantarum* would reduce symptomatology, especially that related to gas formation and pain. Sixty patients with IBS were recruited and before the start of the trial we characterized the patient group regarding symptomatology (22). There was no difference between the two groups regarding symptomatology at the start of the study. The patients were recruited by advertisements in the local press and therefore represent a group of patients with milder symptoms than patients already attending an outpatient clinic. During the study period there was an improvement in the symptoms of flatulence, pain, and defecation in both groups. It has been observed previously that a placebo response in IBS patients is common but variable, ranging from <20% to 50% (23, 24), which was observed in our study as well. However, the decrease in flatulence was significantly greater in the test group than in the placebo group and there was a gradual increase of this effect during the first 2 wk. Thereafter conditions remained stable for 2 wk. The effect on abdominal pain paralleled the effect on flatulence to some extent and it is reasonable to assume that the relief in pain was mainly due to reduced flatulence. Effects on the number of defecations and fecal consistency were more variable. The patients’ assessment of the overall symptomatology was, however, in favor of a test solution, compared with the placebo. The reasons behind the reduced flatulence in these patients are not obvious. Microbiological analysis showed colonization by *Lb. plantarum* in the test group and these bacteria were not present in any group before the start of therapy. Previous tests with *Lb. plantarum* have shown that the bacteria are a dominating part of the mucosal lactobacilli flora 10 days after administration in both jejunum and rectum. We did not see any obvious change in sulfite-reducing clostridia or *Enterobacteriaceae*. However, we quantitatively analyzed only a few of the major groups of bacteria that occur in the feces and rectal mucosa and there may have been quantitative shifts between different fractions within the groups that were not detected in this analysis. It can be argued that because IBS is a chronic, sometimes lifelong condition, the length of the study trial should have been longer than 4 wk. It is, however, very difficult to restrict the intake of live bacteria over a prolonged period, as this means that severe dietary restrictions must be imposed on the patients that might influence the study in other and uncontrollable ways. The effects of altering the gut mucosal microflora will also last for some time after the end of administration, and we did not use a crossover design to avoid this carryover effect. Furthermore, it is easier to reach statistical significance in a crossover study.

The influence of factors other than bacteria must be considered. The fiber content of the test solution was minimal and it is unlikely that the fiber content could have had any effect. Rose-hip was administered in both groups and is not known to have any effect on the GI function.

The patients in the test group exhibited a continuous improvement in overall GI function at 12 months compared with patients receiving placebo 12 months earlier. Although the intake of fermented products was slightly lower in the test group at the 12-month follow-up than in the placebo group, there seemed to be a remaining improvement in overall GI function in these patients compared with patients receiving placebo 12 months earlier. The reasons behind these long-term effects are difficult to explain in detail. One
possible explanation is that the dose of lactobacilli administered 12 months earlier was sufficiently high to bring about a lasting alteration in the gut flora. Another explanation may be the continuously higher intake of fermented products in the test group. The effect may, however, also be secondary to other factors in addition to bacteria. Patients in the test group may have changed their dietary habits after the trial to a larger extent than the patients in the placebo group. Because the patients in the test group experienced an improvement in their symptoms during the trial, they may have become more aware of food intake once the code was broken.

In summarizing the results of the study, it can be argued that the design of the trial was not ideal in all respects, but the results certainly show an effect of administration of lactobacilli to patients with mild forms of IBS. This has not been shown previously. This type of therapy should undergo further testing in other groups of patients with IBS.

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