Clinical evaluation of *Lactobacillus paracasei* subsp. *paracasei* F19 with gluco-oligosaccharides in the short-term treatment of irritable bowel syndrome

LUCIO LOMBARDO, ANNALISA VERNETTO & ILENIA BLANCO

Abstract

Objective: The aim of the present research was to evaluate, in an open, short-term study, the efficacy and tolerability of a probiotic containing *Lactobacillus paracasei* subsp. *paracasei* F19 in irritable bowel syndrome (IBS). Patients and methods: Studies were carried out on 100 patients with IBS (38 male, 62 female, age range 18-68 years), 52 with diarrhoea and 48 with constipation, recruited in accordance with the Rome II diagnostic criteria. All patients were administered Genefilus F19 (Siffra Pharmaceuticals SpA, Florence, Italy), a commercial preparation containing *L. paracasei* subsp. *paracasei* F19, at a dose of one sachet (dissolved in water) twice daily for 14 days. Each sachet contained $12 \times 10^9$ genetically stable microorganisms of *L. paracasei* subsp. *paracasei* F19, combined with 750 mg gluco-oligosaccharides plus vitamins B1, B5 and B6. Symptoms were evaluated, based upon a daily diary, before and after treatment. In addition, in a series of 20 consecutive patients, *L. paracasei* subsp. *paracasei* F19 content was evaluated in faeces before and after treatment. Results: Following treatment, abdominal pain was no longer present in 94% of the patients with IBS with diarrhoea or in 87% of those with IBS with constipation. Diarrhoea was no longer present in 88% of the patients, while constipation was no longer present in 83% of the patients. Abdominal distension had disappeared, or considerably improved, in 95% of the study population. These percentages remained practically unchanged at follow-up, 2 weeks after the end of treatment. Tolerability was excellent – no significant side effects or drop-outs were recorded. The results of microbiological evaluations performed on faeces revealed a low microbial *L. paracasei* subsp. *paracasei* F19 load before treatment and a marked increase in load following treatment, with a minimum of 2, to a maximum of 10, cfu/g faeces. Conclusions: In this short-term study, Genefilus F19 was confirmed to be efficacious and well tolerated in patients with IBS presenting with diarrhoea or with constipation and, therefore, useful in everyday clinical practice. Double-blind controlled studies, with placebo, including clinical evaluations at medium- and long-term follow-up, are now under way with a larger series of patients to confirm these promising findings.

Key words: *L. paracasei* subsp. *paracasei* F19, irritable bowel syndrome, constipation, diarrhoea

Introduction

Irritable bowel syndrome (IBS), a functional motor disorder of the intestine, is characterized by the triad of abdominal pain, constipation and/or diarrhoea, absence of organic intestinal lesions. The precise characteristics have been well defined by the Rome III criteria, referring to a duration of at least 3 months, not necessarily consecutive, within a 1-year period.

The social impact of the condition is quite high, affecting approximately 20% of the population, accounting for approximately 20% of general practitioners’ medical activity and, economically, leading to high direct and indirect costs (1).

The prevalence of IBS has increased over the last 50 years in countries in which a Western-style diet has been prominent and a number of patients (20-65%) attribute their symptoms to something in food that activates an abnormal response (2,3).

The aetiology remains to be fully elucidated but the recognized causes have been reported to be due not only to psychological factors, but also to sensitive and motor disorders of the visceral nerves, as well as previous episodes of enteritis and changes in the bacterial ecosystem of the intestine. Probiotics may suppress the low grade inflammation associated with IBS or restore normal local immune function (4).
In the scientific world, considerable attention has recently been focused, in particular, on this latter aspect. This is not surprising considering that eating habits have a marked effect upon the immune system and that a large part of this system is indeed located in the intestine (5). Hundreds of thousands of molecules (short-chain fatty acids, vitamins, antioxidants, peptides, etc.) are released in the intestine, on account of bacterial fermentation (4).

These substances are produced, therefore, by a 'bacterial digestive system', rich in approximately 300 000–2 000 000 genes, representing at least four-to five-fold the genetic patrimony contained in the human body (65 000), thus indicating an enormous complex amount of information (6).

The majority of the beneficial bioactive effects are due to the symbiotic action of the prebiotics (fibre) and the probiotics (lactobacilli), with a ‘scavenging’ action on the free radicals. However, this effect has been demonstrated only for four bacteria: Lactobacillus plantarum, Leuconostac mesenteroides, Pediococcus pentosaceus and Lactobacillus paracasei subsp. paracasei F19 (7). Only the latter is of human origin. So far, clinical studies on the effects of probiotics on IBS have been surprisingly scarce.

