Morphological Changes in the Brain of Mice with Systemic Candidiasis Treated with Composition of Amphotericin B and Oxidized Dextran

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We observed morphological manifestation of encephalitis 3, 7, 10 and 28 days after intravenous infection of adult male CBA mice with *Candida albicans*. Compounds were administered intraperitoneally every other day starting from the next day postinfection. Untreated animals (100%) died over the period between days 18 and 20 postinfection; 60% animals receiving oxidized dextran alone survived by day 28 of observation. All animals treated with amphotericin B and composition of amphotericin B and oxidized dextran survived. On day 3 postinfection, the count of macrophage infiltrates and granulomas in the cerebral interstitium of mice treated with amphotericin B was equal to that in untreated mice, but was sufficiently lower in animals treated with the composition or oxidized dextran alone. On day 10, this index was similar in all groups and was approximately 5 times lower than in untreated animals on day 3. On day 28, macrophage infiltrates and granulomas were absent in the brain of all treated mice. These data suggest that oxidized dextran produced a therapeutic effect, which manifested earlier than the effect of amphotericin B and potentiated its effect, probably due to its competition with *Candida albicans* for mannose receptors on the brain-blood barrier endothelium.

Key Words: *brain-blood barrier; Candida albicans; amphotericin B; oxidized dextran; composition of amphotericin B and oxidized dextran*

High incidence of mycoses caused by *Candida albicans* in 78-85% cases is recorded in oncological clinics as a consequence of intensive chemotherapy programs and in infant intensive care units, probably due to high level of mannose receptor expression during the first postnatal week [6], which provides adhesion and translocation of the infectious agent. Drug transport across the blood-brain barrier (BBB) is also a problem in the treatment of mycoses. Higher efficacy of a composition (C) consisting of amphotericin B (amphB) and oxidized dextran (OD) compared to amphotericin alone, in the treatment of systemic candidiasis was previously shown [2,3,11,14].

The aim of this study is to investigate structural changes in the brain of mice with generalized candidiasis after treatment with C.

MATERIALS AND METHODS

The study was performed on 170 male CBA mice weighing 20-25 g (groups 5), obtained from the nursery of the Institute of Cytology and Genetics (Siberian Division of the Russian Academy of Sciences, Novosibirsk). The animals were kept on a standard laboratory diet with free access to water and food. Generalized

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candidiasis was modeled in mice of groups 2, 3, 4 and 5 by single injection of a 1-day-old high-virulent culture of *C. albicans* (strain RKPGY-1129/13, obtained from Institute of Medical Mycology of St-Petersburg Medical Academy of Postgraduate Studies) in a dose 12.5×10^6 microbial bodies in 0.5 ml isotonic NaCl solution in the caudal vein. Group 1 mice (non-infected and untreated mice) received single intravenous injection of 0.5 ml isotonic NaCl. Group 2 mice were not treated. Group 3 animals received amphB in a dose 250 ml U/kg in 0.2 ml 5% glucose solution, group 4 received 0.2 ml oxidized dextran with a molecular weight of 35-45 kDa [13], group 5 mice received C prepared as described previously [13] in doses given for groups 3 and 4. All substances were administered intraperitoneally starting from the next day postinfection; the course consisted of 10 injections on every other day. Ten mice from each group were used for morphological examination. The animals were sacrificed by cervical dislocation under ether anesthesia. Brain samples for light microscopy (5-mm frontal sections from the parietal region) obtained on day 3, 7, 10, 28 postinfection were fixed in 10% neutral formalin, dehydrated in ascending alcohols, and embedded in paraffin. Sections (5 μ) were prepared on Microm rotation microtome, stained with hematoxylin and eosin, Schick reagent (detection of fungi), and by the method of Nissl (glia visualization). Immunohistochemical reaction with monoclonal antibodies to GFAP (Abcam) followed by staining with Mayer hematoxylin was performed in order to reveal astrocyte bodies and processes. Examination of brain sections was done using AxioImager A1 light microscope. Morphometry was performed using AxioVision 4.7 software and 100-point ocular grid. We determined volume density (Vv) of transversely and tangentially cut blood vessels per test area of cerebral tissue 6.76×10^6 μ^2, pericellular and perivascular edema regions, astrocytes with processes, neurons, and numerical density of infiltrates and granulomas. Significance of differences between the means was evaluated by Student's *t* test, the differences were significant at $p<0.05$.

RESULTS

All mice infected with *C. albicans* and receiving no treatment died during the period between days 18 and 20 postinfection. Among OD-treated animals 40% died by day 28 postinfection. Macrophage infiltrates and granulomas with *C. albicans* inside them were found in the brain since day 3. On day 3 of amphB therapy, the number of granulomas (major manifestation of visceral mycoses) did not significantly differ from the group of untreated animals, but this index was significantly lower in mice treated with OD and C (Fig. 1). On day 7 postinfection, granuloma concentration in untreated animals did not significantly differ from the previous term, but it decreased several times in amphB-, OD-, and C-treated animals and no significant differences between groups were observed. On day 10, granuloma count was equally low in all groups, which determined high variability of this parameter within the groups (Fig. 1), and by day 28 they were not observed in survivors (Fig. 1).

