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ORIGINAL ARTICLE

Influence of *Saccharomyces boulardii* on the intestinal permeability of patients with Crohn's disease in remission

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Abstract

Objective. Crohn's disease (CD) is characterized by a reduction in mucosal integrity that permits antigen penetration into the intestinal tissue. The administration of probiotics has been suggested to improve the barrier function of the mucosa. The objective of this study was to evaluate the influence of *Saccharomyces boulardii* on the intestinal permeability in CD. ***Material and methods.*** Thirty-four patients were randomized according to the Vienna classification for treatment with either placebo or *Saccharomyces boulardii*. Baseline medications (mesalamine, azathioprine, prednisone, metronidazole and/or thalidomide) were maintained. Intestinal permeability (lactulose/mannitol ratio) was evaluated immediately before the beginning of treatment and at the end of the first and third treatment month. Fifteen healthy volunteers were also submitted for the intestinal permeability test. ***Results.*** In volunteers, the lactulose/mannitol ratio was 0.005 ± 0.0037, whereas this value was 0.021 ± 0.01 in patients with CD ($p = 0.001$). In the placebo group, there was an increase in lactulose/mannitol ratio by 0.004 ± 0.010 ($p = 0.12$) at the end of the third month. In the *S. boulardii* group, there was an improvement in intestinal permeability, with a decrease in the lactulose/mannitol ratio by 0.008 ± 0.006 ($p = 0.0005$) in the same period. ***Conclusions.*** Patients with CD in remission present alterations in the integrity of the intestinal mucosal barrier according to lactulose/mannitol ratio. *S. boulardii* added to baseline therapy improved intestinal permeability in these patients, even though complete normalization was not achieved.

Key Words: *Crohn's disease, intestinal permeability, probiotics*

Introduction

Crohn's disease (CD) is characterized by chronic inflammation affecting any site of the digestive tract. Its etiology is not completely understood and treatment is generally effective in relieving symptoms but is not curative.

On the basis of histopathological findings, CD is believed to result from a sustained immunological response. However, it has been questioned whether an unknown pathogen causes an appropriate inflammatory response, or whether the inflammatory reaction is an inappropriate response to an initially harmless stimulus [1]. Antigen penetration caused by the breakdown of the mucosal barrier is one of the main factors implicated in the pathogenesis of CD; however, it is also not known whether this event is primary or secondary to immunological alterations that favor the inflammatory process in CD [2–4].

Although the prevention of recurrences is one of the main objectives of treatment, the long-term outcomes are unfavorable and patients are frequently submitted to high corticosteroid doses for a prolonged period of time, a fact that markedly increases treatment-associated morbidity. Since patients with CD are known to present antigens in the intestinal microbiota in addition to altered intestinal permeability, and since probiotics may exert a beneficial effect on this microbiota, emphasis has

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been placed on the use of this therapeutic option in intestinal diseases [5–8].

The application of non-invasive tests, such as the intestinal permeability test, may permit a more precise and sensitive monitoring of the therapeutic response than clinical observation in patients with CD, since these tests are able to detect repercussions of alterations intimately associated with the inflammatory processes present in the disease [9,10]. Thus, the objective of the present study was to evaluate the integrity of the intestinal mucosa in patients with CD in remission and the influence of *Saccharomyces boulardii* on the intestinal permeability of these patients compared to a placebo group.

Material and methods

From August 2006 to January 2007, 34 patients with a diagnosis of CD in remission, followed-up prospectively for a period of 3 months at the Intestine Outpatient Clinic of the Alfa Institute of Gastroenterology (IAG), University Hospital, Federal University of Minas Gerais (HCUFMG), were included in the study. Three patients interrupted their medication in the first week of treatment without previous consultation with the researchers. The 31 remaining patients (18 M (58.1%), 13 F (41.9%), age range 19 to 54 years, mean 37 years) completed the treatment. Disease remission was defined using the Crohn's disease activity index (CDAI) [11]. Patients were blindly randomized into two groups, according to the Vienna classification, and the baseline medications were maintained during the follow-up period (Table I) [12]. The mean CDAI score at the beginning of treatment was 62.8 ± 44.6 points (median: 56.0) in the placebo group and 50.7 ± 36.9 points (median: 52.0) in the *S. boulardii* group ($p = 0.66$). All patients had been diagnosed for more than 3 years and were in remission for more than 6 months. To date, none of the patients had remission induced by surgery and the last surgical procedure was performed 6 years ago.

