IMMUNOLOGY AND MICRIBIOLOGY

Study of Antimycotic Activity of Lyticase

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Laboratory studies of lyticase (enzymatic drug) as an antimycotic agent were carried out. The enzyme reduced optical density of *Candida albicans* test culture, inhibited adhesion of yeast-like fungi on vaginal epitheliocytes, stimulated the formation of germinative tubes, and made *Candida albicans* more available for phagocytosis.

Key Words: *candidiasis; Candida albicans; lyticase; adhesion; phagocytosis*

According to WHO data, every fifth human suffers from diseases caused by yeast-like fungi (YLF). Resistance to fluconazole and amphotericin B in a part of YLF population makes complete eradication of the agent virtually impossible.

The opportunistic nature of candidal infection necessitates the search for new drugs aimed at elimination of the agent from microecological niches by preventive, etiotropic, and antirelapse therapy.

For this reason, the effect of lyticase enzyme on YLF cells attracts special interest. This enzyme is produced by *Micrococcus luteus* and is used as a reagent destroying YLF cell wall [3]. The cell treated with lyticase is transformed into a spheroplast analog sensitive to fluctuations in osmotic pressure and available for gene engineering manipulations. Due to this, some companies manufacture lyticase produced by not only its natural producers, but also recombinant *E. coli* strains [6].

Here we studied the effect of lyticase on clinical strain of *Candida albicans*, its viability, activity of adhesion on the vaginal epithelium (VE), morphogenesis, and its phagocytosis by macrophages.

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MATERIALS AND METHODS

Candida albicans clinical isolate was obtained from a pregnant woman with symptoms of candidal vaginitis.

Yeast-like fungi were identified by culturing in Nickerson's chromogenic medium (HiMedia) and subsequent testing in the API RAT system. The 24-h YLF culture was treated with lyticase (Difco) with the initial activity of 20,000 U/g dry weight of lyophilized sample. Working solutions with lyticase concentrations of 2 and 10 U/ml were prepared.

The lythic effect of the enzyme was evaluated by reduction of optical density of YLF suspension after 1-h treatment [3,5]. The effect was expressed in percent of optical density of intact YLF suspension. The viability of YLF after fermentation was evaluated by inoculation in Saburo solid medium by counting the CFU in comparison with the control (*Candida albicans* CFU before lyticase treatment).

Changes in the capacity of lyticase-treated *Candida albicans* to adhere on VE from clinically healthy women (who gave written informed consent to participation in the study) was evaluated by two parameters: percentage of VE cells to which YLF adhered in experiment and control (YLF without lyticase treatment) and by adhesion index (number of YLF on one VE cell) [3].

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The effect of lyticase on morphogenesis of *Candida albicans* was studied by induction of tube formation as follows. Lyticase-treated (experiment) and untreated (control) suspension of YLF washed from culture medium was transferred into medium 199. After 30- and 90-min incubation at constant shaking, the percent of YLF with germinative tubes was counted in the smears. In order to detect the effect of lyticase on macrophage capacity to capture YLF, a 24-h culture of these fungi was washed, resuspended in buffered saline (pH 7.2), and diluted to a concentration of 10^6 cell/ml. Lyticase (1 ml) in a concentration of 2 U/ml was added to 1 ml suspension (experiment) and saline was added to 1 ml YLF suspension in the other tube (control). Macrophages were isolated from ascitic fluid obtained after preinjection of 3 ml sterile meat-peptone broth into the abdominal cavity to outbred mice [2]. The cells were precipitated by 10-min centrifugation at 1000 rpm. The supernatant was discarded and macrophages in the precipitate were twice resuspended in medium 199. Finally, 0.3 ml YLF suspension and 0.15 ml bovine serum (Venture for Bacterial Preparations Manufacture, Ekaterinburg) were added to 0.2 ml macrophage suspension. The components were mixed and the resultant mixture was incubated for 30 min at 37°C. After incubation, 5 ml isotonic NaCl was added to the tube, the mixture was shaken and centrifuged (10 min, 1500 rpm); the supernatant was discarded and smears were prepared. The suspension was again incubated at 37°C for 90 min more (summary incubation 120 min). After 120-min incubation, the preparations were made from the precipitate and samples were collected for inoculation. Viable *Candida albicans* were counted by the number of CFU before and after exposure of YLF culture with macrophages.

