World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v18.i44.6452

World J Gastroenterol 2012 November 28; 18(44): 6452-6460
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Effectiveness of *Saccharomyces boulardii* in a rat model of colitis

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Received: July 15, 2012 Revised: October 8, 2012
Accepted: October 22, 2012
Published online: November 28, 2012

Abstract

AIM: To investigate the effects of *Saccharomyces boulardii* (*S. boulardii*) in an experimental rat model of trinitrobenzene sulfonic acid (TNBS)-induced colitis.

METHODS: Thirty-two Wistar albino female rats were categorized into five groups. On the first day of the study, 50 mg TNBS was administered *via* a rectal catheter in order to induce colitis in all rats, except those in the control group. For 14 d, the rats were fed a standard diet, without the administration of any additional supplements to either the control or TNBS groups, in addition to 1 mg/kg per day *S. boulardii* to the *S. boulardii* group, 1 mg/kg per day methyl prednisolone (MP) to the MP group. The animals in the *S. boulardii* + MP group were coadministered these doses of *S. boulardii* and MP. During the study, weight loss, stool consistency, and the presence of obvious blood in the stool were evaluated, and the disease activity index (DAI) for colitis was recorded. The intestines were examined and colitis was macro- and microscopically scored. The serum and tissue levels of tumor necrosis factor-α (TNF-α) and nitric oxide (NO) were determined, and fungemia was evaluated in the blood samples.

RESULTS: The mean DAI scores for the MP and *S. boulardii* + MP groups was significantly lower than the TNBS group (3.69 ± 0.61 *vs* 4.46 ± 0.34, $P = 0.018$ and 3.77 ± 0.73 *vs* 4.46 ± 0.34, $P = 0.025$, respectively). While no significant differences between the TNBS and the *S. boulardii* or MP groups could be determined in terms of serum NO levels, the level of serum NO in the *S. boulardii* + MP group was significantly higher than in the TNBS and *S. boulardii* groups (8.12 ± 4.25 μmol/L *vs* 3.18 ± 1.19 μmol/L, $P = 0.013$; 8.12 ± 4.25 μmol/L *vs* 3.47 ± 1.66 μmol/L, $P = 0.012$, respectively). The tissue NO levels in the *S. boulardii*, MP and *S. boulardii* + MP groups were significantly lower than the TNBS group (16.62 ± 2.27 μmol/L *vs* 29.72 ± 6.10 μmol/L, $P = 0.002$; 14.66 ± 5.18 μmol/L *vs* 29.72 ± 6.10 μmol/L, $P = 0.003$; 11.95 ± 2.34 μmol/L *vs* 29.72 ± 6.10 μmol/L, $P = 0.002$, respectively). The tissue NO levels in the *S. boulardii*, MP and *S. boulardii* + MP groups were similar. The mean serum and tissue TNF-α levels were determined to be 12.97 ± 18.90 pg/mL and 21.75 ± 15.04 pg/mL in the control group, 18.25 ± 15.44 pg/mL and 25.27 ± 11.95 pg/mL in the TNBS group, 20.59 ± 16.15 pg/mL and 24.39 ± 13.06 pg/mL in the *S. boulardii* group, 9.05 ± 5.13 pg/mL and 24.46 ± 10.85 pg/mL in the MP group, and 13.95 ± 10.17 pg/mL and 24.26 ± 10.37 pg/mL in the *S. boulardii* + MP group.

Significant differences in terms of the levels of serum and tissue TNF-α and the macroscopic and microscopic scores were not found between the groups. *S. boulardii* fungemia was not observed in any of the rats. However, *Candida* fungemia was detected in one rat (14%) in the TNBS group, two rats (28%) in the *S. boulardii* group, three rats (50%) in the MP group, and three rats (42%) in *S. boulardii* + MP group.

CONCLUSION: *S. boulardii* does not demonstrate considerable effects on the DAI, pathological scores, or cytokine levels but does decrease the tissue NO levels.

Key words: *Saccharomyces boulardii*; Rat; Trinitrobenzene sulfonic acid; Tumor necrosis factor-α; Nitric oxide; Fungemia

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Soyturk M, Saygili SM, Baskin H, Sagol O, Yilmaz O, Saygili F, Akpinar H. Effectiveness of *Saccharomyces boulardii* in a rat model of colitis. *World J Gastroenterol* 2012; 18(44): 6452-6460 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i44/6452.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i44.6452

INTRODUCTION

It is currently speculated that inflammatory bowel disease (IBD) develops in genetically prone individuals as a result of defective immune responses against enteric bacterial flora antigens. Antibiotics and immunomodulatory therapies are believed to play an important role in the treatment of this disease[1].

