Effect of Dialdehyde Dextran on Structural Changes in the Liver and Lungs during Chronic BCG Granulomatosis

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Male BALB/c mice were intraperitoneally infected with BCG vaccine. Cytomorphological changes in BCG granulomas of the liver and lungs were compared during spontaneous tuberculous inflammation and after intraperitoneal injection of dialdehyde dextran for 5 months. Administration of dialdehyde dextran to mice infected with mycobacteria of BCG vaccine was followed by a decrease in the number and size of BCG granulomas in organs, contributed to the increase in the count of fibroblasts in hepatic and pulmonary granulomas, decreased the severity of destructive changes in the liver parenchyma, promoted reparative processes in hepatocytes, and reduced the degree of fibrosis in the liver and lungs due to a decrease in fibroplastic activity of fibroblasts in BCG granulomas.

Key Words: *BCG granulomatosis; dialdehyde dextran; liver; lungs; lysosomotropism*

The development of tuberculous granulomatosis is associated with incomplete phagocytosis, which results from the ability of mycobacteria tuberculosis (MBT) to prevent phagosome-lysosome fusion [6] and suppress oxidation in the internal space of phagosome [8,10]. These features determine the necessity of intravacuolar (intralysosomal) transport of drugs to modulate these processes.

This can be achieved by the development of prolonged intravacuolar compositions, which are tropic for phagocytic cells (*e.g.*, in tuberculous granulomas). Previous studies suggest that the effectiveness of dextran compositions [6,8] is partly associated with properties of the dextran matrix. The matrix is preoxidized before conjugation with isonicotinic acid hydrazide, which promotes the formation of a strong chemical bond.

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Here studied the effects of activated (oxidized) dextran during chronic BCG granulomatosis.

MATERIALS AND METHODS

Experiments were performed on male BALB/c mice aging 2 months. BCG granulomatosis in each animal was induced by intraperitoneal injection of BCG vaccine (single dose 0.5 mg, Allergen) [9] in 0.9% aqueous solution of NaCl [3,5,8].

Disseminated tuberculous inflammation developed 1 month after infection. Morphological signs included the formation of tuberculous granulomas in internal organs and visceral membranes. The animals were divided into 2 groups. Group 1 consisted of infected untreated animals (control). Group 2 mice received intraperitoneal injections of 70-kDa dialdehyde dextran (DAD, twice a week) 1 month after infection. DAD was obtained by chemical (periodate-induced) oxidation of dextran [5]. The single dose was 0.5 ml.

The liver and lungs were sampled from 10 animals of each group. The mice were killed by cer-

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vical dislocation under ether anesthesia 6 months after infection with BCG vaccine (5 months after the start of therapy). Comparative morphological study was performed for BCG granulomas from animals with spontaneous tuberculous inflammation (group 1) and from DAD-treated mice. The following parameters were recorded: numerical density (ND) of granulomas, diameter of granulomas, volume density of destructive changes in the liver parenchyma, ND of binucleated hepatocytes, and volume density of fibrous tissue in the liver and lungs. ND and fibroplastic activity of fibroblasts were measured. Fibroplastic activity was calculated as the ratio of the volume density of collagen fibers to the number of fibroblasts in granuloma.

Tissue samples for light microscopy were prepared by the standard method [1]. The significance of differences between the means was estimated by Student's *t* test. These differences were significant at $p<0.05$.

RESULTS

ND and diameter of granulomas serve as morphological criteria for the therapeutic effectiveness of antituberculous drugs [3,5,8]. These cytomorphological parameters reflect the persistence of live MBT in the vacuolar apparatus of granuloma phagocytes and induced chemoattractant gradient [6,7].

Injection of DAD decreased the number of hepatic and pulmonary granulomas in mice by 37 and 25%, respectively, compared to untreated animals. Under these conditions, the diameter of hepatic and pulmonary granulomas decreased by 23.7 and 15.4%, respectively (Table 1).

These data suggest that DAD obtained after activation (oxidation) of dextran is capable of increasing the frequency of phagosome-lysosome fusion. Probably, DAD gains properties of the polycation during oxidation. Similarly to dextran with the same molecular weight, DAD contributes to activation of plastic processes in macrophages (*e.g.*, in granulomas) [4,9]. DAD probably increases microbicidal activity of macrophages and, therefore, reduces the number of pathogenic agents and induced chemoattractant gradient in granulomas. This conclusion is derived from the decrease in the number and size of granulomas (Table 1).

This property of DAD holds much promise for the prevention of drug resistance. Death of MBT in phagocytes under the influence of lysosomotropic compound DAD [9] is not associated with the resistance to antituberculous drugs, but occurs spontaneously due to increase in the natural resistance, which is induced by lysosomal hydrolases of phagocytic cells in the blood and granulomas. This process probably involves reactive oxygen metabolites, whose production increases in activated macrophages [2].