The smears were fixed and stained with methylene blue. At least 100 cells were analyzed, among which the phagocytic cells (phagocytic number) and total number of YLF adhered to the surface of a phagocyte (phagocytic index) were estimated.

The numerical data were analyzed biometrically using Student's *t* test. The data of some experiments were processed by regression analysis using Advanced Grapher 2.11 and Excel software.

RESULTS

One-hour treatment of YLF suspension with lyticase in a concentration of 10 U/ml led to reduction of optical density of the suspension to 53% of the control. The lythic effect depended on the enzyme dose used in the experiment (Fig. 1, *a*). The detected relationship can be described by the following equation:

$$Y(x)=-10.5497856\times\ln(x)+67.1981181,$$

where Y is the decrease in optical density of YLF suspension after treatment in comparison with the control and x is lyticase dose at standard deviation equal to 5.128.

The effect of YLF culture lysis was recorded also by the results of subsequent inoculation of the surviving viable cells (Fig. 1, *b*). The enzyme added to the system destroys the cell wall and causes lysis of some YLF. Inoculation in Saburo's medium showed that the decrease in the percentage of CFU conformed to a similar relationship and could be described by the following equation (Fig. 1, *b*):

$$Y(x)=-11.6411733\times\ln(x)+83.5368459,$$

where Y is the percentage of viable cells in comparison with the control and x is lyticase dose at standard deviation equal to 5.945.

It remained unclear how much lyticase could modify the characteristics of surviving YLF. In order to detect the possible effect of the enzyme on *Candida albicans* morphogenesis, YLF treated (2 U/ml) and not treated with lyticase were inoculated in medium 199. Some yeast forms were transformed into mycelium forms, the percentage of tube-forming cells being significantly higher among samples obtained after 30- and 90-min incubation with the enzyme. The percentage of tube-forming cells after 30-min incubation with lyticase was 3.7 times higher than among intact cells (results of 3 experiments). The same trend was observed after 90-min exposure.

Surface mannoprotein complex is involved in YLF adhesion to target cells, being the key factor of colonization. Presumably, destruction of this complex modifies adhesion characteristics of YLF. We evaluated adhesion activity of *Candida albicans* YLF isolated on day 14 of the menstrual cycle to VE before and after their treatment with the enzyme [4]. The mean YLF adhesion index without enzyme treatment was 4.2±0.4, which significantly surpassed that of enzyme-treated YLF (1.6±0.1). Significant decrease in adhesion index of *Candida albicans* culture to VE depended on lyticase dose (Fig. 2). The regression curve in this case is described by the following equation:

$$Y(x)=-0.3618888\times\ln(x)+3.6518707,$$

where Y is adhesion index (number of YLF per VE cell) and x is lyticase activity at standard deviation equal to 0.330.

These data indicate that pronounced decrease in adhesion characteristics of cultured *Candida albicans* is detected after its treatment with lyticase in a concentration of 0.15 U/ml and higher.

Lyticase treatment partially lyzed the culture and reduced viability of surviving YLF cells. The cell wall structure was impaired, which manifested in a decrease

