Morphological Study of the Efficiency of Isoniazid and Dialdehyde Dextran Composition in the Treatment of Mice with BCG Granulomatosis

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Male BALB/c mice were intraperitoneally injected with BCG vaccine. After 1 month therapy was started: isoniazid or composition of isoniazid with dialdehyde dextran (CID) obtained by chemical and radiochemical methods. The therapeutic efficiency evaluated by the number granulomas in the liver and lungs and granuloma size was higher in mice treated with CID obtained by the radiochemical method in comparison with mice treated with isoniazid and with CID obtained by the chemical method. Hepatotoxicity evaluated by volume density of degenerative hepatocytes and necrotic zones was higher in mice treated with CID obtained by the radiochemical method than in animals treated with isoniazid, because CID contained free isoniazid and isoniazid bound to dialdehyde dextran.

Key Words: *BCG granulomatosis; isoniazid; dialdehyde dextran; liver; lungs; hepatotoxicity*

By its prevalence, risk for patient's life, and social significance tuberculosis ranks one of the leading diseases among those the morphologically manifesting in granulomatosis [4,11].

The formation of granulomas in tuberculosis is a morphological manifestation of delayed hypersensitivity to persistence of *Mycobacterium tuberculosis* in the vacuolar lysosomal system of phagocytic cells constituting the basis of granulomas. This fact impedes drug delivery to the site of agent's location. A possible solutions of the problem is the development of lysosomotropic compositions, permitting the creation of effective concentrations of antibacterial agents at the site of agent's persistence [10].

An experimental solutions of the problem was proposed previously: creation of compositions by means of chemical conjugation of the basic antituberculous drug isoniazid (isonicotinic acid hydrazide) with dextran oxidation product (dialdehyde dextran; DAD), mol. weights 30-40 and 65-75 kDa. These compositions contained isoniazid only in the bound form, and it was released after hydrolysis of DAD matrix in cell pino- and phagolysosomes [7]. These lysosomotropic compositions have some advantages over free isoniazid, presumably due to characteristics of the matrix (DAD) [4,10].

It is known that the number of tuberculous granulomas is primarily determined by the content of agents circulating in the blood and lymph and the content of resident macrophages capable of phagocytosing and effectively destroying them [8].

All these facts prompted creation of an antibacterial composition containing free isoniazid and this drug in long-acting intralysosome form. Dextran with a mol. weight of 70 kDa served as the matrix; after activation by the radiochemical method, dextran was converted into DAD and bound up to 40%

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free isoniazid [5]. It was expected that the use of this composition would lead to reduction in the number of mycobacteria (which would be seen from lesser number of granulomas) due to suppression of the circulating part of mycobacterial population by free isoniazid, while the part of mycobacterial population persisting in the granulomatous phagocytes would be destroyed by isoniazid released as a result of DAD hydrolysis and due to stimulation of the phagosomal/lysosomal fusion [8] at the expense of the DAD polycationic characteristics.

We compared the efficiencies of compositions of isoniazid with DAD (CID) with mol. weight of 70 kDa, one of which was obtained by the chemical method and contained only bound isoniazid (CIDc) and the other was obtained by combination of chemical and radiation methods (by irradiation of elementary particles in an accelerator) and contained free and bound isoniazid (CIDr).

MATERIALS AND METHODS

The study was carried out on male BALB/c mice aged 2 months (20-22 g). Disseminated tuberculous granulomatous inflammation was induced by injection of BCG vaccine [2,11] (N. F. Gamaleya Institute, Moscow) in a single intraperitoneal dose of 0.5 mg in 0.9% aqueous solution of NaCl [10].

Disseminated tuberculous inflammation developed after 1 month [8]. The animals were then divided into 3 groups: 1) no treatment; 2) isoniazid treatment: 2 times a week, intraperitoneally, 14 mg/kg in 0.9% NaCl for 5 months; and 3) CIDc [6,7] or CIDr treatment according to the same protocol during the same periods; the content of free and bound isoniazid in CIDr constituted 60% [5]. Single and course isoniazid doses for groups 2 and 3 were identical. Specimens of the liver and lungs were collected 3 and 6 months after infection with BCG vaccine (untreated group) and 3 and 6 months after infection (after 2 and 5 months of treatment, respectively; treated groups) from 10 animals per term and group which were sacrificed by cervical dislocation under ether narcosis.

