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TREATMENT OF DIARRHEA-PREDOMINANT IRRITABLE BOWEL SYNDROME WITH MESALAZINE AND/OR *SACCHAROMYCES BOULARDII*

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ABSTRACT - ***Context*** - Irritable bowel syndrome (IBS) is a functional bowel disease characterized by abdominal pain and altered intestinal habits. The pathophysiology of IBS remains unclear. Recent studies have demonstrated that some IBS patients, especially in diarrhea-predominant IBS (IBS-D), display persistent signs of minor mucosal inflammation and a modified intestinal microflora. The mesalazine has known intestinal anti-inflammatory properties. *Saccharomyces boulardii* is a probiotic used for a long time in treatment of diarrhea, including infectious diarrhea. ***Objective*** - Evaluate the effects of mesalazine alone, combined therapy of mesalazine with liophylised *Saccharomyces boulardii* or alone on symptoms of IBS-D patients. ***Methods*** - Based on Rome III criteria, 53 IBS-D patients (18 year or more) were included. To exclude organic diseases all patients underwent colonoscopy, stool culture, serum anti-endomisium antibody, lactose tolerance test and ova and parasite exam. Patients were divided in three groups: mesalazine group (MG) - 20 patients received mesalazine 800 mg t.i.d. for 30 days; mesalazine and *Saccharomyces boulardii* group (MSbG) - 21 patients received mesalazine 800 mg t.i.d. and *Saccharomyces boulardii* 200 mg t.i.d. for 30 days and; *Saccharomyces boulardii* group (SbG) – 12 patients received Sb 200 mg t.i.d. for 30 days. Drugs that might have any effect on intestinal motility or secretion were not allowed. Symptom evaluations at baseline and after treatment were performed by means of a 4-point likert scale including: stool frequency, stool form and consistency (Bristol scale), abdominal pain and distension. Paired *t* test and Kruskal-Wallis test were used for statistical analyses. ***Results*** - Compared to baseline, there were statistically significant reduction of symptom score after 30 th day therapy in all three groups: MG ($P<0.0001$); MSbG ($P<0.0001$) and in SbG ($P = 0.003$). There were statistically significant differences in the symptom score at 30 th day therapy of the MG, MSbG and SbG groups ($P = 0.03$). There were no statistical differences between MSbG and MG symptom score at 30th day therapy ($P = 0.9$). ***Conclusions*** - The use of mesalazine alone, *Saccharomyces boulardii* alone or combined treatment with mesalasine and *Saccaromyces boulardii* improved IBS-D symptoms. The improvement of the symptom score was greater with mesalazine alone or combined with Sb as compared with Sb treatment alone. These preliminary results suggest that mezalazine may be useful in treatment of IBS-d patients, and warrant further larger studies.

HEADINGS - Irritable bowel syndrome. Diarrhea. Mesalamine. *Saccharomyces boulardii.*

INTRODUCTION

Irritable bowel syndrome (IBS) is a common, functional intestinal disorder that affects a heterogeneous group of patients. It is characterized by chronic and recurrent abdominal pain associated with altered bowel habits (Figure 1). From 10%-20% of adults have symptoms suggestive of IBS; prevalence is higher in women than in men and is equally prevalent all races as a function of race and geographic distribution[2].

The pathophysiology of IBS remains unclear. Alterations in intestinal motor function, visceral hypersensitivity and gut-brain axis activity have been found in some patients with IBS. More recently, the presence of continuous mucosal inflammation and alterations in the intestinal microflora have been reported, particularly in diarrhea-predominant IBS (IBS-d). The putative role of immune cells, microflora and environmental factors (e.g. chronic stress), in the generation and perpetuation of this inflammatory process is not known. It has been suggested that interactions between intestinal flora and intestinal cells could produce intestinal inflammation in various gastrointestinal diseases and possibly in IBS. The imbalance between inhibiting factors of inflammatory response and antigenic stimuli could lead to the perpetuation of the inflammatory response[19, 24, 38, 51].