Injection of DAD was accompanied by an increase in the number of fibroblasts in the liver and lungs (by 61 and 32%, respectively, compared to the control; Table 1). Fibroplastic activity of fibroblasts decreased in the liver (by 55% compared to untreated animals), but remained practically unchanged in the lungs (Table 1). The total volume of fibrous tissue in the liver and lungs of DAD-treated mice was lower than in control specimens (by 18 and 13.2%, respectively). These differences are of particular importance due to specific features of gas exchange in the lungs.

TABLE 1. Morphometric Parameters of Structural Changes in BCG Granulomas of the Lungs and Liver in Male BALB/c Mice 6 Months after Infection (*M*±*m*)

Parameter	Lungs		Liver	
	group 1	group 2	group 1	group 2
ND of granulomas in the section area (5.4×10^5 μ^2)	0.680±0.075	0.410±0.067*	2.93±0.23	1.840±0.143*
Diameter of granulomas, μ	69.41±1.26	58.74±1.43*	52.09±1.48	39.75±1.26*
Volume density of fibrous tissue in organs, %	22.44±1.14	19.48±1.06*	25.28±1.67	20.76±2.31*
ND of fibroblasts in granuloma	9.36±0.19	13.79±0.40*	2.970±0.179	7.65±0.32*
Fibroplastic activity of fibroblasts in granuloma	1.120±0.062	1.050±0.063	3.820±0.012	1.700±0.063*
Volume density of areas with hepatocyte degeneration, %	—	—	47.04±1.12	34.52±0.28*
Volume density of areas with hepatocyte necrosis, %	—	—	8.84±0.29	6.78±0.33*
ND of binucleate hepatocytes	—	—	14.12±0.62	27.48±1.29*

Note. *$p<0.05$ compared to group 1.

DAD-induced activation of macrophages probably results in attraction and proliferation of macrophages and production of IFN-γ and IFN-β, which suppresses collagen synthesis [11,13]. The decrease in the total volume of fibrous tissue in organs is probably associated with not only the influence of these cytokines, but also reduction of tuberculous granulomas and increase in collagenolytic activity of activated macrophages due to the effect of lysosomal hydrolases and matrix metalloproteinases [12]. This assumption requires further investigations.

The disadvantage of polychemotherapy for tuberculosis is high hepatotoxicity of antituberculous drugs. Previous studies showed that the composition of isonicotinic acid hydrazide and DAD reduces hepatotoxicity of xenobiotics and MBT toxins [5,6,8]. The volume density of destructive changes in the liver of DAD-treated mice was 24% lower than in animals with spontaneous tuberculous inflammation. The number of binucleated hepatocytes in mice of the DAD group increased by 48.6% compared to the control (Table 1). Hence, DAD stimulates plastic and reparative processes in the liver parenchyma [4]. This agent probably contributes to elimination of toxic MBT metabolites by activated macrophages.

Our results indicate that DAD has a biological effect on macrophages in tuberculous granulomas and, probably, increases microbicidal activity of these cells. These changes are associated with reduction of liver and lung fibrosis, decrease in the hepatotoxic effect of MBT metabolites on the liver parenchyma, and stimulation of reparative processes.

REFERENCES

1. O. V. Volkova and Yu. K. Eletskii, *Bases of Histology and Histological Technique* [in Russian], Moscow (1971).
2. E. B. Men'shchikova, V. Z. Lankin, N. K. Zenkov, *et al*., *Oxidative Stress. Prooxidants and Antioxidants* [in Russian], Moscow (2006).
3. P. N. Filimonov, V. A. Shkurupiy, Yu. N. Kurunov, *et al*., *Probl. Tub*., No. 1, 63-65 (1999).
4. V. A. Shkurupiy, *Byull. Eksp. Biol. Med.*, **105**, No. 5, 613-616 (1988).
5. V. A. Shkurupiy, Yu. N. Kurunov, and T. G. Chernova, RF Inventor's Certificate No. 2087146 (1997).
6. V. A. Shkurupiy, P. N. Filimonov, and Yu. N. Kurunov, *Probl. Tub.*, No. 6, 63-65 (1998).
7. V. A. Shkurupiy, Yu. N. Kurunov, and N. N. Yakovchenko, *Lysosomotropism. Problems of Cellular Physiology and Medicine* [in Russian], Novosibirsk (1999).
8. V. A. Shkurupiy, Yu. N. Kurunov, M. A. Kozyaev, and G. N. Shorina, RF Inventor's Certificate No. 2163120 (2001).
9. V. A. Shkurupiy, M. A. Kozyaev, and A. P. Nadeev, *Byull. Eksp. Biol. Med.*, **141**, No. 4, 474-477 (2006).
10. D. Clemens and M. Horwitz, *J. Exp. Med*., **181**, No. 1, 257-270 (1995).
11. M. R. Duncan and B. Berman, *Ibid*., **162**, No. 2, 516-527 (1985).
12. *Granulomatous Infections and Inflammations: Cellular and Molecular Mechanisms*, Ed. D. L. Boros, Washington (2003).
13. O. C. Turner, R. J. Basarabd, A. A. Frank, and I. M. Orme, *Granulomatous Infections and Inflammations: Cellular and Molecular Mechanisms*, Ed. D. L. Boros, Washington (2003), pp. 65-84.