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EFFECT OF *LACTOBACILLUS* STRAINS (*L. CASEI* AND *L. ACIDOPHILLUS STRAINS* CERELA) ON BACTERIAL OVERGROWTH-RELATED CHRONIC DIARRHEA

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Abstract Small bowel bacterial overgrowth and related diarrhea is a condition that frequently accompanies anatomic disorders, surgically created blind loops or strictures with partial small bowel obstruction and although it is often controlled with antimicrobial therapy, alternative treatment may be needed. The aim of this study was to evaluate the efficacy of an oral probiotic preparation of 2 viable lyophilized strains of lactobacilli (1.5 g each) compared with placebo.Twenty two patients with proven overgrowth and chronic diarrhea are described. In random order and double-blind fashion, 2 groups of patients received identical capsules with both *Lactobacillus casei* and *L. acidophillus strains* CERELA (12 patients) (LC) and placebo (10 patients) (P) during three consecutive periods of 7 days each followed by a similar three periods of control after withdrawal. At the end of each period the mean daily number of stools, glucose breath H_2 test, and symptoms were considered. *Lactobacillus* were investigated in feces in both groups at day 0 (baseline), on day 21 of treatment with LC and P and on day 21 after withdrawal. Compared with P a significant reduction in mean daily number of stools was achieved with LC ($p< 0.005$) at 15 days, and ($p< 0.0005$) at 21 days and the effect was sustained at 7 days and 15 days ($p< 0.005$) after withdrawal. With respect to breath H_2 level a significant decrease in H2 concentration was noted at 7 days ($p< 0.005$) at 15 days, and 21 days ($p< 0.0001$) with LC and only a significant decrease ($p< 0.005$) was observed at 7 days after withdrawal. No significant changes were observed with respect to symptoms .The *Lactobacillus* CERELA strains were isolated from the feces in all patients LC (n=12) on day 21, and by contrast no *Lactobacillus* were observed except in two patients out of seven patients after withdrawal. In summary, this study provides evidence that LC are effective for treatment of bacterial overgrowth - related chronic diarrhea, and suggest that probiotics must be used with continuity.

Key words: probiotic, bacterial overgrowth, chronic diarrhea

Resumen ***Efecto de cepas de* Lactobacillus (L. casei, L. acidophillus *CERELA*) *sobre la diarrea crónica debida al sobrecrecimiento bacteriano.*** La diarrea crónica debida al sobrecrecimiento bacteriano es una condición que frecuentemente acompaña a las alteraciones anatómicas, asa ciega o estrecheces post quirúrgicas del intestino delgado, con parcial obstrucción, y aunque con frecuencia se controla con antimicrobianos, tratamientos alternativos pueden ser necesarios. El propósito de este estudio randomizado, doble ciego, fue determinar si la asociación de dos cepas liofilizadas de *Lactobacillus casei* (1.5 g) y *Lactobacillus acidophillus* (1.5g) desarrollada por CERELA (Centro de Referencia para Lactobacilos) es más efectiva que un placebo, en pacientes con diarrea crónica debida a un sobrecrecimiento bacteriano del intestino delgado. Fueron estudiados 22 pacientes; 12 recibieron lactobacilos (L) y 10 placebo (P) en cápsulas idénticas, 2 veces por día durante tres períodos consecutivos de 7 días seguido por 3 períodos similares post tratamiento. Al final de cada período se evaluó el promedio diario del número de heces, la presencia de sobrecrecimiento por el empleo de la prueba de concentración de H_2 post carga con glucosa en el aire espirado y la persistencia de los síntomas clínicos. La presencia de lactobacilos en las heces también fue investigada en ambos grupos en el período basal, a los 21 días de tratamiento y 21 días post tratamiento. Comparado con P una significativa reducción en el número diario de heces fue observado con L a los 15 días ($p< 0.005$) y a los 21 días ($p< 0.0005$) y este efecto se sostuvo a los 7 y 15 días ($p< 0.005$) post tratamiento. Respecto al H_2 una significativa reducción fue observada a los 7 días ($p< 0.005$) a los 15 y 21 días ($p< 0.0001$) con L durante el tratamiento y solo a los 7 días ($p< 0.0005$) posterior al tratamiento. No hubo cambios significativos con respecto a los síntomas. Las cepas CERELA se aislaron de las heces de todos los pacientes tratados con L (n=12) recogidas a los 21 días de tratamiento pero en cambio solo se aislaron de 2 pacientes (n=7) a los 21 días post tratamiento. Se concluye que el empleo de cepas de lactobacilos (*L. casei, L. acidophillus* CERELA) son efectivas en el tratamiento de la diarrea crónica debida a un sobrecrecimiento bacteriano del intestino delgado a cuyo fin se sugiere una admistración continuada de estos probióticos.

