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Oral Bacteriotherapy as Maintenance Treatment in Patients With Chronic Pouchitis: A Double-Blind, Placebo-Controlled Trial

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See editorial on page 584.

Background & Aims: **Pouchitis is the major long-term complication after ileal pouch–anal anastomosis for ulcerative colitis. Most patients have relapsing disease, and no maintenance treatment study has been performed. We evaluated the efficacy of a probiotic preparation (VSL#3) containing 5 × 10^{11} per gram of viable lyophilized bacteria of 4 strains of lactobacilli, 3 strains of bifidobacteria, and 1 strain of *Streptococcus salivarius* subsp. *thermophilus* compared with placebo in maintenance of remission of chronic pouchitis.** ***Methods:*** **Forty patients in clinical and endoscopic remission were randomized to receive either VSL#3, 6 g/day, or an identical placebo for 9 months. Patients were assessed clinically every month and endoscopically and histologically every 2 months or in the case of a relapse. Fecal samples were collected for stool culture before and after antibiotic treatment and each month during maintenance treatment.** ***Results:*** **Three patients (15%) in the VSL#3 group had relapses within the 9-month follow-up period, compared with 20 (100%) in the placebo group ($P < 0.001$). Fecal concentration of lactobacilli, bifidobacteria, and *S. thermophilus* increased significantly from baseline levels only in the VSL#3-treated group ($P < 0.01$).** ***Conclusions:*** **These results suggest that oral administration of this new probiotic preparation is effective in preventing flare-ups of chronic pouchitis.**

Pouchitis, a nonspecific inflammation of the ileal reservoir, is the most common long-term complication after pouch surgery for ulcerative colitis. Its cumulative frequency depends largely on the duration of the follow-up and is approximately 50% after 10 years at the major referral centers.[1–4]

Pouchitis is characterized clinically by increased stool frequency, urgency, abdominal cramping, and discomfort. Bleeding, low-grade fever, and extraintestinal manifestations may also occur.[5,6] Endoscopic findings of inflammation in the pouch include edema, granularity, loss of vascular pattern, contact bleeding, erosions, and ulcerations[7]; biopsies show an acute neutrophilic inflammatory infiltrate with crypt abscesses and ulceration in addition to the normal chronic inflammatory infiltrate, the latter of which is almost universal and probably represents an unavoidable response to fecal stasis.[8,9]

The cause of pouchitis is still unknown, but it seems that a history of ulcerative colitis and increased bacterial concentration are main factors.[10–12] The importance of bacteria is further emphasized by the evident efficacy of antibiotics.[10]

In most cases, patients have multiple attacks.[3,13,14] So far, no studies have focused on the maintenance of remission.

Probiotics are living microorganisms that belong to the natural flora and are important to the health and well-being of the host.[15] Recent observations support their role in the treatment of inflammatory bowel diseases. The administration of *Lactobacillus* spp. prevented the development of spontaneous colitis in interleukin (IL)-10–deficient mice, and continuous feeding with *Lactobacillus plantarum* attenuated established colitis in the same knockout model.[16,17]

Pouchitis has recently been shown to be associated with reduced counts of lactobacilli and bifidobacteria, suggesting that this syndrome may be the result of an unstable microflora.[18]

The aim of this study was to evaluate the efficacy of a new oral probiotic preparation, containing very high bacterial concentrations of 8 different bacterial strains

Abbreviations used in this paper: GI, gastrointestinal; IL, interleukin; PDAI, Pouchitis Disease Activity Index.

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compared with placebo in the maintenance treatment of chronic relapsing pouchitis.