Since mucosal inflammation and imbalance in

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intestinal microflora might both play a role in IBS, it is reasonable to hypothesize that anti-inflammatory and/or probiotic agents would improve IBS symptoms. Mesalazine, a derivative of 5 – aminosalicylic acid, has intestinal anti-inflammatory properties that are likely to be a function of topic exposure. It has been used in inflammatory bowel disease for many years. Recent reports showed good clinical results of mesalazine treatment in IBS patients[1, 3].

Saccharomyces boulardii, isolated from litchi fruit by Henri Boulard in the 1920s, belongs to the *Saccharomyces* genus, being commonly used in several food processes that produce beverages or require fermentation. This yeast is frequently prescribed in a lyophilized form as a biotherapeutic agent. *Saccaromyces boulardii* (*S.boulardii*) has been used in treatment of several intestinal diseases including IBS[5, 21, 28, 29, 45, 49].

Accordingly, the aim of this study was to assess the effects of mesalazine alone, combined therapy of mesalazine with *S. boulardii* or *S.boulardii* alone on IBS symptoms, through a small pilot study in IBS-D patients.

METHODS

Ethical Considerations

This study was approved by the Research Ethical Committee of Hospital Geral de Goiânia, and was done according to Helsinki Declaration. All patients gave their consent to participate.

A prospective study was realized with fifty-three (53) IBS-d patients (18 years old or more). Patients were included, based on the Rome III criteria (Figure 1 and 2). In order to exclude organic diseases, all patients were screened by colonoscopy, stool culture, serum anti endomysium antibody, lactose tolerance test and ova and parasite stool test.

Recurrent abdominal pain or discomfort** at least 3 days/month in the last 3 months associated with two or more of the following:
1. Improvement with defecation
2. Onset associated with a change in frequency of stool
3. Onset associated with a change in form (appearance) of stool

*Criterion fulfilled for the last 3 months with symptom onset at least 6 months prior to diagnosis
** "Discomfort" means an uncomfortable sensation not described as pain.

FIGURE 1. Diagnostic Criterion * for irritable bowel syndrome
In pathophysiology research and clinical trials, a pain/discomfort frequency of at least 2 days a week during screening evaluation is recommended for subject eligibility.

1 IBS-C – hard or lump stolls a at least 25% and loose (mushy) or watery stools b <25% of bowel movements*
2. IBS-D – loose (mushy) or water stolls b at least 25% and hard or lumpy stool a <25% of bowel movements*
3. IBS-M) – hard or lump stolls a at least 25% and loose (mushy) or watery stools b <25% of bowel movements*
4. Unsubtyped IBS – insufficient abnormality of stool consistency to meet for IBS-C D, or M*

FIGURE 2. Subtyping IBS by predominant stool pattern
IBS: irritable bowel syndrome; IBS-C: IBS with constipation; IBS-D: IBS with diarrhea; IBS-M: IBS mixed; *In the absence of antidiarrheal or laxative use

Study Design

Patients were stratified into one of three groups: 1) Mesalazine Group (MG) - 20 patients received mesalazine (M) 800 mg three times a day (t.i.d.) for 30 days; 2) Mesalazine and *S. boulardii* Group (MSbG) - 21 patients received M 800 mg t.i.d. and *S. boulardii* (Sb) 200 mg t.i.d. for 30 days; and 3) *S. boulardii* Group (SbG) – 12 patients received Sb 200 mg t.i.d. for 30 days. Drugs that influence intestinal motility or secretion were not allowed.

Symptom evaluation at baseline and after treatment was performed by means of a 4 point likert scale assessing: stool frequency, stool form and consistency (based on Bristol Scale), abdominal pain and abdominal distension (table 1).

The differences between symptom scores at baseline and after treatment were compared using paired t test and Kruskal-Wallis test for statistical analyses.

