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Original/*Alimentos funcionales*

The effect of *Saccharomyces boulardii* in patients eligible for liver transplantation

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Abstract

Objective: **The aim of this study was to evaluate the influence of *Saccharomyces boulardii* on the intestinal permeability, laboratory parameters and MELD and Child-Pugh severity scores in cirrhotic patients eligible for liver transplantation.**

Methods: **Eighteen patients followed in a Transplant Outpatient Clinic were evaluated immediately before the beginning of treatment, after a 30-day period of treatment period with probiotics and at the end of the second study month (after a thirty-day period without probiotics). Fifteen healthy controls also underwent the intestinal permeability test (lactulose/mannitol).**

Results: **Before the probiotic, the median lactulose/mannitol ratio was greater in the cirrhotic patients (0.0209, range 0.0012-0.1984) compared to the healthy controls (0.0030, range 0.0020-0.0013) ($p < 0.05$). Eight of fifteen patients, half of whom had ascites, showed increased intestinal permeability above the higher value observed in the controls. No significant association was found between the severity scores for liver disease, age, presence of ascites and intestinal permeability immediately before the beginning of study. After treatment with *S. boulardii*, there was no improvement in intestinal permeability or significant differences in the laboratory parameters for the three evaluations.**

Conclusions: **Patients eligible for liver transplants presented with increased intestinal permeability compared to healthy controls. A thirty-day treatment with *S.***

EL EFECTO DE *SACCHAROMYCES BOULARDII* EN PACIENTES CANDIDATOS A TRASPLANTE HEPÁTICO

Resumen

Objetivo: **Evaluar la influencia del *Saccharomyces boulardii* en la permeabilidad intestinal, parámetros bioquímicos, de MELD y de Child-Pugh en pacientes cirróticos candidatos al trasplante de hígado.**

Métodos: **Dieciocho pacientes seguidos en ambulatorio de Transplantes fueron evaluados inmediatamente antes del inicio del tratamento con probióticos, después de período de 30 días con probióticos y al final del segundo mes de estudio (período de treinta días sin probióticos). Quince controles sanos también se sometieron a la prueba de permeabilidad intestinal (lactulosa/manitol).**

Resultados: **Antes del probiótico, la relación de lactulosa / manitol media fue mayor en los pacientes cirróticos (0,0209; 0,0012-0,1984) en comparación con los controles sanos (0,0030; 0,0020-0,0013) ($p < 0,05$). Ocho de los quince pacientes, la mitad de los cuales tenía ascitis, presentaron con aumento de la permeabilidad intestinal por encima del valor más alto observado en los controles. No se observó asociación significativa entre los critérios de Meld, Child-Pugh y la edad, la presencia de ascitis y la permeabilidad intestinal inmediatamente antes del inicio del estudio. El tratamiento con *S. boulardii*, no resultó en mejoría de la permeabilidad intestinal, de los critérios de Meld y Child-Pugh así como de los los parámetros bioquímicos a lo largo de las tres evaluaciones.**

Conclusiones: **Pacientes en lista de espera para transplantes hepático presentan mayor permeabilidad intestinal en comparación con los controles sanos. El tratamien-**

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***boulardii* did not improve this intestinal permeability or the severity scores, nor did it impact the laboratory parameters.**

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Key words: *Cirrhosis. Intestinal permeability. Probiotic. Severity scores.*

to de treinta días con *S. boulardii* no resultó en mejoria de la permeabilidad intestinal, del MELD y Child-Pugh, asi como de los parâmetros bioquímicos.

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Palabras clave: *Cirrosis. Permeabilidad intestinal. Probióticos. Criterios de gravedad.*

Abbreviations

ALT: Alanine aminotransferase.
AST: Aspartate aminotransferase.
HPLC: High-performance liquid chromatography.
INR: International normalized ratio.
MELD: Model for End-Stage Liver Disease.
TB: Total bilirubin.

Introduction

Increased intestinal permeability has been reported in many studies involving cirrhotic patients[1-10]. This condition, which is associated with bacterial overgrowth and failure of the immune defense mechanisms, promotes bacterial translocation[11] and consequently leads to cirrhosis complications, such as infections and circulatory dysfunction[12]. Generally, these problems worsen the patient quality of life and decrease survival rates[13]. Therefore, strategies that might reduce the occurrence of such problems should be developed.

Probiotics, which are defined as live organisms that when ingested in sufficient amounts have beneficial effects on the overall health of the host[14], may modulate the intestinal microbiota and the immune system and promote the integrity of the mucosal barrier. Thus, microbial translocation and their complications may be prevented.

