Modulatory Effect of the Lectin-Binding Component, Added to Gel, on Adhesion of Yeast-Like *Candida* Fungi *In Vivo*

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The lectin-binding component added to gel was studied *in vivo* as a potential therapeutic and preventive agent in experimental vaginal candidiasis. Laboratory studies demonstrated its antimycotic effect in relapsing vaginal candidiasis in mice.

Key Words: *lactobacilli; lectin-binding component; vaginal candidiasis; Candida albicans; Hispagel*

Studies of surface lactobacillus structures carried out in 2006-2008 showed that some lactobacillus species released a glycoprotein substances selectively reacting with concanavalin A, a natural lectin, into the culture fluid [1,2]. *In vitro* experiments on vaginal epitheliocytes of clinically healthy women confirmed the hypothesis according to which the lectin-binding component (LBC) of the lactobacillus cell wall shielded the receptor system of the eukaryotic cell and prevented or reduced significantly adhesion of pathogenic and opportunistic flora to target cells [3,4].

We continued the studies of the modulatory effect of *L. fermentum* strain 90 TS-4 (21) LBC *in vivo*.

MATERIALS AND METHODS

Candidal vaginitis was induced in mice by the method developed at Department of Microbiology and Virology of Medical Faculty of Peoples' Friendship University of Russia [6].

The following microorganism strains were used. Yeast-like *Candida albicans* fungi were isolated from albino mouse vagina when testing animal's vagina purity at the beginning of the study and identified as *C. albicans* in Nikerson's chromogenic medium (HiMedia). The fungi were cultured in Sabouraud's commercial liquid and solid media (HiMedia). The culture was injected into the vagina in a concentration of 5 U.

Lactobacillus fermentum strain 90 TS-4 (21) was obtained from the collection of L. A. Tarasevich Institute of Standardization and Control. The bacteria were cultured in commercial liquid and solid MRS (HiMedia). The culture was injected into the vagina in a concentration of 5 U.

The lectin-binding component was obtained by culturing *L. fermentum* strain 90 TS-4 (21) and purified from low-molecular components by ultrafiltration on AMICON cells (200 ml) using Diaflo PM-20 membranes and Millipore sterilizing membranes with 0.02 μ pores [2]. Protein concentration in culture fluid was measured by the method of Lowry [5]. The reactivity of culture fluid components with concanavalin A was evaluated in annular precipitation test [2].

The LBC isolated from culture fluid was introduced in Hispagel in a concentration of 6 mg/ml and pH 6.0-6.5. The LBC was dissolved in calculated volume of pure water and mixed with Hispagel base, after which the system was mixed on an ECPOS-8100

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turbomixer at 50-80 rpm until homogenous gel was obtained. The resultant gel was left until completion of the structure formation for 5-6 h.

Each animal was "standardized" by the ovulation cycle with Messalin (the animals were subcutaneously injected with 200 µl drug solution, 200 µl hormone/ml saline, for 5 days [6]).

Vaginal contamination was evaluated by collecting lavage fluid from the vaginal mucosa every other day throughout the experiment. Saline (25 µl) was pipetted through an individual tip into the vagina. The collected material was inoculated in Sabouraud's solid nutrient medium (HiMedia), cultured for 24-48 h at 37°C, and CFU were then counted [6].

The study was carried out on 6 groups of animals, 10 per group. In group 1 the development of relapsing vaginal candidiasis (RVC) was prevented with LBC added to the gel. Group 2 mice served as controls for animals treated from RVC. In group 3 RVC was treated with LBC in gel in the same dose as in group 1. Group 4 mice served as controls for evaluating the effects of gel on RVC. Group 5 mice were treated with LBC (6 mg/ml) in gel and the native culture of *Lactobacillus fermentum* strain 90 TS-4 (21) in a dose of 5 U. In group 6 mice, the treatment was carried out during the acute period of vaginal candidiasis (AVC) with LBC (6 mg/ml) added to the gel.

The protocol of the experiment is shown in Table 1.

The data were statistically processed using MS Office Excel 2007 software.

RESULTS

Administration of 25 µl Hispagel with LBC on days 39 and 42 of the experiment led to reduction of *C. albicans* contamination of the vaginal mucosa as soon as on day 48, while complete elimination of the fungus was recorded on day 56 (Fig. 1).

In group 4 mice receiving intravaginally Hispagel without LBC, the contamination of the mucosa with *C. albicans* decreased only on day 56, while complete elimination was recorded on day 62 (Fig. 2).

In order to prevent RVC, group 1 mice were injected with 25 µl gel with LBC on days 35-38. The LBC introduced in the gel did not lead to statistically significant prevention of the infection, but reduced vaginal mucosa contamination with *C. albicans* on day 48 (Fig. 3). The results obtained in group 1 were lower in comparison with the results in group 3 treated with the gel with LBC.

