Therapeutic Efficacy of a Composition of Amphotericin B and Dialdehyde Dextran in Kidney Damage in Mice of Various Strains with Systemic Candidiasis

A. A. Pristavka*, A. P. Nadeev*, M. A. Travin*, and V. A. Shkurupiy

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The therapeutic efficacy of a composition of amphotericin B and dialdehyde dextran was much higher than that of amphotericin B in the therapy for systemic candidiasis. This conclusion was derived from the earlier and progressive decrease in the number and size of candidal granulomas in the kidneys. The composition of amphotericin B and dialdehyde dextran was more potent than amphotericin B in decreasing the nephrotoxic effect of *C. albicans*. The opposite strains of CBA and C57Bl/6 mice differed in morphological signs of granulomatosis in the kidneys, but not in the nephrotoxic effect of *C. albicans* metabolites.

Key Words: *candidal granulomatosis; kidneys; amphotericin B; dialdehyde dextran*

Highly efficient polyenic antibiotic amphotericin B (AmB) is extensively used in clinical practice for the therapy of systemic candidiasis. This drug decreases the mortality rate of patients by several times [4,5]. AmB in therapeutic doses has cumulative properties, which is manifested in strong hepatotoxicity and nephrotoxicity [4,14]. The variability of these symptoms is genetically determined [8]. The composition of AmB and dialdehyde dextran (CAD) has lysosomotropic activity, exhibits a strong therapeutic effect on the liver and lymph nodes in mice with systemic candidiasis, and reduces the severity of destructive processes in organs [11,13].

The therapeutic efficacy and nephrotoxicity of CAD were studied in biologically opposite mice (CBA and C57Bl/6) with systemic candidiasis.

Research Center of Clinical and Experimental Medicine, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk; *Novosibirsk State Medical University, Russian Ministry of Health, Russia. ***Address for correspondence:*** nadeevngma@mail.ru. A. P. Nadeev

MATERIALS AND METHODS

Experiments were performed on male CBA and C57Bl/6 mice aging 2 months, weighing 20-22 g, differing in several genetically determined parameters, and exhibiting opposite reactions to various infectious agents [2,3,8]. The animals were obtained from the nursery of the Institute of Cytology and Genetics (Siberian Division of the Russian Academy of Sciences, Novosibirsk). The mice were divided into 4 groups (10 animals per group) each. Group 1 animals received one injection of a 1-day-old culture of *C. albicans* (2.5×10^9 microbial bodies intraperitoneally) in 0.2 ml isotonic aqueous solution of NaCl [7]. AmB in a dose of 250 U/kg was dissolved in 0.2 ml 5% glucose and injected intraperitoneally to group 2 animals on day 1 after infection with *C. albicans* in the same dose. Group 3 animals were intraperitoneally injected with 250 U/kg CAD in 0.2 ml dialdehyde dextran on day 1 after *C. albicans* infection. The animals received 10 injections of AmB and CAD at 1-day intervals. Group 4 included intact animals of each strain.

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CAD was synthesized at the Institute of Nuclear Physics (Siberian Division of the Russian Academy of Sciences, Novosibirsk). Dextran with a molecular weight of 30-40 kDa was subjected to radiation oxidation and mixing with AmB. The composition consisted of free or dialdehyde dextran-bound AmB. It was obtained by radiochemical oxidation of dextran.

The kidneys were isolated on days 10, 28, and 56 after infection of animals. The mice were killed by cervical dislocation under ether anesthesia. The samples were fixed in 10% neutral formalin, dehydrated with alcohols of increasing concentrations, and embedded into paraffin. Histological sections (5-7 μ) were stained with hematoxylin and eosin and PAS reagent [1]. The numerical density of granulomas in renal tissue was estimated morphometrically (test area 1.56×10^5 μ^2). Morphometric study was used to evaluate the volume density of destructed cells in the renal parenchyma (focal micronecroses; and degeneration of proximal tubular epitheliocytes, PTE).

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

RESULTS

Candidal inflammation in mice was manifested in the formation of macrophage/epithelioid cell granulomas and macrophage infiltrates in the liver, lungs, regional lymph nodes, and kidneys (systemic inflammation). Histological study of the kidneys from group 1 mice revealed severe plethora of renal glomeruli, decrease in the lumen of the Bowman's capsule, medium- and large-vacuolar degeneration of PTE, focal micronecroses of epitheliocytes, and desquamation of the brush border in PTE (Fig. 1). Epithelioid cell granulomas, macrophage granulomas, and interstitial infiltrates were found in CBA mice, while only solitary granulomas were found in C57Bl/6 mice. On days 28 and 56, only solitary macrophage granulomas and epithelioid cell granulomas were seen in the kidneys of group 2 CBA mice, in C57Bl/6 mice no granulomas were found. Plethora of renal glomeruli, interstitial edema, small-vacuolar degeneration of PTE, and focal micronecroses of epitheliocytes were found in the kidneys of group 3 CBA and C57Bl/6 mice. Individual macrophage infiltrates were revealed in CBA mice, but not in C57Bl/6 mice. During systemic candidiasis, the severity of granulomatous inflammation in the kidneys is much lower than in other organs [11,13]. It is probably related to lower content of resident macrophages in the kidneys (sites of granuloma formation).

