Structural Changes in the Liver and Content of Steroid Hormones in the Blood and Adrenal Glands of Mice with Systemic Candidiasis Treated with A Composition of Amphotericin B and Dialdehyde Dextran

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In CBA mice infected with *C. albicans*, phasic pattern of granulomatosis development was observed. In all groups, the number of granulomas in the liver was minimum on day 56 after infection. Treatment with free amphotericin B and its composition with dialdehyde dextran (CA) reduced the number of infiltrations and granulomas in the liver, the changes were more pronounced in animals receiving CA. A different pattern of cyclic fluctuations of cortisol content in the blood and adrenal glands and progesterone content in the adrenal gland was observed. By the end of observation (day 84), cortisol content in the blood and adrenals of mice treated with CA was considerably lower than in untreated mice and animals receiving amphotericin B.

Key Words: *granulomatosis; liver; treatment; adrenal glands; corticosteroids*

Systemic mycoses characterized by intracellular persistence in macrophages of dimorphic commensals *C. albicans* fungi causing up to 70% fungal diseases are now becoming a pressing problem of modern medicine [4,6] along with tuberculosis, and other diseases manifesting in granulomatous inflammation with the involvement of all components of the mononuclear phagocyte system [7]. This dictates the need of studying the pathogenesis of systemic mycoses characterized by pronounced changes in the function of the pituitary—adrenocortical system [3]. More comprehensive understanding of intersystem changes in the organism in these pathologies are required for the development of pathogenetically substantiated drugs with targeted delivery to the sites of agent persistence, *i.e.* cells of the mononuclear phagocyte system.

Here we studied structural changes in the liver and functional state of the adrenal cortex (AD) in male CBA mice with systemic candidiasis treated with free amphotericin B (AmB) or CA.

MATERIALS AND METHODS

The study was performed on 175 male CBA mice obtained from Nursery of Institute of Cytology and Genetics, Siberian Division of Russian Academy of Sciences (Novosibirsk). The animals were divided into four groups. Group 2-4 mice were intraperitoneally infected with *C. albicans* (2.5×10^9 microbial bodies per mouse in 0.2 ml 0.9% isotonic aqueous solution of NaCl). The development of systemic candidiasis was verified by the presence of macrophage infiltrations and macrophage-epithelioid cell

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granulomas in visceral organs [8,9]. Group 1 mice (controls) received the same volume of 0.9% aqueous solution of NaCl. Treatment with AmB (group 3) and CA (group 4) was started 1 day after infection. Group 2 mice received no treatment. The drugs were injected intraperitoneally every other day (10 injections) in a dose of 250 U antibiotic per 1 kg body weight in 0.2 ml 5% glucose. The material for the study was obtained on days 10, 28, 56, and 84 after infection.

The content of cortisol in the serum and the content of cortisol and progesterone in AD were measured by radioimmune and enzyme immune assays [3]. The total volume density of macrophage infiltrations and macrophage-epithelioid cell granulomas in the liver was determined morphometrically and served as the indicator of antimycotic efficiency of the applied drugs.

The data were processed statistically using Kruskal—Wallis dispersion analysis and nonparametric Mann—Whitney test (with the use of Bonferroni correction for multiple comparisons) [1]. The differences were significant at $p<0.05$.

RESULTS

Oscillations in blood cortisol concentration were observed in untreated mice in the dynamics of systemic candidiasis development (Fig. 1, *a*) with its drop on day 10 after infection, which agreed with previous findings [3]. In mice with systemic candidiasis treated with free AmB, cortisol content did not decrease at this term, but on day 28 an elevation of blood hormone concentration was noted followed by its gradual decrease throughout the experimental period. In mice with experimental candidiasis treated with CA, the oscillations of blood cotrisol concentration disappeared and a monotonous decrease in hormone level was observed.

