Effectiveness of Bombesin and *Saccharomyces boulardii* Against the Translocation of *Candida albicans* in the Digestive Tract in Immunosuppressed Rats

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**Abstract**

**Purpose.** We investigated the effects of bombesin on disseminated candidiasis, and compared the effectiveness of bombesin with *Saccharomyces boulardii* against *Candida albicans* translocation from the gastrointestinal tract in immunosuppressed rats.

**Methods.** Sixty rats were divided into five groups of 12. Group 1 was given only a laboratory pellet diet and water during the experiments; the other four groups were orally inoculated with *C. albicans*; and groups 3, 4, and 5 were also given prednisolone intraperitoneally. The treatment groups consisted of group 4, given *S. boulardii* orally, and group 5, given bombesin subcutaneously. The rats were killed after 10 days, and the large bowel, liver, spleen, and kidneys were removed for microbiological and histopathological examination. Blood samples were taken to measure tumor necrosis factor-α (TNF-α) and interleukin-1 β (IL-1β) levels, and the results were compared.

**Results.** The number of translocated *C. albicans* colonies from the gastrointestinal tract and the serum TNF-α and IL-1β levels were significantly lower in groups 4 and 5 than in group 3 (*P* < 0.05). Histological analysis revealed that the bombesin-treated group (group 5) had significantly less mucosal ulceration and submucosal inflammation in the large bowel, less inflammation and necrosis in the liver, and less inflammation of the Bowman capsules in the kidney than the *S. boulardii*-treated group (group 4) (*P* < 0.05).

**Conclusions.** These findings show that both *S. boulardii* and bombesin inhibit the translocation of *C. albicans* from the gastrointestinal tract, although mucosal ulceration, submucosal inflammation in the large bowel, and dissemination in the liver and kidneys were significantly less severe in the bombesin-treated immunosuppressed rats.

**Key words** Systemic candidiasis · *Candida albicans* · *Saccharomyces boulardii* · Bombesin

**Introduction**

The opportunistic fungal pathogen *Candida albicans* can cause life-threatening infections in cancer patients, organ or bone-marrow transplant recipients, and patients with congenital or acquired immunodeficiencies. Indwelling central venous catheters and broad-spectrum antibiotics can also predispose patients to this infection.¹

The host immune defense system, together with an ecologically balanced gastrointestinal microflora and the physical barrier of an intact intestinal mucosa, comprise the defense mechanisms that inhibit *C. albicans* translocation from the gastrointestinal tract.² The intestinal mucosa is covered with a mucus gel layer, which acts as lubrication and a diffusion barrier for small molecules and protects against colonization by pathogenic microorganisms and their toxins, and against luminal protease arising from microorganisms and mucosal cells. The major structural components of mucus are mucins: high-molecular-weight glycoproteins produced by the goblet cells of the surface epithelium.³ It is speculated that *Candida* proteinases may act as a virulence factor by allowing fungal access and adherence to epithelial cells, invading the host tissues, and interfering with the host defense mechanism.⁴ T-helper cells play a central role in regulating immune responses to the fungus *C. albicans* by secreting cytokines that modulate the development and activity of immune effectors.⁵ Tumor necrosis factor (TNF)-α is highly sensitive to the infectious agent *C. albicans*,⁶ and interleukin (IL)-1β is released into the gastrointestinal mucosa by lamina propria-activated immune cells during inflammation.⁷ Moreover, Cohan et al.⁸ reported that IL-1β induced...
mucin release from explanted cultures of mouse duodenum. 

*Saccharomyces boulardii* is a thermophilic, nonpathogenic yeast, given orally as a lyophilized preparation to treat acute infectious gastroenteritis and antibiotic-associated diarrhea. In immunosuppressed mice, oral *S. boulardii* has been found to inhibit *C. albicans* translocation to the mesenteric lymph nodes, liver, and kidneys.

Bombesin is a tetrapeptide isolated from the skin of a European frog, which is chemically and biologically homologous to gastrin-releasing peptide. Bombesin plays a critical role in the integration of exocrine and endocrine secretory and smooth-muscle contractile functions in the gastrointestinal tract. Bombesin and gastrin-releasing peptide are exclusively found in nerve tissue and in the gastrointestinal tracts of various mammals, where they mediate diverse secretory motor functions and release peptides such as neuropeptide Y, motilin, insulin, cholecystokinin, secretin, and glucagon. Plaisancié et al. showed that bombesin provoked a dramatic mucin discharge in the rat colon. Therefore, we investigated the effects of bombesin on the translocation of *C. albicans* in the digestive tract and compared these effects with those of *S. boulardii* in immunosuppressed rats.