Palabras clave: probióticos, sobrecrecimiento bacteriano, diarrea crónica

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Bacterial overgrowth is defined as 10^4 of predominantly Gram negative anaerobes of colonic origin per ml of small bowel fluid[1].

The syndrome is mainly caused by stasis or an anatomic abnormality and it is characterized by the presence of chronic diarrhea and/or malabsorption and weight loss[1-3].

The diagnosis of bacterial overgrowth can be adequately screened by demonstrating an early peak in breath hydrogen concentration after administration of an oral glucose load[4,5] and a treatment with broad-spectrum antibiotics either for sporadic or on a continuous basis is often necessary[6].

Beneficial therapy may also involve probiotics agents, components of the natural flora including lactic acid bacteria such as lactobacilli, streptococci and bifidobacteria and new probiotics such as yearst (e.g., *Saccharomyces boulardii*) and entirely unrelated bacteria (*Clostridium, Bacillus subtilis*). Defined as living microorganisms, which upon ingestion in certain number exert health benefits beyond inherent basic nutrition, improving the properties of indigenous microbiota[7], the clinical importance of probiotics is supported by many observations.

Lactobacillus spp have been shown to be able to prevent the development of spontaneous colitis and attenuate established colitis in IL-10 knockout mice[8,9]. Two controlled studies comparing a nonpathogenic strain of *Escherichia coli* versus mesalamine in patients with ulcerative colitis registered no significant changes in relapse rates[10,11]. Also, probiotic preparations with the presence of a mixture of different bacterial species have been shown to be beneficial in the maintenance and treatment of chronic pouchitis[12] and have been effective in the prevention of relapses in patients with ulcerative colitis[13].

Lactobacilli alleviate viral[14] or bacterial[15] induced diarrhea, especially in infants and with reference to bacterial overgrowth, probiotics were evaluated successfully in children with short bowel syndrome[16]. *Saccharomyces boulardii* has proved to be effective too, though recent reports have reached an opposite conclusion at least when it has been used for a short time[17].

Lactobacilli has never been tested in a controlled study for reduction of small bowel bacterial overgrowth and related diarrhea. Non controlled trials have been performed studying long treatments or following those with probiotic withdrawal. We initiated a double-blind clinical trial to explore those effects of lactobacilli using Lactobacillus casei (CRL 431) and *Lactobacillus acidophilus* (CRL 730) strains, from CERELA, culture collections.

Materials and Methods

Patients. Twenty four consecutive ambulatory patients with chronic diarrhea and conditions that predisposed them to bacterial overgrowth of the small bowel, referred to inpatients consultative services were enrolled in our study from March 1993 to June 1997. All patients were dependent on antibiotic therapy as evidenced by exacerbations of disease after withdrawal of medication or reduction in dose. Six were being maintained on indefinite treatment schedules whose doses had been adjusted individually to stabilize the clinical symptoms. Diabetic autonomic neuropathy was present in 3 patients, Billroth II gastrectomy with or without vagotomy in 5 patients; idiopathic intestinal pseudo-obstruction in 1 patients; intestinal pseudo-obstruction after abdominal radiotherapy for gynecologic cancer in 3 patients; atrophic gastritis in 2 patients; prolonged treatment with H_2 receptor antagonist in 2 patients; ileocecal resection in 5 patients; jejunoileal bypass in 2 patient and scleroderma in 1 patient.

Chronic diarrhea was defined as a change in bowel habits consisting of at least 3 loose watery stools per day for a minimun of 3 months and bacterial overgrowth was based on a positive glucose ingestion hydrogen breath test.

Informed written consent was obtained from all patients and the study performed in accordance with the Declaration of Helsinki was approved by the medical ethics committee of Hospital ELMA (Empresas Líneas Marítimas Argentinas) and Hospital Español , Buenos Aires.

