

The study of the therapeutic effectiveness of the associated inactivated and subunit vaccines based on *Candida albicans* and *Candida tropicalis* fungi

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Received 14 December 2019 ♦ Accepted 13 January 2020 ♦ Published 7 January 2021

Citation: Rybalkin M, Khokhlenkova N, Azarenko Yu, Kaliuzhnaia O, Podolsky I (2021) The study of the therapeutic effectiveness of the associated inactivated and subunit vaccines based on *Candida albicans* and *Candida tropicalis* fungi. Pharmacia 68(1): 89–91. <https://doi.org/10.3897/pharmacia.68.e49384>

Abstract

Candidiasis can be in various forms, the most dangerous of them are systemic and visceral candidiases. Many researchers believe that using drugs that are able to stimulate a protective immune response against candidal infections, i.e. immunobiological drugs, is a promising direction in the fight against candidiasis, and that these drugs are an alternative to antifungal agents. The aim of the work was to compare the therapeutic properties of the inactivated and subunit vaccines of cells of *C. albicans* and *C. tropicalis* fungi. Previously, animals were infected with the candidal infection. In 5 days, after infection the vaccines studied were injected to mice intramuscularly in the volume of 0.2 ml in the upper part of the rear right paw. In 14 days, these vaccines were injected to mice in the volume of 0.2 ml in the upper part of the rear left paw. In 14 days, the body protection functions were determined by the titer of *C. albicans* specific antibodies when performing the enzyme-linked immunosorbent assay (ELISA). According to the results of the research conducted it has been found that the use of the inactivated and subunit vaccines stimulates the eight times growth of the titers. Therefore, both vaccines are effective. However, the subunit vaccine has the maximum purification from ballast substances; therefore, it is promising to use exactly this vaccine for further studies.

Keywords

antigen, candidiasis, immunity, therapy, vaccine

Introduction

The number of patients with candidiasis dramatically increases; it is associated with the irrational use of antibiotics, hormones, steroid drugs, as well as with deterioration of the environment, increased pathogenicity of *Candida* genus fungi and development of comorbidities (LeibundGut-Landmann et al. 2012). Candidiasis can be in various forms, the most

dangerous of them are systemic and visceral candidiasis (Holubka 2011). They are characterized by a long course of the disease, have diverse clinical manifestations and frequent relapses. These forms of candidiasis are difficult to treat by modern medicines, including antifungal antibiotics (Holubka 2011; LeibundGut-Landmann et al. 2012).

In modern practice of domestic physicians the species of *Candida* fungus as a causative agent of the disease are

identified very rarely. Candidiasis caused by *Candida* genus fungi is most often diagnosed without closer definition of its species. It should be noted that, even if required, the identification of the species of *Candida* fungus is not always possible to do because of the lack of reagents, equipment and experience of laboratory technicians. Moreover, the long-term use of the same antifungal drugs has led to the loss of sensitivity of many species of *Candida* fungi or their separate epitopes to them (Holubka 2011).

Some researchers believe that using drugs that are able to stimulate a protective immune response against candidal infections, i.e. immunobiological drugs, is a promising direction in the fight against candidiasis (Cassone 2008; D'Argenio and Wilson 2010; Grover et al. 2010; Han and Rhew 2012), and that these drugs are an alternative to antifungal agents (Rybalkin et al. 2017). Today, there are several classifications of vaccines depending on the production method and constituents (Zhukova and Krivosheeva 2013). However, there is still no consensus among researchers which vaccine is the most promising. It should be noted that a promising direction in development of modern immunobiological drugs stimulating the body's defense mechanisms is creation of combined drugs against several pathogens (Skibinski et al. 2011; Rybalkin 2014). Therefore, we decided to compare the therapeutic properties of the associated inactivated and subunit vaccines of cells of *C. albicans* and *C. tropicalis* fungi as the main causative agents of candidiasis. Inactivated vaccines are important in the prevention of various infections. The necessary microorganisms – causative agents – are used to create a vaccine. The microorganism is subjected to such treatment, which leads to the required loss in the ability to reproduce (replication, propagation), but retains antigenic and immunogenic properties. Subunit vaccines are produced by cell disruption and subsequent ultrafiltration (Rybalkin 2014).

The aim of the work

Was to compare the therapeutic properties of the inactivated and subunit vaccines of cells of *C. albicans* and *C. tropicalis* fungi.

