Evaluation of Destructive and Reparative Processes in the Liver in Experimental Chronic Granulomatosis of Mixed (Silicotic and Tuberculous) Etiology

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Experimental silicosis and silicotuberculosis induced by intravenous injection of silicon dioxide particles are characterized by extensive degenerative and necrotic processes in the liver parenchyma. After 6 months, fibrosis of portal tracts in silicotuberculosis and silicosis was 2.8- and 1.4-fold more pronounced that in BCG granulomatosis and silicosis, respectively. Depression of cellular and intracellular regeneration processes in the liver parenchyma was also observed.

Key Words: *granulomatosis; silicotuberculosis; regeneration; fibrosis; liver*

Fibrosis is the most prevalent complication of tuberculosis and silicosis. This complication is better studied in granulomas, primarily in the lungs [8,11]. Silicosis is a tuberculosis-predisposing factor; it impairs the prognosis and aggravates the course of tuberculosis, similarly as *M. tuberculosis* infection in silicosis patients [10,12]. At the same time, both these diseases often have systemic manifestations [5,6,9]. However, the pathological manifestations of these diseases, especially in silicotuberculosis, in other organs (except the lungs) remain poorly studied. Of particular interest in this context is the liver, the major compartment of the system of mononuclear phagocytes (MPS, the cells participating in granuloma formation), and the target organ for glucocorticoid hormones regulating their biogenesis and functions. High destructive potential of phagocytes accumulated in their lysosomes [6,7] considerably increases in these pathologies due to production of reactive oxygen species (ROS) by granuloma macrophages [3]; liver destruction in tuberculosis is aggravated by aggressive polychemotherapy [1,6]. This determines the possibility of extensive injuries to the liver parenchyma in silicotuberculosis and development of postdestructive complications considerably altering the structure and function of this central organ of homeostasis maintenance in mammals.

Here we studied the peculiarities of destructive and reparative processes in the liver of mice with chronic silicotuberculosis.

MATERIALS AND METHODS

The study was performed on 2-month-old male CBA mice (n=210) weighing 20-22 g obtained from Nursery of Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Sciences (Novosibirsk). The animals were divided into 4 groups: 3 groups consisted of 60 mice each and group included 30 mice (10 animals per point). The animals fed standard ration and had free access to water and food. In group 1 animals, BCG granulomatosis was modeled by intraperitoneal injection of 0.5 mg BCG vaccine (Allergen) in 0.2 ml 0.9% NaCl aqueous solution. In group 2 mice, granulomatous inflammation was induced by a single injection of SiO_2 suspension (S-563

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grade, 1-5-μ particles, Sigma) in a dose of 100 μg/kg body weight in 0.5 ml sterile 0.9% NaCl aqueous solution into the caudal vein (90% particles had a diameter of 0.9-1.5 μ). Group 3 mice first received intravenously SiO_2 suspension in the same dose as group 2 mice and after 10 days they received an intraperitoneal injection of 0.5 mg BCG vaccine in the same dose as group 1 mice. Liver samples were taken on days 3, 10, 28, 56, 120, and 180 after administration of BCG vaccine (groups 1 and 3) and SiO_2 suspension (group 2). Group 4 mice (controls) intravenously received 0.2 ml sterile 0.9% NaCl. The samples were fixed in 10% neutral formalin, dehydrated in ascending alcohols, and embedded in paraffin. Histological sections (5-7 μ) were prepared on a rotation microtome (Microm HM3555S; Carl Zeiss), stained with Mayer's hematoxylin and eosin and after van Gieson, and examined under an AxioStar Plus light microscope (Carl Zeiss). For detection of argyrophilic fibers, the sections were stained after Sweet and Gordon [2]. The volume densities (Vv) of necrotic and degenerative changes in hepatocytes, their ratio, and volume density (Vv) of binuclear hepatocytes were determined morphometrically. Volume densities (Vv) of collagen and argyrophilic fibers were determined and their sum was calculated. The significance of differences between the means was evaluated using Student *t* test, the differences were significant at $p<0.05$.

