Therapeutic Efficacy and Hepatotoxicity of a Composition of Isoniazid and Dialdehyde Dextran Obtained by Radiochemical Oxidation of Dextran

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Tuberculous granulomas were found in all parenchymal organs of mice infected with mycobacteria of BCG vaccine. The number and size of hepatic granulomas decreased, while the count of degenerated and necrobiotic hepatocytes in infected animals increased 3 months after the start of therapy with a composition of isoniazid and dialdehyde dextran. The composition of isoniazid and dialdehyde dextran obtained by radiochemical oxidation of dextran had greater therapeutic efficacy and lower hepatotoxicity.

Key Words: *tuberculous granulomatosis; hepatotoxicity; isoniazid; dialdehyde dextran*

Tuberculous granulomatosis is induced by mycobacteria tuberculosis (MBT), which persist in the vacuolar apparatus of blood phagocytes and granulomas due to the incompleteness of phagosome-lysosome fusion. Hence, the pathogenic agent becomes insusceptible to the natural hydrolase system of phagocytes. The medicinal agents for therapy of this disease should have the following properties: tropism for the vacuolar-lysosomal apparatus of macrophages, intravacuolar prolongation, and ability to prevent the incompleteness of phagosome-lysosome fusion.

This composition was developed by conjugation of isoniazid (isonicotinic acid hydrazide, INAH) and dialdehyde dextran (DD), chemically oxidized dextran with a molecular weight of 30-40 kDa, and did not contain free INAH [4]. To simplify the method for preparation of this composition, we used INAH and radiochemically oxidized dextran with the same molecular weight. However, this composition was not purified from DD-unbound INAH [3]. The INAH/DD ratio was 40:60.

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Here we studied the therapeutic efficacy and hepatotoxicity of an INAH-DD composition obtained by radiochemical oxidation of dextran.

MATERIALS AND METHODS

Experiments were performed on 45 female 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). The animals fed a standard diet and had free access to water and food. Liver samples were obtained in the morning time. The animals were killed by cervical dislocation under ether anesthesia.

The liver was chosen as the object of the study because it is damaged in generalized tuberculosis and long-term polychemotherapy with the basic hepatotoxic drug INAH. Moreover, tuberculous granulomas consist of cells that belong to the mononuclear phagocyte system. Resident liver macrophages (Kupffer cells) constitute the major component of this system and serve as the site for granuloma formation.

The therapeutic efficacy was estimated from the number of granulomas, since granuloma formation depends on the chemoattractant gradient indu-

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ced by live MBT. It was assumed that the size of granulomas reflects the value of this gradient.

The animals received intraperitoneal injections of BCG vaccine (1 mg, Allergen) in 0.5 ml isotonic solution of NaCl. Group 1 mice (n=15) received no therapy. In group 2 and 3 animals, therapy with INAH preparations (50 mg/kg intraperitoneally) was started 2 weeks after infection and lasted for 3 months. The animals received 1 injection per 4 days. Group 2 mice received INAH. Group 3 mice received a composition of free INAH and INAH bound to DD by a strong chemical bond (40:60 ratio).

For morphological study, the liver was sampled after 3-month therapy. Liver samples were fixed in 10% aqueous solution of neutral formalin, dehydrated with alcohols of increasing concentrations, and embedded into paraffin. Liver sections (5-7 μ) were stained with hematoxylin and eosin and examined under a light microscope. We estimated the volume density of hepatocyte degeneration and necrosis, numerical density and size of BCG granulomas, and relative number of granuloma cells. The total number of granuloma cells was taken as 100%.

RESULTS

Tuberculous granulomas were found in all organs of MBT-infected animals, which reflects the development of generalized inflammation. The numerical density and size of BCG granulomas were highest in untreated animals (Fig. 1). INAH therapy had a strong therapeutic effect, which was manifested in a decrease in the number and size of BCG granulomas (by 100 and 22%, respectively, compared to untreated animals). Therapy with INAH-DD composition was followed by a more significant decrease in the number and size of BCG granulomas (by 2.8 times and 37%, respectively, compared to untreated animals; Fig. 1).