Exclusion criteria were: patients younger than 18 years or older than 65 years, women who were pregnant or breastfeeding, patients with renal failure, cirrhosis, congestive heart failure, nephrotic syndrome, diabetes and thyroid diseases that interfered with absorption, flux of water and solutes and intestinal motility, in order to avoid interference with the intestinal permeability tests.

Treatments were allocated at the outpatient clinic using a computer-generated randomization list issued by a pharmacist, who knew the results of randomization. The first group, consisting of 19

Table I. Treatment randomization of patients with CD between placebo and *S. boulardii* groups according to the Vienna classification and according to the medications used during the remission phase of CD.

	Placebo N	*S. boulardii* N	*p*-value
Vienna classification – %			
Age			
A1 (<40) – 80.6	13	12	0.66
A2 (≥40) – 19.4	4	2	
Location			
L1 (terminal ileum) – 77.4	13	11	0.96
L3 (ileocolon) – 22.6	4	3	
Behavior			
B1 (non-stricturing, non-penetrating) – 16.1	3	2	
B2 (stricturing) – 35.5	6	5	0.27
B3 (penetrating) – 48.4	8	7	
Treatment – %			
Mesalamine – 35.5	4	6	0.44
	13	8	
Azathioprine – 32.3	5	5	1.00
	12	9	
Azathioprine/mesalamine/prednisone – 12.9	4	0	0.11
	13	14	
Azathioprine/mesalamine – 6.5	2	0	0.49
	15	14	
Thalidomide – 3.2	0	1	0.45
	17	13	
Thalidomide/metronidazole – 3.2	1	0	1.00
	16	14	

Abbreviation: CD = Crohn's disease.

patients, received a placebo every 8 h as an oral capsule containing 200 mg cellulose, 6 mg sucrose and 2.4 mg magnesium stearate. The second group, consisting of 15 patients, received *S. boulardii* every 8 h as an oral capsule formulation which contained 200 mg lyophilized *S. boulardii*-17 (about 4×10^8 cells), 6 mg sucrose and 2.4 mg magnesium stearate (Floratil®). A third group, consisting of 15 healthy volunteers (aged between 23 and 47 years, mean age 36 years) who agreed to take part in the study, within the ethics norms of human research, were submitted to intestinal permeability tests in order to establish control values. They were not assigned to any kind of treatment.

Intestinal permeability was evaluated immediately before treatment (T0) and one month (T1) and 3 months (T3) after the beginning of treatment. At the same time, the patients were submitted to clinical assessment and laboratory tests including a complete blood count for determination of the CDAI. Two substances, lactulose (Sigma-Aldrich, Steinheim, Germany) and mannitol (Sigma, São Paulo, Brazil), were used for the intestinal permeability test and the excretion rate of these products was quantified in urine.

For the intestinal permeability test, the patients and healthy volunteers were seen at the hospital in the morning after a 10-h fast and were asked to eliminate any eventual residual urine. An isomolar solution (120 ml) containing 6.0 g lactulose and 3.0 g mannitol was then ingested by the patient, and urine was collected into a sealed flask over a period of 6 h. At the end of this period, a 2.5 ml urine aliquot was stored in a second smaller flask and 0.6 mg thimerosal was added to prevent bacterial growth. The samples were stored in liquid nitrogen.

