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Probiotic Supplementation Affects Pulmonary Exacerbations in Patients With Cystic Fibrosis: A Pilot Study

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Summary. Objective: Probiotics reduce intestinal inflammation in, and *Lactobacillus GG* (LGG) reduces pulmonary exacerbation rate cystic fibrosis (CF) patients. We intended to determine the effect of a mixed probiotic preparation on pulmonary exacerbations and inflammatory characteristics of the sputum in CF patients. Study Design: A prospective pilot study of 10 CF patients with mild–moderate lung disease and *Pseudomonas aeruginosa* colonization, treated with probiotics for 6 months. Pulmonary function tests (PFT's), sputum cultures with semi-quantitative bacterial analysis, and sputum neutrophil count and interleukin-8 (IL-8) levels were compared to pre-treatment and post-treatment values. The rate of pulmonary exacerbations was compared to 2 years prior to the study. Results: The exacerbation rate was significantly reduced in comparison to the previous 2 years and to 6 months post-treatment ($P = 0.002$). PFT's have not changed at the end of treatment and during 6 months post-treatment. No change in sputum bacteria, neutrophil count, and IL-8 levels was observed. Conclusion: Probiotics reduce pulmonary exacerbations rate in patients with CF. Probiotics may have a preventive potential for pulmonary deterioration in CF patients. **Pediatr Pulmonol. 2010; 45:536–540.** © 2010 Wiley-Liss, Inc.

Key words: probiotics; cystic fibrosis; pulmonary; interleukin-8; sputum.

INTRODUCTION

The hallmark of cystic fibrosis (CF) is recurrent severe and destructive pulmonary inflammation and infection, beginning in early childhood and leading to morbidity and mortality due to respiratory failure.[1] The most common pathogen recovered from the sputum of CF patients is *Pseudomonas aeruginosa*.[2] Other bacteria originating from the lungs or gastrointestinal tract may be found as well.[3] Patients colonized with *Pseudomonas* are at increased risk for pulmonary infections and persistent inflammation, and frequently require antibiotic treatment.

Intestinal inflammation is a typical finding in CF patients and bacterial overgrowth may be present.[4,5] Fecal calprotectin concentration and rectal nitric oxide production were found to be increased in CF patients, suggesting a constant intestinal inflammatory state.[4] Probiotics are live bacteria administered orally, that have been successfully used to decrease severity of acute gastroenteritis, and to prevent atopic diseases in children.[6–9] In addition, probiotics have been used as adjuvant therapy in patients with pouchitis[10] and inflammatory bowel diseases.[11] The mechanism of action of probiotics may be through improvement of intestinal barrier function and through modification of immune response.[12–14]

CF patients are constantly exposed to variable medication and large spectrum antibiotics, their intestinal permeability is increased, the microflora is abnormal, and the innate immune mediators are dysregulated.[15,16] These, together with the chronic lung inflammation, lead to the assumption that probiotic treatment may be beneficial for CF patients. Indeed, one previous pilot study reported

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reduced pulmonary exacerbations with the use of Lactobacillus GG (LGG).[17]

The aim of this pilot study is to evaluate the effect of a commercially available mixed probiotic supplementation on aspects of the pulmonary disease of CF patients: rate of respiratory exacerbations, PFT's, bacterial colonization, and inflammatory parameters of the sputum.[18]

PATIENTS AND METHODS

Patients with CF followed in the National Center for Cystic Fibrosis, with mild to moderate lung disease (FEV1 >40%) and chronically infected with *P. aeruginosa*, were screened for this prospective, open, pilot study. The study was approved by the hospital ethical committee. Inclusion criteria included: (1) An average incidence of respiratory infections at least one per year for 2 years prior to the study, requiring intravenous or oral antibiotic treatment for at least 14 days, according to the common treatment practice.[19] (2) The ability to produce sputum. (3) Signing an informed consent by the patient and/or his parents. (4) No other chronic disease except for CF-related diseases is present. (5) Colonization by *P. aeruginosa*, which was defined by at least three consecutive positive sputum cultures within 6 months.[20]

All patients had pancreatic insufficiency diagnosed by steatorrhea or elastase stool levels and clinical symptoms.