Probiotics are live microorganisms that positively affect health when ingested. *Saccharomyces boulardii* (*S. boulardii*) is a thermophilic nonpathogenic yeast that is selectively used to treat antibiotic-associated and traveler's diarrhea[2]. The main mechanisms of action of *S. boulardii* include antimicrobial activities, trophic effects upon the intestinal mucosa, and the modification of the host-signaling pathways that are involved in inflammatory and noninflammatory intestinal diseases. It has been shown that *S. boulardii* inhibits the production of proinflammatory cytokines by inhibiting the main regulators of inflammation, such as nuclear factor κB and mitogen-activated protein kinases, which play crucial roles in the pathogenesis of IBD[3,4]. *S. boulardii* is believed to effectively treat IBD because of its antimicrobial activities and its regulatory effects on enteric flora and the immune system[5]. In a recent study[6], it was shown that treating human colon epithelial cells with *S. boulardii* increases the expression of peroxisome proliferator-activated receptor-c and the secretion of inhibits interleukin-8 (IL-8). In the same study, it was demonstrated that *S. boulardii* decreases intestinal inflammation by reducing the mucosal expression of proinflammatory cytokines in rats with trinitrobenzene sulfonic acid (TNBS)-induced colitis. This earlier study demonstrates that colonic inflammation can be reduced by *S. boulardii* through the regulation of inflammatory gene expression.

Nitric oxide (NO) is an important messenger that is involved in vein permeability and tissue damage. It is possible that in patients with active ulcerative colitis (UC) and Crohn's disease (CD), the inducible activity of NO synthase is elevated in inflamed mucosal epithelial cells. Increased NO levels indicate inflammation and, in turn, the intensity of the disease. Variations in tissue NO levels are important indicators of progression and recovery from IBD. There is also evidence that *S. boulardii* inhibits inducible nitric oxide synthase[7].

In the current literature, there are studies suggesting that *S. boulardii* causes fungemia, particularly in immunosuppressed and intensive care patients[8]. However, controlled studies cannot be used to investigate the effectiveness of *S. boulardii* against IBD, although promising results have been reported in a few studies[9,10].

The primary aim of our present study was to investigate the effects of *S. boulardii* on colonic inflammation and the disease activity index (DAI) in a rat model of TNBS-induced colitis. The secondary purpose of our current analyses was to investigate the risk of fungemia resulting from treatment with *S. boulardii* alone or in combination with corticosteroids.

MATERIALS AND METHODS

Animals

Approval was obtained from the animal ethics council of Dokuz Eylul University Medical Faculty (DEUTF). The DEUTF Hospital Experimental Research Laboratory provided 32 female Wistar albino rats weighing 200-250 g for use in this study which were divided into five groups. The control group included only four rats that were not treated with TNBS. All other groups consisted of seven rats (Table 1).

The rats were maintained in a room at a temperature of 23 ± 2 ℃ under a 12-h light/dark cycle at the DEUTF Experimental Animal Laboratory. Prior to and during the study, the rats were fed a standard diet (Yemta; Taris Ltd. Şti., Izmir, Turkey), and their weights were monitored daily. The rats were allowed water *ad libitum*.

Induction of colitis

After 24 h of fasting, 0.5 mL physiological serum was intracolonically administered to the rats in the control group *via* a cannula that was placed 8 cm proximal to the anus using a rectally inserted flexible polypropylene catheter. To induce colitis in the other groups, the rats were intracolonically treated with 0.5 mL of 100 mg/mL

Table 1 Study groups

Group	*n*	Application (day 1)	Application (days 1-14)
Control	4	Physiological serum	Physiological serum
TNBS	7	TNBS	No treatment
S. boulardii	7	TNBS	*S. boulardii*
MP	7	TNBS	MP
S. boulardii + MP	7	TNBS	*S. boulardii* + MP

TNBS: Trinitrobenzene sulfonic acid; *S. boulardii*: *Saccharomyces boulardii*; MP: Methyl prednisolone.

TNBS that was dissolved in 50% ethanol and administered *via* a cannula. Prior to catheter insertion, short-term sedation was provided *via* ether anesthesia. After TNBS administration, no rats developed perforation or exitus due to the formation of ulcerations in the colon.

Experimental design

As shown in the Table 1, 32 Wistar albino female rats were divided into five groups. The rats in the control group (n = 4) were not treated with TNBS after the intracolonic administration of physiological serum (*via* a cannula placed 8 cm proximal to the anus using a rectally inserted polypropylene catheter, similar to the administration of TNBS). After the administration of TNBS, the rats in the TNBS group (n = 7) were not treated. *S. boulardii* (Reflor; Biocodex laboratories, Gentilly, France) was prepared in its lyophilized form (282.5 mg/sachet with a biological activity of 5×10^9 viable cells) by the manufacturer. *S. boulardii* (1 mg/kg per day) was suspended in distilled water and added to the water supply of the rats in the *S. boulardii* group (n = 7) in the morning and evening starting on day 0. The rats in methyl prednisolone (MP) group (n = 7) were treated with MP (Prednol; Mustafa Nevzat, Istanbul, Turkey) at a dosage of 1 mg/kg per day, while the rats in the *S. boulardii* + MP group (n = 7) were treated with both *S. boulardii* and MP at the previously defined dosages using the previously discussed techniques.

Disease activity index

TNBS-induced colitis was scored according to the DAI proposed by Murthy *et al*[11] (Table 2). Scoring was calculated according to body weight loss (as a percentage), differences in stool consistency, and the occurrence of rectal bleeding. Fecal occult blood testing (FOBT) of stool samples (Hemoccult II; Beckman Coulter Inc., Fullerton, CA, United States) was used to detect obscure bleeding.