Study medication. Identical capsules cointaining 3 g of maize starch (placebo) and 2 strains of *Lactobacillus* CERELA (*L. casei, L. acidophillus*) containing 1.5 g of viable lyophhilized bacteria each per capsule, were administered orally twice daily for 21 days.The taste and smell of the active drug or placebo were not really identifiable. Sufficient compliance was assumed when patients took more than 90% of the prescribed study medication.

Study design. This study was a randomized, double-blind, placebo-control.

After 4 weeks without antibiotics or other drugs affecting gastrointestinal function, came three 7 day periods of treatment followed by 3 similar periods of control after withdrawal periods. Prior to treatment and every period thereafter, patients were matched for sypmtoms and degree of symptoms severity, the mean daily number of stools, and a glucose hydrogen breath test between the two groups.

Patients were asked to keep a record of stool frequency on daily cards, and the evaluation of symptoms, on the basis of its percived intensity, consisted of a questionnaire concerning the presence and severity of four symptoms: abdominal cramp/colicky, flatulence, borborygmi and bloating. They were graded according to the following scale 0= absent, 1=mild (ocassional symptoms), 2= moderate (frequent discomfort that was not treatment), 3=severe (frequent discomfort with a painful sensation that was treatment). Any medication taken, and any side effects were also, recorded. Patients were allowed to eat their prescription diet including milk (2 cup) and wine (1 cup)/24h. The medications were dispensed by one of the inves-tigators (DG) at each visit, and no concurrent treatments were allowed.

Breath H_2 testing. Breath H_2 concentration was measured after 12 hours fasting, and to minimize basal H_2 excretion, the dinner meal on the day before the test consisted of rice and meat. None of the patients smoked and all were sedentary throughout the test. The lactulose test was performed prior to the other test to ensure that all participants harbored a bacterial flora able to generate a significant sustained rise in H_2 in end-expiratory air

of more than 10 ppm. All participants fulfilled this criterion. End-expiratory breath samples were collected before and at 15 min intervals for 2 hours after ingestion of 50g of D-glucose as previously described[5]. An increase in breath hydrogen concentration of more than 10 ppm above baseline value after glucose, at any sampling time, was considered a positive result on at least 2 samples. The tests were performed at baseline and on the last day of each 7-day period. End-expiratory breath samples were analyzed using Quintron CM model (Milwaukee WI). Minimal detectable H_2 concentration was 1 ppm.

Stool culture. Stool specimens were collected and inmediately frozen and stored at -20°C until they were assayed (within 10 days), at baseline, at day 21 with treatment, and at day 21 after withdrawal in both groups of patients.

Lactobacillus spp strains CERELA were analysed as described earlier[15].

Stool specimens or rectal swabs of the patients were collected in Lapt soft agar. Adequate dilutions were plated onto LBS -raffinose agar (LBS agar is a selective medium for lactobacilli). The final pH was 6.5. A solution of glucose, sterilized by filtration,was added at 1% final concentration. After 48 h of incubation at 37 °C under CO_2 atmosphere, the number of colony forming units per gram (CFU/g), was determined.

Statistical analysis. Population global profile was defined throughout the analysis of the means, the standard deviations and the frequency of distribution. Discrete variables were assessed with the use of the Chi-squared test. Continuous data from both groups of subjects were compared with the use of the two-sided T-test or in case that the data had no normal distribution the Wilcoxon-Mann-Whitney rank-sum test was used. Wilcoxon-rank-sum test or paired T-test were used for paired data.

A p value of less than 0.05 was considered as statistically significant. All data were collected on an Excel spreadsheet and then analysis by the Statistica/W and EPI info programs.

Results

Patients. Twenty-eight patients were screened and twenty-four were eligible; 12 were randomly assigned to receive *Lactobacillus* spp strains CERELA and 12 to receive placebo. Twenty -two patients completed the study because 2 patients belonging to the placebo groups were excluded during the 1st week of treatment for non-compliance. Both study groups and their dates with respect to age, sex, and duration of diarrhea are shown in Table1.

