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The effects of *Saccharomyces boulardii* on bacterial translocation in rats with obstructive jaundice

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ABSTRACT

INTRODUCTION The aim of this study was to investigate the effect of *Saccharomyces boulardii* treatment on preventing bacterial translocation in an obstructive jaundice animal model.

MATERIALS AND METHODS Sixty adult rats were divided into five groups: group 1 – the sham-operated group; group 2 – the common bile duct ligation group; group 3 – the *S. boulardii* group; group 4 – the ampicillin-sulbaktam group; and group 5 – the *S. boulardii* plus ampicillin-sulbaktam group. The saline, antibiotics and *S. boulardii* were given, respectively, for a 7-day period as a single dose per day via temporary orogastric intubation. Seven days following the obstructive jaundice, the animal had laparatomy under sterile conditions. Segments of ileum were removed for histopathological examination. Blood, liver, spleen and mesenteric lymph nodes were taken for microbiological culture.

RESULTS Bacterial translocation rates were 0% in the sham-operated group, 83% in group 2, 42% in group 3, 42% in group 4 and 33% in group 5. Bacterial translocation significantly increased in group 2 compared to groups 3, 4 and 5 ($P = 0.001$). The bacterial counts (CFU/g) of group 2 were significantly higher than those of groups 3, 4 and 5 ($P = 0.001$). Histopathological examination of ileum specimens revealed a significant decrease in the heights of villi in groups 2–5 compared to the sham-operated group ($P = 0.001$). The mean villus height in groups 3 and 5 was significantly higher than that of group 4 ($P = 0.001$).

CONCLUSIONS *S. boulardii* was found to be effective in the successful control of translocation and improvement of intestinal barrier function.

KEYWORDS

Saccharomyces boulardii - Bile – Obstructive – Jaundice – Translocation

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Bacterial translocation is defined as the passage of viable indigenous bacteria from the gastrointestinal tract to extra-intestinal sites, such as the mesenteric-lymph-node complex, liver, spleen and bloodstream. Three major mechanisms promote bacterial translocation: intestinal bacterial overgrowth, deficiencies in host immune defences and increased permeability or damage to the intestinal mucosal barrier.[1] Most transmigrating organisms are subsequently subject to phagocytosis by macrophages, but some are found to be free in blood and lymph vessels.[2] Increased intestinal permeability and bacterial translocation were demonstrated following both experimental biliary obstruction and in jaundiced patients.[3,4]

Saccharomyces boulardii is non-pathogenic yeast that is used for the prevention and treatment of antibiotic-associated diarrhoea, acute and chronic enterocolopathies, trophic intestinal effects and for the treatment of pseudomembranous colitis.[5–7] Clinical trials and experimental studies have demonstrated that oral treatment with a lyophilised preparation of *S. boulardii* has beneficial effects in preventing the occurrence of complications linked to changes in the normal gut flora and significantly increased the intestinal secretion of secretory component of immunoglobulins and secretory IgA concentration in the gut.[8–12]

According to our review of the literature, this is the first *in vivo* study of the relationship between *S. boulardii* and the development of bacterial translocation following obstructive jaundice in rats. The aim of this study was to investigate the effect of *S. boulardii* treatment on preventing bacterial translocation in an obstructive jaundice animal model.

Materials and Methods

Animals

This study was approved by the Animal Ethics Committee of the Dicle University Hospital and performed following standard guidelines for the care and use of laboratory animals. A total of 60 adult Sprague-Dawley male rats (250–300 g weight) were housed under constant temperature (22°C) and humidity, with 12-h dark/light cycles and allowed tap water and rat pellets *ad libitum* before and after the operation.

Surgery

Rats were randomised into 5 groups containing 12 rats each. All rats were initially anaesthetised by intramuscular injection of ketamine (25 mg/kg) and xylasine (5 mg/kg). All procedures were performed under sterile conditions. After midline abdominal incision, the common bile duct was identified and mobilised. It was then doubly ligated using 5-0 silk and divided. Sham-operated group rats had a similar incision followed by mobilisation of the common bile duct, without ligation or division. At the end of the experiment, rats were killed by an overdose of intravenous pentobarbital.