Materials and methods

The therapeutic effect of the associated inactivated vaccine of *C. albicans* and *C. tropicalis* fungi with the concentration of $10 + 10$ ($\times 10^6$ of cells/ml) and the associated subunit vaccine 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 1:1 was studied 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 studies the animals acclimatized themselves under the experimental room conditions. The animals were infected intraperitoneally by the suspension of *C. albicans* fungi of CCM 335-867 strain in the amount of 20×10^6 of

cells and *C. tropicalis* of ATCC 20336 strain in the amount of 60×10^6 of cells in the volume of 1 ml. In 5 days, the inactivated vaccine was injected intramuscularly in the volume of 0.2 ml in the upper part of the rear right paw to mice of one group, while the second group received the subunit vaccine. In 14 days, 0.2 ml of the inactivated vaccine was injected again to mice of one group in the upper part of the left rear paw, while the second group was injected the subunit vaccine. The animals of the control group were injected with the physiological saline solution. In 14 days, the body protection functions were determined by the titer of *C. albicans* specific antibodies when performing the enzyme-linked immunosorbent assay (ELISA) according to the State Pharmacopoeia of Ukraine (SPhU, ed. I, art. 2.7.1, p. 55–57). For this purpose, the immunoassay kit for detection of antibodies from G to *C. albicans* class with the “Vector-Best” ELISA test system was used.

Results and discussion

The results of the study showed that before infection the antibody titers of healthy animals were in the range of 1:400. It can be explained by the possible contact with the fungus of *Candida* genus during the life of mice or a possible carrier state of the genus fungi since they are part of the normal microflora of animals.

After infection of mice the antibody titers were 1:800, indicating development of the immune response of animals to cells of *C. albicans* and *C. tropicalis* fungi.

With the first injection of the associated inactivated vaccine the antibody titers of *C. albicans* fungi were observed at the level of 1:800, indicating the insufficient immune response of a single injection since the antibody titers remained at the level of infected animals without their growth. After a double injection of the associated inactivated vaccine with an interval of 14 days there was an increase in the antibody titers by eight times (1:3200) compared to the titers in healthy animals and by four times compared to the infected animals. In 1 and 3 months of the studies the antibody titers remained at the same level and were 1:3200. The results of the studies are presented in Table 1.

After the first injection of the associated subunit vaccine the antibody titers of *C. albicans* fungi were observed at the level of 1:800, indicating the insufficient immune response of a single injection. After a double injection of the associated subunit vaccine with an interval of 14 days there was an increase in the antibody titers by eight times (1:3200) compared to the titers in healthy animals and by four times compared to the infected animals. The studies conducted in 1 and 3 months demonstrated that antibody titers were eight times increased compared to the original data and were 1:3200 (Table 1).

Therefore, both vaccines provide the increase of the titers by eight times. For further studies the associated subunit vaccine was selected since it had the maximum purification and was without ballast substances. Ballast

Table 1. Comparison of the therapeutic action of the associated inactivated and subunit of cells of *C. albicans* and *C. tropicalis* fungi.

| Associated vaccines | After the 1st injection | After the 2nd injection | Antibody titers | | |
|---------------------|-------------------------|-------------------------|-----------------|---------------|--------------|
| | | | In 1 month | In 3 months | Control |
| Inactivated vaccine | 1: 800 ± 173 | 1: 3200 ± 792 | 1: 3200 ± 758 | 1: 3200 ± 761 | 1: 800 ± 152 |
| Subunit vaccine | 1: 800 ± 168 | 1: 3200 ± 731 | 1: 3200 ± 723 | 1: 3200 ± 749 | 1: 800 ± 171 |

substances can provoke adverse immune reactions that will have a negative impact on the vaccine.

To determine the vaccine priority the study on the effectiveness of its action should be conducted, i.e. determine the number of animals remained alive after introduction of the vaccine.

The antibody titers in the control group increased twice. The experimental animals showed no signs of the disease. The animals of the control groups showed the signs of 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. In an advanced form of the disease there was 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 and 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 observed.

Conclusion

According to the results of the studies conducted it has been found that the use of the associated subunit and inactivated vaccines stimulates the eight times growth of titers compared to the original data, and these titers are 1:3200.

Thus, both vaccines provide immune responses. However, the subunit vaccine has the maximum purification from ballast substances, which can give adverse immune reactions. Therefore, it is promising to use the associated subunit vaccine 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 1:1 for further studies.

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