GENERAL PATHOLOGY AND PATHOPHYSIOLOGY

Effects of Modified Amphotericin in Experimental Systemic Candidiasis

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Lysosomotropic composition of dialdehyde dextran and amphotericin B had a greater therapeutic effect in mice with systemic candidiasis compared to free amphotericin B. This composition normalized glucocorticoid function of the adrenal glands and decreased the severity of liver destruction at late terms of granulomatous inflammation.

Key Words: *candidiasis; granulomatous inflammation; liver; glucocorticoids*

According to WHO reports, a variety of infectious diseases induced by opportunistic pathogens (*e.g.*, fungi) will dominate in the current century. The key elements of the pathogenesis of systemic mycoses are immunodeficiency and intracellular persistence of fungi in the vacuolar apparatus of macrophages forming granulomas, which considerably complicate drug therapy.

Polyenic antibiotics, including amphotericin B (AmB), are effective drugs for *C. albicans*. However, the use of AmB is limited due to high hepatotoxicity and nephrotoxicity. At the same time, lysosomotropic forms of drugs allow creation of effective concentration of these compounds at the site of persistence of the infectious agent (vacuolar apparatus of granuloma phagocytes) [5-7]. Taking into account these data, a lysosomotropic composition of AmB and dialdehyde dextran (DD) was developed by the method of radiation synthesis [8].

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Here we studied therapeutic activity and other effects of a DD+AmB composition in experimental animals with systemic candidiasis.

MATERIALS AND METHODS

Experiments were performed on 110 male C57Bl/6 mice obtained from the nursery of the Institute of Cytology and Genetics (Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk). The animals were divided into 4 groups. Group 1 mice (controls) received 0.2 ml isotonic NaCl. The mice of groups 2-4 were infected with *C. albicans* (single intraperitoneal injection of 2.5×10^9 microbial bodies per mouse, 0.2 ml) [3,4]. AmB (AKO Sintez) and DD+AmB were administered to group 3 and 4 mice with candidiasis, respectively, starting from the 1st day after infection. The animals received 10 intraperitoneal injections of the test compounds at 1-day intervals (250 U/kg antibiotic, 0.5 ml). The samples were taken on days 10, 28, and 56 after infection.

Liver samples were examined under a light microscope: the numerical density of granulomas and total volume density of destruction zones (necrotic

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and dystrophic hepatocytes) were evaluated [6]. Cortisol concentration in blood plasma and adrenal glands (AG) was measured by radioimmunoassay [2].

The differences between mean values were estimated by analysis of variance, Student's *t* test, and Mann—Whitney test [1].

RESULTS

Macrophage granulomas were mainly detected in the liver of mice 10 days after infection. They were also found in the lungs and lymph nodes under similar experimental conditions [4]. These changes attest to the development of systemic inflammation. Zones of hepatocyte dystrophy and necrosis were found in the greater part of these structures. The number of granulomas increased by 1.3 times on day 10 after infection, but 2-fold decreased by the 56th day (Fig. 1, *a*). Macrophage-epithelioid cell granulomas prevailed in the last two periods of the study. Volume density of destructive changes in the liver parenchyma progressively increased (Fig. 1, *b*). Blood cortisol concentration in *C. albicans*-infected mice was low in all periods of the study. Cortisol concentration in AG tended to decrease in these animals (Fig. 2).

On days 10 and 28, numerical density of hepatic granulomas in group 3 mice did not differ from that in group 2 animals. By the 56th day, only few mice had hepatic granulomas (Fig. 1, *a*). Volume density of zones of dystrophy and necrosis in the liver parenchyma increased on days 10 and 28. By the 56th day, this parameter in mice of the treatment group was 20% lower than in untreated animals (Fig. 1, *b*).

In group 3 mice blood cortisol concentration tended to increase on days 10 and 28 after infection, but decreased by the 56th day (3-fold lower than in control animals; Fig. 2, *a*). Cortisol concentration in AG of these mice did not differ from the control in all periods of the study.

Treatment with DD+AmB decreased numerical density of mycotic granulomas and size of destruction zones in the liver parenchyma on day 10 after infection (by 4 and 1.2 times, respectively, compared to untreated animals; Fig. 1). At later terms, hepatic granulomas were found in only few animals. On days 28 and 56, total volume density of zones of dystrophy and necrosis in these mice was lower than in animals receiving AmB alone (by 2.3 and 1.4 times, respectively; Fig. 1, *b*). The data indicate that DD+AmB composition exhibits a greater therapeutic effectiveness and lower hepatotoxicity than AmB.

In group 4 mice blood cortisol concentration decreased 10 days after infection and was minimum on day 28. By the 56th day, blood cortisol concentration in these mice increased and did not differ from the control. Cortisol concentration in AG of group 4 mice tended to decrease on day 10 after infection, but returned to normal by the 28th day (Fig. 2).