On day 10 after infection, the numerical density of renal granulomas in CBA mice of group 3 was 3-fold lower than in group 1 animals (0.22±0.03 and 0.60±0.07, respectively). The numerical density of renal granulomas in CBA mice of group 2 (0.32±0.04) was 50% higher than in group 3 animals. Since macrophage granulomas and infiltrates were rare in C57Bl/6 mice (even in group 1 specimens), morphometric data were not used for statistical analysis. However, previous experiments showed that the number of candidal granulomas in the liver is high in animals of this strain. During spontaneous inflammation, the number of granulomas on day 56 was 2-fold lower than on day 10 after infection [13]. The concentration of resident macrophages significantly differs in various organs. This parameter reaches maximum in the liver. Moreover, CBA and C57Bl/6 mice differ by the number of Kupffer cells in the liver under normal conditions (higher content in C57Bl/6 mice) and during infection with *M. tuberculosis* [3]. After intraperitoneal infection with the culture of *C. albicans*, the pathogenic agent is probably engulfed by resident macrophages in the liver and lungs (but not in the kidneys). These features probably contribute to low number of sites for granuloma formation in the kidneys (fungus-engulfing resident macrophages).

The volume and dynamics of PTE destruction (degeneration and necrosis) in CBA and C57Bl/6 mice were similar during all periods. Moreover, no intergroup differences were found in animals receiving CAD and AmB (Table 1).

Our findings attest to high toxicity of *C. albicans* metabolites. The toxic effects of these metabolites are manifested in destructive processes in various organs [11,13] and not associated with the presence and number of granulomas. They are probably related

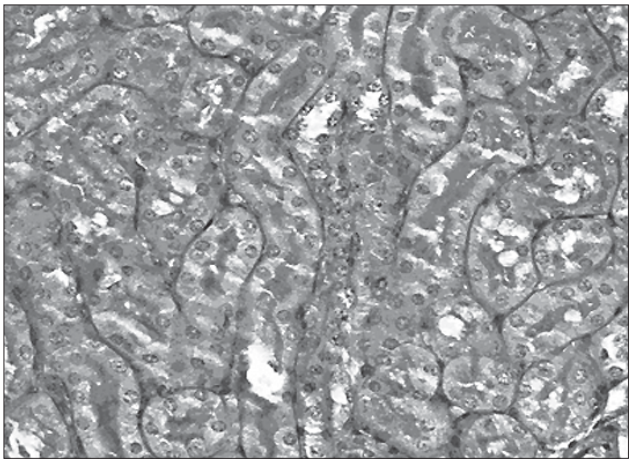

Fig. 1. Medium- and large-vacuolar degeneration of PTE; and desquamation of the brush border in PTE. PAS staining, ×400.

TABLE 1. Volume Density of Destructive Changes (Degeneration and Necrosis of PTE) in the Kidneys of CBA and C57Bl/6 Mice during Experimental Systemic Candidiasis and Therapy with AmB and CAD (*M*±*m*)

Group	Mouse strain	Period, days		
		10	28	56
1	CBA	64.37±1.84[a]	58.65±1.44	47.77±0.80
	C57B1/6	60.88±3.46[a]	58.47±2.08	49.98±1.70
2	CBA	55.55±2.02*[a]	50.96±0.84*	37.83±0.79*[+]
	C57B1/6	54.68±2.59[a]	48.16±1.79*	43.26±1.38
3	CBA	31.73±1.06*[ab]	34.91±0.79*[ab+]	31.78±0.65*[b]
	C57B1/6	29.63±2.06*[ab]	25.10±1.35*[b]	30.76±1.52*[b]
4	CBA	8.54±0.23	—	—
	C57B1/6	11.01±0.64	—	—

Note. $p<0.05$: *compared to group 1; [a]compared to group 4; [b]compared to group 2; [+]compared to C57Bl/6 mice.

to the influence of fungal metabolic products and manifested in modulation of steroidogenesis [10,12]. In our experiments, AmB did not exhibit nephrotoxicity. The volume of destructive changes in AmB-receiving mice of both strains did not increase, but even decreased compared to that in group 1 animals (Table 1). Similar results were obtained in the study of destructive changes in the liver [13] and lymph nodes of these animals [11]. CAD therapy in mice of both strains was associated with a decrease in the volume of destructive changes in renal PTE during all periods of the study (by 25-50%, Table 1).

We conclude that the therapeutic efficacy of CAD is much higher than that of AmB. It is manifested in a significant decrease in the number and size of *C. albicans*-induced granulomas in group 3 CBA mice compared to group 2 animals. These features reflect the gradient of chemoattractants, which is induced by the pathogen and revealed in granuloma cells. The reduction of destructive changes in CAD-receiving animals did not result from the decrease in AmB toxicity, but was associated with fungal elimination from granuloma cells of other organs. [11,13]. The concentration of *C. albicans* in these organs was higher before the start of therapy. Hence, the toxic effect of *C. albicans* decreased under these conditions. The severity of granulomatous inflammation is strongly determined by genotypic characteristics. They contribute to specific structural and functional parameters of organs and systems, which play a role in granuloma formation during systemic candidiasis.

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