Pathological examination

After 14 d, blood was drawn from the abdominal aorta under ether anesthesia following 24 h of fasting, and then the rats were sacrificed due to hypovolemia. Decapitation was performed after tissue samples were collected for pathological examination. The abdominal cavity was opened *via* a midline incision, and the whole small and large intestines were harvested from the pylorus to the rectum. The intestinal lumen was washed with physiological serum containing phosphate buffer (PBS), and the intestinal materials collected from the opened lumen was fixed in formaldehyde. A pathologist who was blind to the groups conducted the pathological examinations of the intestinal samples.

Table 2 Disease activity index analysis[1]

Score	Weight loss (%)	Stool consistency	Rectal bleeding
0	-	Normal	-
1	1-5	Loose stool	Occult blood in stool
2	5-10	Loose stool	Occult blood in stool
3	10-20	Loose stool	Occult blood in stool
4	> 20	Watery stool	Obvious blood in stool

[1]Data are reported by Murthy *et al* [11].

The scoring method defined by Wallace *et al*[12] was used to evaluate damage due to colonic inflammation. Fixed intestinal tissue samples were microscopically examined (5 × magnification) and scored from 0-10 according to various inflammation markers, such as the diameters of any developing ulcers, thickening of the intestinal wall, and hyperemia. While intestinal tissues without any evidence of lesions were scored as 0, intestinal tissues with serious ulcerations were scored as 10. Subsequently, histological sections, including the peripheral normal mucosa, were prepared from gross ulcerative lesions. Approximately 1-cm sections were obtained from the intestines and transported on ice to the Department of Clinical Microbiology (DEUTF, Izmir, Turkey) for homogenization before fixation in formaldehyde. The tissues were fixed in formaldehyde, embedded in paraffin, and stained with hematoxylin and eosin. For the microscopic evaluation, we employed the defined scoring system described by Ameho *et al*[13].

Tissue homogenization

Prior to sacrifice, blood samples were drawn from the vena cava under ether anesthesia, and the serum was separated by centrifugation and stored at -70 ℃ until use. Homogenization of the intestinal tissues was performed in accordance with current methods[14]. The intestinal tissues were first homogenized in an ice-cold buffer [0.1 mol/L potassium phosphate (pH 7.5) and 20 mmol/L ethylene diamine tetra acetic acid (EDTA), 1:10 w/v] using a mechanical homogenizer (Potter B. Braun; Gemini, Apeldoorn, The Netherlands) and then in an ultrasonic homogenizer on ice. The resulting lysates were centrifuged at 14 000 rpm for 10 min followed by an additional spin at 14 000 rpm for 20 min. The proteins were purified using zinc sulfate (300 g/L) at a 1:20 ratio, and the final sample concentration of 15 g/L was obtained *via* centrifugation. The final products were centrifuged at 4 ℃ for 20 min at 2000 rpm, and 100-μL samples were subsequently prepared for evaluation of cytokine and NO levels.

NO analysis

The 100-μL intestinal tissue lysates and serum samples

were mixed with an equal volume of 100 μL of Griess reagent (Ingredient A: 0.1% naphthalene diamine dihydrochloride at a final concentration of 5 mmol/L; Ingredient B: 1% sulfanilamide at a final concentration of 5 mmol/L in orthophosphoric acid) in a 96-well microtiter plate (Maxisorb Immunoplate; NUNC, Roskilde Denmark). After incubation for 10 min at room temperature, the absorbance at 540 nm was measured using a microplate reader (Reader Model 230S; Organon Teknika, Boxtel, The Netherlands). For each measurement, 2-fold increases in sodium nitrite in PBS, from 0-128 mmol/L, were used to generate a standard curve[15].

Tumor necrosis factor-α analysis

Serum and tissue tumor necrosis factor (TNF)-α levels were measured using a commercially available rat-specific enzyme-linked immunosorbent assay kit according to the manufacturer's instructions (Invitrogen; Life Technologies, Camarillo, CA, United States).

Fungal isolation and identification

Blood samples were cultured in Petri dishes containing the following culture media: Sabouraud dextrose agar (BD Difco; Becton, Dickinson, NJ, United States) supplemented with 100 mg/L chloramphenicol and selective CHROMagar Candida (CHROMagar Candida; CHROMagar, Paris, France). Following incubation at 30 ℃ for 72 h, the yeast colonies were visually quantified, if they existed. A specimen from each colony variant was placed in a tube containing Sabouraud dextrose agar for storage and identification. Identification was based on the results of filament production tests; the production of the germinative tube, ascospores, urease, and phenoloxidase; and zymogram, auxanogram, and growth rates at different incubation temperatures, as recommended by Boekhout *et al*[16,17].

Statistical analysis

Statistical analyses were conducted using SPSS software for windows 11.0 (SPSS, Chicago, IL). Data are expressed as the mean ± SD. Quantitative data, such as the macroscopic and microscopic scores and NO and TNF-α levels, were compared between groups using the Kruskal-Wallis and Mann-Whitney *U* tests. Differences between the mean were considered statistically significant when $P < 0.05$.

