



The study of protective properties of associated antigens of candida albicans and candida tropicalis

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ABSTRACT

Causative agents of candidiasis are yeast fungi of *Candida* genus, *Cryptococcaceae* family. The most widespread causative agent of candidiasis is *C. albicans*, which causes 90 % of surface forms and 50 – 70 % of deep forms. Another causative agent by prevalence is *C. tropicalis*. The aim of our work was to study the ability of the associated antigens of *C. albicans* and *C. tropicalis* to activate together the body's protective properties against candidal infection. The suspensions of cells of *C. albicans* and *C. tropicalis* fungi were subjected to the action of ultrasound, then filtered through a "Vladipore" membrane MFA-MA No.3 providing separation of the biological material with the size of 10 kDa; prefiltration and sterilizing filtration were carried out. The resulting purified antigens of *Candida albicans* fungal cells with the protein concentration of 3 mg/ml and *Candida tropicalis* with the protein concentration of 5 mg/ml were mixed in the ratios of 30:70; 70:30; 40:60; 60:40; 50:50. The associated antigens of cells of *C. albicans* and *C. tropicalis* fungi in the volume of 0.2 ml were injected to mice intramuscularly. In 14 days the procedure was repeated. The animals of the control group were injected with the sterile 0.9 % isotonic saline solution. In a month and 3 months some groups of the tested animals were infected intra-abdominally with the suspensions of *C. albicans* fungi in the amount of 20 million of cells and *Candida tropicalis* in the amount of 60 million of cells in the volume of 1 ml. After that in 14 days the animals were examined and the results were determined. As the result of the research conducted it has been found that application of the associated antigens of *C. albicans* with the protein concentration of 3 mg/ml and *C. tropicalis* with the protein concentration of 5 mg/ml in the ratio of 1:1 (50:50) provide the protective effect in 100 % of mice for 3 months when injected intramuscularly in the volume of 0.2 ml.

Key words: candidiasis, antigen, vaccine, immunity, protein

INTRODUCTION

Causative agents of candidiasis are yeast fungi of *Candida* genus, *Cryptococcaceae* family. At present about 160 species of *Candida* genus fungi are known, but only approximately 20 of them are capable to cause candidiasis in human. Most often 8 species are distinguished; they are *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, *C. kefyr*, *C. guilliermondii*, *C. lusitanae*. The most widespread causative agent of candidiasis is *C. albicans*, which causes 90 % of surface forms and 50 – 70 % of deep forms. Another causative agent by prevalence is *C. tropicalis* [1-2].

C. albicans has a high sensitivity to antifungal drugs, at the same time resistance to them can be rapidly developed. In recent years due to the active use of fluconazole the fungi of *Candida* genus are often insensitive to it. Another world problem is increasing prevalence of different species of *Candida* genus fungi (non-albicans), the part of which from the very beginning is insensitive to azoles, but it is common practice to start the treatment with them [3-4].

To fight candidal infection in recent years vaccines with the immunomodulatory properties are being actively investigated both in the CIS countries and in the countries of Europe and America [5-7]. It should be noted that currently no domestic vaccine is produced in Ukraine and no imported vaccines have been registered for prevention and treatment of candidiasis. Therefore, development of a vaccine against candidal infection is the topical issue of modern pharmacy and medicine.

According to the data of various researchers whole cells, cell walls, proteins and polysaccharides of *C. albicans* possess the antigenic properties; they activate the complement system by the alternative way [8-11]. Of a wide range of possible variants of vaccines different authors research traditional dead vaccines or more modern subunit vaccines most often. However, there is no consensus. Subunit vaccines consist of the antigen fragments that are capable to provide the adequate immune response [12-14]. These vaccines can be presented both as particles of microbes and as those obtained in the laboratory conditions by using genetic engineering technologies. The examples of subunit vaccines with the fragments of microorganisms used are vaccines against *Streptococcus pneumoniae* and meningococcus type A.

Multivalent or associated or combined vaccines are used for simultaneous immunization against a number of infections. They can include both homogeneous antigens (for example, anatoxin), and antigens of different nature (particle and molecular, living and dead antigens) [15-18]. The example of the associated vaccine of the first type can be hexa-anatoxin against tetanus, gas gangrene and botulism, of the second type – DTP vaccine, which includes tetanus and diphtheria toxoids, pertussis particle vaccine. The living multivalent associated polio vaccine contains living vaccines of polio virus types I, II, III strain.

The associated vaccine contains antigens in doses that do not cause mutual competition in order to form the immunity to all antigens including in the composition of the vaccine.