Current evidence suggests that probiotics benefit some factors related to liver disease, eventually leading to encephalopathy reversal[15;16] and improved liver function[17]. However, no studies have investigated the role of these microorganisms on intestinal permeability in cirrhotic patients. Therefore, the aim of this study was to determine the influence of *S. boulardii* on intestinal permeability, severity scores and laboratory parameters in cirrhotic patients eligible for liver transplantation.

Methods

Patients

This prospective study examined the effect of probiotics in liver transplant candidates who were followed at the Instituto Alfa de Gastroenterologia - Transplant Outpatient Clinic at Universidade Federal de Minas Gerais (UFMG), Brazil. The study was conducted in accordance with the Declaration of Helsinki, was approved by the UFMG Ethics Committee (registration number: ETIC 0609.0.203.000-09; date of issue: april 8, 2010) and is registered with ClinicalTrials.gov, number NCT01762748. All patients and volunteers provided written informed consent before starting the study.

Patients with hepatic cirrhosis of viral, alcoholic or cryptogenic etiology were included. The following exclusion criteria were applied: aged younger than 18 years or older than 65 years, end-stage renal failure, congestive heart failure, nephrotic syndrome, diabetes and thyroid diseases that interfered with absorption, flux of water and solutes and intestinal motility to avoid interference with the intestinal permeability tests. Furthermore, patients who had ingested substances that affected intestinal permeability, such as non-steroidal anti-inflammatory drugs and alcohol, in the seven days prior to the test, were also excluded.

The patients were initially assessed and then followed-up for a two-month period. The follow-up was divided into three periods: immediately before the beginning of treatment (T0), after a thirty-day treatment period with probiotics (T1) and at the end of the second study month (after a thirty-day washout period) (T2).

Probiotic

The patients received *S. boulardii* three times per day for 30 days as an oral capsule formulation containing 200 mg of lyophilized *S. boulardii*-17 (approximately $1x10^9$ cells), 6 mg of sucrose and 2.4 mg of magnesium stearate (Floratil®). The patients were instructed to ingest the capsules before breakfast, lunch and dinner and to not consume fiber supplements (prebiotics) or other probiotics during the study.

Baseline medications were maintained during the follow-up period.

Patient assessments

The patients were scheduled for three follow-up visits (T0, T1 and T2) at which they underwent clinical assessments, routine laboratory tests and the intestinal permeability test.

The severity of cirrhosis was assessed by Child-Pugh scores[18] and by the Model for End-Stage Liver Disease[19].

The intestinal permeability test was carried out in the following manner. The patients and volunteers were seen at the hospital in the morning after an 8-h fast and were asked to eliminate any residual urine. The patient then ingested an isomolar solution (120 mL) containing 6.25 g of lactulose (Sigma-Aldrich, Steinheim, Germany) and 3.0 g of mannitol (Sigma, São Paulo, Brazil). They continued fasting for an additional 2 h after which food intake was permitted. Urine was collected into a sealed flask over a 5-hour period for the patients and over six hours for the healthy controls. At the end of this period, a 2.5 mL urine aliquot was stored in a second smaller flask and 0.6 mg of thimerosal was added to prevent bacterial growth. The samples were stored in liquid nitrogen until the analysis.

The excretion rate of these products was quantified in urine using high-performance liquid chromatography (HPLC) with a Shimadzu® system (Japan). The substances were separated using a Rezek RHM-monosaccharide H+® (8%) column (Phenomenex, Torrence, USA). The final test result consisted of the ratio between the substances (obtained by dividing the excretion rate of lactulose by the mannitol excretion rate.

The results for the intestinal permeability of the patients before probiotic treatment were compared to the data from 15 healthy controls who were not assigned to any kind of treatment to verify whether intestinal permeability in the cirrhotic patients had increased.

Statistical analysis

The Mann-Whitney U-test was used to compare the medians in the independent samples. To assess the association between variables, Spearman's correlation coefficient and Mann-Whitney or Kruskal-Wallis were used, as needed. The level of significance was set at 5% ($p < 0.05$). Wilcoxon was carried out with the Bonferroni correction applied, resulting in a significance level set at $p < 0.02$ for within-group (before/after) comparisons. Statistical analyses were performed using the Statistical Package for Social Sciences version 17.0 (SPSS Inc., Chicago, IL).

