Structural Changes in the Liver Parenchyma and Granulomas of Mice with Chronic BCG Granulomatosis during Therapy with Composition of Isoniazid and Dialdehyde Dextran

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Male BALB/c mice were intraperitoneally infected with BCG vaccine. The therapy with isoniazid or composition of isoniazid with dialdehyde dextran (intraperitoneally, twice a week for 5 months) was started 1 month after infection. The retention time of the isoniazid-dialdehyde dextran composition in hepatocytes was much longer compared to that of isoniazid. The mice receiving the composition of isoniazid and dialdehyde dextran were characterized by a more significant decrease in the number and size of BCG granulomas, lower severity of destructive changes in the liver parenchyma, and more pronounced reparative regeneration (compared to animals of the isoniazid group).

Key Words: *BCG granulomatosis; liver; destruction; reparative regeneration; prolonged lysosomotropic isoniazid*

Persistence of mycobacteria tuberculosis (MBT) in the vacuolar (lysosomal) apparatus of macrophages determines specific reaction of the organism, which is manifested in granulomatous inflammation. Therapeutic difficulties in this disorder are associated with the search for new anti-MBT agents, methods of drug targeting, and achievement of the effective concentration of medicinal preparations at the site of pathogen occurrence. These problems can be solved by the development of prolonged intravacuolar (intralysosomal) antituberculous drugs, which are engulfed by macrophages of tuberculous granulomas.

Structural changes in the liver of mice with chronic disseminated BCG granulomatosis were studied during therapy with the composition of isonicotinic acid hydrazide (isoniazid) and dialdehyde dextran (DAD, molecular weight 65-75 kDa).

MATERIALS AND METHODS

Chronic disseminated BCG granulomatosis was induced by intraperitoneal injection of BCG vaccine (single dose 0.5 mg, N. F. Gamaleya Institute) in 0.9% aqueous solution of NaCl [2,10]. Experiments were performed on male BALB/c mice weighing 20-22 g and obtained from the nursery of the Institute of Cytology and Genetics (Siberian Division of the Russian Academy of Sciences). Group 1 consisted of BCG-infected mice. Group 2 mice received isoniazid 1 month after infection with BCG vaccine. Isoniazid (14 mg/kg) was dissolved in 0.9% aqueous solution of NaCl and injected intraperitoneally twice a week for 5 months. In group 3 mice, therapy with the composition of isoniazid and DAD (CID) was started 1 month after BCG infection. The

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weight content of isoniazid in this composition was 6.79-9.16% (isoniazid/DAD molar ratio 34.1-47.3). The substances were bound via a strong C-N chemical bond. The concentration of free and bound isoniazid was measured spectrophotometrically [6,7]. The single and course doses of isoniazid were similar in group 2 and 3 mice. In each group of animals and period of the study, the liver samples were obtained from 10 animals. The mice were killed by cervical dislocation under ether anesthesia 2, 3, 4, and 6 months after infection (1, 2, 3, and 5 months after the start of therapy, respectively).

Disseminated tuberculosis is always accompanied by liver damage. The liver has a well-developed compartment of the mononuclear phagocyte system. Hence, the concentration of hepatic granulomas is high. Moreover, hepatic parenchymal injury occurs in spontaneous and polychemotherapy-induced disseminated tuberculosis. For example, this disorder is observed during therapy with isoniazid (essential component of drug treatment for tuberculosis). Liver samples for light microscopy were prepared by the standard method [1].

The numerical density and diameter of granulomas were estimated morphometrically and served as the morphological criteria for evaluation of the effectiveness of therapy. MBT induce a gradient of chemoattractants, which is required for granuloma formation. The size of granulomas reflects the value of this gradient [3,8,9]. The severity of parenchymal destruction (dystrophy and necrobiosis) served as the criterion for hepatotoxicity of MBT metabolic products and drugs. The numerical density (concentration) of binucleated hepatocytes reflected the degree of reparative regeneration [8].