Patients and Methods

Patients

The study was performed in accordance with the Declaration of Helsinki and was approved by the ethical committee of our hospital; written, informed consent was obtained from the patients. Eligible patients were between 18 and 65 years old and had chronic relapsing pouchitis, defined as at least 3 relapses per year. In addition, patients were in clinical and endoscopic remission, defined as score 0 after 1 month of combined antibiotic treatment, in the clinical and endoscopic portion of the Pouchitis Disease Activity Index (PDAI) by Sandborn et al.,[19] which includes clinical, endoscopic, and acute histologic criteria (Table 1). No concurrent treatments were allowed. Patients with perianal disease, including abscess, fistula, fissure, stricture, or anal sphincter weakness, were excluded.

Study Medication

VSL#3 (Yovis; Sigma-Tau, Pomezia, Italy) consisted of 3-g bags each containing 300 billion viable lyophilized bacteria per gram of 4 strains of *Lactobacillus* (*L. casei, L. plantarum, L. acidophilus,* and *L. delbrueckii* subsp. *bulgaricus*), 3 strains of *Bifidobacterium* (*B. longum, B. breve,* and *B. infantis*), and 1 strain of *Streptococcus salivarius* subsp. *thermophilus*.

The placebo consisted of identical bags each containing 3 g of maize starch. The VSL#3 and placebo were administered orally twice a day. The taste and smell of the active drug were not readily identifiable.

Table 1. Pouchitis Disease Activity Index

Criteria		Score
Clinical		
Postoperative stool frequency	Usual	0
	1–2 stools/day more than usual	1
	3 or more stools/day more than usual	2
Rectal bleeding	None or rare	0
	Present daily	1
Fecal urgency/abdominal cramps	None	0
	Occasional	1
	Usual	2
Fever (temperature > 100°F)	Absent	0
	Present	1
Endoscopic		
Edema		1
Granularity		1
Friability		1
Loss of vascular pattern		1
Mucus exudate		1
Ulcerations		1
Acute histological		
Polymorph infiltration	Mild	1
	Moderate + crypt abscess	2
	Severe + crypt abscess	3
Ulcerations per low-power field (average)		1
	<25%	
	25%–50%	2
	>50%	3

Study Design

This was a randomized, double-blind, placebo-controlled study.

Patients, whose conditions were in clinical and endoscopic remission (with a score of 0 in the clinical and endoscopic portion of PDAI) after 1 month of antibiotic treatment with 1 g ciprofloxacin plus 2 g rifaximin daily (Alfa-Wasserman, Bologna, Italy), were randomized to receive VSL#3 (6 g/day) or placebo for 9 months.

Assignment to therapy or placebo was determined according to a computer-generated randomization scheme.[20] Randomization was done by the clinical trial's pharmacist, who kept the codes until completion of the study. None of the staff or patients had access to the randomization codes during the study. The medications were dispensed by the investigator at each visit; compliance was assessed by counting returned bags and questioning the patients.

Evaluation and Scheduling

Symptoms were assessed, medical histories were taken, and physical examinations were performed at baseline and every month thereafter. Endoscopic examination of the ileal pouch and the ileum for a few centimeters proximal to the pouch, with mucosal biopsies, was performed at baseline and every 2 months thereafter, and histologic assessment of biopsy specimens was performed at entry and every 2 months thereafter. Laboratory studies, including a complete blood count and blood chemistry measurements, were performed at baseline and at the end of treatment.

Relapse was defined as an increase of at least 2 points in the clinical portion of PDAI, confirmed by endoscopy and histology.

Microbiological Determinations

Stool cultures were performed before and after antibiotic treatment and every month during maintenance treatment. Collection of specimens, anaerobic culture techniques, isolation procedures, and identification methods were performed according to the *Wadsworth Anaerobic Bacteriology Manual* (5th edition).[21] Fecal specimens were collected into sterile plastic containers and stored at −20°C until they were assayed (within 7 days). Fecal samples were homogenized and serially diluted in an anaerobic cabinet (Anaerobic System, model 2028; Forma Scientific Co, Marietta, OH) with half-strength Wilkins Chalgreen anaerobic broth (Oxoid, Basingstoke, England). Plates were incubated in triplicate using the appropriate media for enumeration of total aerobes (nutrient agar; Oxoid), total anaerobes (Schaedler agar; Oxoid), enterococci