Urinary lactulose and mannitol were analyzed by high-performance liquid chromatography (HPLC) using a Shimadzu® system which consisted of an injector pump, an auto-injector, a controller integrated with workstation software for interpretation of the results and a refractive index detector. For HPLC, urine was filtered through a micropore filter (0.22 µl. Millex, São Paulo, Brazil), passed through an ion-exchange resin (Mixed-bed resin TMD-8, Sigma, USA), and then 50 µl was injected into the chromatograph with the auto-injector. MilliQ water was used as the mobile phase at a predetermined flow rate of 0.6 ml/min. A Supelcogel 33H® pre-column (Supelco, St Louis, MO, USA) and a Rezek RHM-monosaccharide H^{+}® (8%) (Phenomenex, Torrence, CA, USA) column were used for separation of the substances. In the refractive index module, different amplitudes of the waves generated by the sample containing lactulose and mannitol were captured and interpreted by the workstation, and the results were transformed into graphs. For standardization of the test and adequate interpretation of the data reported as g/l, an equation was generated using the areas under the curves calculated by the workstation and a line was drawn for the determination of the two substances. The ratio of administered test probes is a more accurate indicator of permeation because the premucosal and postmucosal factors can influence the probes equally, and, therefore, the urinary excretion ratio is not likely to be affected [9]. We did not specifically examine the aspect of the tight junctions.

The χ^2 test and, when necessary, the Fisher exact test were used for the analysis of frequency distribution. Analysis of variance was used for comparison of means in independent samples, and when the data showed a non-Gaussian distribution, the medians were compared using the Kruskal-Wallis test. Student's paired *t*-test was used for within-group (before/after) comparisons. The level of significance was set at 5% ($p < 0.05$).

The study was approved by the Ethics Committee of the Federal University of Minas Gerais and was conducted in accordance with the Declaration of Helsinki. All patients and volunteers signed a free informed consent form before the beginning of the study.

Results

Two patients, in the placebo group, who interrupted the use of medication, complained of abdominal pain, vomiting and diarrhea, events that they associated with the introduction of the new medication. The third patient, who belonged to the *S. boulardii* group, chose to be excluded from the study protocol.

The intestinal permeability test was similar between the two groups at T0, also (0.020 ± 0.006 and 0.024 ± 0.013 for placebo and *S. boulardii* group, respectively).

Fifteen healthy volunteers were also submitted to the intestinal permeability test. In this group, the mean urinary lactulose excretion was $0.14 \pm 0.094\%$ (median: 0.14%), the mean urinary mannitol excretion was $22.6 \pm 3.13\%$ (median: 23%), and the mean lactulose/mannitol ratio was 0.005 ± 0.0037 (median: 0.003). Comparison of these values with those obtained from patients with CD before the beginning of treatment revealed a significant difference in the median urinary lactulose excretion and in the median lactulose/mannitol ratio, while no difference in the median urinary mannitol excretion was observed between the two groups (Table II).

Next, the mean differences in urinary lactulose and mannitol excretion and in lactulose/mannitol ratio were compared between T1 and T0, T3 and

Table II. Comparison of the results of the intestinal permeability test between healthy volunteers and patients with CD in remission before the beginning of treatment.

Permeability intestinal test	N	Mean%	Standard deviation	Median% (variation)	*p*-value
% Urinary lactulose					
Healthy volunteers	15	0.14	0.09	0.14 (0.04–0.28)	0.001
CD patients	31	0.45	0.20	0.43 (0.15–1.02)	
% Urinary mannitol					
Healthy volunteers	15	22.60	3.13	23.0 (18.0–28.0)	0.56
CD patients	31	21.44	4.87	21.0 (13.0–29,0)	
Lactulose/mannitol ratio					
Healthy volunteers	15	0.005	0.0037	0.003 (0.002–0.013)	0.0001
CD patients	31	0.021	0.01	0.021 (0.007–0.046)	

Abbreviation: CD = Crohn's disease.

T0, and T3 and T1 in order to evaluate the influence of *S. boulardii* on intestinal permeability (Tables III, IV and V). The reduced lactulose/mannitol ratio values in T1 and T3 in the *S. boulardii* group indicate an improvement in barrier function. In the placebo group there was no any difference in worsening.