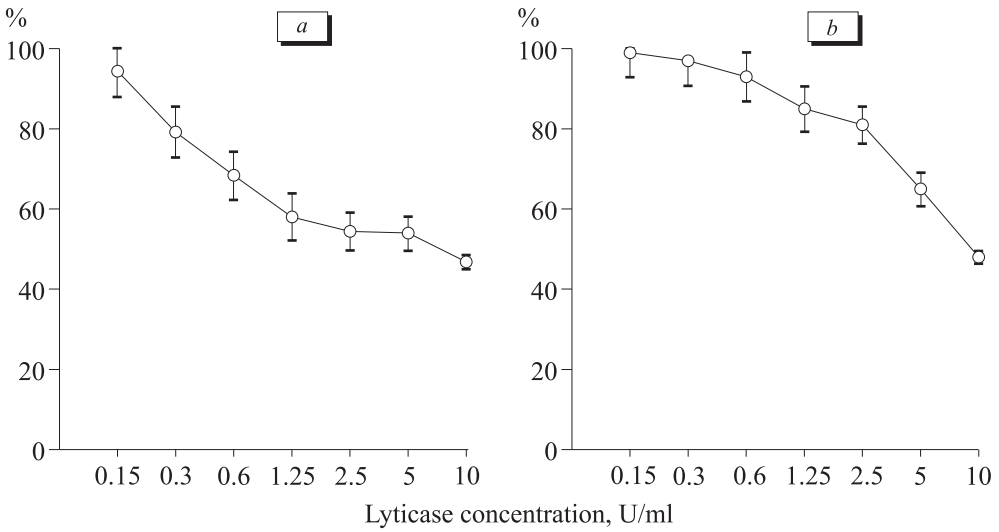

Fig. 1. Changes in YLF concentration in response to lyticase treatment of *Candida albicans* culture in different doses. *a*) changes in optical density of the culture; *b*) changes in the concentration of viable cells.

of YLF adhesive characteristics. These changes could modulate the development of the infectious process.

It is assumed that one of the main mechanisms inhibiting candidal infection is activity of macrophages, which, in turn, largely depends on characteristics of phagocytized object. It was therefore important to evaluate the capacity of peritoneal macrophages to phagocytize intact and lyticase-treated *Candida albicans*.

Evaluation of the phagocytosis index after 30-min exposure showed 47.0±0.7% macrophages with captured YLF cells, the phagocytic number being 1.80±0.16%. These parameters were significantly higher after macrophage interactions with lyticase-treated *Candida albicans* cells: the phagocytic index reached 79.0±0.4%, the phagocytic number was 2.70±0.18.

Hence, changes in the surface structure of YLF cell wall by fermentation of the mannane layer stimulated their capture by phagocytes. As YLF are poorly digested in phagolysosomes after their capture by macrophages, it was interesting to compare the resistance of intact and lyticase-treated *Candida albicans* to intracellular digestion. After exposure of native YLF with macrophages, 41.0±0.4% cells were digested, while after pre-fermentation of YLF this value increased to 48.0±1.0%. These data indicate that the digestive phase of phagocytosis is more effective after cell wall damage by lyticase.

Our findings suggest that the effect of lyticase can be caused by YLF lysis as a result of destruction of their cell wall. Incomplete degradation of mannane is associated with reduction of *Candida albicans* viability and impairment of its adhesion to VE. The agent under these conditions is more sensitive to the phagocytic attack of the host. On the other hand, it was found that lyticase-treated YLF were easier transformed into the mycelium. Further experiments on mouse model of candidiasis will show to what degree this phenomenon is related to YLF pathogenicity.

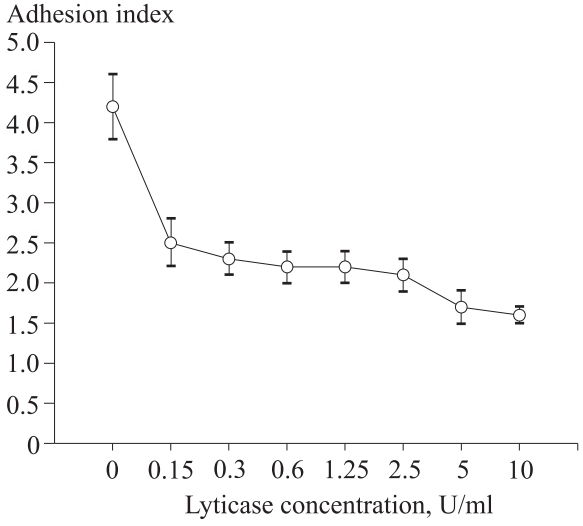

Fig. 2. Changes in *Candida albicans* index of adhesion to VE cells.

REFERENCES

1. V. N. Prilepskaya and G. R. Bairamova, *Rus. Med. Zh.*, **6**, No. 5, 301-308 (2005).
2. *A Method for Evaluation of Phagocytic Activity of Peripheral Blood Neutrophils in Living Organisms*, Description of Invention for Patent of the Russian Federation. Registration No. of application: 2003107776/15 of 20.12.2004.
3. P. Ferrer, *Microb. Cell Fact.*, **5**, 10 (2006).
4. F. M. Klis, *Yeast*, **10**, No. 7, 851-869 (1994).
5. K. Saeki, J. Iwata, S. Yamazaki, *et al.*, *J. Ferment. Bioeng.*, **78**, 407-412 (2005).
6. A. M. Ventom and J. A. Asenjo, *Enzyme Microb. Technol.*, **13**, 71-75 (2007).