Hence, administration of AmB to mice with systemic candidiasis prevents the development of hypocorticism at the early terms of inflammation. However, during the follow-up period (day 56) blood cortisol concentration in AmB-treated mice decreases and becomes lower compared to untreated animals. These changes probably result from enhanced metabolism and elimination of steroid hormones

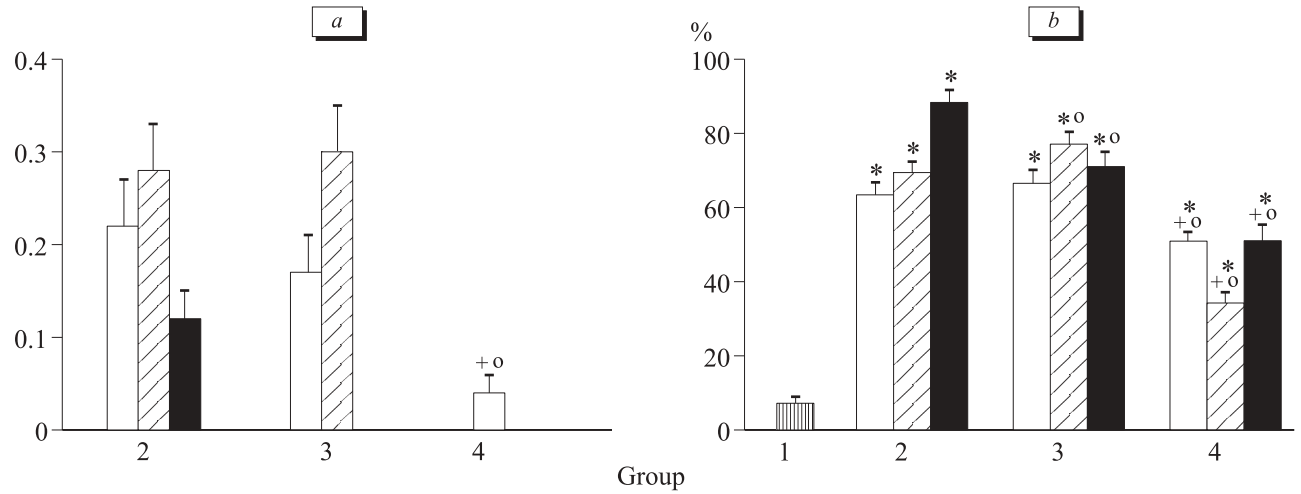

Fig. 1. Numerical density of granulomas (*a*) and volume density of zones of parenchymal dystrophy and necrosis (*b*) in the liver of mice with *C. albicans*-induced granulomatous inflammation during therapy with AmB and DD+AmB. (*a*) Ordinate, number of granulomas per 5.63×10^5 μ^2. Here and in Fig. 2: light bars, 10 days; shaded bars, 28 days; dark bars, 56 days. $p<0.05$: *compared to the control; +compared to group 2 mice; °compared to group 3 mice.

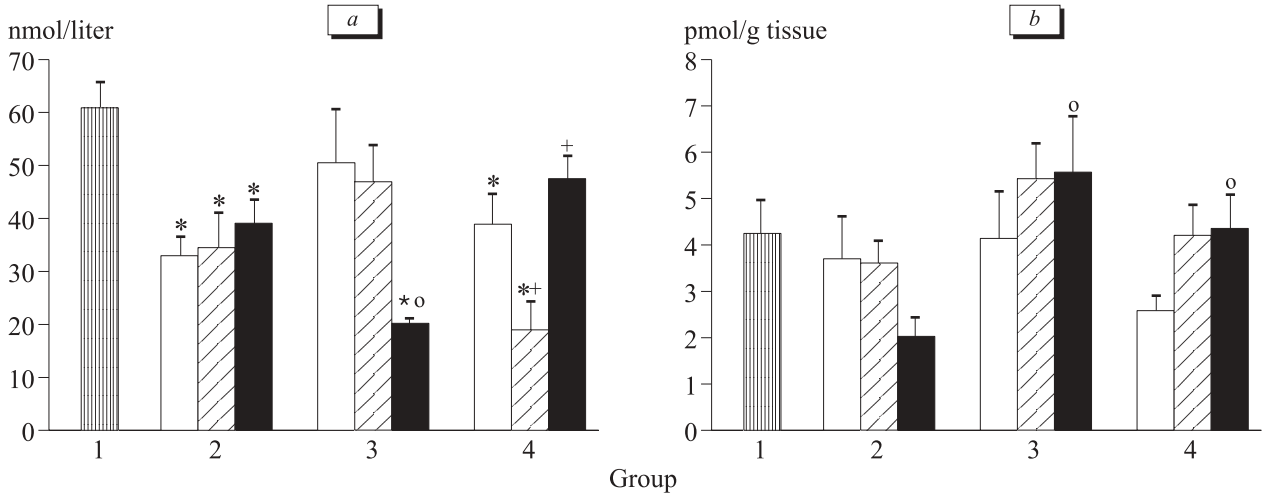

Fig. 2. Cortisol concentration in the blood (*a*) and AG (*b*) of mice with *C. albicans*-induced granulomatous inflammation during therapy with AmB and DD+AmB.

under conditions of damage to most hepatocytes due to decreased synthesis of transport proteins, transcortins, and albumin, maintaining certain concentration of non-metabolized cortisol in the blood. By contrast, administration of DD+AmB to mice with systemic candidiasis does not prevent the decrease in glucocorticoid function of AG during the initial period of the study, but promotes its recovery during the late period, which is consistent with lower severity of destructive processes in the liver of these animals. The data suggest that destructive processes in the liver parenchyma determined by persistence of *C. albicans* and toxic effect of AmB are associated with variations in blood cortisol concentration. The positive effect of therapy with DD+AmB on AG develops more slowly compared to that of AmB. The observed features are related to lysosomotropic activity, since the release of AmB from this complex after hydrolysis of the DD matrix takes longer time.

Our results indicate that administration of lysosomotropic composition DD+AmB to mice with systemic candidiasis promotes elimination of fungi (judging from the decrease in the number of granulomas). This composition is less hepatotoxic than AmB, which significantly increases the range of its therapeutic activity.

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