Discussion

To evaluate the effects of administration of *S. boulardii*, intestinal permeability was measured in a serial manner at three different time-points (T0, T1 and T3). The 33.33% decrease in the lactulose/mannitol ratio in the *S. boulardii* group ($p = 0.0006$) and the lack of a difference in the placebo group demonstrated that *S. boulardii* associated with the maintenance treatment of patients with CD in remission improved barrier function, without normalizing it. These data resulted from reduced lactulose excretion, since mannitol excretion was not affected. The main objective of the simultaneous use of lactulose and mannitol in intestinal permeability tests is to suppress the effects that variations in gastric emptying or even in intestinal transit time may exert on the excretion of either one of the sugars when analyzed separately.

Comparison of the results of the intestinal permeability test between healthy individuals and patients with CD before treatment showed that the barrier function of the intestinal mucosa was compromised in the latter group, a fact that might help explain the recurrent course of the disease. Similar differences were reported by others [13–15]. In CD patients, the lactulose/mannitol ratio was four times greater than that in healthy volunteers. The lactulose urinary excretion and lactulose/mannitol ratio variations were also much greater in CD patients than in healthy volunteers. In short, CD patients showed intestinal permeability values that ranged from normal to high and this was reflected by broader standard deviations than those observed in healthy controls. Wide variations in intestinal permeability in CD in remission have been observed and higher values correlated with recurrence [13,14].

Indirect evidence indicates that components of the microbiota of the terminal ileum and colon contribute to the pathogenesis of CD [16–21]. Over the

Table III. Comparison between mean differences in the urinary lactulose excretion of patients with CD (Crohn's disease) treated with *S. boulardii* or placebo for a period of 3 months.

	N	Mean differences	Standard deviation	*p*-value
Δ% L1-L0				
Placebo	17	+0.066	0.159	0.10
S. boulardii	14	−0.079	0.098	0.009
Δ% L3-L0				
Placebo	17	+0.105	0.272	0.13
S. boulardii	14	−0.182	0.151	0.0006
Δ% L3-L1				
Placebo	17	+0.038	0.301	0.61
S. boulardii	14	−0.103	0.074	0.0002

Δ% L1-L0: difference in the mean urinary lactulose excretion between the end of the first month and the beginning of treatment.
Δ% L3-L0: difference in the mean urinary lactulose excretion between the end of the third month and the beginning of treatment.
Δ% L3-L1: difference in the mean urinary lactulose excretion between the end of the third and first month of treatment.

Table IV. Comparison between mean differences in the urinary mannitol excretion of patients with CD (Crohn's disease) treated with *S. boulardii* or placebo for a period of 3 months.

	N	Mean differences	Standard deviation	*p*-value
Δ% M1-M0				
Placebo	17	−0.76	3.96	0.44
S. boulardii	14	−0.28	2.92	0.72
Δ% M3-M0				
Placebo	17	+1.53	4.11	0.14
S. boulardii	14	−0.23	2.54	0.74
Δ% M3-M1				
Placebo	17	+1.53	4.11	0.14
S. boulardii	14	−0.23	2.54	0.74

Δ% M1-M0: difference in the mean urinary mannitol excretion between the end of the first month and the beginning of treatment.
Δ% M3-M0: difference in the mean urinary mannitol excretion between the end of the third month and the beginning of treatment.
Δ% M3-M1: difference in the mean urinary mannitol excretion between the end of the third and first month of treatment.

years, the mechanisms proposed to explain the beneficial effects of probiotics have relied on the capacity of these substances to suppress the growth of pathogenic microorganisms [22]. Recently, however, it has been suggested that probiotics and their secretions also exert a modulating effect on the adaptive or non-adaptive immune response of the host [23–26]. The biological properties of *S. boulardii* are determined by the vitality of the species after lyophilization and by reaching high concentrations in the intestine without the occurrence of colonization [27]. Analysis of the kinetics of the fecal clearance of viable *S. boulardii* in adult subjects taking lyophilisate showed an equilibrium state reached by the third day of administration. *S. boulardii* disappears from the feces 2–5 days after treatment has been stopped [27]. The modulating effect of this probiotic is based on the inhibition of the activation of NF-κB and inhibition of the production of inflammatory cytokines by intestinal cells infected with *Escherichia coli*. *S. boulardii* also prevents the migration of CD4+ T cells and induces apoptosis of infiltrating cells [28]. In addition, *S. boulardii* contains substantial concentrations of polyamines, specifically spermidine and spermine, which are essential for cell division and differentiation [29,30]. Endogenous polyamines are derived from the decarboxylation of ornithine. The data are controversial, but the activity of ornithine decarboxylase is decreased in patients with inflammatory bowel disease [31,32]. So, it is possible that *S. boulardii* exerts some effect on mucosal epithelial regeneration, too.