The liver and lungs were selected as the objects of the study because they are always involved in generalized tuberculosis and contain the greatest compartment of the mononuclear phagocyte system cells forming the basis of granulomas.

Liver and lung specimens for light microscopy were prepared routinely [1]. The numerical density of granulomas and their diameters were evaluated by morphometry and served as the morphological criteria of treatment efficiency, because living mycobacteria initiate the creation of chemattractant gradient (an obligatory condition for the formation of granulomas) and the size of granulomas indicates the extent of this gradient [3,8,10]. The degree of lesions (degenerative and necrotic) in the liver parenchyma was evaluated as the criterion of hepatotoxicity of mycobacterial metabolites and the drugs used [8].

The therapeutic efficiency and hepatotoxicity were evaluated by comparing the parameters of the liver and lungs from animals treated with CIDc and CIDr and from animals treated with free isoniazid.

The significance of differences between the means was evaluated using Student's t test. The differences were considered significant at $p<0.05$.

RESULTS

Judging from the results of morphometry of BCG granulomas in the liver and lungs of mice, both compositions were much more effective than isoniazid (Figs. 1, 2).

After 2 months of CIDc and CIDr treatment, the number of granulomas in the liver was lower by 27.6 and 50%, respectively (Fig. 1), the diameters of granulomas were lower by 16 and 27%, respectively, than after isoniazid treatment (Fig. 2). During this period of the experiment, the number of granulomas in the lungs was 16% lower after CIDc treatment and 33% lower after CIDr treatment (Fig. 1), the diameters being 6 and 15.8% lower, respectively, in comparison with isoniazid therapy (Fig. 2).

After 5 months of CIDc therapy, the number of granulomas in the liver was by 38% and in the lungs by 15% lower than in the isoniazid group, while after CIDr therapy the number of granulomas in the liver and in the lungs was lower by 51.2 and 39%, respectively (Fig. 1). After CIDc treatment, the diameters of granulomas in the liver and in the lungs during this period of the experiment were lower by 25 and 11.6% in comparison with isoniazid group. In the CIDr group, the corresponding values were 46.2 and 21.8% (Fig. 2).

These data indicate higher therapeutic efficiency of CIDr in comparison with isoniazid and higher than of CIDc, judging from the significant decrease in the number of granulomas and their size. Presumably, this difference is due to the fact that CIDr contains free isoniazid suppressing mycobacteria circulating in the blood and lymph directly after the drug injection, and not only after the conjugate matrix hydrolysis in pinolysosomes of cells which captured it [8]. As a result, the percentage of mycobacteria fixed in macrophages was presumably also lower, which determined the differences in the number and size of granulomas.