Our findings suggest that the therapeutic effect of amphB was not yet realized on day 3, as was seen from the number of infiltrates and granuloma in the brain, whereas OD, with consideration for mortality rate and granulomas concentration, exhibited individual therapeutic effect, which manifested earlier and potentiated the action of amphB during combined treatment (Fig. 1).

It is known that *C. albicans* penetration across the blood-brain barrier is an obligatory condition for the development of candidal encephalitis; the main phases of this process are receptor-mediated adhesion to endothelium and transcytosis [7]. Cell wall of *C. albicans* contains glycosylated polysaccharides carrying mannose residues (N-mannose residues), so called mannanes [6,9], recognized by mannose receptors [15], while Toll-like receptor 4 binds O-bound mannosyl residues [4,10]. They participate in *C. albicans* adhesion to endothelial surface, probably BBB, along with other cell wall components. Thus, according to previous studies [5,8], mannose receptors were found on cerebral blood vessel endothelium. In addition, they are expressed on macrophages [8] actively phagocytizing

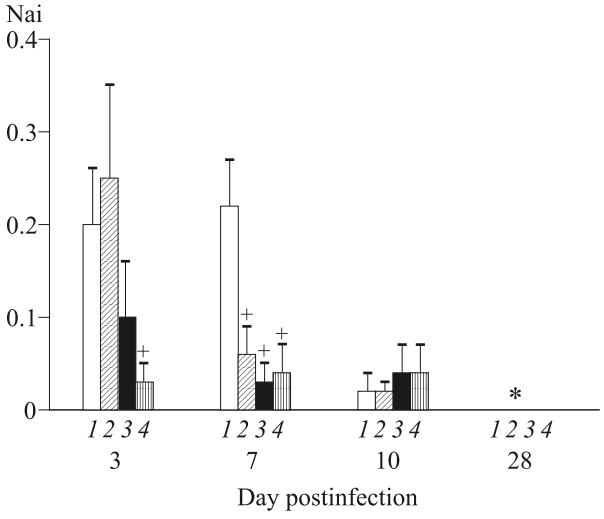

Fig. 1. Numerical density (Nai) of infiltrates and granulomas in the brain of male CBA mice with systemic candidiasis during treatment. *1*) untreated mice with systemic candidiasis; *2*) mice treated with amphB; *3*) treated with C; *4*) treated with OD. $^+p<0.05$ compared to *1*. Here an on Fig. 2: *granulomas were absent in all treated animals, untreated animals died during the period from day 18 to day 20 postinfection.

fungi. Endocytosis of dextrans actively captured by macrophages is also mediated by mannose receptors [8]. Thereby the positive results of OD and OD composition in our experiments are probably caused by OD competition with *C. albicans* for mannose receptors on the surface of BBB blood vessel endothelium. This can manifest in blockade of *C. albicans* adhesion to BBB endothelium and its subsequent transcytosis through BBB into the brain, and provide transcytosys of OD and amphB linked to it into the brain. The same phenomenon could reduce the intensity of macrophage migration across BBB in sites of BBB endothelium damage produced by *C. albicans* transcytosis under the effect of its phospholipases and proteinase activity. This could probably determine initially (on day 3) low level of macrophage infiltrates and granulomas in OD- or C-treated mice, because monocytes and macrophages, along with neutrophiles, are the major infiltrate- and granuloma-forming cells in candidiasis at the early stages. It could also be hypothesized that lower number of viable *C. albicans* captured by macrophages got into the brain, but they were more efficiently eliminated from the blood by macrophages activated by OD, which promotes phagocytosis completion at the stage of phagosome-lysosome fusion [1]. It should be noted that under similar experimental conditions, the concentration of infiltrates and granulomas and the therapeutic effect of C in the liver, lungs, kidney, and lymph nodes were sufficiently higher than in the brain, probably due to different conditions for blood vessel crossing by *C. albicans,* macrophages, and C [2,3,11,14].

We observed cerebral vessel plethora in all groups, which manifested in the blood vessel lumen enlargement, especially in untreated mice (Table 1); we observed desquamation of vascular endothelium (one of the pathways of *C. albicans* and phagocytes transmigration across BBB, which is not mediated by receptors) and fibrinoid swelling and necrosis of vascular walls. The changes of BBB blood vessel lumen together with changes mentioned above can be considered as an indicator of their damage and also of treatment success. Thus, volume density of blood vessels in mice, treated separately with amphB, OD or C, was

TABLE 1. Morphometry of Brain Tissue in Male CBA Mice after Hematogenic Infection with *C. albicans* and during Treatment (*M*±*m*)

