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The Study of the Effect of “Candidocyde” Drug on T-, B-Lymphocytes and the γ -Globulin Fraction.

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ABSTRACT

Year by year the number of patients with candidiasis has increased; it is associated with weakening of the human immunity and increased pathogenicity of a causative agent. At the premises of the National University of Pharmacy at the Biotechnology Department and the Department of Microbiology, Virology and Immunology a potential vaccine – the immunobiological drug “Candidocyde” solution for prevention and treatment of candidal infection has been developed. The aim of this work is to study the values of T-, B-lymphocytes and γ -globulin in rabbits when introducing the solution of the immunobiological drug “Candidocyde”. Blood analysis was performed in several groups of animals: 1) intact animals, 2) healthy animals received the solution of the immunobiological drug “Candidocyde”, 3) infected animals, 4) infected animals received the solution of the immunobiological drug “Candidocyde”. The state of cellular and humoral immunity was assessed by the number of T-, B-lymphocytes and γ -globulin. The blood values in healthy rabbits and rabbits with candidiasis after introduction of the solution of the immunobiological drug “Candidocyde” are characterized by elevation of T-, B-lymphocytes and γ -globulin. The analysis of the data obtained indicates activation of the humoral and partially cellular immunity.

Keywords: candidiasis; antigen, vaccine, immunity; T-, B-lymphocytes, γ -globulin

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INTRODUCTION

Candidiasis is a common fungal infection that affects the nails, skin, hair, mucous membranes and internal organs. Year by year the number of patients with candidiasis has increased; it is associated with weakening of the human immunity and increased pathogenicity of a causative agent [1]. Currently vaccines against candidal infection are actively being developed abroad since the traditional antifungal therapy is inefficient [2-5].

At the premises of the National University of Pharmacy at the Biotechnology Department and the Department of Microbiology, Virology and Immunology a potential vaccine – the immunobiological drug solution “Candidocyde” for prevention and treatment of candidal infection has been developed. The technology of its preparation is as follows. The fungal cells of *Candida albicans* and *Candida tropicalis* were incubated separately in the test-tubes with the Sabouraud agar at 25 ± 2 °C within 48 h, and the fungal cells were washed with 10 ml of the sterile 0.9 % isotonic saline solution. The suspensions of the fungal cells of *Candida albicans* and *Candida tropicalis* obtained separately were transferred to the flasks with the Sabouraud agar, incubated at 25 ± 2 °C within 6 days, and the fungal cells were washed with 25 ml of the sterile 0.9 % isotonic saline solution. The microbiological purity of the suspension of the fungal cells of *Candida albicans* and *Candida 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 suspensions of cells of *C. albicans* and *C. tropicalis* fungi in the volume of 10 ml were subjected separately 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/cm^2 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 cold water into the surrounding container [6]. Then filtration was carried out through a “Vladipore” membrane MFA-MA No.3 providing concentration of the biological material with the molecular weight of more than 10 kDa. The substances obtained were transferred to phosphate buffer with pH 7.2 ± 0.2 . After that prefiltration and sterilizing filtration using filters with the pore diameter of 0.45 μm and 0.22 μm were carried out. Proteins and polysaccharides possessing the antigenic properties are in the composition of the cell extract of *Candida* fungi. According to the requirements of the SPbU (State Pharmacopoeia of Ukraine) determination of the active substance in such case is conducted by protein. The sterile antigens obtained from *Candida albicans* fungi with the protein concentration of 3 mg/ml and *Candida tropicalis* fungi with the protein concentration of 5 mg/ml in the ratio of 1:1 [7-10] with the simultaneous addition of phenol in such amount that its concentration in the solution was 0.25 % were mixed by a paddle mixer with the rotation speed of 100 rpm for 10 min [11]. The safety of the drug developed was proven [12].

The aim of this work is to study the values of T-lymphocytes, B-lymphocytes and γ -globulin in laboratory animals when introducing the solution of the immunobiological drug “Candidocyde”.

Objects and methods of research

The study was performed in rabbits with six animals in each group; they were kept in the same conditions on a standard diet. Prior the research the animals acclimatized themselves under experimental room conditions. The state of cellular and humoral immunity was assessed by such blood values as T-, B-lymphocytes and γ -globulin. Blood analysis was performed in several groups of animals: 1) intact animals, 2) healthy animals received intramuscularly 0.2 ml of the solution of the immunobiological drug “Candidocyde” twice with an interval of 14 days, 3) animals infected with candidal infection in 5 days after infection, 4) animals infected with candidal infection that in 5 days after infection received intramuscularly 0.2 ml of the solution of the immunobiological drug “Candidocyde” twice with an interval of 14 days. The blood from the ear marginal vein was taken in animals in the volume of 1 ml for determination of blood values. To infect animals the cultures of *Candida albicans* and *Candida tropicalis* fungi incubated for 48 h were used. The suspension with cultures of *Candida albicans* fungi in the dose of 3×10^9 cell/ml in the volume of 10 ml was injected intramuscularly to rabbits, and in an hour the suspension with cultures of *Candida tropicalis* fungi in the dose of 5×10^9 cell/ml in the volume of 10 ml was injected intramuscularly. In 5 days after infection manifestations of the disease were recorded in animals. Animal study was taken after the prior approval of Animal Ethics Committee.