Treatment

The rats were divided in to five groups: group 1 – the sham-operated group; group 2 – the common bile duct ligation group; group 3 – the *S. boulardii* group (Reflor sase®, Sanofi, Istanbul, Turkey; 100 mg/day); group 4 – the ampicillin-sulbaktam group (Alfasid suspension®, Fako AS, Istanbul, Turkey; 50 mg/kg/day); group 5 – the *S. boulardii* plus ampicillin-sulbaktam group (Reflor sase®; 100 mg/day plus Alfasid suspension®; 50 mg/kg/day). The saline, antibiotics and *S. boulardii* were given respectively for a 7-day period as a single dose per day via temporary orogastric intubation. There was no death in any group after 7 days. Seven days following the obstructive jaundice, the animal had laparatomy under sterile conditions. Samples of systemic blood, liver, spleen and mesenteric lymph nodes (MLNs) were taken for microbiological culture under sterile conditions. Segments of ileum were removed for histopathological examination. Liver, spleen and MLNs were taken for microbiological culture.

Collection of tissues and bacterial translocation

Blood samples were obtained from the portal vein and cultured aerobically and anaerobically using the BacTec™ Peds battles (Becton-Dickinson Diagnostic Inc., Sparks, MD, USA). Blood cultures were continuously monitored for 7 days. Positive cultures were plated out on blood agar, chocolate agar, eosin methylene blue (EMB) agar or Sabouraud-dextrose agar. Identification was performed by the Sceptor microdilution method. At the same time, MLNs, spleen and the right lobe of the liver were removed and placed in sterile glass bottles containing sterile brain-heart infusion media. The bottles were re-weighed and tissue homogenates were prepared in 2 ml brain-heart infusion using a sterile mortar and pestle. A portion (0.1 ml) of each of homogenates was cultured on blood agar, chocolate agar, EMB agar and Sabouraud-dextrose agar . All the plates were examined after 24 h and 48 h of incubation at 37°C. Individual colonies were identified and quantified as colony-forming units (CFUs) per gram tissue. The results of all CFUs were averaged and expressed as mean Log_{10}.

Assessment of ileal villus heights

Following retrieval of the solid organs at laparotomy, a 2-cm segment of terminal ileum was removed. The bowel was stripped from its mesentery, each of the segments was opened along its length, and rinsed in a cold solution. The specimens of terminal ileum were fixed in 10% formalin in 0.15 M phosphate buffer (pH 7.2), embedded in paraffin and then 5-μm sections were cut. The specimens were stained with hematoxylin and eosin and examined under the light microscope (Olympus BH-2, Tokyo, Japan). An independent pathologist who had no knowledge of the experimental groups from which the specimens were derived performed histological evaluation. Morphometric analysis was fulfilled using an eyepiece micrometer (Olympus). For each rat, 10 randomly chosen mucosal regions were traced and villus height (μm) was measured and the mean was calculated.

Statistical analysis

Statistical analyses were made using analysis of variance (one-way ANOVA) with Tukey HSD honestly significant test for *post hoc* multiple comparisons on an IBM-compatible personal computer using SPSS v10.0 software. A P value of < 0.05 was considered to be statistically significant.

Results

The predominant bacteria obtained from blood, liver, spleen and MLNs samples were *Escherichia coli* (29%) *Klebsiella* spp. (21%), *Enterobacter cloaca* (13%), *Proteus mirabilis* (6%), *Enterococcus faecalis* (3%) and mixed cultures (28%). The effects of the treatments on bacterial translocation are presented in Figure 1. Bacterial translocation rates were 0% in the sham-operated group, 83% in group 2, 42% in group 3, 42% in group 4 and 33% in group 5. Bacterial translocation significantly increased in group 2 compared to groups 3, 4 and 5 ($P = 0.001$). There was no significant difference among groups 3, 4 and 5 for bacterial translocation rates ($P > 0.05$). The bacterial counts (CFU/g) of group 2 were significantly higher than those of groups 3, 4 and 5 ($P = 0.001$; Table 1).