All patients received two commercially available probiotic tablets per day for 6 months, each tablet containing a mixture of 6×10^9 CFU/day bacteria: *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Bifidobacterium bifidum*, *Streptococcus thermophiles* (Bio-plus, Supherb, Israel). Nine of 10 patients received regular preventive antibiotic treatment with Azithromycin prior to and during the study. They were instructed to take the probiotics at least 2 hr apart from the antibiotics, following the manufacturer instructions.

Patients' follow-up was performed in the pulmonary division every 4 weeks, and included in addition to history and physical examination PFT's, measurements of weight and height and body mass index (BMI), pill count and collection of empty medication packages. Sputum studies were performed prior to probiotic treatment, and at weeks 12 and 24 of therapy. Additional sputum cultures were obtained according to clinical symptoms. Patients' follow-up was recorded for 6 months post-probiotic treatment.

Sputum Studies

Sputum samples were rapidly transferred to the microbiology laboratory, within less than 2 hr.

The sample quality was evaluated according to the following criteria: WBC >25 per high power field and squamous cells <10 per high power field, to verify that the sputum was recovered from the lower respiratory tract. A Gram stain was performed on each sample.

Samples were cultured on non-selective media: Blood Agar (BA)(Hy Lab, Rehovot, Israel), Mocconkey Agar (MAC) (Hy Lab), and Chocolate Agar (CHOC) (Hy Lab). After overnight incubation in 35°C in room air for MAC, and in 35°C with 5% CO_2 air for BA and CHOC, a semi-quantitative evaluation was performed as follows: (1) Semi-quantitative analysis for the presence of *P. aeruginosa*, *Bukhalderia cepacia*, *Klebsiella pneumonia*, *Escherichia coli*, graded +1 to +3.

An additional sputum sample was obtained for white blood cell count with differential count, and interleukin-8 (IL-8) concentration.[21–27]

Outcome Measures

Primary Outcomes

1. The number of episodes of pulmonary exacerbation in the treatment period compared to the exacerbation rates during two 6 months periods matching the same months of the year, in the 2 years prior to the study (e.g., when the patients' treatment period was January to June, the same months in the prior 2 years were chosen for comparison). Pulmonary exacerbations were diagnosed by increase of pulmonary symptoms and secretions (CF foundation criteria), requiring oral or intravenous antibiotics.
2. Forced expiratory volume in 1 sec (FEV1) measured prior to and at the end of probiotic treatment. For each test three expiratory maneuvers were performed and the best result was selected.[28]

Secondary Outcomes

1. Change in qualitative and quantitative sputum bacteria.
2. IL-8 level in the sputum as a marker of inflammation in CF patients.

Statistical Analysis

The data were analyzed using BMDP.[29] Results are presented as mean ± SD. Since the sample size was small and not all the variables had Gaussian distributions, we compared the results, over time, using the Wilcoxon signed ranks test. A *P*-value of ≤0.05 was considered significant.

RESULTS

Ten patients were recruited to the study from December 2005 to December 2006. All patients completed the study. The demographic data of the participating patients is presented in Table 1. Compliance to treatment, evaluated by pill count, was 89% overall.

TABLE 1— Clinical Characteristics of CF Patients

Gender (M/F)	7/3
Age (years)	26.2 ± 12.9
Age of *Pseudomonas* colonization (years)	16.0 ± 12.8
Genetic mutations (# patients)	
ΔF508 homozygous	3
ΔF508/W1282X	3
W1282X homozygous	2
A455E heterozygous	1
Y1092X heterozygous	1
FEV1 at baseline (%)	63.8 ± 19.8

Pulmonary Evaluation

During probiotic treatment none of the patients experienced pulmonary exacerbations, and no additional antibiotic treatment was prescribed. The exacerbation rate was significantly reduced in comparison to the previous 2 years and to 6 months post-treatment ($P = 0.002$). The PFT's have not changed at the end of treatment and during follow-up 6 months post-treatment. There was no change in BMI during and after the study period (Table 2).