RESULTS

Histological examination of the liver from mice in all three groups showed, similarly to our previous studies [6], macrophage-epithelioid cell granulomas; the number and size of these granulomas as well as the degree of fibrosis of portal tracts increased with increasing the time elapsed after administration of both granulomagenic factors. It should be noted that on days 120 and 180 after individual and combined systemic administration of both granulomagenic factors, the granulomas primarily consisted of argyrophilic and collagen fibers and contained very few MPS cells (primarily fibroblasts). Moreover, hepatocytes in the state of vacuolar degeneration and necrosis were seen without certain linkage to microanatomical zones of the hepatic lobules. Destructive processes in the liver parenchyma were very extensive in mice of groups 1, 2, and 3 starting from day 3 after administration of both pathogenic factors (Table 1). In animals receiving SiO_2 in the beginning of the experiment, the percent of necrotic hepatocytes was higher by 50% than in mice receiving BCG mycobacteria alone. After 180 days, the percent of necrotic hepatocytes in mice of all groups was somewhat higher than in the beginning of the experiment. The intensity of these processes in animals of all groups observed at the beginning of the experiment and did not tend to decrease even after

TABLE 1. Volume Density (Vv) of Hepatocytes in the State of Vacuolar Degeneration and Necrosis in the Liver of CBA Mice with Chronic Granulomatoses of Different Etiology (*M*±*m*)

Parameter	Day after infection	Experimental conditions			
		group 1 (BCG)	group 2 (silicosis)	group 3 (silicotuberculosis)	group 4 (control)
Degeneration (Vv)	3	75.06±0.74	64.31±0.72*	66.44±0.68*+	11.71±0.98
	10	64.80±0.69	59.68±0.73*	65.20±0.69+	–
	28	62.26±0.66	54.57±0.68*	57.82±0.87*+	–
	56	57.91±0.86	54.62±0.97*	59.28±0.79+	–
	120	60.80±0.77	55.95±0.73*	60.57±0.74+	–
	180	66.26±0.69	51.20±0.90*	59.15±0.77*+	–
Necrosis (Vv)	3	20.26±0.74	31.20±0.70*	25.64±0.60*+	0.75±0.28
	10	25.64±0.83	33.28±0.72*	25.28±0.72+	–
	28	30.40±0.72	38.44±0.65*	35.20±0.77*+	–
	56	33.73±0.84	38.40±0.97*	36.26±0.81*+	–
	120	33.73±0.78	35.95±0.82*	35.91±0.81*	–
	180	26.66±0.77	38.84±0.88*	36.13±0.83*	–

Note. Here and in Table 2: $p<0.05$ compared to: *group 1, +group 2.

180 days of observations (Table 1). In group 3 mice (silicotuberculosis), the intensity of necrotic changes in hepatocytes were lower than in mice receiving SiO_2 alone (Table 1). Destructive processes in the liver parenchyma in tuberculosis are related to toxic effects of *M. tuberculosis* metabolites and with ROS produced by activated macrophages in granulomas phagocytising mycobacteria and SiO_2 particles [3]. In case of SiO_2, the extensive destructive processes in hepatocytes can hardly be explained solely by the effects of ROS due to activation of their production by granuloma macrophages capturing these particles: their effects are very local due to short life-time of these molecules [3]. A possible explanation of the destructive action of SiO_2 on hepatocytes in the analyzed situation, apart from ROS effects, can be adsorption pinocytosis of the nanosized fraction of the administered SiO_2 particles by hepatocytes leading to damage of the lysosomal membranes.

In BCG granulomatosis, the fibrous connective tissue does not form by the substitutive principle even under conditions of treatment with hepatotoxic isoniazid and is located primarily in granulomas despite extensive destructive processes in the liver parenchyma [6]. This is confirmed by the results of examination of the connective tissue in periportal zones of the hepatic lobules (Table 2). In silicotuberculosis and silicosis (180 days after injection of SiO_2), fibrosis of portal tracts was 2.8- and 1.4-fold more pronounced than after infection with BCG mycobacteria. In all types of granulomatosis, fibrosis of periportal zones of the liver developed early and after 180 days fibrosis of these zones was maximum in the liver of mice with silicotuberculosis: the fibrous connective tissue in these animals constituted ~20% of liver sample volume (Table 2). Judging from the site of fibrosis development (primarily portal tracts and granulomas, which are not structural elements of the liver), this process did not have the substitutive reparative nature. Taking into account spreading pattern of fibrosis (periportal zones and granulomas), we can classify it as a pathological process, fibrosis.