Combined injection with LBC in gel and native culture of *L. fermentum* strain 90 TS-4 (21) in group 5 led to a sharp increase in vaginal mucosa colonization by *C. albicans* on day 42 of the experiment. On day 48, the level of *C. albicans* CFU per ml decreased almost 20-fold, reaching the level of *C. albicans* CFU per ml of lavage fluid from the vaginal mucosa in group 3.

This result seemed to confirm that the use of probiotic preparations based on lactobacillus cultures during exacerbation of candidal infection can aggravate the course of vaginal candidiasis and enhance mucosal

TABLE 1. Experiment Protocol

Day of the experiment	Manipulations	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
1-5	"Standardization" of animals by ovulation cycle [6]	+	+	+	+	+	+
1-5	Dysbacteriosis simulation [6]	+	+	+	+	+	+
8	Simulation of AVC [6]	+	+	+	+	+	+
8-32	Spontaneous cleansing of vaginal mucosa [6]	+	+	+	+	+	+
35-38	RVC simulation [6]	+	+	+	+	+	+
35-38	Prevention of RVC development by vaginal injection of gel with LBC (6 mg/ml)	+					
39/42	Vaginal injection of gel with LBC (6 mg/ml)			+			
39/42	Vaginal injection of gel without LBC				+		
39/42	Vaginal injection of gel with LBC (6 mg/ml) and native lactobacillus culture					+	
39/42	Vaginal injection of gel with LBC (6 mg/ml) for AVC treatment						+
45-68	Registration of vaginal mucosa cleansing	+	+	+	+	+	+

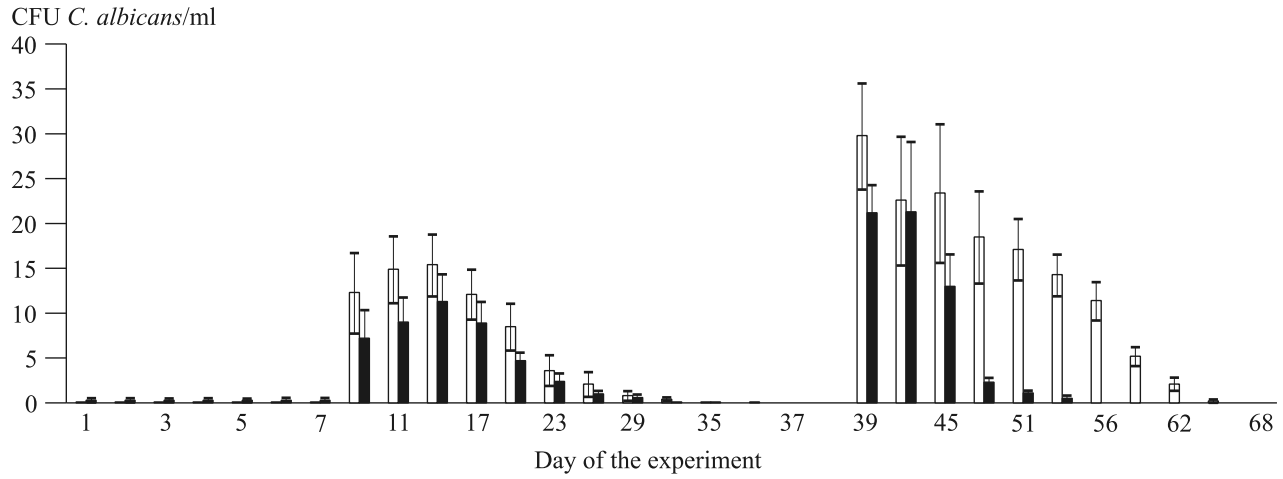

Fig. 1. Therapeutic effect of LBC (6 mg/ml) introduced in Hispagel. Open bars: control; dark bars: gel treatment.