Table 2. Demographic and Clinical Characteristics

	VSL#3 n = 20	Placebo n = 20
Mean age (*yr*)	32.8	34.2
Sex (M/F)	11/9	12/8
Months of pouch function; median (range)	46 (8–108)	49 (5–134)
Duration of disease (*mo*); median (range)	37 (4–96)	43 (3–118)
No. of yearly relapses; mean	3.8	3.5

(Azide maltose agar; Biolife, Milan, Italy), coliforms (MacKonkey agar; Merck, Darmstadt, Germany), *Bacteroides* (Schaedler agar plus vancomycin and gentamycin; Oxoid), bifidobacteria (PYG, plus polymyxin [50 mg/mL] and kanamycin [50 mg/mL]), and *Clostridium perfringens* (O.P.S.P.; Oxoid). Plates were incubated aerobically or anaerobically as appropriate. The lower limit of detection was 1000 microorganisms per gram of feces.

Statistical Analysis

Based on their experience, clinical investigators thought it was reasonable to expect a 25% response in the placebo group and a 75% response in the therapy group, and such difference is relevant from a clinical point of view. Accordingly, for $\alpha = 0.05$ (2-tailed test) and $\beta = 0.20$, a sample size of more than 19 patients per group was estimated.

Baseline characteristics of patients after randomization in the 2 groups were compared using the χ^2 test or the Student *t* test for independent samples as appropriate. The primary study variable (number of patients who relapsed) was tested using the χ^2 test with the Yates correction.

Survival analysis was used to analyze the data set with respect to relapse. The Kaplan–Meier method was used to estimate the survivor function, and comparison of cumulative relapse rates between treatment groups was tested by the log-rank test.

The results of microbiological tests (secondary study variable) have been submitted to comparative multivariate analyses of variance. The significance of contrasts and multiple pairwise comparisons was tested using the 2-tailed Student *t* test. The level of significance was adjusted using the Bonferroni correction for multiple comparisons.

Results

Patient Characteristics

Forty-three patients were screened, and 40 were eligible; 20 were randomly assigned to receive VSL#3 and 20 to receive placebo; and 3 patients were excluded because they refused consent. Study groups were well matched with respect to age, sex, duration of follow-up, duration of pouchitis, and number of yearly relapses (Table 2).

The basal median PDAI score was 0 (range, 0–1) in both groups (median clinical portion score, 0 [range, 0–0]; median endoscopic portion score 0 [range, 0–0]; and median histologic portion score, 0 [range, 0–1]). Median stool frequency was 10 (range, 8–13) before antibiotic treatment and 4 (range, 3–7) after antibiotic treatment.

Clinical Results

Life-table analysis of the relapses in the 2 groups is shown in Figure 1.

Of the 20 patients who received the placebo, all had relapses, 8 within 2 months, 7 within 3 months, and 5 within 4 months. Of the 20 patients treated with VSL#3, 17 (85%) were still in remission after 9 months ($P < 0.001$) (Figure 2); all 17 of these patients had relapses within the 4 months after the conclusion of active treatment, and the median duration of remission was 2 months (range, 1–4).

The median total PDAI score of the 20 relapsed patients treated with placebo was 12 (range, 8–18); this score was the result of a significant increase in clinical (median 4 [range, 3–6]), endoscopic (median 4 [range, 3–6]), or histologic (median 4 [range, 3–5]) scores on the PDAI; median stool frequency was 9 (range, 7–11).