In AD of mice with systemic candidiasis receiving no treatment, oscillations of cortisol and progesterone content were also observed, moreover, changes in the concentrations of these hormones were in antiphase: the concentration of progesterone decreased with increasing cortisol level and vice versa (Fig. 1, *b, c*). These relationships are naturally determined, because progesterone is a precursor in

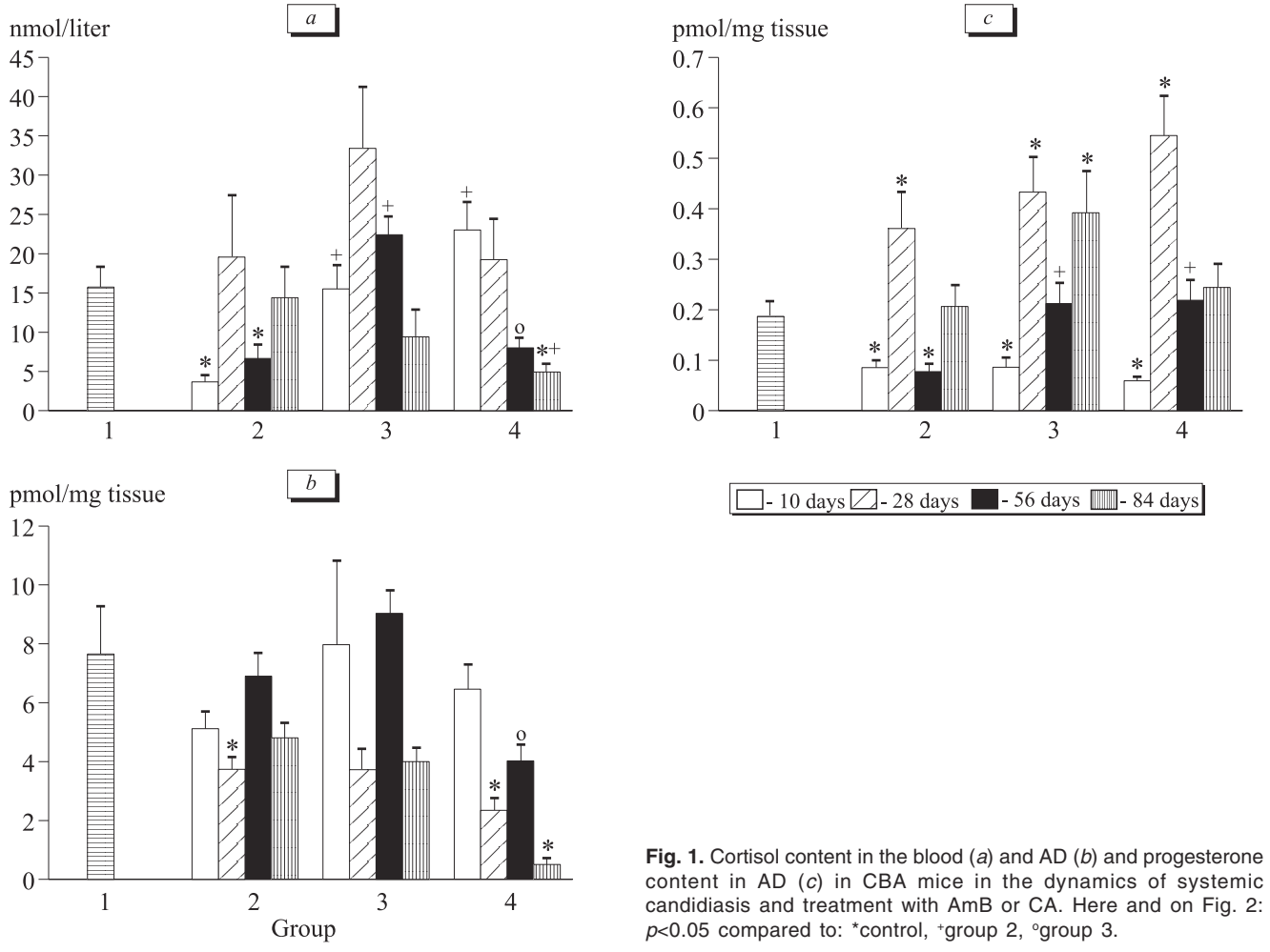

Fig. 1. Cortisol content in the blood (*a*) and AD (*b*) and progesterone content in AD (*c*) in CBA mice in the dynamics of systemic candidiasis and treatment with AmB or CA. Here and on Fig. 2: $p<0.05$ compared to: *control, +group 2, °group 3.

