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Original Article

Investigation of therapeutic effect of Saccharomyces boulardii and translocation in immunosuppressed rats infected with Shigella sonnei

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ABSTRACT

Aim: To investigate the therapeutic effects of *Saccharomyces boulardii* (*S. boulardii*) and detect blood and tissue penetrations of *S. boulardii* and *Shigella sonnei* (*S. sonnei*) in immunosuppressed rats infected with *S. sonnei*.

Methods: Forty rats were divided into four groups: Group A (immunosuppressed, not-treated); Group B (immunosuppressed, treated with *S. boulardii*); Group C (immunosuppressed, infected with *S. sonnei*, treated with *S. boulardii*); Group D (immunosuppressed, infected with *S. sonnei*). After taking samples for blood cultures, the rats were sacrificed. The large bowel, liver, spleen and mesenteric lymph nodes (MLN) were removed for microbiological examination.

Results: *S. boulardii* in group B and *S. sonnei* in group D were isolated from blood in some rats. Statistical analysis of our data, showed that the numbers of translocated colonies in the liver and spleen were relatively higher for *S. boulardii* in Group B and for *S. sonnei* in Group D, without reaching levels of statistical significance. For MLN, colony counts in Group B was higher than Group C and A showing statistical significance.

Conclusion: The administration of *S. boulardii* showed promising results for the therapy of *S. sonnei* infection in immunosuppressed rats, but therapeutic usage of *S. boulardii* should be carefully assessed by taking into consideration the risk it poses versus potential benefits in risk groups.

Keywords: *Saccharomyces boulardii, Shigella sonnei*, immunosuppression, rat, translocation.

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Introduction

Intestinal infections due to *Shigella spp*. are worldwide endemic but it mainly occurs in developing countries. Four species of *Shigella* (*S*); *S. dysenteriae, S. flexneri, S. boydii* and *S. sonnei* are the causative agents of shigellosis. Nearly two-thirds of the infections *are caused by S. flexneri* in low and middle-income countries.

S. sonnei is the leading species in high-income countries and the second most common species in low and middle-income countries. Immunodeficiencies lead to more severe clinical manifestations of Shigella infection including persistent or recurrent intestinal disease and bacteriemia [1]. Antimicrobial agents used as effective options in the treatment of shigellosis became limited due to global drug resistance [2].

Saccharomyces boulardii (S. boulardii) is a non-pathogenic yeast used in many countries in the treatment of non-specific diarrhea and in cases of gut flora impairment. Several mechanisms such as fungal antagonism, diminution of the pathogenic effects of bacterial toxins, stimulation of intestinal immune defenses and increased intestinal disaccharidase activity can possibly explain the actions of *S. boulardii* in diarrhea**.** The activity of *S. boulardii* has been extensively studied in the contexts of gastroenteritis and antibiotic-associated diarrhea. The good tolerability of this yeast was shown and no serious adverse reactions have been reported despite a very slight potential risk of blood penetration of *S. boulardii* in immunodeficient individuals [3].

This study was conducted with the aim of investigating the therapeutic effects of *S. boulardii* in immunosuppressed rats infected with *S. sonnei* and detecting the presence of translocations of *S. boulardii* and *S. sonnei* in vulnerable hosts.

Materials and methods

These experiments were performed with the approval of the Ethics Committee of Duzce University School of Medicine (Decision no: 100-019). The animals which were used in the study were provided by Duzce University. The procedures were conducted according to routine animal care guidelines, and all experimental procedures complied with the Guide for the Care and Use of Laboratory Animals (1996).

In this study, forty male Wistar albino rats weighing 200±20 g were divided into four groups of 10 animals each: Group A (immunosuppressed, not treated), Group B (immunosuppressed, treated with *S. boulardii*), Group C (immunosuppressed, infected with *S. sonnei,* treated with *S. boulardii*) and Group D (immunosuppressed, infected with *S. sonnei*).

All rats were housed individually in stainless steel cages in an animal room at 20^0 C with a 12-hour light-dark cycle. All the rats were fed with a laboratory pellet diet and were allowed to have free access to water during the course of the experiments. Rats were decontaminated from antibiotics for 4 days by adding 2 mg/ml of streptomycin sulphate (Sigma, St. Louis, MO, USA) and 1500 units/ml penicillin-G (Sigma) to their water, which was prepared daily. Reduction of the gastrointestinal flora was confirmed by microscopic examination of Gram stained smears of fecal pellets and with aerobic cultures of feces for Gram-negative enteric bacteria.

The animals in all groups were given cyclophosphamide (Baxter Oncology, Germany) intraperitoneally at a dose of 200mg/kg. Group A was the immunosuppressed rats with no infection. On the fourth day after immunosuppression, Groups C and D were inoculated with 0.1 ml of *S. sonnei* containing $9x10^8$ viable cells by gavage route after having cultures of the bacteria in Brain Heart Infusion broth (HiMedia Laboratories, India) for 12 hours [4]. On the third day after the inoculation, *S. sonnei* was isolated from the fecal samples of all rats.