RESULTS

Clinical evaluation

We did not observe diarrhea or bloody stool in the four rats in the control group during the course of this study but these effects were observed in 27 rats starting on day 1, plus one rat on day 2, following the administration of TNBS. In the TNBS and *S. boulardii* groups, a loose stool was noted on day 7 and never returned to normal and diarrhea persisted for a total of 14 d over the course of this study. In the MP group, a loose stool was observed on day 3 and had returned to normal by day 9. However, in the *S. boulardii* + MP group, the stool returned to normal by day 10 after becoming loose on day 2. The MP and *S. boulardii* + MP groups demonstrated 9 and 10 d of diarrhea, respectively (Figure 1A).

While defecation in the TNBS and *S. boulardii* groups macroscopically continued for an average of 9 and 7 d, respectively, in the following days the FOBT results were positive until day 14. In the MP group, bloody defecation was observed for an average of three days, while for the following six days the FOBT results were positive even without clearly noting bloody defecation. In addition, the blood in the stool was, on average, not observed until day 9. In the *S. boulardii* + MP group, bloody stool was observed for an average of two days, and the FOBT results were positive for seven days; however, there was no traceable blood in the stool samples after day 9 (Figure 1B).

The rats in the control group gained an average of 4.3 g in weight, those in the TNBS and *S. boulardii* groups gained an average of 11.9 and 2.4 g, respectively, and the rats in the MP and *S. boulardii* + MP groups lost 3 and 5.9 g, respectively. No significant differences in body weight changes could be determined between the *S. boulardii*, MP, and *S. boulardii* + MP groups. There were significant differences in body weight changes for the MP and *S. boulardii* + MP groups compared with the TNBS group ($P = 0.01$ and 0.02, respectively) (Table 3).

Based on the scoring system that was previously suggested by Murthy *et al*[11], DAI scores were 1 ± 0 for the control group, 4.46 ± 0.34 for the TNBS group, 4.07 ± 0.77 for the *S. boulardii* group, 3.69 ± 0.61 for the MP group, and 3.77 ± 0.73 for the *S. boulardii* + MP group. The DAI scores of the MP and *S. boulardii* + MP groups were significantly lower compared with the TNBS group ($P = 0.018$ and 0.025, respectively). Regarding the other groups, no significant differences in DAI could be determined (Figure 1C).

Pathological evaluation

Macroscopic ulceration was not observed in the control group, although inflammation was observed at the microscopic level. Upon macroscopic examination, a 14-mm ulcer in the intestinal tissue was observed in one rat in the TNBS group, while 3, 2, and 3 larger ulcers (> 2 cm) were observed in the *S. boulardii*, MP, and *S. boulardii* + MP groups, respectively. After macroscopic and microscopic scoring, of all the groups that were induced to form colitis, the lowest score was observed in the TNBS group. When the treated groups were examined, the *S. boulardii* group demonstrated the lowest scores, although significant differences were not found between the MP and *S. boulardii* + MP groups (Figure 1D).

Serum and tissue NO levels

The mean serum NO levels in the control, TNBS, *S. boulardii*, MP, and *S. boulardii* + MP groups were determined to be 5.92 ± 3.65 μmol/L, 3.18 ± 1.19 μmol/L, 3.47 ± 1.66 μmol/L, 8.22 ± 6.28 μmol/L, and 8.12 ± 4.25