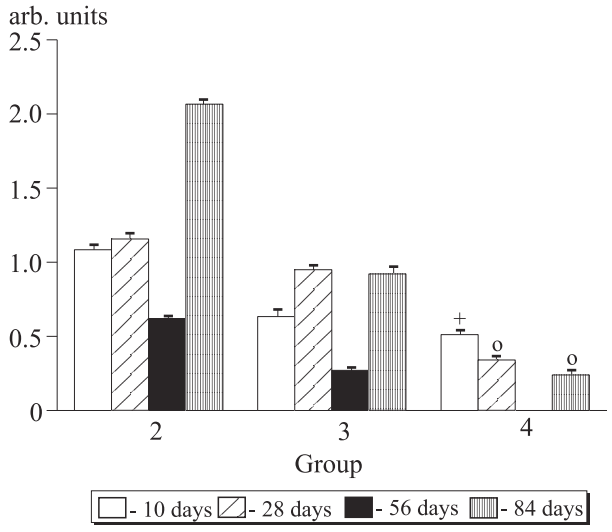

Fig. 2. Total volume density of mycotic infiltrates and granulomas in the liver of CBA mice with systemic candidiasis treated with AmB or CA.

the synthesis of cortisol in AD cortex and enhanced synthesis of cortisol leads to a decrease in progesterone content.

It is known that stimulation of corticosteroid production during stress or after administration of ACTH triggers activation of all stages of their synthesis. The fact that the content of cortisol and progesterone in AD did not increase synchronously in the dynamics of candidiasis can be explained by disturbances of steroid biosynthesis in AD caused by fungal metabolites. These microorganisms synthesize signal substances of higher animals, in particular, steroid hormones [10], and increased blood content of these hormones can modify the synthesis of endogenous steroid hormones. The dynamics of cortisol content in the blood of untreated animals with systemic candidiasis was similar to that for progesterone in AD. This also confirms that the phasic pattern of changes in corticosteroid content in the blood and AD in systemic candidiasis is mediated by external (with regard to the pituitary-adrenocortical system) factors.

The mice treated with AmB and CA demonstrated the phasic pattern of the dynamics of cortisol and progesterone content in AD similar to that in untreated animals. It should be noted that in mice treated with CA, the content of cortisol in AD sharply decreased on day 84 after infection, which coincided with the most pronounced decrease in the blood concentration of this hormone.

Thus, these findings suggest that treatment with antibiotics prevented the decrease in blood cortisol content in mice with candidiasis on day 10 of the disease. The total area of macrophage infiltrations and macrophage-epithelioid cell granulomas in the liver of AmB- and CA-treated also decreased at this term (Fig. 2) compared to that in untreated animals, which attests to antimycotic activity of both drugs. CA was more effective, because the number of infiltrates and granulomas in the liver of CA-treated mice was lower than in AmB-treated mice at all terms of the study (Fig. 2).

Hence, disturbances in the functional state of the liver in treated mice were less pronounced, which, in turn, can contribute to maintenance of normal concentration of non-metabolized cortisol due to the maintenance of the concentration of transcortin and other steroid-binding proteins.

The count of infiltrates and granulomas in the liver, the organ with the highest content of macrophages in the system of mononuclear phagocytes, in untreated animals and animals receiving AmB and CA also underwent cyclic changes (Fig. 2), which differed from cyclic changes in the function of AD.

The lowest total area of infiltrates and granulomas was found in mice treated with the antibiotics on day 56 after infection, the mice receiving CA had practically no infiltrates and granulomas in the liver. This also attest to higher therapeutic efficiency of CA compared to AmB, which agree with previous reports [8,9]. The efficiency of both forms of the antibiotic in the treatment of candidiasis in CBA mice was lower than in C57Bl/6 mice opposite to CBA mice by some functional parameters of the pituitary-adrenal and mononuclear phagocyte systems [2,3,5]. For instance, no infiltrates and granulomas were found in the liver of C57Bl/6 on day 56 of AmB treatment, while in animals treated with CA this effect was noted as soon as on day 28 [9].

On day 84 of the experiment, the total volume density of infiltrates and granulomas in untreated mice increased by more than 3 times compared to the corresponding parameter on day 56. In mice treated with antibiotics we also observed repeated growth of the number of infiltrates and granulomas, but is was less pronounced than in untreated mice, especially in case of CA treatment (Fig. 2). Since antibiotics were administered only during the first 20 days of the disease, it can be hypothesized that this duration of treatment was insufficient for complete elimination of the infectious agent; under these conditions, the yeast-like population of *C. albicans* was probably replenished at the expense of their micellar population. The period of repeated growth of infiltrates and granulomas coincided with further decrease in cortisol content in the blood, especially in mice treated with CA, which attests to clear-cut relationship between the intensity of can-

didiasis inflammation with changes in steroidogenesis. This fact one more time confirms the above assumption on the essential role of *C. albicans* metabolites in modulation of the functional state of the pituitary—adrenocortical system in CBA mice.

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