In the group treated with VSL#3, the 3 patients who had relapses during the 9 months of follow-up had a median total PDAI score of 11 (range, 9–17; median clinical portion score 3 [range, 2–5]; median endoscopic portion score 4 [range, 3–5]; and median histologic portion score 4 [range, 3–5]). Median stool frequency in these patients was 8 (range, 6–11) at the time of relapse. The 17 patients who remained in remission had a median total PDAI score of 0 (range, 0–1; median clinical portion score 0 [range, 0–0]; median endoscopic portion score 0 [range, 0–0]; and median histologic portion score 0 [range, 0–1]). The median stool frequency in these patients did not increase significantly compared with that obtained after antibiotic treatment (4 [range, 3–6]). Median stool frequency increased slightly within 15 days after cessation of active treatment (5 [range, 2–6]) and was 7 (range, 6–11) at the time of relapse.

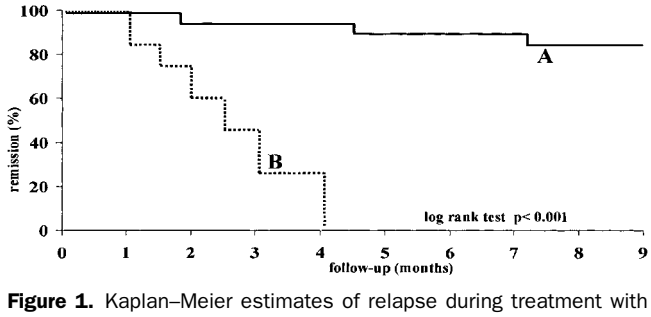

Figure 1. Kaplan–Meier estimates of relapse during treatment with VSL#3 (A) or placebo (B).

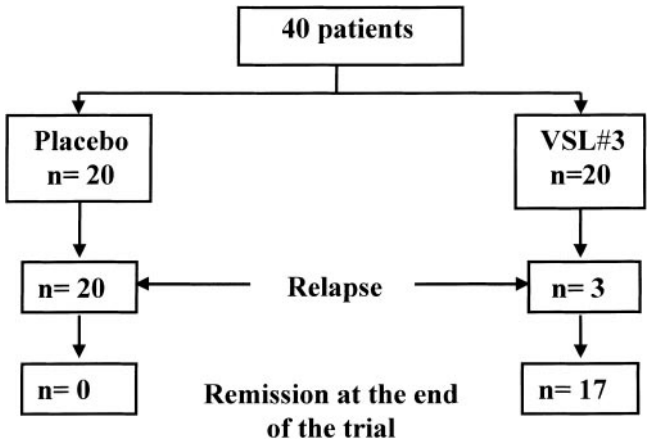

Figure 2. Clinical outcome of patients according to treatment received.

Microbiological Results

In patients treated with VSL#3, fecal concentrations of lactobacilli, bifidobacteria, and *Streptococcus salivarius* increased significantly ($P < 0.001$) compared with concentrations present both before and after antibiotic treatment and remained stable throughout the study (Figure 3). No significant changes were registered for concentrations of *Bacteroides*, coliforms, clostridia, enterococci, and total aerobes and anaerobes compared with basal levels. One month after discontinuation of VSL#3, fecal concentrations of lactobacilli, bifidobacteria, and *Streptococcus salivarius* subsp. *thermophilus* had reached levels similar to basal levels again.

In the group treated with placebo, fecal concentrations of all species evaluated remained similar at all intervals to those measured before antibiotic treatment.

Safety

No side effects and no significant changes from baseline values in any of the laboratory parameters examined were registered in either group of patients.

Discussion

This is the first controlled trial of maintenance treatment of pouchitis. Oral administration of VSL#3 was effective in the prevention of relapses in patients with chronic pouchitis; the efficacy of this new probiotic preparation may be related to the increase in concentrations of protective bacteria, as shown by the microbiological data, and in their metabolic activities.