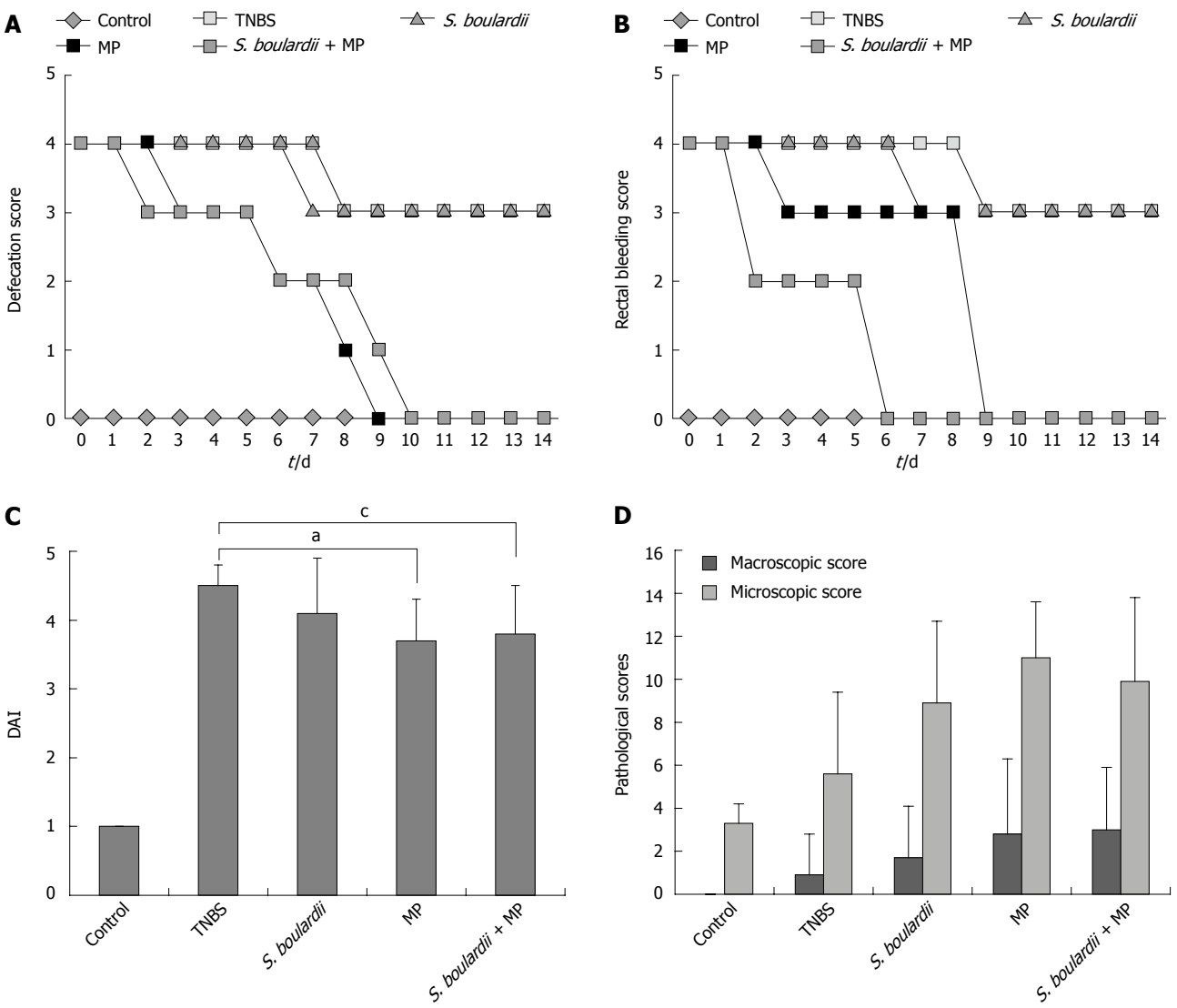

Figure 1 Daily defecation trends, rectal bleeding scores, disease activity index analyses, macro- and microscopic pathological scores of the experimental groups. A: Daily defecation trends; B: Rectal bleeding scores; C: Disease activity index analyses; The results are shown as the mean ± SD. [a]*P* = 0.018 between trinitrobenzene sulfonic acid (TNBS) and methyl prednisolone (MP) group; [c]*P* = 0.025 between TNBS and *Saccharomyces boulardii* (*S. boulardii*) and TNBS group; D: Macro- and microscopic pathological scores. The results are shown as the mean ± SD. DAI: Disease activity index.

μmol/L, respectively. The lowest serum NO levels were observed in the TNBS and *S. boulardii* groups. The serum NO levels in *S. boulardii* + MP group were higher than those in the TNBS and *S. boulardii* groups ($P = 0.013$ and 0.012, respectively).

The mean tissue NO levels in the control, TNBS, *S. boulardii*, MP, and *S. boulardii* + MP groups were determined to be 7.95 ± 0.50 μmol/L, 29.72 ± 6.10 μmol/L, 16.62 ± 2.27 μmol/L, 14.66 ± 5.18 μmol/L, and 11.95 ± 2.34 μmol/L, respectively. The highest tissue NO level was observed in the TNBS group. The tissue NO levels in the *S. boulardii*, MP, and *S. boulardii* + MP groups were significantly lower in comparison with the TNBS group ($P = 0.002$, 0.003 and 0.002, respectively). However, the tissue NO levels in the *S. boulardii*, MP, and *S. boulardii* + MP groups were similar (Figure 2A).

Serum and tissue TNF-α levels

The mean serum TNF-α levels were determined to be 12.97 ± 18.90 pg/mL in the control group, 18.25 ± 15.44 pg/mL in TNBS group, 20.59 ± 16.15 pg/mL in *S. boulardii* group, 9.05 ± 5.13 pg/mL in MP group, and 13.95 ± 10.17 pg/mL in *S. boulardii* + MP group. The mean tissue TNF-α levels were determined to be 21.75 ± 15.04 pg/mL in the control group, 25.27 ± 11.95 pg/mL in TNBS group, 24.39 ± 13.06 pg/mL in *S. boulardii* group, 24.46 ± 10.85 pg/mL in MP group, and 24.26 ± 10.37 pg/mL in *S. boulardii* + MP group. The serum and tissue TNF-α levels were comparable between groups (Figure 2B).

