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Alimentary Tract

Lactobacillus paracasei F19 *versus* placebo for the prevention of proton pump inhibitor-induced bowel symptoms: A randomized clinical trial

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ABSTRACT

Background: Proton pump inhibitors may foster intestinal dysbiosis and related bowel symptoms.
Aim: To evaluate the effect of *Lactobacillus paracasei* F19 on bowel symptom onset in patients on long-term proton pump inhibitors.
Methods: In this randomized, double-blind, placebo-controlled study, patients with typical gastroesophageal reflux disease symptoms receiving pantoprazole 40 mg/d for six months were randomly assigned to receive: (A) *Lactobacillus paracasei* F19 bid for three days/week for six months; (B) placebo bid for three days/week for six months; (C) *Lactobacillus paracasei* F19 bid for three days/week for three months and placebo bid for three days/week for the following three months; (D) placebo bid for three days/week for three months and *Lactobacillus paracasei* F19 bid for three days/week for the following three months. Bloating, flatulence, abdominal pain and bowel habit were assessed monthly.
Results: 100/312 patients were enrolled. In the parallel groups, the treatment-by-time interaction affected bloating ($p = 0.015$), while *Lactobacillus paracasei* F19 treatment alone affected flatulence ($p = 0.011$). Moreover, the treatment-by-time interaction significantly affected the mean score of bloating ($p = 0.01$) and flatulence ($p < 0.0001$), the mean stool form ($p = 0.03$) and mean stool frequency/week ($p = 0.016$). Analysis of the cross-over groups, limited to the first three months because of carry-over effect, confirmed these results.
Conclusion: Lactobacillus paracasei F19 supplementation prevents bowel symptom onset in patients on long-term proton pump inhibitors.

1. Background

Gastroesophageal reflux disease (GERD) is a widespread condition. Up to 28% of the US population and 26% of the European population suffer from GERD and take proton pump inhibitors (PPIs) to control their symptoms [1]. However, symptoms recur at therapy withdrawal in about 70% of patients who thus require continuous treatment. Moreover, a double dose of PPI is needed to control symptoms in more than 50% of cases [2]. The global sales of these drugs in 2008 were in the order of US$ 26.5 billion [3]. However, the effectiveness and safety of PPIs are being questioned, and adverse effects associated with their chronic use are emerging [4]. Such reports led the USA Food and Drug Administration to issue a number of warnings about all available PPIs [5].

PPIs inhibit gastric acid secretion and increase the pH of the gastric juice, one of the most important defences against exogenous infections, and intimately affect both the quality and quantity of the gut bacteria. An increase of one pH unit in the small intestine corresponds to a 13.8% increase in small bowel microbial counts [6]. Lombardo et al. reported small intestine bacterial overgrowth (SIBO), diagnosed by hydrogen breath tests, in 50% of 200 GERD

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patients receiving PPIs for a median of 36 months [7]. In contrast, a retrospective chart review involving 1191 patients revealed that PPIs were not associated with SIBO diagnosed by the glucose-hydrogen breath test (GHBT) [8]. Finally, a recent meta-analysis of 11 studies revealed an association between PPIs and SIBO only in a subgroup analysis of studies that used duodenal or jejunal aspirate cultures to diagnose SIBO (odds ratio [OR], 7.59; 95% CI, 1.805–31.894) [9]. Differences in study design, sample size, PPI dosage and duration, age of patients, dietary habits, concomitant diseases and, mainly, the lack of a true "gold standard" diagnostic test for SIBO, may account for these different results.

The most common clinical manifestations of SIBO are diarrhoea, abdominal pain and bloating, which resemble the adverse gastrointestinal effects typically observed during PPI treatment [10]. Although considerable evidence supports the relationship between PPI use and SIBO development [7–9], a causal link to symptoms is rather tenuous, and thus far no randomized, prospective trial has addressed this association. In a previous uncontrolled observational study we found that PPI treatment for 6 months was associated with a positive GHBT in 26% of cases, but also with the onset of clinically relevant bowel symptoms, namely bloating, flatulence, but also with abdominal pain in a high percentage of patients (52%, 33% and 24%, respectively) [11]. In agreement with our findings, Jacobs et al., reported that PPI use was an independent risk factor for SIBO in over 50% of 150 patients with unexplained gastrointestinal symptoms [12].

Given the above findings, it is conceivable that PPI-related bowel symptoms result from dysbiosis. Should this be the case, the responsiveness of PPI-induced bowel symptoms to probiotics could identify intestinal dysbiosis as the pathogenetic trigger of this condition. Consequently, the aim of this study was to evaluate whether *Lactobacillus paracasei* subspecies *paracasei* F19 (LP-F19) could prevent the onset of bowel symptoms in GERD patients on long-term PPI treatment.