Clinical Results

Number of stool. Mean daily number of stools (mean + SEM) during each period of 7 days is shown in Fig 1. Compared with placebo a significant reduction ($p< 0.005$) in mean daily number of stools was achieved with Lactobacillus spp. at 15 days (2.27 ± 1.51) and significant differences ($p< 0.0005$) at 21 days (mean 1.55 ± 1.17) and the effect was sustained at 7 days (2.52 ± 1.20 vs 3.70 ± 0.85) and at 15 days (3.18 ± 0.95 vs 3.80 ± 1.16) ($p< 0.005$ respectively) after withdrawal.

No significant changes were registered in mean scores for abdominal cramps, borborigmi, flatulence and bloating at all intervals of treatments or withdrawal.

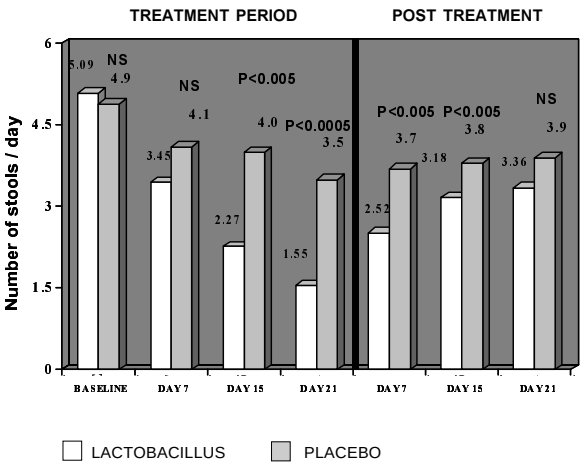

Fig. 1.– Lactobacillus strains CERELA vs placebo. Treatment period and post treatment. Number of stools/day during 7 days of each period. Results are mean +/- SEM.

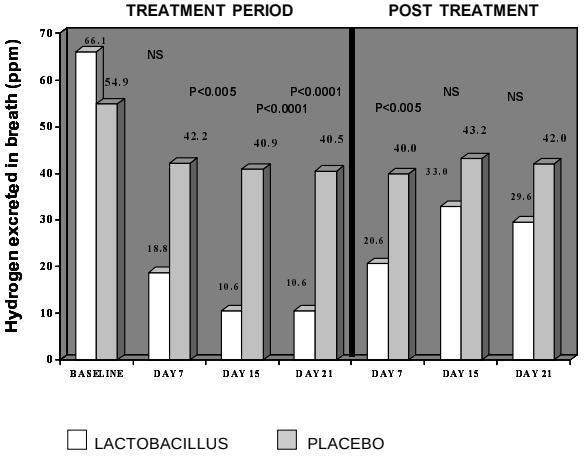

Fig.2.– Lactobacillus strains CERELA vs placebo. Treatment period and post treatment. Breath excreted H_2 concentration (ppm) for 2 hours at the end of each period. Results are mean +/- SEM.

TABLE 1.– *Clinical characteristics of the 2 patients groups for full data set*

Characteristics	*Lactobacillus* (Cerela) N=12	Placebo N=10	p
AGE (ys)[α]	50.83 ± 16.76	48.80 ± 16.35	NS
SEX (M/F)	7 / 5	6 / 4	NS
Duration of diarrhea before inclusion (ys)[α]	3.08 ± 1.98	2.50 ± 1.58	NS

[a] *Mean + / -SEM*

Breath H_2 testing

Treatment periods. Fasting breath H_2 level was similar in concentration at the beginning between both groups but a significant decrease in H_2 excreted was noted between baseline, and at the end of each period (7,15 and 21 days) when was compared the *Lactobacillus* spp. group vs placebo ($p< 0.005$; $p< 0.0001$; $p< 0.0001$, respectively). Fig. 2.

Withdrawal periods. A significant decrease in H_2 excreted was observed only at 7 days when was compared the *Lactobacillus* spp. group vs placebo ($p< 0.005$). There were not significant differences observed at 15 and 21 days. Fig. 2.

Bacteriology

Prior to beginning the study no fecal samples in the different groups yielded colonial forms like *L. casei* and *L. acidophillus* strains CERELA.

On day 21 in all patients (n=12) assigned to probiotic therapy the stool cultures allowed the recovery of lactobacilli in the following concentrations: *L. acidophillus* 10^7-10^8 CFU/g, and *L. casei* 10^6 -10^7 CFU/g, indicating that lactobacilli CERELA have the ability to survive the passage through the gastrointestinal tract. By contrast, none of control group (n=10) excreted *Lactobacillus* strains CERELA.

