Granulomatous Inflammation in the Lungs of Mice with Systemic Candidiasis Receiving a Composition of Amphotericin B and Dialdehyde Dextran

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A composition of amphotericin B and dialdehyde dextran was used for the therapy of male C57Bl/6 mice with systemic candidiasis. The composition was more effective than free amphotericin B. A decrease in the number and size of candidal granulomas in the lungs was more significant after therapy with the study composition (compared to free amphotericin B).

Key Words: *systemic candidiasis; lungs; amphotericin B; dialdehyde dextran*

The incidence of systemic mycoses is high in newborns, children [2], and oncology patients [5]. *C. albicans* fungi are most frequently identified [4]. Candidal inflammation of the lungs is manifested in granulomatous inflammation (GI) and occupies the first place in candidal damage to internal organs [4]. GI during candidiasis is related to persistence of yeast-like *C. albicans* in the vacuolar apparatus of macrophages [8,12], which makes then poorly accessible for drugs, including amphotericin B (AmB), which has several advantages over other preparations of similar action [3]. Therapy with antimycotic drugs that exhibit tropism for the vacuolar-lysosomal apparatus of phagocytes (site for persistence of *C. albicans* fungi) is pathogenetically substantiated [6].

Here we studied the type of GI in the lungs of mice with systemic candidiasis receiving a composition of AmB and dialdehyde dextran (CAD).

MATERIALS AND METHODS

CAD was synthesized by conjugation of AmB and dialdehyde dextran matrix (radiochemical synthesis). Dextran was oxidized by irradiation with an accelerated electron flow [10]. Dialdehyde dextran was obtained from dextran with a molecular weight of 30-40 kDa [4,8,9].

Experiments were performed on male C57Bl/6 mice aging 2 months, weighing 20-22 g, and obtained from the nursery of the Institute of Cytology and Genetics (Siberian Division of the Russian Academy of Sciences, Novosibirsk).

Systemic candidiasis was induced by intraperitoneal injection of a 1-day-old culture of *C. albicans* (single dose 2.5×10^9 microbial bodies) in 0.2 ml 0.9% aqueous solution of NaCl [7,8].

The animals were divided into 3 groups (10 animals per group). The control group (group 1) consisted of untreated mice, which received a 1-day-old culture of *C. albicans*. AmB in a dose of 250 U/kg was dissolved in 0.2 ml 5% aqueous solution of glucose and injected intraperitoneally to group 2 mice on day 1 after infection with *C. albicans*. The animals received 10 injections of AmB at 1-day intervals. Group 3 animals were intraperitoneally injected with the same dose of CAD on day 1 after *C. albicans* infection.

The samples were obtained on days 10, 28, and 56 after infection. Lung samples were fixed in 10% neutral formalin, dehydrated with alcohols of increasing concentrations, and embedded into paraffin. Histological sections (5-6 μ) were stained with hematoxylin and eosin [1]. The numerical density of granulomas and infiltrates was estimated in

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TABLE 1. Numerical Density and Diameter of Granulomas in the Lungs of C57Bl/6 Mice during Experimental Candidiasis and Therapy with AmB and CAD (%, *M*±*m*)

Day of experiment	Group 1		Group 2		Group 3	
	numerical density	diameter, μ	numerical density	diameter, μ	numerical density	diameter, μ
10	3.20±0.30	23.61±0.22	2.80±0.20	23.50±0.22	3.31±0.20	19.71±0.20*
28	5.41±0.50^{o}	23.10±0.22	4.22±0.41^{o}	23.01±0.22	3.40±0.20^{*+}	7.28±0.61^{*+}
56	5.81±0.50	23.71±0.22	3.71±0.33*	23.62±0.23	2.40±0.21^{*+o}	7.04±0.61*

Note. Here and in Table 2: $p<0.05$ *compared to group 1, $^{+}$compared to group 2, ^{o}compared to previous experiment.

a closed test area of 4.62×10^5 μ^2. The diameter of granulomas was measured. We estimated the relative number of neutrophils, macrophages, epithelioid cells, eosinophils, lymphocytes, and fibroblasts in granulomas. The total number of cells in granuloma was taken as 100%.

The significance of differences between the mean values was estimated by Student's *t* test. These differences were significant at $p<0.05$.