2. Patients and methods

2.1. Study design

This was a randomized, double-blind, placebo-controlled, multicentre study that included parallel and cross-over groups. Randomization in the four groups was performed by means of a pre-defined randomization list prepared in blocks of size sixteen and was supervised by the company that donated the probiotic and placebo. Neither the investigators nor the patients were aware of the treatment administered. The randomization schedule was kept at the company's offices until completion of the trial, and all the data were entered into a computer database by people not involved in the study.

All patients were treated with pantoprazole 40 mg/d for 6 months. The probiotic used was a commercial preparation (Gefilus F19®, Siffra Farmaceutici S.r.l., Florence, Italy) containing LP-F19 in sachets to dissolve in water and to take just before a meal. Each sachet contained 12×10^9 CFU/mL. Placebo consisted of a preparation similar to that of the commercial preparation but without microorganisms, and had the same weight, packaging, colour, smell and flavour as the probiotic. Siffra Farmaceutici S.r.l. donated the compounds (probiotics and placebo) used in the study.

Patients were randomly and blindly assigned to one of the following arms: (A) LP-F19 bid for three days/week for six months (LP-F19); (B) placebo bid for three days/week for six months (PBO); (C) LP-F19 bid for three days/week for the first three months and placebo bid for three days/week for the following three months (LP-F19 → PBO); (D) placebo bid for three days/week for the first three months and LP-F19 bid for three days/week for the following three months (PBO → LP-F19). The probiotic treatment (3 days/week) was scheduled based on data showing that LP-F19 survived intestinal transit and colonized the intestinal tract for long periods after ingestion [13]. After the first 3 months, a 2-week washout period was planned for all groups during which only PPI was continued. During the treatment period, patients were instructed to complete daily questionnaires for bowel symptoms and habit assessment. Monthly control visits were scheduled during which the patient received evaluation forms and the investigational products, and returned and discussed the questionnaires with a trained interviewer, who assessed compliance.

The protocol was approved by the Ethics Committee of each recruiting centre. The study was conducted in accordance with Good Clinical Practice and with the Declaration of Helsinki (DoH/Oct2008). All patients gave their written informed consent to participate in the study. All authors had access to the study data, and reviewed and approved the final manuscript. This trial is registered with http://www.ClinicalTrials.gov, number NCT02054455.

2.2. Population

Patients were enrolled at 3 national sites: one secondary and two tertiary care centres. All patients referred because of typical reflux symptoms (heartburn and regurgitation) lasting more than 6 months and occurring at least three times weekly were screened for eligibility. Exclusion criteria were: age <18 or >70 years; pregnancy or breast-feeding; evidence of major concomitant diseases (*i.e.*, malignancies, cardiovascular disorders, hepatic and/or renal failure); PPI or H_2-antagonist use for at least two consecutive weeks in the last three months, use of antibiotics or other probiotics in the 4 weeks before the study, and non-steroidal anti-inflammatory drugs (NSAIDs) on a regular basis 2 weeks prior to screening; history of alcohol abuse 6 months prior to screening; active *Helicobacter pylori* infection; gastric atrophy; irritable bowel syndrome and functional dyspepsia diagnosed according to Rome III criteria; coeliac disease; inflammatory bowel disease; diverticula of the colon; a positive GHBT or lactose hydrogen breath test; previous abdominal surgery; presence of any clinically relevant bowel symptoms.

Patients were evaluated for eligibility by a gastroenterologist at a screening visit after which they entered a 4-week screening period. All eligible patients underwent upper endoscopy and, in the absence of endoscopically visible lesions, ambulatory 24-hour pH-impedancemetry. Biochemical and haematology tests were performed to rule out abnormal results relevant for participation in the study. The patients then received questionnaires regarding bowel symptoms and bowel habits to complete during the screening period. After the screening period, the patients without exclusion criteria and who still fulfilled the inclusion criteria entered the treatment phase of the study. At enrolment, patients were instructed to maintain their eating and life style habits throughout the study.

Compliance to treatment was evaluated based on self-reporting by the patients and on counting the returned sachets at control visits. Patients who took at least 80% of the drugs for at least 80% of the scheduled time were considered compliant. Compliance with the study product was assessed by the presence and number of LP-F19 in faecal samples and cultured in MRS agar plates. Colonies were isolated and the presence of the probiotic strain was confirmed by randomly amplified polymorphic DNA (RAPD)-PCR using the primers LBC-19 for screening and OPA-02 for confirmation of LP-F19. Faecal samples, collected at 3 and 6 months, were immediately frozen, stored at −20 °C and sent in dry ice to an external laboratory that analyzed them at the end of the study.