TABLE 1. The symptom score evaluation submitted to all of tree groups of avaliated patients before and after 30 days of therapy (min score = 4, max score =16)

	Symptom Score			
	1	2	3	4
1. Stool frequency	<2	3	4 a 5	>5
2. Stool form and consistency	<4	5	6	7
3. Abdominal pain	Absent	Mild	Moderate	Severe
4. Abdominal distension	Absent	Mild	Moderate	Severe

RESULTS

Demographic data and symptom characterization at baseline are in table 2. As compared to baseline, statistically significant improvements in symptoms score were seen at the 30 th day therapy for the MG, (P<0.0001), MSbG

TABLE 2. Patient demographic data and symptom score at baseline

	Mesalazine	Mesalazine and Saccharomyces	Saccharomyces
Age (year)	46	50	43
Male	7	7	4
Female	13	14	8
Symptom score Mean (SD)	10.70 (2.34)	10.67 (2.67)	9.75 (1.54)
Total	20	21	12

(*P*<0.0001), and SbG (*P* = 0.003) groups (figures 3, 4 and 5 respectively). Significant differences were also seen after one month when comparing GM, GMSb and GSb (*P* = 0.03) (figure 6). No significant differences were seen between GMSb and GM (*P* = 0.9).

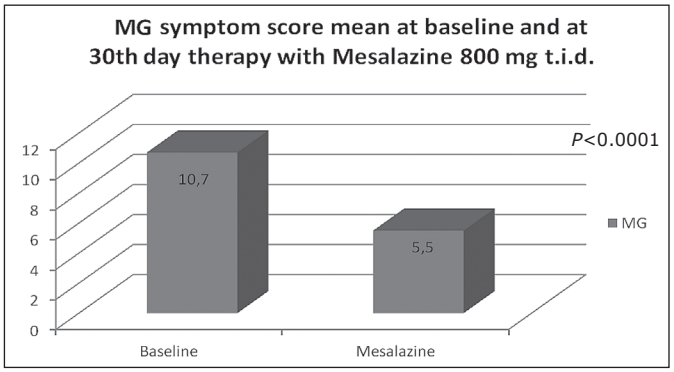

FIGURE 3. Mesalazine group (MG) symptom score mean at baseline (SD: ±2,34) and at 30 th day (SD: ±2,89) therapy with mesalazine (min score = 4; max score = 16)

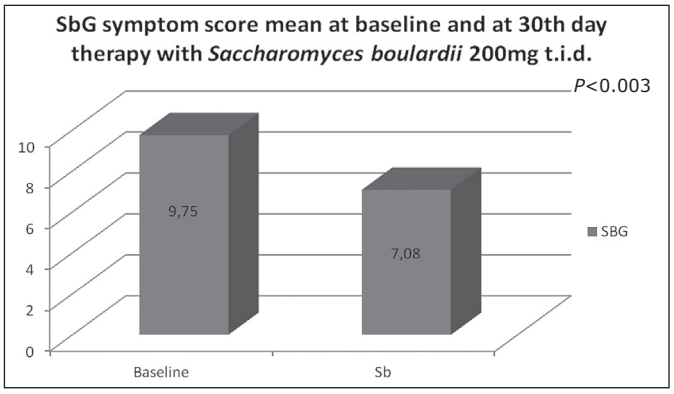

FIGURE 4. *Saccharomyces boulardii* group (SbG) symptom score mean at baseline (SD: ±1,54) and at 30 th day (SD: ±2,64) therapy with *S. boulardii* (min score = 4; max score = 16)
Sb: *Saccharomyces boulardii*

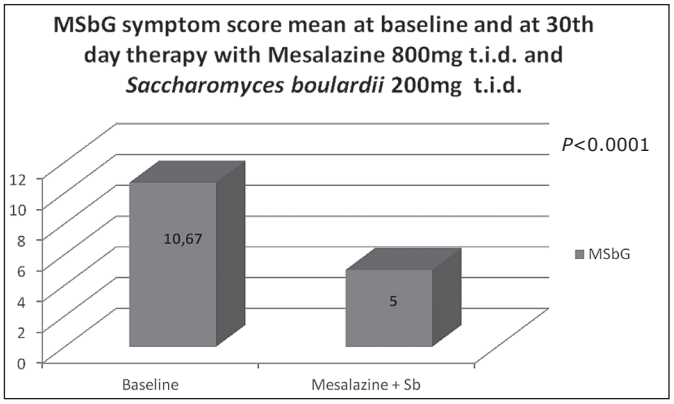

FIGURE 5. Mesalazine and *Saccharomyces boulardii* group (MSbG) symptom score mean at baseline (SD: ±2,37) and at 30 th day (SD: ±1,9) therapy with mesalazine and *S. boulardii* (min score = 4; max score = 16)
SB: *Saccharomyces boulardii*