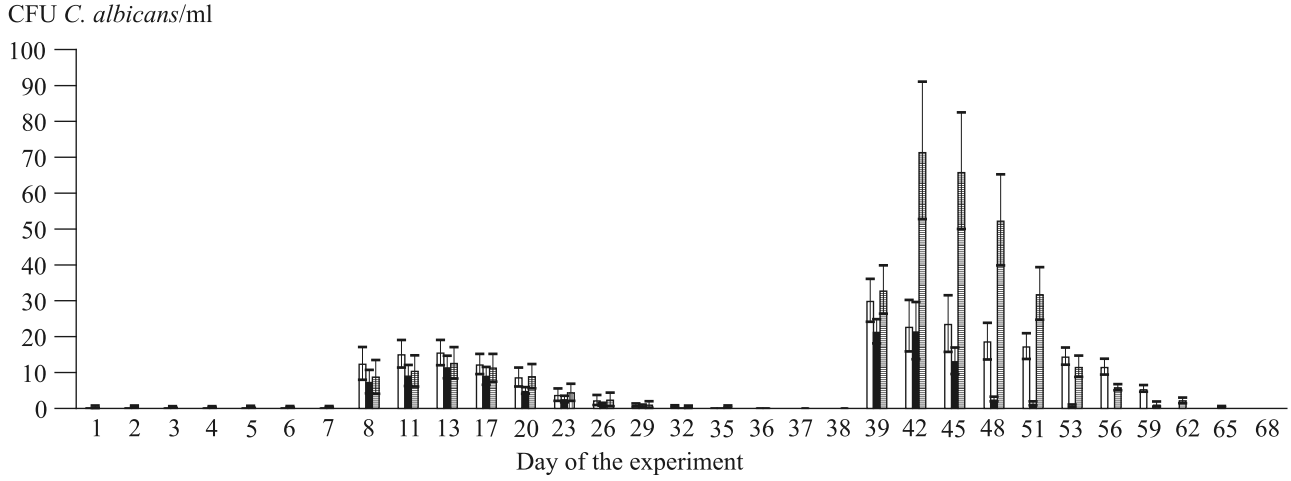

Fig. 2. Therapeutic effect of Hispagel with LBC (6 mg/ml) and Hispagel *per se*. Open bars: control; dark bars: Hispagel+LBC; cross-hatched bars: Hispagel without LBC.

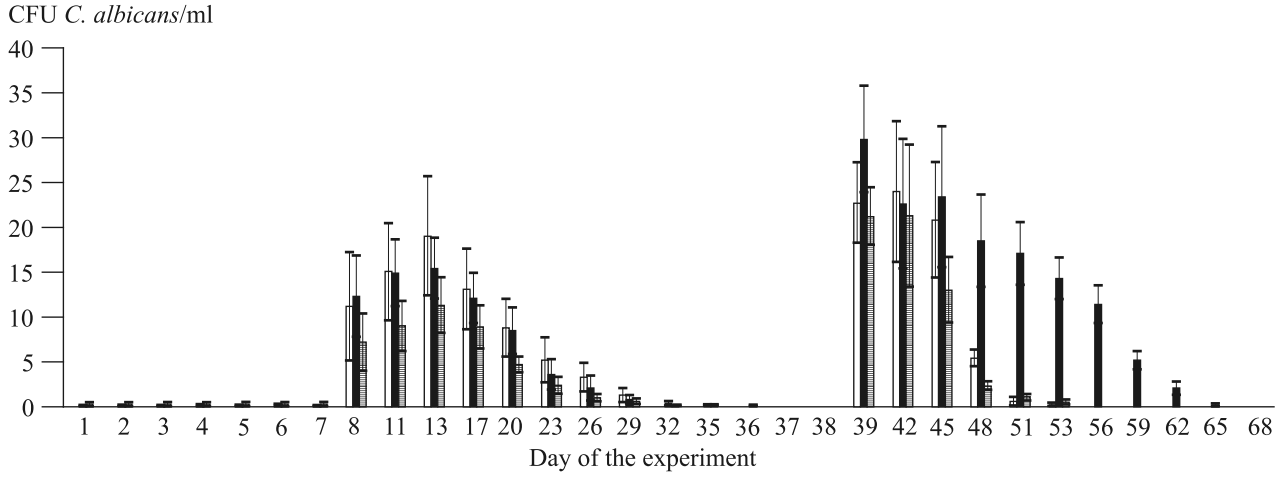

Fig. 3. Preventive effect of LBC (6 mg/ml) in Hispagel on the development of RVC. Light bars: relapse prevention; dark bars: control; cross-hatched bars: Hispagel+LBC.

contamination in the terminal ecological niche. However, therapy with LBC in a concentration of 6 mg/ml reduced the effect developing after inoculation with native culture of *L. fermentum* strain 90 TS-4 (21).

The data indicate that the use of native lactobacillus cultures is not effective in the treatment of AVC and RVC because of aggravation of the infectious process (increase of mucosal contamination with yeast-like *C. albicans*). Therapy for RCV with a combined preparation including lactobacillus culture and the lactobacillus vital activity products (for example, LBC) can reduce the contamination of the vaginal mucosa with yeast-like *C. albicans* fungi due to shielding of the eukaryotic cell receptor system with LBC and in parallel with this enrich the niche with transitory lactobacillus cultures and lead to subsequent accumulation of the host representatives of normal microflora.

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