L. paracasei subsp. paracasei F19 has been developed in accordance with the regulations set down in the World Health Organization Working Team Report, referring, in particular, to a bacterium with proven genetic stability (8). This is particularly important for certain diseases of the digestive tract such as IBS, for which patients require bacteria, probiotics and prebiotics for long periods of time.

**Patients and methods**

**Patients**

A total of 100 patients (62 female and 38 male), age range 18–68 years (mean 39 ± 26 SD), with IBS defined according to the Rome III criteria, were recruited from February 2005 to April 2006. All patients were studied in accordance with the Helsinki declaration and gave informed written consent to take part in the investigation.

Of these patients, 52 presented IBS associated with diarrhoea (5 ± 2 SD bowel movements/day), while 48 presented IBS associated with constipation (0.5 ± 2 bowel movements/week) (Table I).

A semi-quantitative evaluation of pain was made as regards intensity, with a score of 0–3 (0 = no pain, 1 = slight pain, 2 = moderate pain, 3 = severe pain) being obtained. Evaluation of pain frequency was based on the mean number of episodes per day, each lasting for at least 60 s. To gain an overall estimate of the pain, the concept of 'consistency of pain' was introduced, which was obtained by multiplying the mean value of intensity by that of frequency.

This parameter was calculated both before (mean value during the week before treatment) and after treatment (mean of the last week of treatment) and then 1 month after the end of treatment (mean of last week).

All patients had been submitted to total colonoscopy or X-ray enema and oesophago-gastro-duodenoscopy in the previous 4 years, which confirmed the absence of organic intestinal lesions. Patients presenting neoplasia, cirrhosis, clinically relevant liver disease or diverticular disease with complications were excluded from the study. Also excluded were patients who had received antibiotic treatment during the previous 3 months and patients with alarm symptoms requiring further clinical investigations.

**Definitions**

Regular motion: passage of stools with a frequency of 3–12 evacuations per week, with well-formed stools, of soft consistency.

Improvement in diarrhoea: > 50% reduction in the number of daily bowel movements.

Improvement in pain: > 75% reduction in frequency and intensity of pain.

Abdominal bloating: subjective sensation of abdominal fullness and bloating.

**Treatment**

All patients included in the study took one sachet (dissolved in water) of a commercial preparation, i.e. Genefilus F19 (Siffra Pharmaceuticals SpA, Florence, Italy), twice daily for 14 days. Each sachet contained 12 × 10⁹ genetically stable microorganisms of L. paracasei subsp. paracasei F19, associated with 750 mg of gluco-oligosaccharides, 0.7 mg of thiamine (vitamin B1), 3 mg of pantothenic acid (vitamin B5) and 1 mg of pyridoxine (vitamin B6).

In the event that patients had been on concomitant antihypertensive treatment for at least 3 months, these drugs were maintained throughout the study. On the other hand, any anxiolytic drugs were withdrawn at least 2 weeks before the beginning of the study.

**Gastrointestinal symptoms**

Each patient was assigned a diary to be completed daily. Throughout the study, patients regularly made notes in their diary recording the number of evacuations and the characteristics of the faeces (hard, normal, loose), and relevant gastrointestinal symptoms (pain, abdominal bloating).
Table I. Baseline characteristics of patients treated with Genefilus F19.

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Sex</th>
<th>Mean age (years)</th>
<th>IBS + diarrhea</th>
<th>IBS + constipation</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>62 females; 38 males</td>
<td>39 ± 26</td>
<td>52</td>
<td>48</td>
</tr>
</tbody>
</table>

Patients also recorded the number of sachets of *L. paracasei* subsp. *paracasei* F19 consumed each day, to confirm the correct dosage prescribed.

Patients’ opinions were recorded, within 24 hours, indicating the timing and number of eventual episodes.

**Microbiological procedure**

Faecal samples from a series of 20 consecutive non-selected patients (14 male, 6 female) taking part in the study were collected before treatment (time 0) and again after consumption of *L. paracasei* subsp. *paracasei* F19 for 14 days (time 1).

All samples were collected in sterile containers, stored at -70°C, then taken, in anaerobiosis, to the Microbiology Laboratory.

Faecal samples corresponding to the two sample times were diluted in decimal parts in a physiological solution of 9 g/l NaCl, then seeded on Rogosa (Difco, Detroit, MI, USA) to select the total lactobacilli and on Rogosa to which 12 mg/ml vancomycin had been added (Sigma Chemical Co., St Louis, MO, USA) for the selection of vancomycin-insensitive lactobacilli, the group to which the F19 strain under study belongs.