In mice of groups 2 and 3, the processes of cellular reparative regeneration in the liver parenchyma were suppressed (the content of binuclear hepatocytes

TABLE 2. Volume Density (Vv) of Argyrophilic and Collagen Fibers in Periportal Zones of the Liver of CBA Mice with Chronic Granulomatoses of Different Etiology (*M*±*m*)

Parameter	Day after infection	Experimental conditions		
		group 1 (BCG)	group 2 (silicosis)	group 3 (silicotuberculosis)
Argyrophilic fibers	3	0.80±0.13	1.16±0.16	1.33±0.14*
	10	1.56±0.20	1.39±0.14	2.10±0.17*+
	28	2.52±0.13	4.44±0.22*	3.07±0.18*+
	56	4.40±0.19	4.62±0.23	4.75±0.26
	120	3.67±0.14	7.24±0.27*	7.45±0.33*
	180	4.51±0.19	8.34±0.41*	11.90±0.32*+
Collagen fibers	3	0.61±0.13	0.79±0.12	2.26±0.12*+
	10	0.75±0.13	1.02±0.14	2.57±0.14*+
	28	1.43±0.15	2.06±0.17*	3.02±0.13*+
	56	2.76±0.13	2.68±0.15	3.30±0.21*+
	120	1.99±0.14	4.39±0.18*	5.86±0.19*+
	180	2.25±0.21	4.95±0.23*	7.15±0.33*+
Total volume of both types of fibers	3	1.41±0.18	1.95±0.20	3.59±0.18*+
	10	2.31±0.23	2.41±0.19	4.67±0.22*+
	28	3.95±0.19	6.50±0.27*	6.09±0.22*+
	56	7.16±0.23	7.30±0.28	8.05±0.33
	120	5.66±0.19	11.63±0.32*	13.31±0.38*+
	180	6.76±0.28	13.29±0.47*	19.05±0.45*+

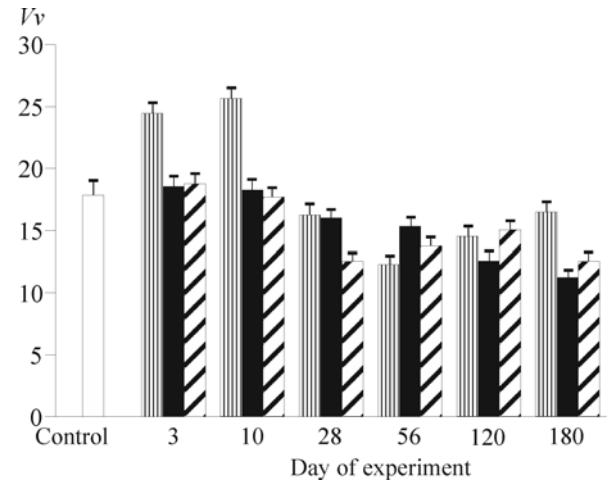

Fig. 1. Volume density (V_v) of binuclear hepatocytes in the liver of CBA mice with chronic granulomatoses of different etiology. Open bars: control; vertical shading: BCG vaccine; dark bars: SiO_2; oblique shading: BCG vaccine+SiO_2.

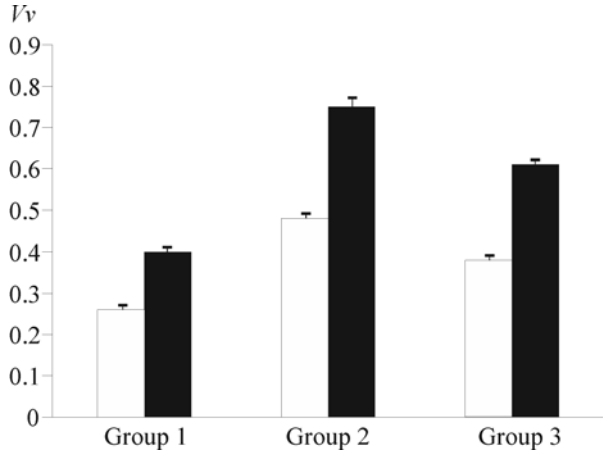

Fig. 2. Relative ratio of volume densities of hepatocytes in the state of necrosis and vacuolar degeneration in the beginning (day 3, open bars) and at the end (day 180, dark bars) of the experiment.