Airway Inflammation

There was no difference in bacterial strains and semi-quantitative analysis of sputum bacteria between pre- and post-treatment sputum. In addition to *P. aeruginosa*, five patients were colonized with *staphylococcus aureus* and two patients with *Aspergillus fumigatus*.

Cell count of neutrophils, eosinophils, macrophages, and lymphocytes has not changed with probiotic treatment. There was a significant decrease in eosinophil number after 12 weeks of probiotic treatment compared to baseline (2.2 ± 1.0% vs. 1.5 ± 1.0%, respectively, $P = 0.03$), but no difference at the end of treatment. Sputum IL-8 concentrations were unchanged during and at the end of treatment (Table 3).

Side Effects

No major complication was encountered during the study. Side effects of treatment included mild flatulence in three patients. None of the patients discontinued the probiotic treatment.

DISCUSSION

This pilot study shows that a mixed probiotic supplementation of *L. acidophilus*, *L. bulgaricus*, *B. bifidum*, *and S. thermophiles* has a protective effect against clinical pulmonary exacerbations in CF patients with mild to moderate pulmonary disease and *Pseudomonas* colonization. Despite the lack of change in PFT's, sputum bacterial load, neutrophil count and IL-8 levels, the number of pulmonary exacerbations requiring antibiotic treatment was significantly reduced during probiotic treatment.

The rationale of treating an infectious state outside the gastrointestinal tract with probiotics is supported by animal studies: *Lactobacillus casei* increased the clearance of *Pseudomonas* from the lungs of young mice, LGG reduced *Pseudomonas* bacteremia in irradiated mice, and *Lactobacillus plantarum* inhibited the pathogenetic activity of *Pseudomonas*.[30,31] Probiotics are administered orally and their mechanism of action is thought to be through modification of the intestinal microflora and effect on the host immune response and intestinal barrier. There is increasing evidence that probiotic species can play a role in the treatment of inflammatory bowel disease and other gastrointestinal disorders characterized by an altered intestinal microbiota.[10,11,32] Intestinal inflammation was found to be a major feature of CF.[4] Probiotics have been shown to reverse epithelial damage produced by cytokines,[32] and to reduce intestinal inflammation in CF patients.[4] Disruption of the intestinal barrier is the pathogenetic mechanism of several inflammatory diseases.[33] Suppression of the gut inflammatory response may lead to a less disrupted intestinal barrier, decrease of bacterial and environmental trigger translocation, and reduced pulmonary inflammation. Such a modification may be potentially achieved by probiotics.

There is only one previous study addressing the use of probitics for prevention of pulmonary exacerbations in CF patients.[17] Bruzzese et al. treated 19 children with CF with *Lactobacillus GG* (LGG, 6×10^9 CFU/day) for 6 months and compared the result with a placebo group, in a prospective cross-over study. A significant decrease in pulmonary exacerbations and hospital admissions was found, together with improvement of FEV1 and BMI. The

TABLE 2— Clinical Course of CF Patients Prior to and Post-Probiotic Treatment

	Pre-probiotic treatment[1]	During probiotic treatment	Post-probiotic treatment[2]	*P*
No. pulmonary exacerbations[3]	1.3 ± 1.0	0	0.6 ± 0.7	0.002
FEV1 (%)	63.8 ± 19.8	59.9 ± 23.2	64.7 ± 22.2	NS
BMI	20.4 ± 2.8	20.5 ± 3.2	20.5 ± 3.1	NS

[1]6 months periods during 2 years prior to the study.
[2]6 months post-treatment.
[3]Including IV and PO antibiotic treatment.