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

RESULTS

The concentration and size of granulomas did not differ 1 and 6 months after administration of BCG vaccine (Fig. 1). Increasing the period of treatment with isoniazid was accompanied by progressive decrease in these parameters. However, the concentration and size of granulomas decreased more significantly in CID-treated mice (Fig. 1). Induction of tuberculous granulomatosis by MBT (BCG vaccine) was not associated with the development of spontaneous necroses in granulomas. Therefore, the decrease in these parameters reflects greater antimycobacterial activity of CID as compared to isoniazid. Administration of both drugs was probably followed by a decrease in the chemoattractant gradient in granulomas and dissociation of macrophageal cells from granulomas [8,9]. The observed changes contribute to the decrease in the size and number of granulomas. This effect is probably related to lysosomotropism and intracellular prolongation of CID due to the presence of DAD matrix [4,8]. Hydrolysis of this matrix is accompanied by the release of isoniazid [8]. The concentration of free isoniazid in the liver decreased by 7 times after 24 h. However, we revealed a 40% increase in the concentration of CID 120 h after intravenous injection (Fig. 2). Hence, CID therapy contributes to greater concentration of isoniazid at the site of MBT persistence. It concerns the vacuolar-lysosomal apparatus of macrophages and granuloma epithelioid cells [4] due to tropism of dextran to the vacuolar

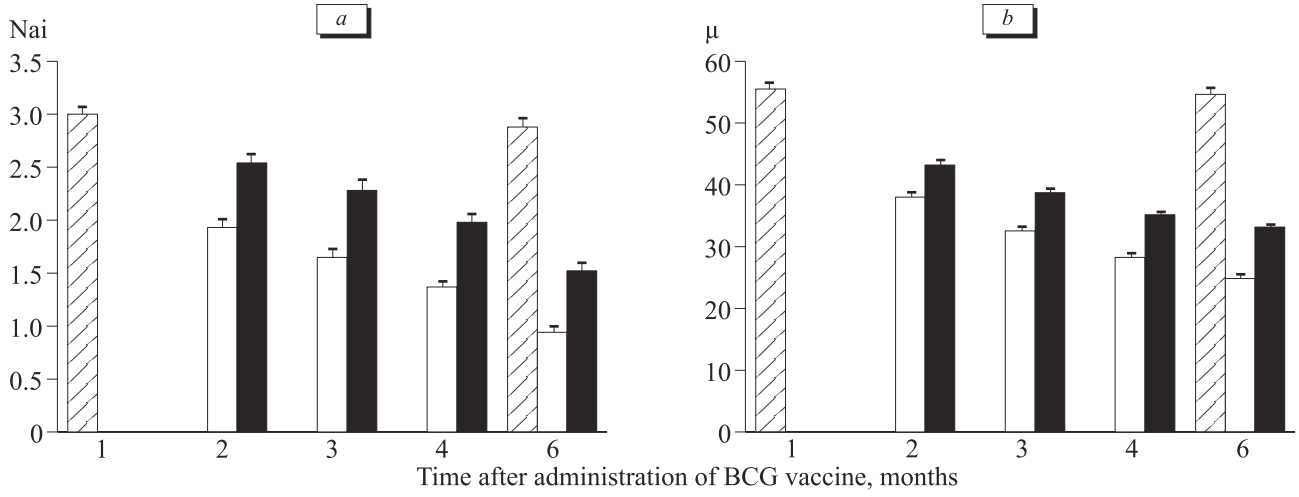

Fig. 1. Numerical density (*a*) and diameter of BCG granulomas in the liver of mice (*b*). Here and in Fig. 3: shaded bars, untreated animals with BCG granulomatosis; light bars, CID-treated animals with BCG granulomatosis; dark bars, isoniazid-treated animals with BCG granulomatosis.