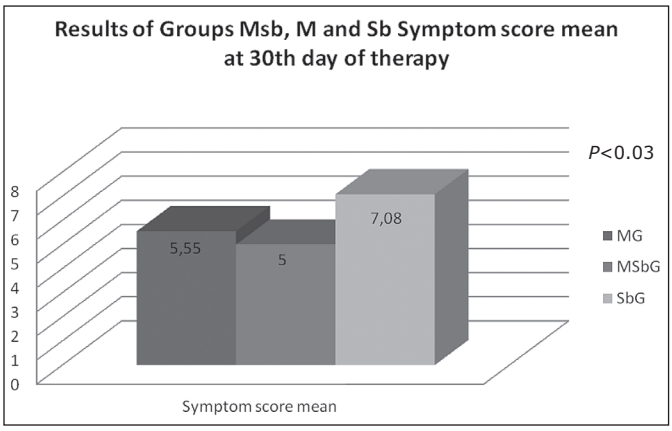

FIGURE 6. Results of MSbG, MG and SbG score mean after 30 th day of therapy (min score = 4; max score = 16)
MSb: mesalazine and *S. boulardii*; M: mesalazine; Sb: *Saccharomyces boulardii*

DISCUSSION

IBS is a complex and variable disorder where prominent symptoms include abdominal pain and altered bowel function, manifested by diarrhea, constipation, a sensation of fullness following evacuation, as well as fecal mucous discharge. No physical or laboratory findings are specific for IBS, and the diagnosis is, therefore, based on symptomatology[1].

A unifying hypothesis to explain the pathogenesis of IBS remains elusive. Alterations in gastrointestinal motor function, enhanced visceral perception of painful stimuli and psychosocial factors are considered as key contributors to symptom generation in IBS[2, 23]. Factors that are receiving recent attention include reduced ability to expel intestinal gas, altered central processing of afferent signals and intestinal inflammation. While routinely performed, histologic examination typically reveals no significant colonic or mucosal abnormalities in the majority of the patients, although quantitative histological, immunohistochemical and ultrastructural analyses sometimes provide evidence of subtle morphologic changes in these patients[2].

Numerous studies performed mucosal biopsies in Post infective (PI)-IBS and IBS-D, and found increased number of CD3+ lymphocytes. Although discrepancies in findings exist, this increase in CD3+ lymphocytes is consistently reported. Also shown were increases in CD25+ lymphocytes, indicating the presence interleukin (IL)-2 receptor, a marker of activated lymphocytes[11]. In addition, studies have examined the presence of mRNA for IL-1β, a macrophage product that has been shown to be increased in both Post infective PI-IBS and IBS-D. These inflammatory cells produce cytokines, which are known to alter enteric neural function and contribute to diarrheal symptoms[8, 20, 22, 56].

The gut has its permeability increased in the presence of inflammatory cytokines, as well as in the context of bacterial gastroenteritis and in PI-IBS and IBS-D[17]. The lamina propria, as well as surface and crypt epithelium have been shown to contain increased numbers of T-lymphocytes in

IBS; the predominant form of diarrhea in IBS is associated with a greater increase in mucosal T-lymphocytes, relative to the predominant form of constipation[12, 16, 50, 54].

Increased numbers of nerve fibres staining positively for neuron specific enolase, substance P and 5-HT (but not calcitonin gene-related peptide) have been demonstrated in biopsies from the terminal ileum and rectosigmoid in patients with both PI-and non-PI-IBS. In addition, positively stained nerve fibres around mast cells are reported to be significantly increased in density in IBS patients compared to controls[58].

The role of mast cells (MC) has also been investigated in a number of studies[15, 23, 39, 42, 60]. In the gastrointestinal tract, as in other mucosal surfaces, MC are part of the allergic response to luminal antigens and of protective, innate immune responses. Increased number of MC, as well as increased concentration of its products, has been described in the terminal ileum and the proximal and distal colon of IBS patients[3, 39, 60].

These findings, associated with increased mast cell degranulation, increased spontaneous release of MC tryptase and histamine 1 and increased proximity of MC to enteric nerves in IBS, suggest a role for MC in the disturbed, sensorimotor function characteristic of this condition[3, 16, 26, 39, 40, 41, 58]. The proximity of MC to enteric nerves suggests that MC mediators have increased potential to activate enteric neurons[39].