The cumulative risk of developing pouchitis increases with time and, in series from centers with the largest experience and the longest follow-up, approaches nearly 50% by 10 years.[3] More than two thirds of patients experience multiple episodes, but most cases will respond to oral antibiotics. The cause is still unknown and is likely to be multifactorial; however, the immediate response to antibiotic treatment suggests a pathogenetic role for the microflora, and recently pouchitis was associated with a decreased ratio of anaerobic to aerobic bacteria, reduced fecal concentrations of lactobacilli and bifidobacteria, and an increase in luminal pH.[18] Treatment of pouchitis is largely empiric, and only a few small placebo-controlled trials have been conducted. Antibiotics have become the mainstay of treatment; metronidazole is the common initial therapeutic approach, and most patients have a dramatic response within a few days, whereas treatment of chronic refractory pouchitis is often difficult and disappointing and may require a prolonged course of antibiotics. Other medical therapies reported to be of benefit in uncontrolled trials include other antibacterial agents such as ciprofloxacin, amoxicillin/clavulanic acid, erythromycin and tetracycline, topical and oral mesalamine, conventional corticosteroid enemas, budesonide enemas, cyclosporine enemas, azathioprine, bismuth carbomer enemas, bismuth subsalicylate tablets, and short-chain fatty acid enemas or suppositories.[22]

The probiotic preparation we used has 2 main innovative characteristics: a very high bacterial concentration (300 billion viable bacteria per gram) and the presence of a mixture of different bacterial species with potential synergistic relationships to enhance suppression of potential pathogens.[23]

Various strains of probiotics can have very different and specialized metabolic activities,[24] such that claims made for one strain of an organism cannot necessarily be applied to another. Theoretically, a composite mixture of a large number of probiotic strains should be most effective. Experiments using anaerobic continuous-flow chemostats that duplicate the normal gastrointestinal (GI) microecology have suggested that a single strain or even a few probiotic strains are unlikely to colonize the GI tract or determine important modifications in the GI microecology.[25]

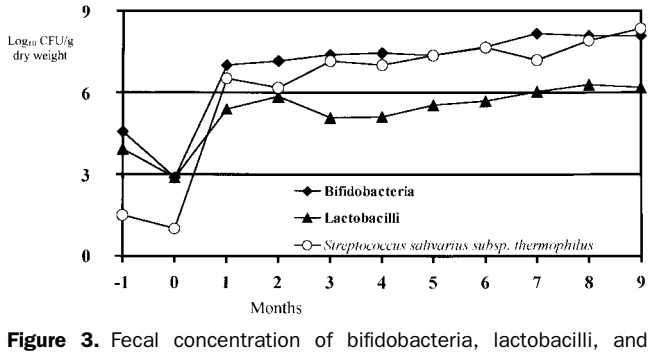

Figure 3. Fecal concentration of bifidobacteria, lactobacilli, and *Streptococcus salivarius* subsp. *thermophilus* before (−1) and after (0) antibiotic treatment and during maintenance treatment in the group treated with VSL#3.

Recent studies have supported the potential role of oral bacteriotherapy in inflammatory bowel disease (IBD). *Lactobacillus* spp. and *Lactobacillus plantarum* have been shown to be able to prevent the development of spontaneous colitis and to attenuate established colitis in IL-10 knockout mice, respectively.[16,17] In 2 controlled studies, patients with ulcerative colitis were given oral mesalamine or capsules containing a nonpathogenic strain of *Escherichia coli* as a maintenance treatment; no significant difference in relapse rates was observed between the 2 treatments.[26,27] Moreover, in an open study VSL#3 was effective in the prevention of relapses in patients with ulcerative colitis who were intolerant or allergic to sulfasalazine or mesalamine.[28] The mechanisms by which probiotics exert their beneficial effects in the host in vivo have not been fully defined; we showed recently that continuous treatment with VSL#3 determines a significant increase of tissue levels of IL-10 in patients with chronic pouchitis.[29]

In conclusion, the results of this study indicate that the use of a highly concentrated mixture of probiotic bacterial strains is effective in maintenance treatment of chronic relapsing pouchitis, further supporting the potential role of probiotics in IBD therapy.[30]

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