TABLE 3—Sputum Inflammatory Characteristics in CF Patients Prior to and at the End of Probiotic treatment

	Pre-probiotics	12 weeks	End of probiotics	*P*
Neutrophils (%)	84.4 ± 7.8	85.8 ± 4.5	87.5 ± 4.5	0.58
Macrophages (%)	4.0 ± 2.1	3.9 ± 1.9	2.9 ± 2.1	0.44
Lymphocytes (%)	9.5 ± 5.9	8.6 ± 3.8	7.8 ± 3.7	1.0
Eosinophils (%)	2.3 ± 1.1	1.6 ± 0.9	1.6 ± 1.1	0.03
IL-8 (pM)	2,597 ± 733	3,025 ± 285	3,039 ± 436	1.0

inflammatory response was evaluated by serum immunoglobulin (Ig) concentrations. IgG levels increased in the placebo and remained stable in the LGG groups, respectively, with no change in other Ig. The current study shows similar results. The patients included in the current study are older than the patients in the first study (mean 26.2 years vs. 12.8–13.7 years), and have a longer mean time of Pseudomonas colonization (10.3 years versus 5.5–6.1 years). Despite the longer disease duration and *Pseudomonas* colonization, there was a significant decrease in clinical pulmonary exacerbations. At the same time, these differences may explain the lack of improvement in FEV1. In the current study we used different probiotic strains than in the Bruzzese study[17]—a commercially available mixture of bacteria compared to LGG alone. Despite the fact that LGG is the most studied and extensively documented probiotic strain, other strains may be as effective in reducing pulmonary exacerbations. The inflammatory state was evaluated in the Bruzzese study by serum IgG levels. Sputum inflammatory markers tests provide a direct evaluation of pulmonary inflammation, and we therefore used sputum neutrophil count and IL-8 levels. The inflammatory response in CF airways may be related to activation of NF-kappaB or to a deficit of lipoxin pathways.[34] *Pseudomonas* lipopolysaccharide induces a time dependent mucosal inflammation indicated by IL-8 release, COX-2 upregulation and neutrophil migration to the upper mucosal layers ex vivo. IL-8 release and neutrophil migration are specific to CF tissues.[34] IL-8 basal production is elevated in CF cells and both the number of neutrophils and IL-8 levels were increased in bronchoalveolar lavage fluid from CF patients as compared to control subjects with negative cultures for common bacterial CF-related pathogens.[18,21] However, we have not found differences in neutrophil count and IL-8 levels in CF patients during probiotic treatment. There was a minor decrease in sputum eosinophil count, which may reflect an anti-inflammatory effect of probiotics, especially in CF patients with asthmatic features. IL-8 and neutropihil count were compared in vitro and in vivo between CF and non-CF epithelium, but no data are available in different groups of CF patients. IL-8 and neutrophil count measurements within CF patients groups in different inflammatory states may not be sensitive enough for evaluation of the level of inflammation. The reasons we were unable to detect differences in the pulmonary variable of inflammation and FEV1 with probiotic treatment may be related to the short period of treatment, and the small group of patients in this pilot study. In addition, measurement of other inflammatory cytokines such as IL-1β, IL-6, and lipid-derived mediators, may be needed in order to evaluate the effect of probiotics on lung inflammation.[27]

Despite those drawbacks, the results of the study suggest, that probiotics may be beneficial in the treatment of CF patients with pulmonary *P. aeruginosa*. Since this is a pilot study with a small number of patients, a prospective large-scale placebo-controlled trial for a longer treatment and follow-up period is needed to evaluate the value of probiotics in treatment and prevention of pulmonary deterioration in CF patients. The design of a prospective study has to take into consideration addition of sputum inflammatory markers for evaluation of the probiotic effect and stool calprotectin, a longer treatment duration, and perhaps a probiotic supplement containing a larger number of probiotic bacteria.

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