MC are also of importance as end effectors of the brain-gut axis (BGA). Upon activation of the BGA by stress, MC releases a wide range of neurotransmitters and other proinflammatory molecules. These mediators include histamine, heparin, chondroitin sulfate, chymase, carboxypeptidase, tryptase, platelet activating factor, prostaglandin (PGD2), leukotriene (LTC4) and a variety of interleukins such as IL-1b, IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-13, IL-16, IL-18, IL-25, TNF-alpha, granulocyte-macrophage colony-stimulating factor (GM-CSF), stem cell factors, macrophage chemotactic peptide (MCP)-1, 3&4, regulated on activation of normal T cell-expressed and secreted protein (RANTES), and eotaxin[25].

Finally, MC are of importance translating stress signals into release of pro-inflammatory mediators that can stimulate gastrointestinal nerve endings and affect its perception, change intestinal motility, and cause intestinal hyperpermeability[4, 52]. Accordingly, MC dysfunctions may be associated with the key symptoms (abdominal pain and/or discomfort and altered intestinal habit) of IBS.

Mesalazine has anti-inflammatory properties and is used in the treatment of inflammatory bowel diseases. Although the exact mechanism of action of mesalazine has still to be elucidated, several potential mechanisms have been suggested, including 5-aminosalicylate-induced inhibition of inflammation by interfering with the metabolism of arachidonic acid, prevention of mucosal generation of leukotrienes and prostaglandins, scavenging of free radicals and mechanisms only recently identified involving inhibition of nuclear factor-kappaB (NFκB) and induction of apoptosis[6, 18, 31, 43, 47, 48, 55, 57, 59]. Additional relevant properties include changes in the production of immune globulins and diminished production of interleukin-1 and partial inhibition of platelet activating factor (PAF) expression, resulting in a decrease in leucocyte trafficking[32]. Moreover there is evidence that mesalazine has a potential inhibition on MC histamine release and was effective to reduce MC infiltration in patients with IBS[13, 19].

Interactions between the intestinal flora and intestinal cells have been identified as determinants of production of intestinal inflammation in various gastrointestinal diseases and possibly in IBS. The imbalance between inhibiting factors of inflammatory response and antigenic stimuli could lead to the perpetuation of the inflammatory response[50]. Based on these points, several probiotics have been used in IBS.

S. boulardii influences the transit of micro-organism in the gastrointestinal tract, being a probiotic agent. During the intestinal transit, *S. boulardii* interacts with resident microflora and intestinal mucosa. Moreover, experimental studies suggest that *S. boulardii* is protective against enteric pathogens, modulating the host immune response, decreasing inflammation and hydro electrolytic secretions, inhibiting bacterial toxin and enhancing trophic factors such as brush border membrane enzymes and nutrient transporters[7, 8, 10, 14, 37, 53, 40, 44, 47].

Controlled clinical trials suggested that oral administration of *S. boulardii* could treat or prevent gastrointestinal diseases such as antibiotic-associated diarrhea, recurrent Clostridium difficile associated diseases, traveler's diarrhea, children acute diarrhea, enteral tube feeding-associated diarrhea, AIDS-associated diarrhea, intestinal bowel disease such as Crohn's disease and ulcerative colitis and IBS[4, 20, 22, 27, 30, 33, 34, 35, 46]. In IBS a double-blind, placebo-controlled study conducted in 34 patients with diarrhea, treatment with *S. boulardii* decreased the daily number of stools ($P<0.05$) and improved their consistency ($P<0.05$)[33].

In the current study, we focused on the putative participation of microbial, intestinal inflammation or both, in IBS. Accordingly, we used two drugs, mesalazine and/or *Saccharomyces boulardii* that may be synergistic. While there are some evidence to support the use of mesalazine or *S.boulardii* alone, the treatment of mesalazine associated with *S.boulardii* in IBS-d patients is lacking.

We found significant improvement in symptoms in the three groups. Nonetheless, we highlight that the placebo effect is relevant in IBS, with a magnitude ranging from 20% to more than 50% in some trials[9, 36]. Other limitations of this study are the small number of patients and the follow-up period of 30 days.