The aim of our work is to study the ability of associated antigens of *C. albicans* and *C. tropicalis* to activate together the body's protective properties against candidal infection.

EXPERIMENTAL SECTION

All studies were conducted in the laminar box maintaining aseptic conditions. To perform the inactivation of the fungal cells of *Candida albicans* of CCM 335-867 strain and *Candida tropicalis* of ATTC 20336 strain, they were preliminary cultivated according to the scheme in the test-tubes on the Sabouraud agar separately at 25 ± 2 °C within 48 hours and the fungal cells were washed with 10 ml of the sterile 0.9 % isotonic saline solution. The suspensions of the fungal cells of *C. albicans* and *C. tropicalis* obtained separately were transferred to the flasks with the Sabouraud agar and incubated at 25 ± 2 °C within 6 days and washed the fungal cells with 25 ml of the sterile 0.9 % isotonic saline solution. The microbiological purity of the suspension of the fungal cells of *C. albicans* and *C. tropicalis* was determined visually and by the method of microscopy. Then centrifugation with the rotation speed of 3000 rpm was conducted for 10 min. The precipitate of the fungi cells obtained was diluted with the sterile 0.9 % isotonic saline solution to $(8.5 - 9) \times 10^8$ in 1 ml, and the suspensions were standardized by fungal count in Goryaev chamber.

The suspensions of cells of *C. albicans* and *C. tropicalis* fungi in the volume of 10 ml were subjected to the action of ultrasound for destruction of the fungal cells on an UZUU-21 device at the frequency of 22 kHz, the intensity of 5 W/cm² and at the temperature of 25 ± 2 °C for 15 min. All the time the temperature of 25 ± 2 °C was controlled with the help of ultrasonication of the suspensions of cells and maintained by adding a cold water into the surrounding container. Then there was filtration through a "Vladipore" membrane MFA-MA No.3 providing separation of the biological material with the size of 10 kDa and its concentration. The filtrate obtained was presented by the mixture of polypeptides and polysaccharides. In each case the protein content was determined according to the requirements of the State Pharmacopoeia of Ukraine (SPhU). Then prefiltration using filters with the pore diameter of 0.45 µm and sterilizing filtration using filters with the pore diameter of 0.22 µm were carried out.

We take into account the fact that proteins and polysaccharides possessing the antigenic properties are in the composition of the cell extract of *Candida* fungi. According to the requirements of the SPhU determination of the active substance in such case is conducted by the substance, which possesses the most expressed antigenic properties, i.e. by protein. The sterile purified solutions of antigens of *C. albicans* with the protein concentration of 3 mg/ml and *C. tropicalis* with the protein concentration of 5 mg/ml were mixed in various ratios with the help of a blade mixer with the rotation speed of 100 rpm for 10 min. In order to substantiate the optimal ratio of antigens of *C. albicans* and *C. tropicalis* several variants of suspensions: 30:70; 70:30; 40:60; 60:40; 50:50 were prepared.

To assess the protective properties of the associated antigens of *C. albicans* and *C. tropicalis* the study was conducted in healthy two-month white mice with the body weight of 18-22 g. There were 6 animals in the control and test groups; they were kept in the same conditions on a standard diet. Before the research the animals acclimatized themselves under experimental room conditions. The associated antigens of cells of *C. albicans* and *C. tropicalis* fungi in the volume of 0.2 ml were injected to mice intramuscularly in the upper part of the rear right paw and separately subcutaneously in the upper part of the rear right paw. In 14 days the associated antigens of cells of *C. albicans* and *C. tropicalis* fungi in the volume of 0.2 ml were injected repeatedly in the upper part of the rear right paw and separately subcutaneously in the upper part of the rear right paw. The animals of the control group were injected with the sterile 0.9 % isotonic saline solution. In a month in one group of the animals under research and in 3 months in another group of the tested animals after introduction of the antigens the animals were infected intra-abdominally. For this purpose the suspensions of *C. albicans* fungi of CCM 335-867 strain in the amount of 20 million of cells and *Candida tropicalis* of ATTC 20336 strain in the amount of 60 million of cells in the volume of 1 ml introduced at one hour intervals were used. After that in 14 days the animals were examined and the results were determined.