Lyophilized *S. boulardii* (Ultra-Levure; BIOCODEX Laboratories, Montrouge, France) was given to each rat at a single dose of 10mg/day by gavage route for Groups B and C. Group A was given 0.1 ml (same amount as other groups) of phosphate buffer saline (PBS), as this group was neither inoculated by *S. boulardii* nor by *S. sonnei*. *S. boulardii* administration was continued for 10 days for group B and for five days for group C. The rats were sacrificed under ether inhalation on day 10.

Prior to being sacrificed, blood samples (2 ml) of rats were collected from inferior vena cava and inoculated into the bottles of BACTEC system (Becton Dickinson, Ireland).

After the rats were sacrificed; their large bowels, liver, spleen and MLN were removed for microbiologic examination. To evaluate the translocation by microbiological methods; the weights of the liver, spleen and nodes were recorded.

To assess *S. boulardii* and/or *S. sonnei* quantitatively, tissue pieces were minced with a scalpel, diluted by tenfold in 0.9% NaCl and homogenized with a handled tissue tearer. (Ultra-Turrax T25, BioSpec Products, Bartlesville, OK, USA).

Diluted organ homogenates were transferred on Sabouraud dextrose agar (SDA) (HiMedia Laboratories, India) and Hektoen Enteric (HE) (HiMedia Laboratories, India) agar plates. The duration of the cultures were as follows: 7 days for blood cultures, 72 hours for SDA and 48 hours for HE agars at 35ºC. Microorganisms were identified by conventional methods and API 32E (BioMerieux, France) and API CAUX (BioMerieux, France) systems. Then, the number of colonies per tissue gram (cfu / g) was determined.

The translocation index shows the number of microorganisms per gram of tissue and was calculated by the following formula [5]:

(Cfu count x dilution coefficient x 10 x 2) / Tissue weight.

Statistical Analysis

The data was expressed as means ± SD. Kruskal-Wallis and Mann-Whitney U tests were used to compare the numbers of colonies between the groups. The differences were considered as being significant at $p<0.05$.

Results

The number of colonies of the microorganisms isolated from the cultures of MLN, liver and spleen specimens and the results of blood cultures were determined for each group.

In Group A, in immunosuppressed rats, blood and tissue cultures were negative and no translocations were seen.

In Group B (immunosuppressed, treated-with-*S. boulardii*), the results showed that *S. boulardii* translocated and it systemically spread to extraintestinal sites of liver, spleen and MLN in some rats. Of 10 rats, one yielded positive results in the liver, spleen, MLN and blood; one yielded positive results in spleen and MLN; one yielded positive results in MLN and blood; and, lastly, four rats yielded positive results in only the MLN cultures. The isolated organism was *S. boulardii* from the MLN, liver and spleen specimens. *S. boulardii* was also isolated from the blood specimens of two rats in which translocations were observed.

In Group C (immunosuppressed, infected-with-*S.sonnei*, treated-with- *S. boulardii*), neither *S. boulardii* nor *S. sonnei* was isolated from the cultures of MLN, liver, spleen and/or blood specimens.

In Group D (immunosuppressed, infected-with-*S. sonnei*), the results of the cultures were as follows: one rat yielded positive results in the liver, spleen, MLN and blood specimens; one yielded positive results in the liver, MLN and blood specimens; and one rat yielded positive results in MLN for *S. sonnei.*

The numbers of translocated colonies are listed in Table 1 and are expressed as cfu. The numbers of translocated colonies in liver and spleen were relatively higher for *S. boulardii* in Group B and higher for *S. sonnei* in Group D, but the difference was not statistically significant *(p>0.05)*. For MLN, colony counts in Group B was higher than in Groups C and A, which was statistically significant *(p=0.005)*. In Group D, colonies of *S. sonnei* was high, but not with statistical significance *(p=0.068)*.

certain agents, including *Giardia intestinalis*, Rotavirus, *Entamoeba histolytica*, *Helicobacter pylori*, *ETEC*, *Cryptosporidium parvum* as well as in the treatment of diarrhea associated with HIV [9-11]. *S. boulardii* has been found to show a protective effect in *Clostridium difficile*

Table 1. Numbers of colonies in the liver, spleen, MLN and blood in the four experimental.

Parameters	A (n=10)	B (n=10)	C (n=10)	D (n=10)
Liver* (cfu/g/10^5)	0.00	363.60±1149.80	0.00	194. 20±409.41
Spleen **(cfu/g/10^5)	0.00	1039.10±2360.01	0.00	210.50±666.65
MLN*** (cfu/g/10^5)	0.00	47273.70±75043.94	0.00	10378.70±17400.4
Bacteriemia/fungemia	0	2	0	2

***n=** number of rats*. ***B:** For S. boulardii*
**** (p=0.001) (Kruscal-Wallis test); (p=0.005) groups A-B, B-C (Mann-Whitney U test)*
***D:** For S. sonnei*
**** (p<0.05) (Kruscal-Wallis test): (p=0.068) groups A-D, C-D (Mann-Whitney U test)*

Discussion

S. boulardii is widely used for clinical conditions and predominantly for the prevention of diarrhea [6,7]. The effects of *S. boulardii* are related to its inhibitory effects on the growth of intestinal microorganisms and to the neutralization of toxins, however the mechanisms underlying such effects could not be fully identified. Furthermore, *S. boulardii* has been shown to increase the activity of brush border disaccharidases in human volunteers and in patients with congenital sucrase isomaltase deficiency. In addition, an enhanced release of secretory IgA into the intestinal lumen supports the immune system [8].