Results

From January 2011 to September 2012, eighteen patients were included in the study (median age 50 years, range 36-65 years; 83% male). Six patients had alcoholic liver cirrhosis, nine were viral (B and C, 3 and 6, respectively), one was alcoholic + viral, and two had cryptogenic cirrhosis. Of these patients, only seven completed all study phases. One patient died, and five interrupted their medication without informing the researchers before the end of the probiotic treatment (before T1). The reasons for this cessation were as follows: two patients chose to be excluded from the study protocol because they did not wish to continue; one interrupted the medication complaining of encephalopathy attributed to constipation (events that they associated with the introduction of the new medication); one patient underwent surgery; and one patient was hospitalized because of disease complications. The other five patients did not attend the T2 follow-up; one of these patients died of causes not associated with disease, and four patients did not attend the follow-up visits because of difficulties in travelling from their cities to the test site.

Laboratory parameters

Table I shows the comparisons of the laboratory values in the probiotic group obtained at the three evaluated times. There was no significant difference in any of the assessed parameters. Although the changes in the individual components of the Child-Pugh score were not expressive, an evaluation of the median Child-Pugh score showed a statistical significance between T0 and T1, with a median increase in the Child-Pugh score after using the probiotics ($p < 0.01$), which was then reduced in the following period without *S. boulardii* (from T1 to T2, $p > 0.02$). Nonetheless, the Child-Pugh classification was maintained (5 B; 2C; 2A) in 9 of 12 (75.0%) patients, improved (from class C to class B) in 1 of 12 (8.3%) patients, and worsened (from class A to class B, n=1; from class B to class C n=1) only in 2 of 12 (16.7%) patients between T0 and T1. However, from T1 to T2, the Child-Pugh class was maintained (4B,1A) in 5 of 7 (71.4%) patients and improved in 2 of 7 (28.6%) patients. The MELD score did not show significant differences in any of the different time periods.

Intestinal permeability

The data were analyzed for only 15 patients. The data from the other three patients could not be considered because it was not possible to read the excretion of lactulose and mannitol.

The results of the intestinal permeability test comparing the patients eligible for liver transplantation before the beginning of treatment with *S. boulardii* (T0) and fifteen healthy controls revealed that the median urinary mannitol excretion and the median lactulose/mannitol ratio were significantly different between the patients and controls (Table II). However, only eight patients showed increased intestinal permeability above the upper limit of normality for the controls (Fig. 1), and half of these patients had ascites. No significant association was found between the Meld and Child-Pugh scores, age, presence of ascites and intestinal permeability of patients (data not shown).

After using the probiotics, it was observed an increase in the median urinary lactulose (T0 0.14%; T1 0.20%) and mannitol (T0 9.01%; T1 12.47%) excretions and the excretion rates of these substances (T0 0.0209; T1 0.0285) in the urine of patients. Then, there

Table I
Comparison of the evaluated laboratory parameters in patients eligible for liver transplantation treated with probiotic

	T0 (n=18) *Median (range)*	*T1 (n=12)* *Median (range)*	*T2 (n=7)* *Median (range)*	*P*		
				T0-T1	*T1-T2*	*T0-T2*
ALT (U/L)	39.0 (21.0-108.0)	36.0 (34.0-112,8)	31.0 (21.0-112,7)	0.50	0.92	0.89
AST (U/L)	46.0 (30.0-169.0)	47.5 (35.0-183.0)	41.5 (26.0-176.5)	0.04	0.46	0.20
TB (mg/dL)	2.2(0.7-9.9)	2.0(0.8-9.9)	2.0(0.8-11.6)	0.42	0.24	0.14
Creatinine (mg/dL)	1.0(0.5-1.9)	1.0(0.5-1.6)	0.9(0.6-1.3)	0.88	0.61	0.83
Urea (mg/dL)	27.0(16-71)	28(16-50)	35(22-54)	0.15	0.11	0.45
Albumin (g/dL)	3.2(2.4-4.2)	3.15(2.90-3.80)	3.49(2.5-4.10)	0.61	0.46	0.92
INR	1.6(1.1-2.4)	1.6(1.2-2.5)	1.6(1.2-2.1)	0.79	0.46	0.33
MELD	16.0(9.0-24.0)	15.5(8.0-23.0)	15.0(12.0-17.0)	0.63	0.16	0.10
Child-Pugh score median	7.5(5.0-11.0)	9.2(5.0-12.0)	8.0(5.0-9.0)	0.01	0.04	0.26
Child-Pugh class (A/B/C)[‡]	4/11/3	2/7/3	1/6/0	-	-	-

ALT, alanine aminotransferase; AST, aspartate aminotransferase; TB, total bilirubin; INR, international normalized ratio; Meld, Model for end-stage liver disease. T0 = immediately before beginning the probiotic treatment; T1 = after a thirty-day treatment period; and T2 = after a thirty-day period without probiotic treatment.
[a]The data are presented as the number of patients classified as A, B or C.