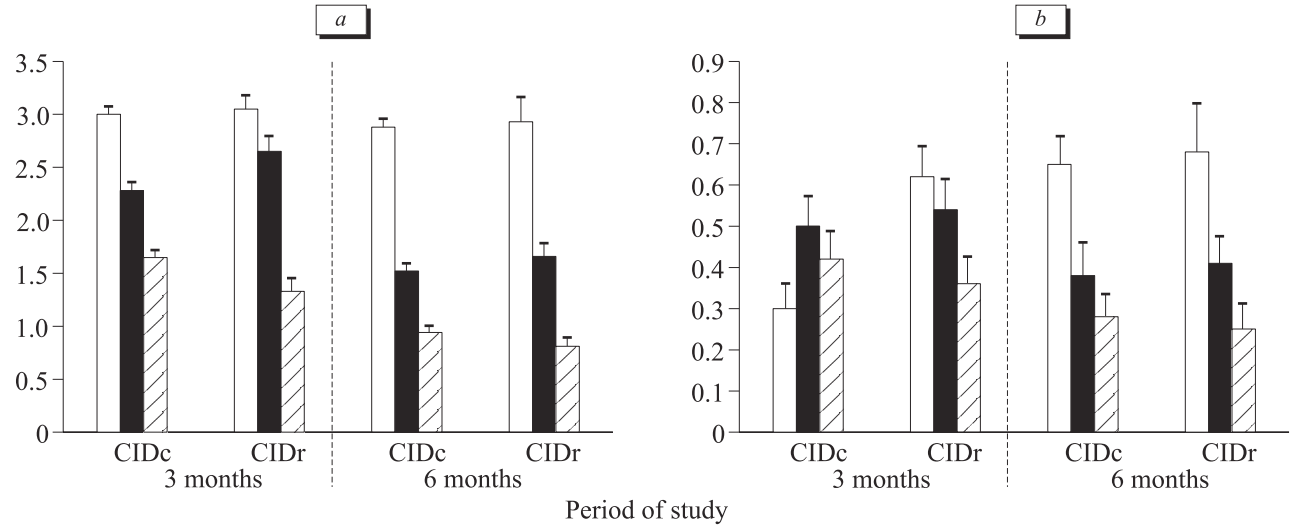

Fig. 1. Numerical density of BCG granulomas in the liver (*a*) and lungs (*b*) of mice with BCG granulomatosis. Here and in Figs. 2, 3: light bars: untreated animals; dark bars: animals treated with free isoniazid; cross-hatched bars: animals treated with CID.

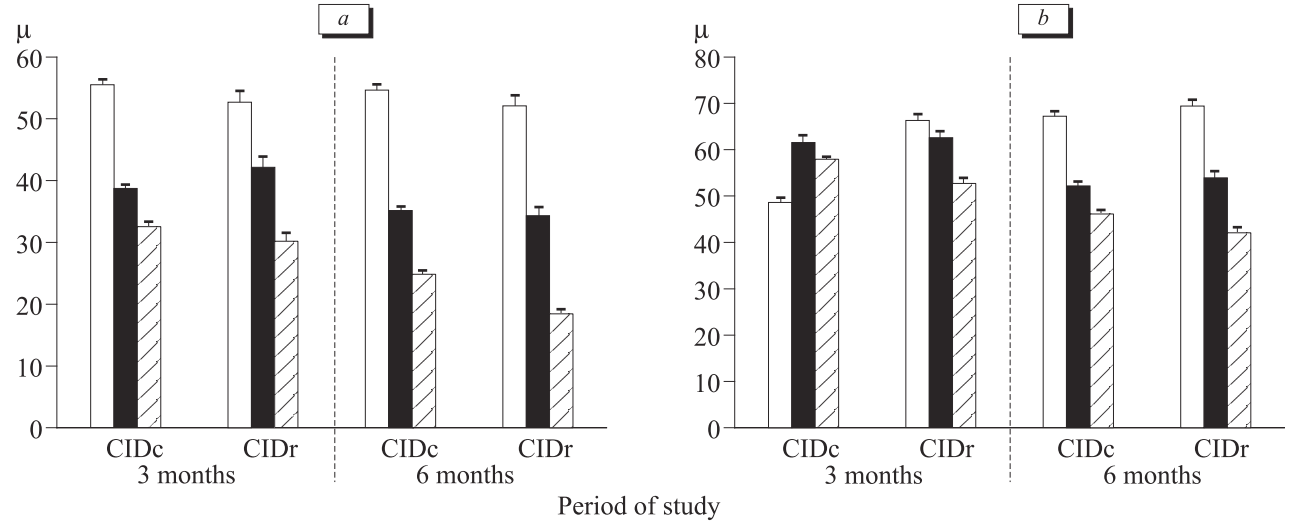

Fig. 2. Diameters of BCG granulomas in the liver (*a*) and lungs (*b*) of mice.