The body’s immunological reactivity was assessed by the following indicators: the total number of T-lymphocytes was determined in the reaction of the rosette formation with the sheep red blood cells, B-

lymphocytes – in the reaction of the rosette formation in the presence of the complement [13]. The total concentration of the serum proteins was determined by the biuret method with the help of a “METHAN 8001” device (Ukraine) at the wavelength of 530 nm [14]. The fractional composition of the blood serum of animals was studied. To study protein fractions of the blood serum of the animals under research a “Cormay gel protein 100” diagnostic kit for electrophoretic separation of blood serum proteins on agarose manufactured by “Cormay” company (Austria) was used. The foregrammes were decoded on a densitometer of the same company [15].

Statistical processing of the digital data obtained was performed using the Statistika software package. When comparing the groups of animals under study by the main indicators the Student t-test was used, and the result was considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The immunological studies showed that after double introduction of the solution of the immunobiological drug “Candidocyde” to rabbits with an interval of 14 days there were significant changes in blood values. First of all, it was found that there was elevation of T-lymphocytes from 37.21 ± 2.82 % to 45.03 ± 2.25 % and B-lymphocytes from 15.12 ± 0.42 % to 11.28 ± 0.34 % in the peripheral blood channels of the immunized rabbits. There was also an increase in the number of γ -globulin from 18.51 ± 1.15 % to 25.32 ± 1.34 %. Therefore, the use of the solution of the immunobiological drug “Candidocyde” provides stimulation of the humoral and cellular immunity, in particular populations of T-, B-lymphocytes and γ -globulin, and it will provide prevention of candidal infection. The results of the study are given in Table 1.

In rabbits infected with candidal infection in 5 days the development of the inflammatory process was observed, primarily it was accompanied by inhibition of T-cell immunity. The number of T-lymphocytes in infected rabbits was less than in clinically healthy animals. The number of T-lymphocytes in healthy animals was 37.21 ± 2.82 % and in infected animals – 32.57 ± 2.31 %, respectively. The number of B-lymphocytes in animals with candidiasis was 11.28 ± 0.34 %, i.e. less than in clinically healthy animals – 21.35 ± 0.43 %. It also indicates the active immunosuppression in the first phase of the inflammatory process. There was reduction of γ -globulin from 18.51 ± 1.15 % to 13.87 ± 0.92 %. Thus, candidiasis in rabbits is accompanied by significant changes in the number of immunocompetent cells in the blood and, first of all, T-component of the immune system and γ -globulin.

Table 1: The values of T-lymphocytes, B-lymphocytes and γ -globulin after introduction of the solution of the immunobiological drug “Candidocyde”

Blood values	Healthy rabbits		Infected rabbits	
	Before immunization	After immunization	Before immunization	After immunization
T-lymphocytes, %	37.21 ± 2.82	45.03 ± 2.25	32.57 ± 2.31	43.45 ± 2.21
B-lymphocytes, %	15.12 ± 0.42	22.63 ± 0.91	11.28 ± 0.34	21.35 ± 0.43
γ -globulins, %	18.51 ± 1.15	25.32 ± 1.34	13.87 ± 0.92	21.38 ± 1.24

Note: $n=6$, $p < 0.5$

In animals infected with candidal infection that in 5 days after infection received intramuscularly the solution of the immunobiological drug “Candidocyde” twice with an interval of 14 days the analysis of the research results showed that in the blood serum of animals the increase in the number of T- and B-lymphocytes, as well as elevation of γ -globulin amount was registered in 14 days after the second injection. The number of T-lymphocytes increased from 32.57 ± 2.31 % to 43.45 ± 2.21 %, B-lymphocytes – from 11.28 ± 0.34 % to 21.35 ± 0.43 %, the level of γ -globulin increased from 13.87 ± 0.92 % to 21.38 ± 1.24 % compared to the values of the infected animals. Therefore, the use of the solution of the immunobiological drug “Candidocyde” in animals with candidal infection provides stimulation of the humoral and cellular immunity, in particular populations of T-, B-lymphocytes and γ -globulin, and it, in turn, provides therapy for candidal infection.

The data obtained indicate that activation of the humoral and cellular immunity factors is observed when introducing the solution of the immunobiological drug "Candidocyde" to rabbits.

CONCLUSION

Thus, when introducing the solution of the immunobiological drug "Candidocyde" to healthy rabbits and rabbits infected with candidal infection the blood values have been determined; their analysis indicates activation of immune-complementary cells of T-, B-lymphocytes and increase of the γ -globulin fraction level. It indicates activation of the humoral and cellular immunity and confirms the presence of the immunogenic properties of the immunobiological drug "Candidocyde" under research both in preventing and in treating candidal infections.

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