RESULTS

Histological study of lung samples from group 1 mice revealed the following signs of suppurative-productive candidal pneumonia [6]: plethora, interstitial edema, degeneration of the bronchial epithelium, perivascular granulomas and infiltrates, and infiltration of interalveolar septa with inflammatory cells. Macrophages, neutrophils, and eosinophils prevailed in candidal granulomas. We identified only individual epithelioid cells and lymphocytes. Similar infiltrates and granulomas were found in the liver, lymph nodes and, to a lesser extent, in the kidneys. These signs reflect the development of systemic candidiasis.

Morphometry revealed progression of candidal GI in the lungs of untreated mice. This conclusion was derived from a 1.7-fold increase in the number

TABLE 2. Cellular Composition (%) of Pulmonary Granulomas in C57Bl/6 Mice during Experimental Candidiasis and Therapy with AmB and CAD (%, *M*±*m*)

Group	Parameter, %	Period, days		
		10	28	56
1	Monocytes/macrophages	64.30±0.31	62.90±0.40	52.31±0.30^{o}
	Neutrophils	22.37±0.60	23.80±0.60	34.01±0.60^{o}
	Eosinophils	11.21±0.10	12.11±0.10	11.16±0.10
	Lymphocytes	1.32±0.10	0.40±0.20^{o}	0.10±0.10
	Epithelioid cells	0.80±0.08	0.80±0.06	0.94±0.20
	Fibroblasts	—	—	1.48±0.10
2	Monocytes/macrophages	64.01±0.51	54.61±0.32^{*o}	51.92±0.43^{o}
	Neutrophils	23.12±0.19	11.40±0.08^{*o}	5.80±0.01^{*o}
	Eosinophils	8.17±0.12*	1.30±0.08^{*o}	1.40±0.10*
	Lymphocytes	1.50±0.80	1.60±0.01	1.60±0.10*
	Epithelioid cells	3.20±0.08	24.08±0.03^{*o}	26.14±0.05^{*o}
	Fibroblasts	—	7.01±0.13	13.14±0.10^{*o}
3	Monocytes/macrophages	24.82±0.46	18.40±0.21^{*+o}	9.30±0.91^{*+o}
	Neutrophils	9.21±0.01^{*+}	4.10±0.38^{*+o}	1.30±0.36^{*+o}
	Eosinophils	1.70±0.11^{*+}	0.70±0.10^{*+o}	0.30±0.10^{*+}
	Lymphocytes	1.60±0.80	1.40±0.10	1.94±0.10
	Epithelioid cells	38.62±0.21^{*+}	47.20±0.37^{*+o}	53.31±0.09^{*+o}
	Fibroblasts	24.05±0.01*	28.20±0.37*	33.85±0.41^{*+o}

of pulmonary granulomas on day 56 (Table 1). The diameter of granulomas slightly decreased by the 56th day (Table 1). Therapy with free AmB and CAD was followed by a decrease in the number of candidal granulomas. CAD was more efficient in this respect. The numerical density of granulomas in group 3 mice decreased more significantly than in group 2 animals (by 46%, Table 1). The size of granulomas in group 3 mice decreased by 3 times on day 28 (Table 1). This effect persisted by the 56th day. Hence, the effect of CAD therapy was observed even on day 56 (delayed period after antimycotic treatment). The size of granulomas decreased with death of *C. albicans* in macrophages and reduction of the chemoattractant gradient in granulomas [11]. These changes provided the conditions for cell migration from granulomas. The cells were probably presented by monocytes and macrophages, which dominated in granulomas of untreated animals during all periods of the study (Table 2). Neutrophils ranked second in abundance in granulomas. Therefore, candidal granulomas differ from granulomas induced by *M. tuberculosis* of BCG vaccine and characterized by the predominance of epithelioid cells [9]. The ratio of neutrophils decreased, while the number of epithelioid cells and fibroblasts increased with increasing in the duration of therapy with both drugs (Table 2). The lower was the number of pulmonary granulomas, the greater was the increase in cell count in granulomas (Table 1, 2).

We conclude that the use of AmB preparations for the therapy of animals with systemic candidiasis was accompanied by changes in the type of lung inflammation. Untreated mice had suppurative-productive inflammation. Treated animals were mainly characterized by productive GI, whose severity depended on antimycotic activity of the drugs. CAD had greater antimycotic efficacy in the lungs of mice with systemic candidiasis. This conclusion was derived from morphologically verified decrease in the number and size of granulomas. The increase in productive processes in candidal granulomas and decrease in the exudative component of inflammation probably reflect progressive reparation. Taking into account the role of the lungs in gas exchange and state of connective tissue structures in the lung interstitium, these changes can be considered as a positive result of therapy.

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