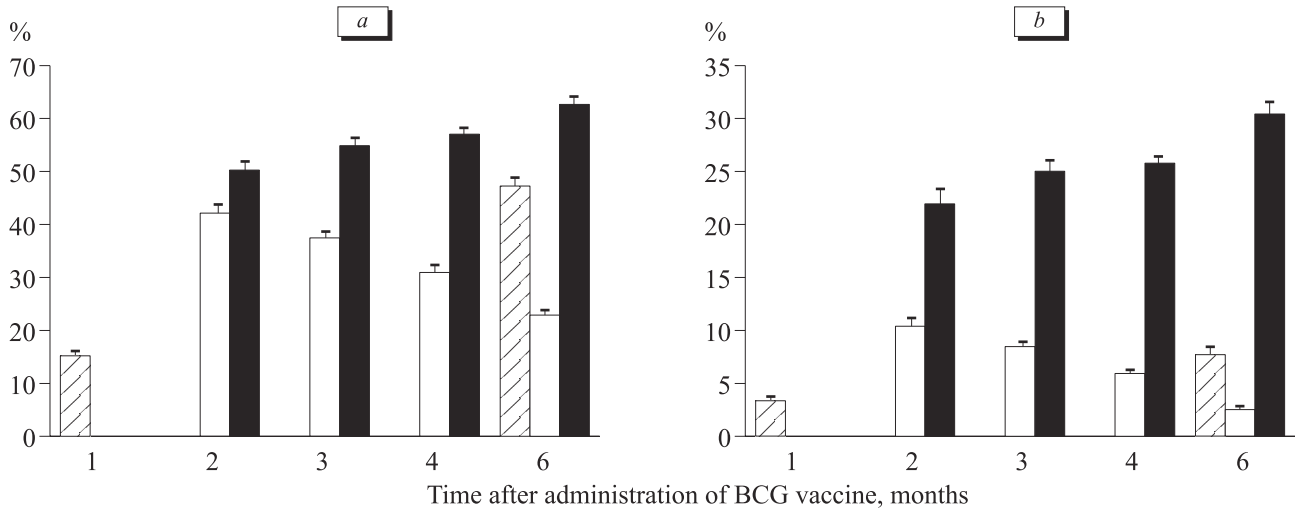

Fig. 2. Volume of destructive changes in the liver of mice. Volume density of hepatocyte degeneration (*a*) and necrosis (*b*).

apparatus [4,8]. Moreover, *in vitro* studies showed that DAD of CID significantly increased the frequency of phagosome-lysosome fusion [5]. The prevention of MBT persistence in macrophages due to incomplete phagocytosis is probably mediated by mechanisms that provide MBT lysis by the hydrolase system after phagosome-lysosome fusion.

The severity of hepatocyte destruction (vacuolar degeneration and necroses) increased with spontaneous development of BCG granulomatosis by the 6th month after infection (Fig. 3). Administration of CID was followed by a significant increase in the degree of these changes (Fig. 3). By contrast, CID therapy reduced the severity of destructive changes (particularly of necroses). They progressively decreased in the follow-up period (Fig. 3). The degree of reparative regeneration in hepatocytes of CID-treated mice was probably higher than in animals with spontaneous BCG granulomatosis and specimens of the isoniazid group. This conclusion is derived from studying the count of binucleated hepatocytes, which reflects the dynamics of mitotic processes in the liver (Table 1).

TABLE 1. Numerical Density of Binucleated Hepatocytes in the Liver of BALB/c Mice (*M*±*m*)

Time after infection, months	Group		
	1	2	3
1	9.47±0.76		
2		14.58±0.69	20.27±1.06*
3		16.04±0.76	26.60±1.01*+
4		19.16±0.88	31.28±0.63*+
6	14.68±1.19+	22.14±0.91o	34.42±0.7*+o

Note. $p<0.05$: *compared to group 2; +compared to the previous period; ocompared to group 1.

The decrease in hepatotoxicity of DAD-conjugated isoniazid is probably related to changes in the "route" of CID. Hepatocytes engulf this composition as dextran [8]. Isoniazid is released from the complex after hydrolysis in pinolysosomes of hepatocytes and phagocytes, enters hepatocyte microsomes through the cytosol, and produces toxic metabolites. As differentiated from CID, isoniazid has a molecular weight <300 Da, easily diffuses through the cell membrane, and is rapidly metabolized by microsomal oxygenases with the formation of toxic products. Therefore, isoniazid treatment is accompanied by acute intoxication. By contrast, toxic metabolites are formed over a long period after

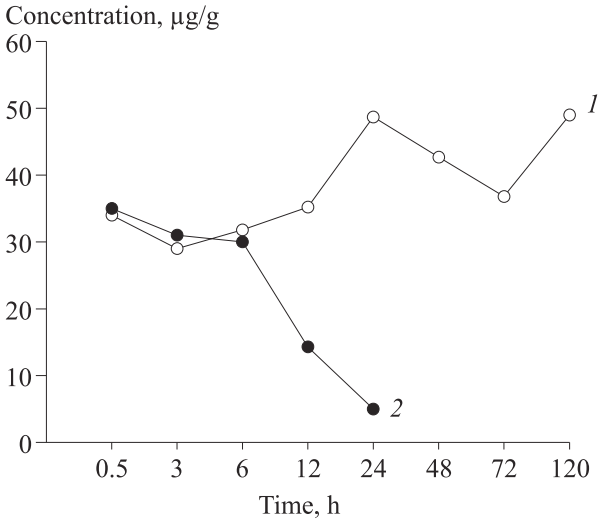

Fig. 3. Concentration of CID (*1*) and free isoniazid (*2*) in the liver of mice after intraperitoneal injection of these compounds.

administration of CID. Dextran serves as a prolonged lysosomotropic matrix in CID. This compound stimulates plastic processes in hepatocytes [8]. It is manifested in high-intensity endomitosis, which results in a greater number of binucleated hepatocytes in CID-treated mice (Table 1). It should be emphasized that CID with 65-75-kDa DAD matrix is more effective that CID with 35-40-kDa DAD matrix [3,9].

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