Experimental conditions	Observation period, days	Volume density, % of area in the test system		Astrocytes
		blood vessel lumen	edema areas in the brain tissue	
Control (0.85% NaCl)		3.56±0.26	1.99±0.13	6.69±0.89
Candidiasis (untreated animals)	3	4.56±0.30^{+}	3.13±0.19^{+}	8.11±0.16
	7	4.03±0.18	3.63±0.18	8.68±0.28^{+}
	10	4.67±0.25^{+}	3.69±0.21^{+}	8.05±0.25
	28	—	—	—
Candidiasis+amphB	3	3.28±0.21*	2.59±0.16*	9.75±0.24^{+*}
	7	3.43±0.25	2.01±0.20*	9.78±0.23^{+*}
	10	3.40±0.19*	2.07±0.13^{+*}	9.85±0.65^{+*}
	28	3.52±0.19^{+}	2.64±0.14^{+}	8.95±0.40^{+}
Candidiasis+OD	3	2.80±0.20*	2.29±0.14*	9.71±0.21^{+*}
	7	2.74±0.18*	2.40±0.15*	10.15±0.24^{+*}
	10	3.48±0.24*	2.81±0.15^{+*}	9.90±0.23^{+*}
	28	3.01±0.22^{+}	2.72±0.16^{+}	10.26±0.29^{+}
Candidiasis+C	3	3.18±0.16*	2.17±0.11*	8.24±0.47
	7	2.86±0.18*	2.58±0.17*	8.73±0.42^{+}
	10	2.86±0.18^{+*}	2.40±0.11^{+*}	10.11±0.25^{+*}
	28	3.47±0.18^{+}	2.40±0.14^{+}	7.16±0.23

Note. $p<0.05$ compared to *untreated animals, $^{+}$control. Dash denotes 100% mortality on day 20 of the experiment.

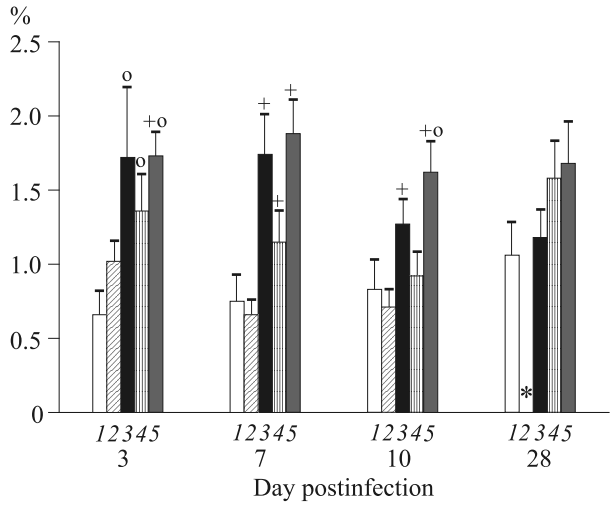

Fig. 2. Volume density (Vv) of cerebral neurons in male CBA mice with systemic candidiasis during treatment. *1*) non-infected mice (control); *2*) untreated mice with systemic candidiasis; *3*) mice treated with amphB; *4*) treated with C; *5*) treated with OD. $p<0.05$ compared to °*1*, +*2*.

lower than in untreated mice. The volume of edema (pericellular and perivascular), a consequence of blood vessel damage, was lower in treated animals (groups 3, 4, 5) on experimental day 10 by 44, 24, and 35% respectively. Edematous events in intact mice seemed to be produced by stress after administration of isotonic NaCl solution (Table 1).

Moreover, changes in the brain of mice infected with C. *albicans* on day 3 manifested in neuron degeneration and necrosis in all untreated mice. Volume density of these cells slightly increased, mainly due to processes of vacuolar degeneration in neurons, but the number of dead neurons was high at this term, and on days 7 and 10 neuronal death lead to decrease of their volume density (Fig. 2). All animals of this group died with neurological disorders by day 20. In mice treated with amphB, processes of vacuolar degeneration dominated in neurons on days 3 and 7; during two subsequent periods they were moderate, which had an impact on their volume density (Fig. 2). In mice treated with C, the intensity of degenerative processes rapidly decreased from day 3 to day 10, which led to a decrease in volume density of degeneration processes, but on day 28 it increased by 1.5 times compared to intact mice. This was probably associated with recovery of their functions, active OD capture and accumulation, which is theoretically not impossible, because neurons possess mannose receptors [5]. However, this phenomenon requires additional studies and other methodological approaches. In animals treated with only OD, the volume density of neurons was equally high at all observation terms (Fig. 2), probably due to active BBB crossing, OD capture and accumulation by neurons, presence of mannose receptors, and blockade of *C. albicans* binding with them, which also requires further investigation.

Astrocytes and their cytoplasmic processes carrying mannose receptors [12], regarded as BBB-forming structures, were enlarged in untreated animals because of vacuolar degeneration, which increased their volume density (Table 1). This index was increased in mice treated with amphB and OD alone, but in C-treated animals it did not significantly differ from the control by day 28 (Table 1). Generally, the increase in astrocyte volume density can also be considered as positive "BBB-strengthening" phenomenon, but processes produce changing this parameter can be determined both by pathogenic properties of *C. albicans* metabolites (degeneration) and by OD capture and accumulation in their vacuolar apparatus; this also requires further investigation.

Thus, our experiments demonstrated higher therapeutic efficacy of C compared to amphB in the treatment of systemic candidiasis, particularly candidal encephalitis, probably by means of OD blockade of mannose receptors on BBB endothelial cells, which could be a barrier for *C. albicans* and macrophages crossing BBB.

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