The decimal dilutions of the samples were then seeded on Rogosa beds and Rogosa plus vancomycin beds. Plates were then incubated in anaerobiosis at 37°C for 48 h in GasPack (Anaerocult, Merck, Darmstadt, Germany), after which the colony-forming units per gram (cfu/g) of faecal material were counted. Colonies displaying a different morphology were removed and studied under light microscopy.

**Identification of the bacterial strain *L. paracasei* subsp. *paracasei* F19**

To identify *L. paracasei* subsp. *paracasei* F19 strain in the faecal samples, 10% of the colonies (with a significant decimal dilution, i.e. 30–300 colonies) grown on Rogosa plus vancomycin were submitted to molecular alpha typing by repetitive extragenic palindromic-PCR (REP-PCR) using the pair of primers REP1R-Dt and REP2-Dt (9). The technique and amplification cycle used were as described by Versalovic et al. (10). The amplification products were analysed by means of electrophoresis run on agarose gel 2.2% for 3 h at 100-150 V.

The *L. paracasei* subsp. *paracasei* F19 strain was submitted to the same technique to obtain a clearly recognizable and useful profile that could be used as a reference during the course of the analysis of the isolates obtained from the faecal specimens under study.

Using the REP-PCR technique and by observing the particular sequences repeated within the bacterial genome, it is possible to identify and type numerous bacteria to strain level (11).

**Results**

**Pain**

At the end of the treatment period, pain was no longer present in 49/52 IBS patients with diarrhoea (94.2%) or in 42/48 of the IBS patients with constipation (87.5%) (Table II).

Intensity of pain, showing an initial mean value of 2.2 ± 0.5 (during the week preceding treatment), dropped to a mean value of 0.5 ± 0.3 (*p* < 0.001), during the last week of treatment.

The mean frequency of pain dropped from an initial value of 6 ± 4 per day to 0.6 ± 0.4 per day (*p* < 0.001). This improvement was practically maintained over the next 4 weeks post-treatment, with a mean frequency of 0.7 ± 0.5 per day and intensity of 0.6 ± 0.5.

Consistency of pain at the beginning of the study was 13.2, while at the end of the treatment period a value of 0.3 was recorded; the difference was statistically significant (*p* < 0.001).

**Bowel behaviour**

In patients presenting IBS associated with diarrhoea, the diarrhoea was brought under control in 46/52 patients (88.4%), improved in 4 and remained unchanged in 2.

Table II. Clinical results in IBS patients treated with Genefilus F19.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result</th>
<th>Absolute no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBS with diarrhoea</td>
<td>Diarrhoea remission 88.4</td>
<td>46/52</td>
</tr>
<tr>
<td></td>
<td>Pain remission 94.2</td>
<td>49/52</td>
</tr>
<tr>
<td>IBS with constipation</td>
<td>Constipation correction 83.3</td>
<td>40/48</td>
</tr>
<tr>
<td></td>
<td>Pain remission 87.5</td>
<td>42/48</td>
</tr>
<tr>
<td>Bloating</td>
<td>Bloating remission 95.5</td>
<td>86/90</td>
</tr>
</tbody>
</table>
In patients with IBS associated with constipation, this was corrected in 40/48 patients (83.3%), with a mean evacuation frequency of 5 ± 2 evacuations per week, but was unchanged in the remaining 8 patients.

**Bloating**

At the end of treatment, bloating was no longer present, or showed considerable improvement, in 95.5% of the 90 patients complaining of this disorder.

No significant side effects that could definitely be attributed to the treatment were observed. Only one case of slight nausea was reported, but this did not require withdrawal from treatment.

**Microbiological analysis of faecal specimens**

At time 0, microbiological results in all 20 patients taking part in the study revealed a low microbial load of *L. paracasei* subsp. *paracasei* F19, while after 14 days of administration of treatment based upon *L. paracasei* subsp. *paracasei* F19 (time 1), an individual variation was observed, with marked presence of lactobacilli, the number of colonies of the F19 strain ranging from a minimum of 2 to a maximum of 10 microbial counts, expressed in cfu/g faeces (Table III). No linear correlation was found between improvement in clinical symptoms and the amount of probiotic found in the faeces, probably on account of the small number of patients studied.

These results demonstrate the survival of *L. paracasei* subsp. *paracasei* F19 during intestinal transit and that, following administration, *L. paracasei* subsp. *paracasei* F19 accounts for a numerically significant presence, over other *Lactobacilli*, in the faeces.