decreased by 30% by the end of the experiments). As soon as on day 3 of observation, the count of binuclear hepatocytes in mice of groups 2 and 3 receiving SiO_2 was lower than in mice with BCG-induced granulomatosis and did not exceed the level observed in intact animals, despite the loss of more than one-fourth of hepatocytes during this period (Fig. 1 and Table 1). In our previous 6-month experiment we found that the decrease in the number of degeneratively changed hepatocytes in the dynamics of BCG granulomatosis was not accompanied by the increase in the level of necrotic hepatocytes [13]. This, according to D. S. Sarkisov [4] concept on intracellular regeneration, can attest to the possibility of this regeneration (in the studied experimental situation) in hepatocytes with moderate changes (vacuolar degeneration). Our findings (Fig. 2 and Table 1) suggest that the processes of intracellular reparative regeneration in the liver parenchyma were suppressed in mice with silicosis and silicotuberculosis, because in these animals shrinkage of zones with degeneratively changed hepatocytes during the period from day 3 to day 180 was accompanied by the increase in volume density of necrotic hepatocytes. In BCG-induced granulomatosis, another relationship was observed (Table 1, Fig. 2).

Thus, silicosis and especially silicotuberculosis are associated with extensive and early developing destructive and fibrotic processes in the liver parenchyma and depression of cellular and intracellular reparative regeneration. These findings attest to high risk of considerable impairment of various liver functions, which should be taken into account during therapy of patients with silicosis and silicotuberculosis implying the use of hepatotoxic antituberculous drugs [1,6].

REFERENCES

1. V. G. Kononenko and V. A. Skurupiy, *Tuberculosis of the Lungs: Epidemiologyand Parenteral Chemotherapy* [in Russian], Novosibirsk (2002).
2. *Microscopic Technique*, Eds. D. S. Sarkisov and Yu. L. Petrov [in Russian], Moscow (1996).
3. *E. B. Men'shikova, V. Z. Lankin, N. K. Zenkov, et al., Oxidative Stress, Prooxidants and Antioxidants* [in Russian], Moscow (2006).
4. D. S. Sarkisov and B. V. Vtyurin, *Electron Microscopy of Destructive and Regeneratory Intracellular Processes* [in Russian], Moscow (1967).
5. E. V. Ulanova, D. V. Fomenko, O. I. Bondarev, and P. V. Zoloeva, *Pat. Fiziol. Eksp. Ter.*, No. 4, 24-26 (2009).
6. V. A. Skurupiy, *Tuberculous Granulomatosis. Cytophysiology and Targeted Therapy* [in Russian], Moscow (2007).
7. V. A. Shkurupiy, Yu. R. Kurunov, and N. N. Yakovchenko, *Lysosomotropism: Problems of Cell Physiology and Medicine* [in Russian], Novosibirsk (1999).
8. J. D. Byrne and J. A. Baugh, *Mcgill J. Med.,* **11**, No. 1, 43-50 (2008).
9. R. F. Hamilton Jr., S. A. Thakur, and A. Holian, *Free Radic. Biol. Med.,* **44**, No. 7, 1246-1258 (2008).
10. G. M. Calvert, F. L. Rice, J. M. Boiano, *et al., Occup. Environ. Med.*, **60**, No. 2, 122-129 (2003).
11. S. L. Cassel, S. C. Eisenbarth, S. S. Iyer, *et al., Proc. Natl. Acad. Sci. USA*., **105**, No. 26, 9035-9040 (2008).
12. D. Rees and J. Murray, *Int. J. Tuberc. Lung Dis.*, **11**, No. 5, 474-484 (2007).
13. V. A. Shkurupiy, M. A. Kozyaev, O. V. Potapova, *Bull. Exp. Biol. Med.*, **146**, No. 6, 853-856 (2008).