The test results were considered according to the number of various manifestations of the disease and were estimated by the following system: (-) – the absence of manifestations of the disease; a mild form of the disease (+) – unkempt appearance, refusal to eat, the body weight loss, dysfunctions of the excretory organs; a moderate form of the disease (+ +) – adynamia, unkempt appearance, refusal to eat, the body weight loss, contractures of the neck muscles, the lateral location of the body, dysfunctions of the excretory organs, when examining the mucous membranes of natural orifices the signs of pathological processes, plating of fungi with feces were revealed; an advanced form of the disease (+ + +) – adynamia, unkempt appearance, refusal to eat, the body weight loss, contractures of the neck muscles, paralysis of the limbs, convulsions, the lateral location of the body, dysfunctions of the excretory organs, during the autopsy when examining the mucous membranes of natural orifices, internal organs of the animals the signs of such pathological processes as microabscesses in the renal cortical layer, lungs, spleen, liver, etc., isolation of retrocultures of fungi from the animals' organs were revealed.

RESULTS AND DISCUSSION

As the result of the research conducted it has been found that 100 % of animals remained healthy in 1 and 3 months after introduction of the associated antigens of cells of *C. albicans* fungi with the protein concentration of 3 mg/ml and *C. tropicalis* with the protein concentration of 5 mg/ml in the ratio of 60:40, 50:50, and 40:60. No statistically significant differences in the efficiency of variants 1, 2 and 3 of the associated antigens of cells of *C. albicans* fungi have been found. Taking into account that the associated extract of *C. albicans* and *C. tropicalis* fungal cells in the ratio of 50:50 (1:1) is prepared somewhat easier than other ratios and, therefore, it simplifies the calculations, then it is this ratio that can be considered to be more expedient for further study. The associated antigens of cells of *C. albicans* and *C. tropicalis* fungi in the ratio of 70:30 protected 84 % of animals from infection in a month after repeated introduction and in 3 months after repeated introduction it protected 67 % of animals from infection. This group of animals had such manifestations of infection that corresponded to the mild form of the disease: unkempt appearance, refusal to eat, the body weight loss, dysfunctions of the excretory organs.

The associated antigens of *C. albicans* and *C. tropicalis* in the ratio of 30:70 protected 67 % of animals from infection in a month after repeated introduction and in 3 months after repeated introduction it protected 50 % of animals from infection. This group of animals had such manifestations of infection that corresponded to the mild and moderate form of the disease: adynamia, unkempt appearance, refusal to eat, the body weight loss, contractures of the neck muscles, the lateral location of the body, dysfunctions of the excretory organs; when examining the mucous membranes of natural orifices the signs of pathological processes, plating of fungi with feces were revealed. The control group of intact animals had the signs that corresponded to the moderate and advanced form of the disease: adynamia, unkempt appearance, refusal to eat, the body weight loss, contractures of the neck muscles, paralysis of the limbs, convulsions, the lateral location of the body, and dysfunctions of the excretory organs. During the autopsy when examining the mucous membranes of natural orifices, internal organs of the animals the signs of such pathological processes as microabscesses in the renal cortical layer, lungs, spleen, liver, etc., as well as isolation of retrocultures of fungi from the animals' organs were revealed. The research results are given in Table 1.

Table 1 The study of the protective properties of *C. albicans* and *C. tropicalis*

The ratio of <i>C. albicans</i> and <i>C. tropicalis</i>	Result						Control
	1	2	3	4	5	6	
	Results in 1 month						
40:60	-	-	-	-	-	-	++
50:50	-	-	-	-	-	-	+++

60:40	-	-	-	-	-	-	+++
70:30	-	-	+	-	-	-	+++
30:70	-	+	-	-	+	-	++
	Results in 3 months						
40:60	-	-	-	-	-	-	+++
50:50	-	-	-	-	-	-	++
60:40	-	-	-	-	-	-	+++
70:30	+	-	-	+	-	-	+++
30:70	-	+	-	++	-	+	++

Note: - absence of the disease, + - a mild form of the disease, ++ a moderate form of the disease, +++ an advanced form of the disease.

CONCLUSION

As a result of the research conducted it has been found that application of the associated antigens of *C. albicans* with the protein concentration of 3 mg/ml and *C. tropicalis* with the protein concentration of 5 mg/ml in the ratio of 1:1 provide the protective effect in 100 % of mice for 3 months when injected intramuscularly in the volume of 0.2 ml. In the future, it is necessary to study the therapeutic properties of the associated antigens of *C. albicans* with the protein concentration of 3 mg/ml and *C. tropicalis* with the protein concentration of 5 mg/ml in the ratio of 1:1.

After determination of the protective and therapeutic properties of the associated antigens of *C. albicans* and *C. tropicalis* it is planned to develop experimentally the composition and technology of the medicine proposed.

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