Detection and identification of fungal pathogens

Fungemia was not observed in the control group but was detected in 1 of 7 rats (14%) in the TNBS group, 2 of 7 rats (28%) in the *S. boulardii* group, 3 of 6 rats (50%) in the MP group (1 rat in the MP group was excluded because sufficient blood samples could not be obtained), and 3 of 7 rats (42%) in the *S. boulardii* + MP group. The

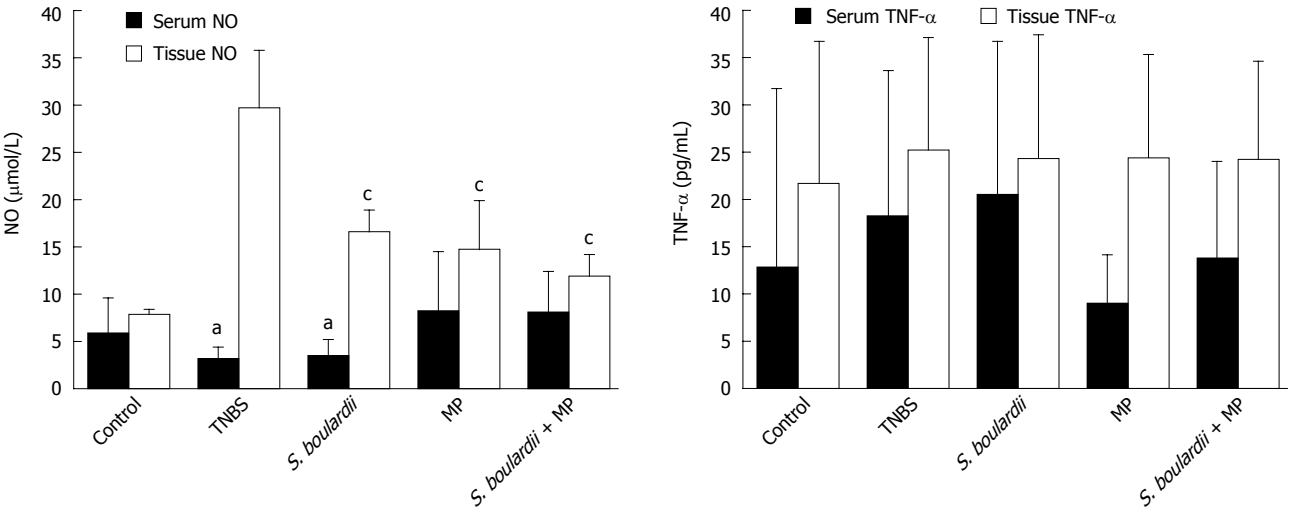

Figure 2 Serum and tissue nitric oxide and tumor necrosis factor-α levels of the experimental groups. A: Serum and tissue nitric oxide levels; B: Serum and tissue tumor necrosis factor (TNF)-α levels. The results are shown as the mean ± SD. [a]*P* < 0.05 *vs Saccharomyces boulardii* (*S. boulardii*) + methyl prednisolone (MP); [c]*P* < 0.05 *vs* trinitrobenzene sulfonic acid (TNBS). NO: Nitric oxide.

Table 3 Changes in body weight in the experimental groups

Groups	Baseline weight	Final weight	Difference in body weight
Control	214.0 ± 18.4	218.25 ± 19.6	4.3
TNBS	218.1 ± 18.6	230.0 ± 17.9	+ 11.9[a,b]
S. boulardii	195.6 ± 9.1	198.0 ± 20.2	+ 2.4
MP	199.3 ± 9.1	196.33 ± 9.1	- 3.0
S. boulardii + MP	197.3 ± 10.7	191.43 ± 9.1	- 5.9

The results are shown as the mean ± SD. [a]*P* = 0.02 *vs Saccharomyces boulardii* (*S. boulardii*) + methyl prednisolone (MP); [b]*P* = 0.01 *vs* MP. TNBS: Trinitrobenzene sulfonic acid.

fungemia was determined to be *Candida* fungi other than *C. albicans* using an identification process as explained in materials and methods section. *S. boulardii* fungemia was not identified in any of the rats.

DISCUSSION

It is thought that IBD develops in genetically prone individuals as a result of defective immune responses against the antigens of enteric bacterial flora. Antibiotics and immunomodulatory therapies are believed to be important for the treatment of these diseases[1]. *S. boulardii* is a probiotic yeast, and it is known that probiotics inhibit pathogenic invasion and also demonstrate regulatory effects on the enteric flora and immune system[2-5]. Although there are several published studies on probiotic bacterial therapies in experimental animals with induced IBD, there are only a few studies on *S. boulardii*. In this study, the effectiveness of *S. boulardii*, DAI, macro- and microscopic pathological ulcer scores, proinflammatory cytokine levels (i.e., serum and tissue levels of TNF-α), and the serum and tissue levels of lipid peroxidation products (i.e., NO) were evaluated.

In our study, the DAI in the groups that were administered individual MP or MP with *S. boulardii* were evaluated at significantly lower levels compared with the TNBS group. Meaningful differences in the DAI scores of the *S. boulardii* group were not determined, although the DAI score of the *S. boulardii* group was lower than that of the TNBS group. As expected, these results show that the treatment of colitis using MP effectively improves the symptoms that are observed in rat models of induced colitis. However, the DAI scores were comparable between the MP and *S. boulardii* + MP groups, suggesting that the application of MP in combination with *S. boulardii* might not be more effective than the individual application of MP. In a recent study[18], *Lactobacillus* and *Bifidobacterium* were used to prevent colitis in rats using dextran sodium sulfate, and DAI scores in the probiotic-treated group were lower than the control group. However there are no other reported studies that have investigated the effects of *S. boulardii* on DAI in an experimental model of colitis.