The hepatotoxicity of CIDr and CIDc was evaluated by the volume densities of hepatocyte zones in a state of vacuolar degeneration and necrotic zones of hepatocytes, which were compared with those in animals treated with isoniazid (Fig. 3). After 2 months of CIDc therapy, the volume densities of hepatocytes with vacuolar degeneration and hepatocyte necrotic zones were lower by 47% and by 3 times than after isoniazid treatment. During the same period of CIDr therapy, the volume densities of hepatocytes with vacuolar degeneration and necrotic zones were lower by 58% and by 2.9 times than after isoniazid treatment. The total volume of destructive changes in mouse liver after 2 months of CIDc treatment was 74% lower and after CIDr therapy by 83% lower than after isoniazid treatment. The hepatotoxicity of CIDc (but not of CIDr) in comparison with isoniazid decreased with prolongation of treatment. After 5-month therapy, the volume density of hepatocytes with vacuolar degeneration was 2.7 times lower in animals treated with CIDc and 2.1 times lower in those treated with CIDr in comparison with isoniazid group. The volume density of hepatocyte necrotic zones during the same period was 12-fold lower in the CIDc group and 8-fold lower in CIDr group in comparison with isoniazid group. The total volume of destructive changes in mouse liver after 5-month CIDc and CIDr therapy was lower by 3.7 and 2.8 times, respectively, in comparison with isoniazid therapy.

Higher hepatotoxicity of CIDr can be explained by higher percent of free isoniazid in its com-

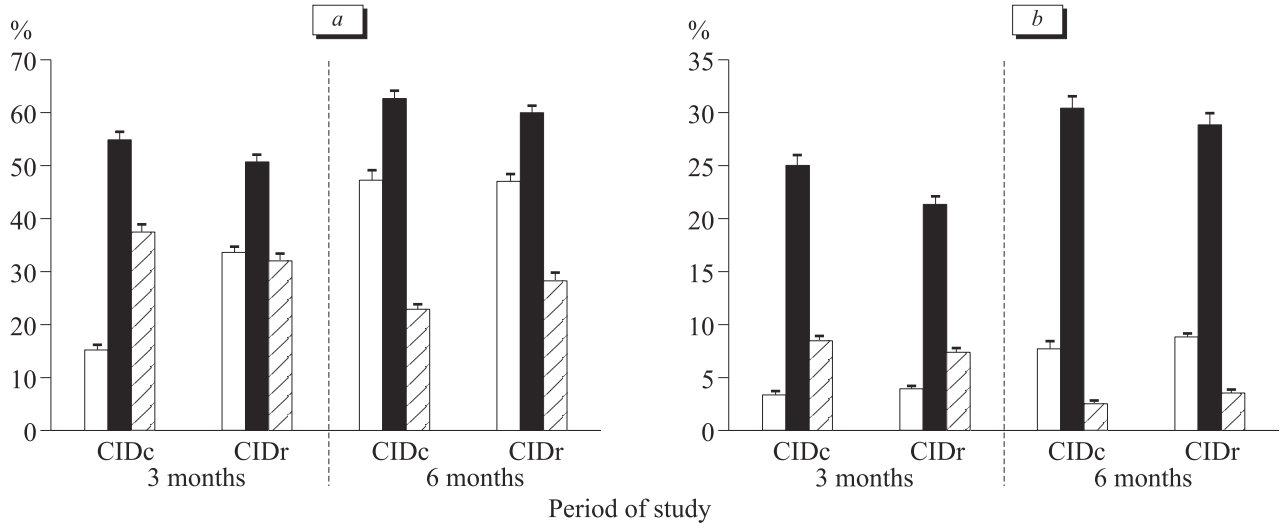

Fig. 3. Volume density of hepatocytes with vacuolar degeneration (*a*) and necrotic zones (*b*) in mouse liver.

position and its rapid entry into the liver, where its toxic metabolites are formed [4,8,10].

The volume of destructive changes in the liver of mice treated with CIDr and CIDc by the end of the experiment was significantly lower than in untreated animals, which was presumably due stimulation of the plastic and reparative processes in hepatocytes by dextran [8].

Hence, both compositions can be considered preferable in comparison with free isoniazid, but CIDr, despite its somewhat higher (in comparison with CIDc) hepatotoxicity, seems to be more promising, due to advantages of technology of its preparation (2 stages *vs.* 10 for CIDc) [5,6].

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