No reports of side effects associated with the use of *S. boulardii* were found in the literature, with the exception of in immunosuppressed patients and one case of fungemia [33]. In this study, patients of the *S. boulardii* group presented no signs or symptoms that could be associated with the microorganism.

Plein & Hotz [34] evaluated the therapeutic effects of *S. boulardii* in 20 patients with oligosymptomatic CD in a randomized, double-blind, placebo-controlled study. *S. boulardii* was administered at a dose of 250 mg three times a day and previous

Table V. Comparison between mean differences in the mean lactulose/mannitol ratio of patients with CD (Crohn's disease) treated with *S. boulardii* or placebo for a period of 3 months.

	N	Mean differences	Standard deviation	*p*-value
$\Delta\%T_{L/M}1\text{-}T_{L/M}0$				
Placebo	17	+0.003	0.007	0.049
S. boulardii	14	−0.004	0.004	0.004
$\Delta\%\ T_{L/M}3\text{-}T_{L/M}0$				
Placebo	17	+0.004	0.010	0.12
S. boulardii	14	−0.008	0.006	0.0006
$\Delta\%\ T_{L/M}3\text{-}T_{L/M}1$				
Placebo	17	+0.001	0.012	0.81
S. boulardii	14	−0.004	0.004	0.0014

$\Delta\%\ T_{L/M}1\text{-}T_{L/M}0$: difference in the mean lactulose/mannitol ratio between the end of the first month and the beginning of treatment.
$\Delta\%\ T_{L/M}3\text{-}T_{L/M}0$: difference in the mean lactulose/mannitol ratio between the end of the third month and the beginning of treatment.
$\Delta\%\ T_{L/M}3\text{-}T_{L/M}1$: difference in the mean lactulose/mannitol ratio between the end of the third and first month of treatment.

medications were maintained. After 7 weeks of treatment, a reduction in the number of evacuations and in the BEST Index was observed in the *S. boulardii* group compared with the control group ($p < 0.01$). The patients were followed-up for an additional 3 weeks after the discontinuation of *S. boulardii* treatment and aggravation of symptoms was observed during this period. In another study using *S. boulardii*, 32 patients with CD in remission were randomized into two groups of 16 patients each [35]. In the first group, patients received mesalamine, while in the second group, *S. boulardii* at a dose of 500 mg twice a day was administered in addition to mesalamine. The patients were followed-up for 6 months. Six relapses of the disease were observed in the first group compared with only one recurrence in the second group ($p = 0.04$).

We could not find any studies in the literature about the influence of probiotics on the intestinal permeability of patients with CD in remission. There was only one report using *Lactobacillus* GG in four children with active CD and the therapeutic response was evaluated by intestinal permeability test [36]. That open study was conducted with maintenance of baseline medications (prednisolone, azathioprine and metronidazole). A dose of 10^{10} CFU (colony-forming units) twice a day was prescribed for 6 months and a permeability test was carried out in weeks 0, 1, 4, 12, 24 and 48 using cellobiose and mannitol. A reduction in the cellobiose excretion rate was observed up to week 24 in all four patients, suggesting that *Lactobacillus* GG improved the barrier function of the intestinal mucosa during this period. However, an increase in the cellobiose excretion rate was observed in week 48. In addition, disease reactivation occurred in three patients within a period of 4 to 12 weeks after discontinuation of *Lactobacillus* GG. One patient required a colectomy and another required ileocecal resection.

Conclusions

Patients with CD in remission present alterations in the integrity of the intestinal mucosa analyzed by the permeability test. Administration of *S. boulardii* reduced lactulose/mannitol ratio in CD patients in remission who were maintained on baseline medications, indicating that an improvement in the intestinal barrier function may have occurred in the study group.

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