After discontinuing the treatment, persistence of lactobacilli was studied in 7 patients of each group. The organisms could be recovered from fecal specimens in only 2 patients (probiotic therapy group), and the viable fecal counts were 10^4-10^5 CFU/g for both *Lactobacillus* (*L. casei*, *L. acidophillus*).

Safety. No side effects were registered in either group of patients.

Discussion

The administration of *Lactobacillus casei* and *Lactobacillus acidophillus* strains CERELA, resulted in the attenuation of bacterial overgrowth related-diarrhea, and reduction in the mean concentration of breath-excreted H_2, significantly.

As some investigators did before, we choose reduction in the number of stools as an objective and clinically relevant endpoint, also we have used breath-excreted H_2 as an indicator of therapeutic efficacy in bacterial overgrowth[4, 5]. Attending the opinion of Madsen et al[8], 21 days of treatment with lactobacilli were selected despite the higher risk of drop-out as a compromise designed to ensure the most efficient length of therapy, the optimal bacterial concentration levels (CFU/g) in the intestine, and to establish the best way to prevent recurrence.

The exact mechanism by which *Lactobacillus* spp. exert protection against the bacterial overgrowth is unknown. However, it is known that certain *Lactobacillus* spp. adhere to mucosal surfaces, and sterically inhibit the attachment of aerobic gram-negative bacteria[15, 18-20]. They secrete inhibitory products that have antimicrobial activity, and reduce pH in the colonic lumen which may decrease the growth of pathogenic bacterial species[21]. In addition to these well-known effects, *Lactobacillus* spp.also induce proliferation of immune cells[22], have nutritional benefits including the induction of growth factor, and enhance synthesis of secretory immunoglobulin A[23]. Lactobacillus spp. provide protection against increase in intestinal permeability[24], and as recent reports have shown, they have a high antagonist effect on intestinal inflammatory disease and prevent the development of colitis[25, 26]. Also disminishe the severity of alcohol-induced liver disease because they reduce gut-derived endotoxemia[27].

An obvious prerequisite for such a beneficial activity is that the peroral administration of *Lactobacillus* strains survives passage through the gastrointestinal tract in humans. Our results showed that *L. casei* and *L. acidophillus* strains CERELA have that property, but also, delayed significantly the mouth-caecum transit time[28], adhere to a human colon carcinoma cell line, and promote clinical recovery from enteral infection[29], demonstrating their ability to modulate complex mechanisms of host defense against pathogens[30]. Such activities are compatible with the notion of a probiotic agent.

The data obtained in this study indicated that bacteriotherapy with *Lactobacillus* strains CERELA was very effective since this treatment reduced the daily stool frequency, showing a relationship between the levels of lactobacilli, and enteric disorders. Also showed that without additional oral bacteriotherapy the diarrhea can be restored, since unfortunately lactobacilli, do not permanently colonize the gastrointestinal tract. Other probiotic therapy (VSL#3), reported to be of benefit in the control of chronic pouchitis, also failed to provide long-term protection as evidenced by recurrence of disease, and returned of fecal lactobacilli and bifidobacterial concentrations to pretreatment levels, 1 month after stopping probiotic treatment[13].

The analysis of these results also suggests that probiotics must be used with continuity because the effects start after a week, so that, the results obtained by Attar et al[17], who did not establish any correlation examining different antimicrobial agent and *Saccharomyces boulardii* in 10 patients, were because their results were based following only 7-day treatment.

There is growing interest in detecting candidate probiotic micro-organisms that may provide protection against few gastrointestinal diseases. This study showed that *Lactobacillus casei* and *Lactobacillus acidophillus*

strains CERELA, significantly reduced both, the number of stools in bacterial overgrowth-related chronic diarrhea, and glucose breath-expired H_2 concentration.

This study raises the possibility that protective actions of bacteriotherapy in the intestinal tract are multifaceted, acting also at least as a bacteriostatic, unless this concept ought to be clearly demostrated, yet.

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La verdad viene de fuera: la observación de los objetos físicos nos dice qué es lo verdadero. Pero lo ético viene de dentro: expresa un "yo quiero", no un "hay".

Hans Reichenbach (1891-1953)

La Filosofía Científica, Fondo de Cultura, Méjico, 1967