Table II
Comparison of the results of the intestinal permeability test between the healthy controls and patients eligible for liver transplantation before the beginning of probiotic treatment

		n	*Median % (range)*	*p*
% Lactulose	Healh control	15	0.07 (0.05-0.28)	0.65
	Patients	15	0.14 (0.01-2.74)	
% Mannitol	Healh control	15	21.00 (18.30-28.00)	0.001*
	Patients	15	9.09 (2.44-24.34)	
Lactulose/Mannitol ratio	Healh control	15	0.0030 (0.0020-0.0013)	0.01*
	Patients	15	0.0209 (0.0012-0.1984)	

*p< 0.05

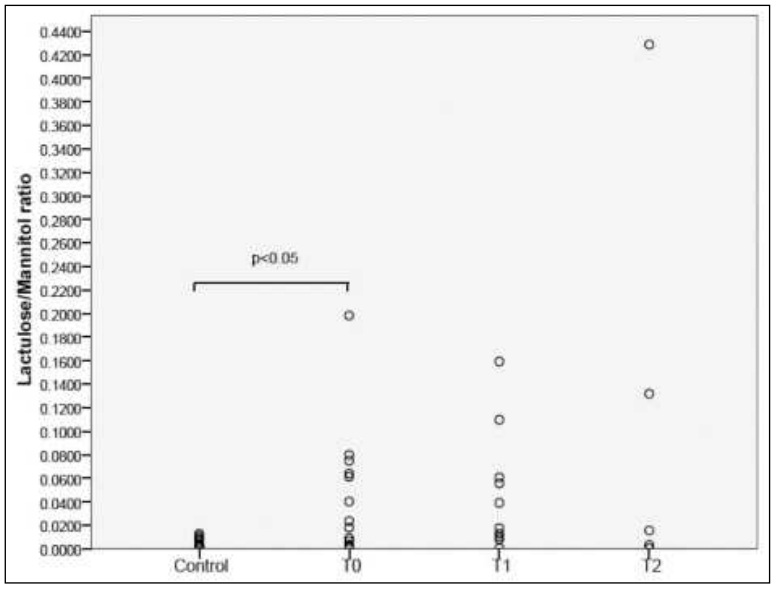

Fig. 1.—Lactulose/Mannitol ratio in healthy controls and cirrhotic patients eligible for liver transplantation in T0 (immediately before the beginning of treatment with probiotic; T1 (after a thirty-day treatment period) and T2 (after a thirty-day period without probiotic treatment).

was a subsequent reduction in T2 after the 30-day washout period (lactulose 0.12%; mannitol 9.87%; and lactulose/manitol ratio 0.0157), without its normalization. These changes were not statistically significant.

During the follow-up period, there were no disease complications, such as spontaneous bacterial peritonitis, variceal bleeding and hepatic encephalopathy. However, five patients (27.8%) complained of intestinal constipation, events that they associated with the introduction of the new medication.

Discussion

Studies assessing intestinal permeability in cirrhotic patients have shown contradictory results. Methodological differences, such as the number of patients evaluated, disease severity, presence of ascites and use of different types of tests to assess intestinal permeability, may be related to these disagreements. However, increased intestinal permeability has been reported in many studies[1-10], which aligned with the results observed in our patients who were eligible for liver transplantation and presented with significantly higher intestinal permeability compared to healthy controls. However, note that only eight out of fifteen patients had increased intestinal permeability above the highest value observed in controls, four of whom had ascites. In a study of 20 cirrhotic patients, it was observed that only a small proportion of them (five patients) presented with increased intestinal permeability, as evaluated by ^{51}Cr-EDTA excretion (one of ten without ascites and four of ten with ascites)[20]. When globally evaluated, as well as in two other studies[3,6], only the patients with ascites and not those without ascites showed significantly higher intestinal permeability compared with the controls. Indeed, intestinal permeability is of particular interest in patients with ascites and justifies their predisposition to spontaneous bacterial peritonitis.

In the present study, intestinal permeability was not significantly associated with any evaluated variable. Similarly, some authors have not found any relation with disease severity scores[21,22], while others have found an association with age as well as statistically greater permeability in cirrhotic Child-Pugh C patients[2,9,10].

As previously described, increased intestinal permeability is related to major complications of liver cirrhosis that favor the increase of morbidity, mortality and health care costs during the period waiting for liver transplant. To prevent such complications, probiotics could be used. We chose *S. boulardii* because it is a nonpathogenic yeast that has been widely used in Europe, Asia, Africa, and Central and South America[23], is rapidly eliminated after discontinuing therapy (2 to 5 days) and is not affected by antibiotics[24,25]. Some studies have already demonstrated its role in stimulating the immune response[26,27], the anti-inflammatory activity[28], the control of microbial translocation[29] and the improvement on intestinal permeability in patients with Crohn's disease under remission[30].

