Effect of Lysosomotropic Form of Amphotericin B on Functional State of Phagocytes in Experimental Candidiasis

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Effect of antifungal preparation amphotericin B and its lysosomotropic composition with dialdehyde-dextran on functional state of phagocytizing cells in the dynamics of granulomatous inflammation induced by *C. albicans* was studied on CBA mice. A stimulating effect of amphotericin B on the production of reactive oxygen species by peritoneal and bone marrow phagocytes was observed, while lysosomotropic form of the antibiotic did not stimulate generation of oxygen radicals.

Key Words: *Candidiasis; granulomatosis; phagocytes; chemoluminescence; lysosomotropic amphotericin B preparation*

Therapy of patients with systemic mycosis remains a complex problem of modern medicine. Clinical studies showed that systemic mycoses develop in immunocompromised individuals, after surgical interventions, and against the background of chemotherapy in individuals with oncological diseases or transplanted organs [4,5,13].

Fungi often induce granulomatous inflammation (GI). The course of GI largely depends on functional state of macrophages [3]. The therapy of mycotic infections is complex because of possible persistence of fungi in macrophages, which modifies functional state of macrophages and complicates the delivery of drugs to the infectious agents. It is well known that the drugs used for the treatment of fungal diseases exhibit not only antimycotic activity, but also produce toxic effect on cells of the body [9,12]. In light of this, targeted delivery of drugs to macrophages in granulomas is the most perspective treatment strategy.

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Here we evaluate the effect of antimycotic drugs amphotericin B (AmB) and its lysosomotropic composition with dialdehyde dextran (CA) on functional state of phagocytes in the dynamics of experimental *C. albicans*-induced GI.

MATERIALS AND METHODS

The study was performed on 81 CBA mice aging 2 months and weighing 20-22 g (nursery of the Institute of Cytology and Genetics, Siberian Division of Russian Academy of Medical Sciences, Novosibirsk). Experimental visceral candidiasis was modeled by single intraperitoneal injection of *C. albicans* (2.5×10^9 microbial bodies per mouse in 0.2 ml isotonic NaCl) [5]. The mice were then treated with AmB (Sintez) and CA obtained by radiation oxidation of dextran [7]. The preparations were injected in a dose of 250 U AmB per 1 kg body weight.

The animals were divided into 4 groups. Group 1 mice (controls) received 0.2 ml isotonic NaCl. Mice of groups 2-4 were infected with *C. albicans*. After 24 h, group 3 mice were treated with AmB and group 4 animals received CA. The course con-

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sisted of 10 injections every other day. Group 2 mice received no treatment. The material for the study was obtained on days 10, 28, 42, 56, and 84 after GI induction.

Peripheral blood, peritoneal lavage fluid, and bone marrow were analyzed [1]. The total number of leukocytes in the blood, peritoneal lavage fluid, and bone marrow was counted in a Goryaev chamber. The cells were counted on cytological preparations stained after Romanovskii—Giemsa.

Functional state of phagocytes in the blood, peritoneal lavage fluid, and bone marrow was evaluated by the method of luminol-dependent chemoluminescence (CL) [14]. CL intensity was measured on a biochemiluminometer SKIF-0301 (Nauka). The total CL response of phagocytes was used as the measure of functional activity of phagocytes; it was expressed as the number of light pulses emitted by the cell over 20 min.

The significance of differences between the means was evaluated using Mann—Whitney test after preliminary Kruskal—Wallis dispersion analysis. The probability of validity of the null-hypothesis was accepted at 5% significance level.

RESULTS

In group 2 mice, the CL response of phagocytes from the blood, peritoneal lavage fluid, and bone marrow gradually increased in the dynamics of GI, attained maximum on days 28, 42, and 56, and then decreased by day 84 (Table 1). Changes in functional activity of phagocytizing cells of the peripheral and central compartments of the mononuclear phagocyte system attest to wave-form and long-lasting course of GI, which was confirmed by morphological analysis [6].

Production of reactive oxygen forms by blood phagocytes increased on day 56 in infected mice treated with AmB (group 3), but not in animals receiving CA (group 4). On day 84 of observation, the CL response of blood phagocytes surpassed the corresponding parameter in group 2 animals by 1.9 times.

In group 3 and 4 mice, the CL response of bone marrow phagocytes on day 42 decreased compared to the corresponding parameter in group 2 mice (by 8.6 and 6.0 times, respectively). On day 56, the CL response of bone marrow phagocytes in both treatment groups increased 2-fold compared to group 2 (Table 1). It should be noted that changes in the level of oxygen radicals produced by bone marrow phagocytes in mice receiving CA were less pronounced than in mice treated with AmB. At the same time, the CL responses of peritoneal phagocytes in group 3 animals on days 10, 28, and 56 were higher than in group 2 mice by 2.1, 1.9, and 1.8 times, respectively. In group 3 mice, the CL response of peritoneal phagocytes on days 10 and 84 was higher than in group 4 animals by 2.1 and 1.7 times, respectively.

TABLE 1. CL-Response of Phagocytes from the Blood, Peritoneal Lavage Fluid, and Bone Marrow of CBA Mice with Experimental *C. albicans*-Induced GI Treated with AmB and CA (*M*±*m*)

Time of observation, day	Group	Total CL-response of phagocytes, pulses/liter/20 min		
		blood	peritoneal lavage fluid	bone marrow
10	group 2 (6)	241.6±60.7	43.1±4.9	69.9±16.4
	group 3 (6)	234.9±48.2	90.1±6.7*	56.0±8.7
	group 4 (6)	377.9±49.4.	42.3±7.9^{+}	43.8±6.7
28	group 2 (5)	375.8±57.1	145.7±19.9	435.5±111.9
	group 3 (7)	498.0±104.6	298.0±36.5*	496.8±80.1
	group 4 (6)	514.5±92.5	209.6±31.8	517.7±44.9
42	group 2 (5)	226.4±43.1	158.2±21.3	316.9±59.0
	group 3 (5)	390.8±64.0	150.5±21.4	36.6±7.5*
	group 4 (5)	278.2±45.8	135.1±24.0	52.7±14.5*
56	group 2 (5)	373.5±54.5	130.8±15.5	105.4±9.3
	group 3 (5)	398.0±36.4	229.2±25.4*	386.8±96.8*
	group 4 (5)	230.9±26.5^{+}	122.5±48.8	205.9±71.5
84	group 2 (5)	279.9±37.6	129.8±23.5	59.0±11.9
	group 3 (5)	544.1±94.3*	106.6±18.9	71.8±22.3
	group 4 (5)	373.3±54.6	61.0±6.1*$^{+}$	43.1±4.6

Note. Number of animals was shown in parentheses. $p<0.05$ compared to: *group 2, $^{+}$group 3.

Some authors showed that AmB produces a systemic anti-inflammatory effect realized via damage to cell membranes and induction of anti-inflammatory cytokine production (TNF-α and IL-β).

The anti-inflammatory effect of AmB on phagocytes in the course of GI development can determine increased production of reactive oxygen species [2]. Comparison of the effect of AmB and CA on functional state of phagocytes showed that CA less markedly increased the production of oxygen radicals by phagocytes, which can be explained by prolonged release of the antibiotic from dialdehyde-dextran matrix.

Thus, CA, in contrast to usual form of AmB, did not increase the production of reactive oxygen species by phagocytes of different compartments of the mononuclear phagocyte system. The dialdehyde-dextran component of the composition potentiated the anti-inflammatory effect of AmB.

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