There are a few clinical studies on the application of *S. boulardii* for the treatment of UC and CD patients. Guslandi *et al*[9] conducted a study using 32 CD patients who were medically treated and had been in remission for 3 mo. For 6 mo, a group was administered only mesalamine (3 g/d) and another group was administered mesalamine (2 g/d) and *S. boulardii* (1 g/d). After 6 mo, 10 patients in the mesalamine-treated group and 15 patients from the mesalamine + *S. boulardii*-treated group were still in remission ($P < 0.04$). The same researchers conducted another pilot study[10] on 25 patients with UC of mild/moderate intensity who could not be treated with corticosteroids. After 3 mo of mesalamine (3 g/d) treatment, the patients were additionally supplemented with *S. boulardii* (250 mg administered 3 × per day) for 4 wk. At the end of treatment, 17 patients (68%) were in remission and had demonstrated an obvious reduction in their clinical activity scores ($P < 0.05$). However, in both studies, *S.*

boulardii was not used alone and a control group was not evaluated.

In our study, macroscopic and microscopic pathological scoring was performed to evaluate the effectiveness of *S. boulardii* against colonic inflammation. Statistically significant differences were not observed between the treated and untreated groups that were induced to form colitis. In addition, no improvement was noted in the colon mucosa following the administration of individual applications of MP and *S. boulardii* or the dual application of MP and *S. boulardii*. However, the use of probiotics, such as *Lactobacillus* and *VSL#3* (contains one strain of *streptococcus thermophilus*, three strains of *bifidobacterium* and four strains of *lactobacillus*), for the treatment TNBS-induced colitis in rat models have demonstrated significant improvements in macroscopic and microscopic scores[19,20]. Surprisingly, in our study, the macroscopic scores in the *S. boulardii*, MP, and *S. boulardii* + MP groups were higher, although they were not significantly different when compared with the TNBS group. In the MP and *S. boulardii* + MP groups, the macroscopic and microscopic pathology scores were higher than in the TNBS group, although these groups also demonstrated significantly lower DAI scores. Thus, these results suggest that the clinical responses are not consistent with the histopathological results. The higher macroscopic and microscopic pathology scores in the treatment groups compared with the TNBS group can be explained by delayed effects in ulcer improvement due to fungal colonization in the gastrointestinal (GI) tract. In our study, non-*Candida albicans* fungemia was detected at considerable frequencies in the treated groups. In a study[21] on inhibiting *Candida* translocation in the GI tract using probiotics, a group of patients with UC and rats with acetic acid-induced stomach ulcers were included. It was shown that *Candida* colonies formed in these groups, which was accompanied by the delayed recovery of stomach ulcers and the persistence of both of gastric ulcers and UC symptoms. An increase in cytokine expression, especially TNF-α and IL-1 levels, was detected in the rats that were inoculated with *Candida*.

IBD is an immunosuppressive disease caused by a defective intestinal mucosal barrier that can be brought on by applied treatments. Thus, during the course of the disease, insidious infections, such as cytomegalovirus and *C. albicans*, can develop. There are reported cases of the development of fungemia caused by *Candida* species, such as *C. parapsilosis*, *C. albicans,* and *Saccharomyces cerevisiae*, in UC patients. *S. boulardii* fungemia was reported in a 33-year-old male patient who was diagnosed with IBD, underwent intestinal surgery, and was in the intensive care unit[22]. In our study, fungemia due to *S. boulardii* did not develop in any of the groups. Accordingly, in the colitis rat model, an increase in the risk of developing *S. boulardii* fungemia was not determined upon the application of *S. boulardii* alone or in conjunction with MP. In this study, while fungemia was not observed in the control group, non-*C. albicans* was observed in the TNBS, *S. boulardii*, MP, *S. boulardii* + MP groups with frequencies of 14%, 28%, 50%, and 48%, respectively. This result is not consistent with previously published reports on the inhibition of *Candida* translocation in the gastrointestinal tract due to the use of probiotics in immunosuppressed rats[21,23].

In this study, the serum NO level in the group treated with *S. boulardii* + MP was high compared with the TNBS and *S. boulardii* groups. In addition, serum NO levels were comparable between the other groups. However, tissue NO levels in all 3 treatment groups were statistically and significantly lower in comparison with the TNBS group. As observed, the serum and tissue NO levels were inconsistent. However, it is known that the serum NO level is affected by systemic events, and tissue NO levels are more reliable. As a result, based on the results of the TNBS group, tissue NO levels are found to be low in all treatment groups. These results suggest that use of *S. boulardii* and MP alone or in combination can reduce the intensity of inflammation and damage to the colitis mucosa. It was also revealed that the addition of *S. boulardii* to MP treatment does not yield a synergistic effect because the tissue NO levels of the MP and *S. boulardii* + MP groups were similar. In another study[7], *S. boulardii* treatment reportedly affected NO levels in a rat diarrhea model that was induced by castor oil. In that study, *S. boulardii* was a successful diarrhea treatment that inhibited inducible NO synthase activity. In addition, other probiotics, especially *Lactobacillus* that has been used in induced colitis models, have been reported to reduce the tissue NO level by inhibiting inducible NO synthase activities[24].