There were no statistical difference between the groups that used mesalazine, but we observed a significant reduction on symptom score when compared groups that used mesalazine to *S. boulardii* alone. It is important to note that mesalazine was not compared to placebo, but with *S. boulardii*, an effective agent in the treatment of IBS-d. Accordingly, we conclude that mesalazine improved key symptoms and has therapeutic properties in IBS-d. These preliminary results warrant further, larger, controlled studies.

Bafutto M, Almeida JR, Leite NV, Costa MBG, Oliveira EC, Resende Filho J. Tratamento da síndrome do intestino irritável tipo diarreia-predominante com mesalazina e/ou *Saccharomyces boulardii*. Arq Gastroenterol. 2013,50(4):304-9.

RESUMO - ***Contexto*** **–** A síndrome do intestino irritável (SII) é uma doença funcional do intestino, caracterizada por dor abdominal e alterações do hábito intestinal, cuja fisiopatologia permanece desconhecida. Estudos recentes sustentam a hipótese de que algumas formas de SII, especialmente a síndrome do intestino irritável tipo diarreia (SII-D), apresentam sinais de uma inflamação de baixo grau persistente da mucosa intestinal e alterações da microflora intestinal. A mesalazina é conhecida por suas propriedades anti-inflamatórias intestinais. O *Saccharomyces boulardii* é um probiótico largamente utilizado para o tratamento da diarreia relacionada à causa infecciosa. ***Objetivo*** **–** Avaliar os efeitos da mesalazina, da terapia com mesalazina combinada ao *Saccharomyces boulardii* e do *Saccharomyces boulardii*, em pacientes com SII-D. ***Método*** **–** Com base nos critérios de Roma III, 53 pacientes com SII-D (maiores de 18 anos) foram incluídos. Para excluir as doenças orgânicas, todos os pacientes realizaram colonoscopia, coprocultura, anticorpo anti-endomísio, teste de tolerância à lactose e exame parasitológico de fezes. Os pacientes foram divididos em três grupos: grupo mesalazina (GM) – 20 pacientes foram medicados com mesalazina via oral 800 mg t.i.d. por 30 dias; grupo mesalazina e *Saccharomyces boulardii* (GMSb) – 21 pacientes foram medicados com mesalazina 800mg t.i.d. e *Saccharomyces boulardii* 200 mg via oral t.i.d. por 30 dias; grupo *Saccharomyces boulardii* (GSb) – 12 pacientes foram medicados com *Saccharomyces boulardii* 200 mg t.i.d. por 30 dias. Não foram permitidas drogas concomitantes com algum efeito sobre secreção ou motilidade intestinal. Os sintomas foram avaliados no basal e após tratamento por meio da escala de Likert de 4 pontos que incluía: frequência de evacuações; forma e consistência das fezes (baseado na escala de Bristol); dor abdominal; e distensão abdominal. A analise estatística foi realizada por meio de teste *t* pareado e do teste de Kruskal-Wallis. ***Resultados -*** Comparados ao basal, observou-se uma redução estatisticamente significativa da pontuação de sintomas após 30 dias de tratamento no GM (P<0.0001); GMSb (P<0.0001); e GSb (P<0.003). Diferença estatisticamente significativa da pontuação de sintomas após 30 dias de tratamento entre GM, GMSb e GSb (P = 0.03). Não foi observada diferença estatisticamente significativa entre GM e GMSb após 30 dias de tratamento (P = 0,9). ***Conclusão*** **–** O uso da mesalazina isolada, do *Saccharomyces boulardii* isolado ou do tratamento combinado com ambos, mesalazina e *Saccaromyces boulardii*, melhoraram os sintomas da SII-D. A melhora dos sintomas foi maior naqueles que usaram mesalazina seja isolada ou em combinação com *Saccharomyces boulardii* quando comparada com o uso de *Saccharomyces boulardii* isoladamente. Estes resultados sugerem que a mesalazina pode ser útil no tratamento de pacientes com SII-D e justificam outros estudos com maior número de pacientes.

DESCRITORES - Síndrome do intestino irritável. Diarreia. Mesalamina. *Saccharomyces boulardii.*

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