Determination of the concentration of low molecular fraction of Candida Albicans proteins by ELISA method at subcutaneous introduction in candidiasis therapy

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The purpose of this work was to determine the C. albicans protein concentration at the subcutaneous introduction. The therapeutic effect of C. albicans proteins has been studied in white mice. Animals were injected intraperitoneally with a suspension of C. albicans fungus. After five days and repeatedly, after 14 days, mice were infected subcutaneously with proteins of Candida cells of volume 0.2 mL. Fourteen days after each injection, the determination of the protective functions of the animal body has been carried out by the titer of specific C. albicans antibodies. According to the data obtained during studies on the treatment of candidiasis, it was found that in the subcutaneous administration after the first and second injection with a concentration of low molecular weight fraction of the C. albicans protein of 1, 2, 3, 4, and 5 mg/mL antibodies titers have doubled, indicating a lack of activation of the body’s protective functions. Proteins of low molecular weight fraction of C. albicans with concentrations 1, 2, 3, 4, and 5 mg/mL do not activate the body’s defence mechanisms.

INTRODUCTION

Today, humanity is experiencing an epidemic of opportunistic infections, among which mycoses are one of the top places. The most common pathogens of mycoses are members of the genus Candida. Candidiasis is an opportunistic mycosis that occurs with lesions of the mucous membranes and skin, in patients with severe immunodeficiency conditions disseminated forms may be found, often with damage to the lungs and organs of the gastrointestinal tract (Grover et al., 2010; Cassone, 2008).

To date, according to mostly foreign researchers, fungi of the genus Candida compete with bacteria for first place among the causative agents of nosocomial infections. For example, in the United States, there are pieces of evidence that fungi of the genus Candida rank 4th among microorganisms secreted from the blood (Skibinski et al., 2011; D’argenio and Wilson, 2010). At the same time, they are characterized by the highest mortality among all septic in-hospital conditions.
Both in developed countries and Ukraine, there are difficulties in the diagnosis and treatment of candidiasis. Among the fatal patients who did not receive adequate treatment, one of the first places is occupied by candidiasis. In Ukraine, the diagnosis of infectious pathology, in most cases, continues to be carried out by seeding on nutrient media even without identification of species (the exception is some gynaecological hospitals), which does not always correspond to the disease, especially in the early stages. This issue is crucial, as specific clinical signs of candidiasis and disseminated candidiasis in the early stages are not described, and given the ability of candidiasis to mask, laboratory diagnosis, especially in our country, is not always timely and effective. Thus, doctors have to make prescriptions for the treatment of candidiasis empirically. Therapeutic problems in the treatment of candidiasis are also not uncommon. Due to the active use of fluconazole in recent years, fungi of the genus Candida are often insensitive to it. The second problem around the world is the growing prevalence of various species of the Candida genus fungi (non-albicans), some of which are initially not sensitive to azoles, from which we usually start treatment (Han and Rhew, 2012; Carvalho et al., 2012).

Many researchers believe that the use of drugs that can stimulate protective immune responses against candidiasis, i.e. vaccines, is a promising area in the fight against candidiasis (Gut-Landman, 2012; Nabel, 2013) and is an alternative to antifungal drugs. It should be noted that today there are several classifications of vaccines depending on the method of production and components. However, there is still no consensus among researchers as to which of them is the most promising. One type of vaccine used by researchers to fight candidiasis is the subunit vaccine. Subunit vaccines play an essential role in the prevention of various infections (Sethu et al., 2012; Vetter et al., 2018). The necessary microorganisms - pathogens are used for their production. Certain physical or chemical factors destroy the microorganism, but individual particles of the microorganism retain antigenic and immunogenic properties.

The authors at the Biotechnology and Microbiology, Virology, and Immunology department of the National University of Pharmacy have developed a method for the disintegration of Candida fungal cells using ultrasonic radiation. The composition of the extract-disintegrate of Candida cells includes proteins and polysaccharides that possess antigenic properties. In this case, according to the requirements of the State Pharmacopoeia of Ukraine, the identification of the active substance is carried out in terms of protein.

Previously, studies were conducted to determine the effectiveness of subcutaneous and intramuscular injections of C. albicans cells disintegrate solution with a molecular mass of antigens greater than 10 kDa in animal experiments for prevention and treatment of candidiasis infection. The studies have found that the antigens of this fraction show immunogenic properties at C. albicans proteins concentration of 3 mg/mL at intramuscular administration and do not exhibit immunogenic properties at subcutaneous administration. Now it is advisable to conduct a study of low molecular weight fractions less than 10 kDa with C. albicans protein concentrations of 1, 2, 3, 4, and 5 mg/mL for immunogenicity at subcutaneous and intramuscular injection by antibody titers in the prevention and treatment of candidiasis.

The purpose of this work was to determine the concentration of low molecular weight fraction of C. albicans fungus proteins at subcutaneous introduction in the therapy of candidiasis.

MATERIALS AND METHODS

All studies were performed in a laminar box, maintaining aseptic conditions. C. albicans strain CCM 335-867 was precultured in test tubes on Sabouraud agar at 25 ± 2 °C for 48 hours and washed the fungal cells with 10 mL of sterile isotonic 0.9% sodium chloride solution. The resulting suspensions of fungal cells were transferred to Sabouraud agar mattresses incubated at 25 ± 2 ºC for six days and washed the fungal cells with 25 mL of sterile isotonic 0.9% sodium chloride solution. The microbiological purity of the C. albicans fungal cells suspension was determined visually and by microscopy. Next, centrifugation was carried out at a speed of 3000 rpm for 10 minutes. The obtained precipitate of fungal cells was brought with sterile isotonic 0.9% sodium chloride solution to (8.5 - 9) x10⁸ in 1 mL.