TNF-α is produced by CD4 + T lymphocytes that are assembled around inflamed mucosa. TNF-α is a strong chemokine that functions in pathological inflammatory signal transduction by directing the migration of neutrophils to inflamed mucosa. Therefore, serum and tissue TNF-α levels are mainly used to evaluate the intensity of inflammation. In many studies conducted using *Lactobacillus* in TNBS-induced colitis models, the tissue TNF-α levels in the groups that were administered *Lactobacillus* were significantly reduced compared with the control group[25,26]. It has been demonstrated that the *Lactobacillus* species used in those studies reduces the number of CD4 + T cells in inflamed mucosa, thus reducing TNF-α production. In addition to these effects, *Lactobacillus* increases the production of anti-inflammatory IL-10 by shifting the T helper1 (Th_1) cellular immune response towards Th_2 and Th_3. By changing the TNF-α/IL-10 ratio, the intensity of inflammation can be reduced[26]. In a recent study[6], it was demonstrated that *S. boulardii* decreases intestinal inflammation by reducing the mucosal expression of proinflammatory cytokines in rats with TNBS-induced colitis. In our study, the serum and tissue TNF-α levels were similar in all groups. These results can be explained by non-*Candida albicans* fungemia, which can cause an increase in cytokine expression[21]. While there are an insufficient number of studies conducted on *S. boulardii*, *S.*

boulardii is believed to be involved in anti-inflammatory effects by affecting various inflammatory mechanisms[5-7].

In conclusion, this study establishes that *S. boulardii* does not improve DAI or colonic inflammation in rats with TNBS-induced colitis and does not reduce serum or tissue TNF-α levels. The only significant effect of *S. boulardii* is reducing tissue NO levels. *S. boulardii*-based fungemia was not detected in any of the rats included in this study.

COMMENTS

Background

It is thought that inflammatory bowel disease (IBD) develops in genetically prone individuals as a result of a defective immune response against the antigens of enteric bacterial flora. Antibiotics and immunomodulatory therapies play an important role in the treatment of these diseases. *Saccharomyces boulardii* (*S. boulardii*) is a probiotic yeast, and it is known that probiotics inhibit pathogenic invasion and demonstrate regulatory effects on the enteric flora and immune system. Although there are several published studies on the use of probiotic bacterial therapy in experimental animals with induced IBD, there are a few studies on the involvement of *S. boulardii*.

Research frontiers

The present study shows that *S. boulardii* is a probiotic agent that demonstrates no effects on the disease activity index (DAI), serum and tissue tumor necrosis factor-α (TNF-α) levels, or pathologic findings in a rat model of trinitrobenzene sulfonic acid (TNBS)-induced colitis. However *S. boulardii* may reduce tissue nitric oxide (NO) levels, which is an important messenger involved in vein permeability and tissue damage. *S. boulardii*-based fungemia was not detected.

Innovations and breakthroughs

There are a few clinical studies on the efficacy of *S. boulardii* for treating IBD. Based on the findings of these published studies, *S. boulardii* appears to be promising. In a recent study, it was shown that treating human colon epithelial cells with *S. boulardii* increases the expression of peroxisome proliferator-activated receptor-c and inhibits the secretion of IL-8. In the same study, it was demonstrated that *S. boulardii* decreases intestinal inflammation by reducing the mucosal expression of proinflammatory cytokines in rats with TNBS-induced colitis. However the effects of *S. boulardii* on DAI and NO were not evaluated. The present study was conducted to investigate the effects of *S. boulardii* on DAI, pathological scores, TNF-α, and NO. Additionally, the risk of fungemia, which could result from treatment with *S. boulardii* alone or in combination with corticosteroids, was also evaluated.

Applications

The present study shows that *S. boulardii* does not improve DAI or colonic inflammation in rats with TNBS-induced colitis or reduce TNF-α levels. These results suggest that *S. boulardii* may not be an effective treatment for patients with IBD. In contrast, the *limited number of studies* conducted on this *issue have reported* some promising results. Therefore, further studies are needed in order to draw a firm conclusion.

Terminology

Crohn's disease and ulcerative colitis, both of which are referred to as IBD, are chronic inflammatory disorders of the gastrointestinal tract that have characteristic clinical, pathological, endoscopic, and radiological features. TNBS-induced colitis is well-established in various animal models of mucosal inflammation that have been used for over 2 decades for the study of IBD pathogenesis and in preclinical studies. Probiotics are live microorganisms that positively affect health when ingested. *S. boulardii* is a live yeast that is extensively used as a probiotic.

Peer review

This study examine the impact of *S. boulardii* on TNBS colitis. This is an excellent experimental study that evaluated the effects of *S. boulardii* on clinical activity scores, TNF-α levels, serum and tissue NO levels, and macroscopic and microscopic pathological scores in a rat model of TNBS-induced colitis.

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S- Editor Gou SX **L- Editor** A **E- Editor** Zhang DN