The only study that assessed the effect of *S. boulardii in vivo* used the probiotic twice daily for three months (each capsule with 1.25 x 10^9 cells). However, in our study, as the patients were very sick, this long time frame would certainly lead to increased losses to follow up, therefore we opted to provide 3 capsules per day (each capsule containing 1 x 10^9 cells) for 30 days.

To our knowledge, this is the first study to assess the effectiveness of *S. boulardii* in cirrhotic patients. Contrary to expectations, we did not find any significant positive result from the probiotic administration. The use of *S. boulardii* was unable to improve intestinal permeability; instead, this permeability was increased after 30 days of treatment with the probiotics and reduced after the 30-day washout period (although not significantly). Furthermore, any significant effect of probiotic administration was observed in laboratory parameters. Likewise, in other study were not observed positive effects on the measurement of albumin, bilirubin, transaminases, ammonia, international normalized ratio (INR) and creatinine in compensated cirrhotic patients treated with *Lactobacillus acidophilus, Lactobacillus bulgaricus, Bifidobacterium lactis* and *Streptococcus thermophiles* throughout six months when comparing to the control group[31]. Regarding median Child-Pugh scores, although the increase in the score reached statistical significance between T0 e T1 without significant change on individual parameters this was explained by only two patients who presented deterioration in classification, while the other ten patients improved or remained stable during the period. Furthermore, the worsening in these patients might be a consequence of disease progression during the study and not caused by using the probiotics. Therefore, the fact that we have not included a control group was a limitation of this study.

In a study with cirrhotic patients treated with symbiotics throughout seven days, there were significant improvements in serum bilirubin and albumin concentrations and international normalized ratio (INR) with consequent improvement in the Child-Pugh classification of 4/9 (44.4%) patients (from class B to class A - n=2; from class C to class B - n=2 and no deterioration in the remaining patients)[3]. Another study did not demonstrate expressive changes in the individual components of the Child-Pugh score and the improved overall Child-Pugh score did not reach statistical significance in cirrhotic patients treated with *E. coli Nissle* for 42 days[32].

Note that in addition to not observing any beneficial effect of the probiotic supplementation, in the present study, some patients complained of intestinal constipation, which they associated with the introduction of the new medication. This result was negative because constipation is considered a precipitating factor for hepatic encephalopathy.

The results observed in this study may be challenged by the potential short treatment time (one month). However, steady state concentrations of *S. boulardii* cells are achieved within a mean of three days after oral administration[24] and other studies have reported important results in cirrhotic patients following short-term treatment. There were significant improvements in Child-Pugh classification after seven days of symbiotic treatment[17] and reversal of minimal hepatic encephalopathy, reduction in endotoxemia and improvement in the Child-Pugh functional classification after 30 days of probiotic treatment[33]. However, the only study that has evaluated the effect of *S. boulardii* on intestinal permeability in patients with Crohn's disease observed positive results[31] when using the yeast for a period of three months.

Beneficial effects after using other probiotics, such as minimal hepatic encephalopathy reversal after probiotic yogurt supplementation[34] and improvement in biochemical and neuropsychological tests in patients treated with Bifidobacterium longum with fructooligosaccharide[35], have also been observed after 60 and 90 days of treatment, respectively. However, as in the present study, Pereg et al.[32] did not observe positive effects in either the clinical or laboratory parameters in compensated cirrhotic patients treated with probiotics over a six-month period. Based on these data, it may be suggested that differences related to the used probiotics, the patients involved and the time of treatment may have led to the different results.

A limitation of our study was the relatively small population, which resulted from two factors: the cost of the drug which prevented us from continuing the inclusion of patients and, mainly, the difficulty in completing the treatment. The latter was caused by the deaths of patients, discontinued drug use by some patients, transportation difficulties in travelling from their cities to the test site and difficulty to attend the examinations due to liver disease, hospitalizations and surgeries prior to transplantation. Therefore, studies with larger number of patients are required to better clarify the effect of *S. boulardii* in cirrhotic. But, future studies should be performed with less severe patients to facilitate adherence to the study and decrease the possibility of disruption of their participation in the study due to the transplant and to complications of the disease.

In conclusion, intestinal permeability of the cirrhotic patients eligible for liver transplantation was increased in relation to healthy controls. The treatment with *S. boulardii* failed to demonstrate any significant effect on this population.

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