Received suspensions of fungal cells in a volume of 10 mL were sonicated for the destruction of fungal cells on the apparatus USUU-21 at a frequency of 22 kHz, intensity 5 W/cm² and at a temperature 25 ± 2 °C for 15 min. Temperature 25 ± 2 °C was monitored continuously during the cell suspensions sonication and maintained by adding cold water to the surrounding tank. Further carried out filtering through the membrane “Vladipore” MFA - MA No. 3, which provides the separation of biological material with the size of 10 kD and its concentration. The filtrate obtained is a mixture of proteins and polysaccharides. In each case, the protein content was determined according to SPU. Because Candida
Table 1: Therapeutic effect of C. albicans fungal cells antigens

| Animals | C. albicans protein content, mg/mL | Method of administration | Titors of C. albicans antibodies in ELISA |
|---------|----------------------------------|--------------------------|-----------------------------------------|
|         |                                  |                          | Healthy animals | Ill after 1st injection | Ill after 2nd injection |
| Mice    | 1                                | s.c.                     | 1: (400 ± 18)  | 1: (600 ± 28)  | 1: (400 ± 18)  |
| Mice    | 2                                | s.c.                     | 1: (200 ± 9)   | 1: (800 ± 35)  | 1: (800 ± 35)  |
| Mice    | 3                                | s.c.                     | 1: (300 ± 15)  | 1: (400 ± 17)  | 1: (600 ± 27)  |
| Mice    | 4                                | s.c.                     | 1: (200 ± 10)  | 1: (400 ± 18)  | 1: (400 ± 17)  |
| Mice    | 5                                | s.c.                     | 1: (300 ± 14)  | 1: (600 ± 27)  | 1: (800 ± 38)  |

fungal cell extract contains proteins and polysaccharides that have antigenic properties, according to the requirements of SPU, the determination of the active substance is carried out by the substance that has more pronounced properties, i.e. by protein. Next, pre-filtration was performed using filters with a pore diameter of 0.45 µm and sterilizing filtration using filters with a pore diameter of 0.22 µm.

The therapeutic effect of low molecular weight fraction less than 10 kD of C. albicans proteins in concentration 1, 2, 3, 4, and 5 mg/mL was studied in two-month-old white mice weighing 18 - 22 g 10 animals in the control and experimental groups, which were kept in the same conditions on a standard diet. The research was conducted based on the State Institution "Il Mechnikov Institute of Microbiology and Immunology". Before the study, the animals were acclimated in the experimental room.

Animals were injected intraperitoneally with a suspension of C. albicans fungus strain CCM 335-867 in the amount of 20 million cells per 1 mL volume. In 5 days mice were injected subcutaneously into the upper part of the right hind paw with proteins of low molecular weight fraction of Candida fungus of volume 0.2 mL. After 14 days, determination of the protective functions of the animal body by the titer of specific C. albicans antibodies was performed during enzyme-linked immunoassay.

To do this, used a set of reagents for enzyme-linked immunosorbent assay of antibodies G to C. albicans using the "Vector-Best" ELISA test system. Fourteen days after the first injection, again, in the upper part of the left hind paw injected proteins of the low molecular weight fraction of Candida fungi with a volume of 0.2 mL subcutaneously. After 14 days, the protective functions of the animal’s body were determined by the specific titer of antibodies to C. albicans Animals in the control group were injected with saline.

RESULTS AND DISCUSSION

According to studies, antibody titers of healthy animals were in the range of 1:200-1: 500. This can be explained by possible contact with a fungus of the genus Candida in the course of life of mice or a possible carriage of this species of fungi, as they are part of the normal microflora of animals.

At subcutaneous administration to animals with candidiasis after the first injection of low molecular weight fraction proteins of C. albicans in concentration 1, 2, 3, 4, and 5 mg/mL antibody titers of C. albicans fungi have doubled compared to titers in healthy animals. The research results are shown in Table 1.

After the second subcutaneous injection of C. albicans proteins with an interval of 14 days, there is no increase in antibody titer when using the protein concentration 1, 2, 3, 4, and 5 mg/mL.

The antibody titer in the control group at the subcutaneous route of administration did not grow.

Comparing the results obtained in previous and present studies, it is safe to say that the intramuscular administration of the high molecular weight fraction of C. albicans fungus antigens more strongly stimulates the formation of antibodies responsible for humoral immunity.

Based on the data obtained, it can be argued that the high molecular weight fraction of C. albicans fungus antigens at intramuscular injection can be a potential antigen for the development of vaccines for the prevention and treatment of candidiasis, in contrast to the low molecular weight fraction at subcutaneous administration.

CONCLUSIONS

According to the results of the studies, it has been found that the low molecular weight fraction of antigens of C. albicans fungi cells with a protein concentration of 1, 2, 3, 4, and 5 mg/mL at repeated subcu-
taneous administration of 0.2 mL does not provide activation of immune mechanisms in the treatment of candidiasis. For further studies, it is advisable to use a high molecular weight fraction of C. albicans fungus cells antigens with a protein concentration of 3 mg/mL at intramuscular injection, based on which it is planned to develop a vaccine to prevent and treat candidiasis.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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