Table 1 **Log colony forming unit (CFU) ratios in the second group and treated groups**

	Group 2 Log_{CFU}	Group 3 Log_{CFU}	Group 4 Log_{CFU}	Group 5 Log_{CFU}
1	5.9	5.3	5.1	3.7
2	5.8	3.8	4.7	3.8
3	6.1	3.3	2.6	3.0
4	4.3	5.0	2.9	3.2
5	5.0	4.6	3.5	0
6	5.5	0	0	0
7	5.8	0	0	0
8	5.6	0	0	0
9	5.7	0	0	0
10	5.8	0	0	0
11	0	0	0	0
12	0	0	0	0
Mean (± SD)	4.6 (± 2.2)	1.8 (± 2.3)	1.6 (± 2.1)	1.1 (± 1.7)

Serum bilirubin levels, alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) activities were significantly higher in groups 2, 3, 4 and 5 compared to group 1 ($P < 0.001$); however, there was no significant difference among groups 2, 3, 4 and 5 ($P > 0.05$; Table 2). The mean ileal villus heights in the sham-operated group, and groups 2, 3, 4 and 5 were 484.5 ± 32.1 μm, 328.1 ± 22.7 μm, 442.8 ± 29.7 μm, 344.2 ± 34.5 μm and 462.7 ± 29.6 μm, respectively. Histopathological examination of ileum specimens revealed a significant decrease in the heights of villi in groups 2–5 compared to the sham-operated group ($P = 0.001$). The mean villus height in groups 3 and 5 was significantly higher than that of group 4 ($P = 0.001$). However, there was no statistically significant difference between group 3 and group 5 for mean villus height ($P > 0.05$).

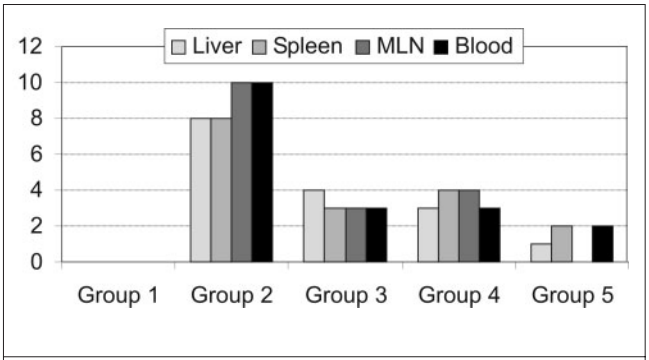

Figure 1 The number of rats with bacterial translocation for each group.

Discussion

Infectious complications such as biliary sepsis, wound infections, intra-abdominal abscess formation, and renal failure frequently occur during obstructive jaundice.[2,3,13,14] Bacterial translocation from the intestinal mucosal barrier implicated in the pathophysiology of complications has been associated with obstructive jaundice.[3,4] Several factors have been proposed as promoters of bacterial translocation. These include alterations in gastrointestinal microbiota, impairment of gut barrier function, and deficiencies in the host immunity.[15,16] Experimental obstructive jaundice induces regional loss of occluding expression in the intestinal epithelium, which may be a key factor contributing to the disruption of the mucosal barrier.[17] Treatment with growth hormone and insulin-like growth factor I in rats with experimental obstructive jaundice has been shown to reduce endotoxin and to improve liver histopathology.[13] Intestinal absorption of endotoxin is increased in patients with obstructive jaundice that causes endotoxaemia in the portal blood and depression of reticulo-endothelial functions.[18]

Table 2 **The average of serum bilirubin, ALT, AST and ALP values of the groups**

	Bilirubin (mg/dl)	ALT (IU/l)	AST (IU/l)	ALP (IU/l)
Group 1	0.4 ± 0.3*	69.7 ± 14.9*	65.9 ± 12.2*	99.6 ± 13.4*
Group 2	8.8 ± 0.9	360 ± 15.6	402.4 ± 48.4	384.3 ± 20.1
Group 3	8.7 ± 0.8	358.8 ± 15.1	421.9 ± 17.5	379.9 ± 21.2
Group 4	8.5 ± 0.6	353.5 ± 16.4	407.4 ± 27.2	378.0 ± 22.8
Group 5	8.9 ± 0.7	371.5 ± 16.3	414.1 ± 32.6	384.5 ± 20.5

*$P = 0.000$ group 1 compared with groups 2–5.