These data confirm the fact that the chemoattractant gradient is induced by live MBT. INAH in bacteriostatic doses caused death of some MBT, which was manifested in a decrease in the number and size of granulomas. These changes were probably associated with migration of macrophages and epithelioid cells from granulomas. No other processes could result in a decrease in the number and size of granulomas (necrosis and apoptosis). It should be emphasized that the decrease in the relative number of macrophages and monocytes was more significant than the increase in the relative number of epithelioid cells. The macrophage/monocyte/epithelioid cell ratio in mice of experimental groups was 1.0:0.3:0.4 and 1.0:1.5:1.9, respectively. Hence, the decrease in the number of monocytes and macrophages in granulomas of treated animals was not accompanied by an increase in the ratio of epithelioid cells. Our findings contradict the hypothesis that epithelioid cells are formed due to macrophage differentiation [2]. The role of epithelioid cell migration from granulomas remains unclear.

Examination of liver sections from mice of all groups revealed vacuolar degeneration or necrobiosis of some hepatocytes. Therapy with both preparations was accompanied by an increase in the volume density of these hepatocytes (Table 1). However, these parameters were much lower in mice receiving a composition of INAH and DD (Table 1). Three months after the start of therapy, the incidence of vacuolar degeneration and necrobiosis of hepatocytes in group 3 mice was lower than in group 2 animals (by 2.5 and 2.3 times, respectively).

Our results indicate that the composition of INAH and DD has several advantages over INAH.

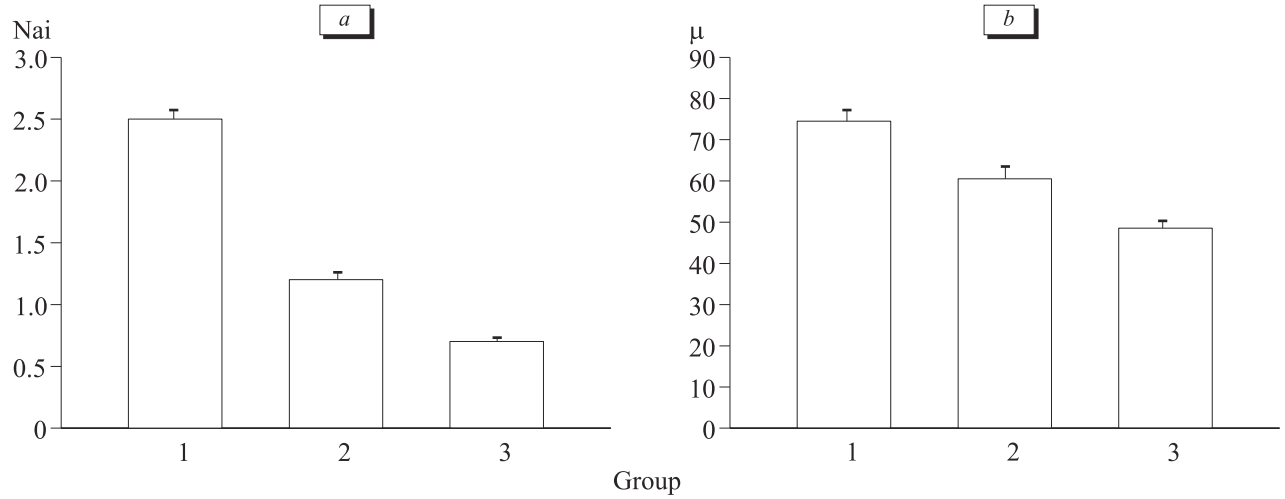

Fig. 1. Morphometry of BCG granulomas. Numerical density (*a*) and size of granulomas (*b*).

TABLE 1. Destructive Changes in Liver Parenchyma (% of Liver Section Area, *M*±*m*)

Group	Zones of degeneration			Zones of necroses		
	1 month	2 months	3 months	1 month	2 months	3 months
2	3.80±0.18	6.30±0.31	10.1±0.5^{+}	2.50±0.12	2.90±0.14	3.70±0.17
3	1.60±0.07*	2.60±0.11*	3.90±0.19*$^{+}$	0.70±0.03*$^{+}$	1.20±0.06*	1.60±0.08*

Note. $p<0.05$: *compared to group 2; $^{+}$compared to 1 month.

They are associated with lysosomotropism and intravacuolar prolongation of a DD matrix. This matrix serves as an INAH carrier at the site of MBT persistence. Moreover, DD contributes to the fusion of MBT-containing phagosomes and macrophage lysosomes [5]. These properties abolish the ability of pathogenic agents (MBT) to prevent the completeness of phagocytosis by macrophages [1,7,8]. Such mechanism of natural resistance in the host organism (mammals) and ability of MBT to suppress these processes were formed during coevolution.

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