Many studies have been conducted to investigate the activity of *S. boulardii* against associated colitis [12,13]. Studies showed a protective effect against *Salmonella typhimurium* and *Shigella flexneri* in the intestinal tracts of conventional or gnotobiotic mice [14]. The effects of orogastric administration of *S. boulardii* on the jejunal villi was studied by Dias et al. [15] in rats infected with *Vibrio cholerae* and their data showed the inhibition of the action of the cholera toxin on enterocytes by *S. boulardii*. Sheele et al. [16] showed the efficiency of *S. boulardii* in patients in whom the duration and severity of cholera was reduced. Zbinden et al. [17] investigated the influence of *S. boulardii* on *Salmonella typhimurium* and *Yersinia enterocolitica* under in vitro conditions and their results showed that *S. boulardii* inhibited either the growth of both bacteria or their invasion into HeLa cells, so they suggested to study these effects in vivo as well.

In recent years, invasive fungal infections have been frequently reported worldwide in parallel to with the increase in risky population such as patients with chronic or debilitating diseases, who receive immunosuppressive drugs broad spectrum antibiotics, and parenteral nutrition and who were administered central venous catheter [18].

Microbial translocation is defined as the passage of viable microbes from the gastrointestinal (GI) tract to extraintestinal sites, such as the MLN, spleen, liver, kidneys, and blood [19]. Overall, *S. boulardii* is considered to be a safe and well tolerated agent, but recently, numerous studies reported fungemia after *S. boulardii* treatment for risk groups [17,20-28]. Some studies emphasize the importance of blood penetration after *S. boulardii* usage in immunosuppressed patients resulting in *S. boulardii* associated fungemia [29,30]. For this reason, the therapeutic usage of probiotics should be carefully evaluated by taking into consideration its risks as well as its potential benefits.

In a study investigating the ability of orally administered viable *S. boulardii* in inhibiting translocation of *Candida albicans* from the gastrointestinal tract in antibiotic-decontaminated, specific pathogen-free mice, orally administered *S. boulardii* was shown to decrease the incidence of *Candida albicans* translocation to the MLN, liver and kidneys [19].

Peret Filho et al. [31] studied the translocation and histological alterations in the terminal ileum, liver and spleens of immunosuppressed mice under *S. boulardii* treatment. The results of this study showed that *S. boulardii* administration decreased the bacterial translocation to the liver and spleen in a dose dependant manner. Low *S. boulardii* translocation to MLN was observed in some animals. In our study, in Group B, translocation to MLN was significantly higher *(p=0.005)*. The translocation to spleen and liver was also high, but the difference was not statistically significant *(p>0.05)*. In evaluation of the blood cultures, two rats (20%) in Group B developed fungemia due to *Saccharomyces cerevisiae* representing the translocation of *S. boulardii*. Comparing our results with Peret et al. [31] our findings also support the relative protection with *S. boulardii* in immunosuppressed rats. However, reported cases in clinical trials and development of fungemia in two immunosuppressed rats showed the importance of probable fungemia during treatment.

Shigellosis is currently an important public health problem. Shigella bacteriemia is rare but associated with a high mortality rate. Immunosuppressed patients, malnourished children, and elderly people are at risk of serious complications and bacteriemia [2,32]. In experiments on white mice, *S. sonnei* strains were shown to be capable of penetrating into the blood for a short period of time [33]. Over the past decades, Shigella strains have progressively become resistant to most of the widely used antimicrobials [2]. For this reason, alternative therapeutic approaches are under investigation. In our study, we investigated the therapeutic effects of *S. boulardii* in *S. sonnei* infection, which is the predominant cause of Shigellosis in our country and the translocation rate of *S. sonnei* and *S. boulardii* in immunosuppressed hosts. The results of our study showed that in some rats in the D group *S. sonnei* cause bacteremia and caused translocation in the MLN, liver and spleen. These results showed the importance of bacterial translocation in immunosuppressed rats. In group C, in which the immunosuppressed rats were infected with *S. sonnei* and treated with *S. boulardii*, no translocation and no growth in blood cultures was observed.

Conclusion

Both *S. boulardii* and *S. sonnei* caused translocation resulting in fungemia/ bacteriemia in immunosuppressed rats when they were applied individually; but *S. boulardii* administration to *S. sonnei* infected rats seemed to result in the inhibition of translocation and bacteriemia. *S. boulardii* administration is found to be effective in the treatment of *S. sonnei* infections in immunosuppressed rats under in vivo conditions, but potential risk of developing fungemia in risk groups must always be considered during the therapeutic use of *S. boulardii.*

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