Many authors have studied the effects of different drugs on preventing bacterial translocation in animal models of obstructive jaundice. Berg *et al.*[19] demonstrated that a combination of antibiotics and immunosuppressive drugs promotes the systemic spread of bacterial translocation, resulting in lethal sepsis. According to Reid *et al.*,[20] bacterial colonisation or infection of the intestine by bacteria such as *Escherichia, Clostridium*, *Klebsiella*, *Salmonella*, *Shigella*, *Campylobacter*, *Pseudomonas*, *Streptococcus*, *Enterococcus*, *Staphylococcus aureus*, and coagulase-negative staphylococci increases the risk of necrotising enterocolitis. Bile salts are known to inhibit the growth of intestinal bacteria and may contribute to the regulation of the indigenous gut microbiota. Absence of intraluminal bile salts and their anti-endotoxic effects may result in overgrowth of bacteria.[2,3] In the present study, we also showed significant increase in bacterial translocation in the liver, spleen, MLNs and blood as well as significant reduction in mean villus height in jaundiced rats. Bacterial translocation after obstructive jaundice may be due to the inhibition of bile salts, reduction of villus height or disruption of the ecological balance of the normal indigenous microbiota.

S. boulardii is a yeast presently used as a lyophilised powder in the prevention and treatment of diarrhoea associated with antibiotic use.[21] *S. boulardii* has been used in the treatment of intestinal disorders in recent studies.[22–25] Berg *et al.*[19] reported that bacterial translocation is caused by oral antibiotics due to the disruption of the gastrointestinal ecological equilibrium, leading to intestinal overgrowth. However, they also reported that *S. boulardii* is widely used as a probiotic which decreases the incidence of *Candida albicans* translocation to the MLNs, liver, and kidneys. The mucosal damage in the gut may increase the invasion of bacteria through the disrupted mucosal barrier; it is possible that the suppression of bacterial translocation is simply caused by a decrease in the severity of intestinal lesions induced by *S. boulardii*.

Kakkos *et al.*[26] reported that administration of non-absorbable antibiotics had a positive effect on bacterial and endotoxin translocation after extended hepatectomy, and related this to reduction of colonic bacterial load as an intraluminal effect of antibiotics. Our study has demonstrated that ampicillin-sulbaktam administration reduces the level of bacterial translocation. In addition, *S. boulardii* also has shown a significant suppression against the increase of bacterial translocation as well as ampicillin-sulbaktam and additionally *S. boulardii* preserved intestinal mucosal integrity. It should be noted from the present study that both *S. boulardii* and ampicillin-sulbaktam exhibited a significant suppression of bacterial translocation.

Morphometric evidence of ileal mucosal injury with reduction in villus height and total thickness in jaundiced rats have also been reported.[10,27] The effect of *S. boulardii* on intestinal trophic architecture in pigs was indicated by increased villus length in the small intestine.[28] In this study, mean villus height in groups 1, 3 and 5 was higher than that of group 2. Obstructive jaundice may contribute to the breakdown of gastrointestinal barrier functions, thus promoting bacterial translocation. Additionally, bacterial overgrowth can promote bacterial translocation. This study showed that *S. boulardii* preserved mucosal integrity. On the other hand, the effect of *S. boulardii* may inhibit overgrowth of pathogenic organisms.

Conclusions

The administration of *S. boulardii* to rats suffering from obstructive jaundice is effective in the successful control of translocation and improvement of intestinal barrier function. *S. boulardii* is a non-toxic preparation and has been found to be experimentally effective in decreasing bacterial translocation. Further studies are needed for use in humans.

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