**Discussion**

In our experience, the product Genefilus F19 has been demonstrated to be clinically useful in patients presenting IBS, whether associated with diarrhoea or constipation. In particular, abdominal pain disappeared completely or showed considerable improvement (reduction > 75%) in 94.2% of the patients with IBS associated with diarrhoea and in 87.5% of the patients with IBS associated with constipation. The exact pathophysiological mechanism responsible for this result remains to be defined. As is well known, the placebo effect may be of considerable importance in these clinical settings, but in our opinion could not, in itself, completely account for the results obtained.

A return to normal of bowel movements was confirmed not only in a large percentage of patients presenting IBS associated with diarrhoea (88.4%) but also in those with IBS associated with constipation (83.3%), together with a substantial numerical increase in *L. paracasei* subsp. *paracasei* F19 in the faeces, following treatment. This finding is in keeping with the hypothesis of a beneficial role of *L. paracasei* subsp. *paracasei* F19 on intestinal microbiota activity and on its effective capacity to enhance colonization, possibly with a positive effect upon the immunomodulation described in the literature (12,13). The mechanisms responsible for these positive results remain to be fully elucidated. It is tempting to suggest that the generation of nitric oxide (NO), a gas with immunomodulating and antibacterial properties, by lactobacilli using nitrate and nitrite, could play an important role (14).

For the form of IBS associated with diarrhoea, it would appear feasible to suggest that the substitution of ‘pathogenic’ or at least ‘irritating’ bacteria, in the intestinal lumen with other ‘non-pathogens’ of demonstrated usefulness, is able to remove or replace the ‘irritative’ cause and to restore the intestinal mucosa to its normal functions. For the constipation-related form, however, this mechanism is not completely exhaustive and immediately ‘acceptable’. In the latter case, in fact, it is necessary to hypothesize at least a double intermediate passage, which foresees the possibility of improvement/normalization in cell tropism of the intestinal mucosa with positive effects upon the neuro-motor and neuro-sensitive loco-regional transmission. Neurotrophic effects, promoted by vitamins such as B1, B5 and B6, may produce beneficial effects on enteric motor regulation (15). This hypothesis, despite being supported by physiological assumptions, nevertheless still remains to be confirmed. Psychological factors may play an important role in improvement of symptoms, including diarrhoea and constipation. Even if the results obtained appear, in our opinion, to be of considerable interest and worthy of due clinical attention, we are clearly aware that the present open ‘pilot’ study cannot be considered conclusive and prompts criticism related to the subjective

---

**Table III. Total lactobacilli cfu/g faeces determined on Rogosa and Rogosa plus vancomycin cultures (mean ± SD).**

<table>
<thead>
<tr>
<th>Method</th>
<th>cfu T₀</th>
<th>cfu T₁</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rogosa</td>
<td>4.5 ± 2.0 x 10⁶</td>
<td>6.5 ± 3.0 x 10⁶</td>
</tr>
<tr>
<td>Rogosa + vancomycin</td>
<td>2.5 ± 1.7 x 10⁴</td>
<td>3.7 ± 1.8 x 10⁴</td>
</tr>
<tr>
<td>REP-PCR</td>
<td>0</td>
<td>15 ± 5</td>
</tr>
<tr>
<td>F19</td>
<td>0</td>
<td>6 ± 4</td>
</tr>
</tbody>
</table>

Colonies were identified by the REP-PCR technique (mean number ± SD). *L. paracasei* subsp. *paracasei* F19 cfu/g faeces (mean number ± SD); T₀ before treatment; T₁, after 14 days of treatment with Genefilus F19 (T₁) in 20 patients affected by IBS.
evaluation. Albeit, following these promising data, a double-blind study vs placebo is currently under way, with a larger number of patients, so as not only to confirm the results of the present research, but also to extend the study to include clinical evaluations at medium- and long-term follow-up.

In conclusion, Genefilus F19, evaluated in this investigation, was well tolerated by all the patients taking part in the study, with excellent compliance and displaying a significant improvement in symptoms during treatment, which was maintained for at least 2 weeks after the end of the treatment period.

These results are in agreement with those of other authors from a smaller, double-blind study on patients with IBS, employing Lactobacillus plantarum (16).

In our opinion, therefore, the product evaluated in this study should be taken into consideration as a new probiotic, clearly demonstrating the capacity to survive gastric transit and to resist biliary secretions, improving intestinal microflora and may be useful in the outpatient setting in the short-term treatment of IBS, whether associated with diarrhoea or constipation. More detailed evaluations are currently under way with studies on clinical intestinal pathophysiology and controlled clinical trials with double-blind vs placebo, taking into consideration also the medium- and long-term effects.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References