**Materials and Methods**

These experiments were done with the approval of the Osmangazi University ethics committee at Osmangazi University Surgical Research Center. Sixty male Wistar albino rats weighing 270 ± 30 g were divided into five groups of 12 animals each. Group 1 was given only a laboratory pellet diet and water during the experiments. All rats were housed individually in stainless steel cages in an animal room at 20°C with a 12-h light–dark cycle. All of the rats were fed a laboratory pellet diet and allowed free access to water during the experiments. Rats were antibiotic-decontaminated for 4 days by adding 2 mg/ml of streptomycin sulfate (Sigma, St. Louis, MO, USA) and 1500 units/ml penicillin-G (Sigma) to their water, which was prepared daily. Reduction in the gastrointestinal microflora was confirmed by microscopic examination of Gram-stained smears of fecal pellets and aerobic culturing of feces for Gram-negative enteric bacteria. The *C. albicans* strain (laboratory number, 4821/2001), isolated from the blood of a patient with candidiasis, was donated by Professor N. Kiraz of the University of Osmangazi. It was cultured overnight at 37°C with stirring in brain–heart infusion broth (BHI), pH 5.7, containing 2 mg/ml streptomycin and 1500 units/ml penicillin. The culture was prepared daily and given to groups 2, 3, 4, and 5 at a dose of ~10⁹ cells/ml in BHI in their drinking water on days 5, 6, and 7. The animals in groups 3, 4, and 5 were also given prednisolone (Sigma) intraperitoneally, at a dose of 100 mg/kg on days 0, 2, 4, 6, 8.

Lyophilized *S. boulardii* (Ultra-Levure; BIOCODEX Laboratories, Montrouge, France) was suspended in a 5% concentration [5 × 10⁸ colony forming unit (cfu)/ml] in the BHI containing the overnight culture of *C. albicans* (10⁵ *C. albicans* cells/ml). The culture was prepared daily and given orally to the rats in group 4 on days 3, 4, 5, 6, and 7.

Lyophilized bombesin 1 mg (Sigma B-4272) was suspended in 0.05 M acetic acid stored at −20°C. Bombesin was given to group 5 at a dose of 15 μg/kg per 8-h period by subcutaneous injection, on days 3, 4, 5, 6, and 7. The rats were killed by nitrogen gas inhalation on day 10.

To measure the serum cytokine levels, blood samples (4 ml) were collected by cardiac puncture on day 10. The serum TNF-α and IL-1β levels were measured in duplicate with a commercially available rat-specific enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer’s instructions (Endogen, Woburn, MA, USA). The lowest limit of sensitivity for TNF-α and IL-1β in this assay was 3 pmol/ml.

After the rats were killed, the large bowel, liver, spleen, and kidneys were removed for microbiological examination. The liver and spleen were divided into equal parts and sections of the liver, spleen, and right kidney were used to assess *C. albicans* quantitatively. Tissue pieces were minced with a scalpel, diluted tenfold wet weight, in 0.9% NaCl, and homogenized with a handled tissue tearer (Ultra-Turrax T25, BioSpec Products, Bartlesville, OK, USA). Dilutions of the organ homogenates were plated on Sabouraud dextrose agar (SDA), and incubated for 7 days at 35°C; then, the number of cfu/g wet weight was determined.

The *C. albicans* count per gram of tissue was calculated by the following formula:

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\text{cfu count per gram of tissue} = \frac{\text{cfu count} \times \text{dilution coefficient} \times 10^{-2}}{\text{tissue weight}}
\]

For histological examination, samples were taken from the large bowel, left kidney, another part of the liver, and the spleen, fixed in 10% neutral buffered formalin, and then sectioned into 4- to 5-μm-thick sections and stained with periodic acid Schiff (PAS), and hematoxyline. The sections were examined by a histologist blinded to the protocol for light microscope studies, who evaluated and graded the following: mucosal ulceration, inflammation of the lamina propria and submu-