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Cytomorphological Study of the Development of Fibrotic Complications in Chronic SiO_2 Granulomatosis in the Liver during Radon Treatment

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Granulomatosis was induced in male Wistar rats by intravenous injection of SiO_2. The course of SiO_2 granulomatosis was cyclic in animals receiving radon baths: phase 1 was characterized by an increase in the number and size of granulomas, while during phase 2 the intensity of the inflammatory process decreased and fibrosing of granulomas progressed. No trends to alleviation of the inflammatory process were noted in rats with SiO_2 granulocytosis receiving tap water baths.

Key Words: *silicon dioxide; radon; granulomatous inflammation; fibrosis; liver*

The nature of positive effects of radon exposure [2,7] is still disputed; radon procedures are mainly used in patients with destructive processes in the locomotor system and chronic inflammatory processes, one of the complications of which is excessive formation of fibrous tissue. The model of chronic granulomatous inflammation induced by injection of SiO_2 can be used for studies of fibrosis and interrelationships between cells largely determining this process in any organ: lymphocytes, macrophages, and fibroblasts [9]. High clearing activity of the liver towards microcorpuscular objects due to high concentration of resident macrophages (Kupffer cells) in it [9] makes this organ convenient for studies of fibrotic processes induced by injection of a lysosomotropic factor, for example, SiO_2 [9].

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We studied the effect of radon on the development of chronic SiO_2 granulomatosis and its complication (fibrosis).

MATERIALS AND METHODS

The study was carried out on 36 male Wistar rats (300-350 g) in which granulomatosis was induced by a single injection (into the caudal vein) of 0.8 ml of microcrystal SiO_2 suspension (1-5-μ particles) in 0.85% NaCl (SiO_2 dose 35 mg/kg).

The animals were divided into 3 groups (12 per group): group 1 consisted of untreated rats; groups 2 and 3 rats received tap water baths and baths of silicon nitrate radon-containing water from Belokurikha Health Resort, respectively (3, 10, or 20 daily baths). The animals were plunged completely, except the heads, in water (22-24°C) for 30 min; radon concentration was 8 nCi/liter.

Material for analysis was collected on days 3, 10, and 20 after the start of balneotherapy, which

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corresponded to days 13, 20, and 30 after SiO_2 injection. The animals were sacrificed by decapitation under ether narcosis.

Liver specimens for light microscopy were stained with hematoxylin and eosin and by van Gieson method. The following parameters were studied: density (N_{ai}) and diameters (in μ) of SiO_2 granulomas in the liver, cell composition of granulomas (percent from the total number of cells in the granuloma), volume density (V_v) of fibrous connective tissue in granulomas, fibroplastic "activity" of fibroblasts, which was expressed through V_v of collagen fibers per fibroblast in the granuloma, fibrous tissue concentration in the organ (product of fibrous tissue V_v in granulomas and N_{ai} of granulomas in the organ).

The significance of differences between the means was evaluated using Student's *t* test, the differences were considered significant at $p<0.05$ [1,11].

RESULTS

Granulomas formed in the livers of animals of all groups on day 13 after SiO_2 injection (day 3 after the start of balneotherapy). The N_{ai} was maximum in the livers of groups 1 and 2 rats (Table 1); the

TABLE 1. Morphometric Parameters of Structural Changes in the Liver of Animals with Chronic SiO_2 Granulomatosis after Balneotherapy (*M*±*m*)

Parameter	Balneotherapy duration, days	Group 1	Group 2	Group 3
N_{ai}	3	0.520±0.073	0.450±0.015	0.300±0.025^{+o}
	10	0.65±0.04	0.500±0.017^{+}	0.800±0.038^{*+}
	20	0.720±0.061	0.630±0.056*	0.710±0.049
Diameter of granulomas, μ				
	3	29.53±0.74	30.52±1.07	25.08±1.15^{+o}
	10	35.58±1.05*	34.27±1.26*	50.28±0.87^{*+}
	20	36.95±1.05	37.18±0.91*	35.40±1.56
Cell composition of granulomas, %				
macrophages	3	87.40±1.90	85.40±0.77	71.78±1.29^{+o}
	10	87.90±0.89	84.12±1.54^{+}	70.15±2.04^{o}
	20	84.30±1.19	84.31±1.33	75.17±1.62^{*+o}
lymphocytes	3	5.25±0.025	6.14±0.043	13.43±0.63^{+}
	10	4.100±0.029	6.250±0.052	10.21±0.15^{+}
	20	4.760±0.037*	5.450±0.063	3.500±0.038^{+o}
neutrophils	3	2.180±0.018	1.61±0.03^{+}	7.430±0.071^{+}
	10	2.280±0.016*	1.980±0.017*	6.400±0.025^{+}
	20	1.96±0.03	1.79±0.08	1.19±0.07^{+o}
fibroblasts	3	5.170±0.074	6.850±0.036	7.360±0.067^{+}
	10	5.720±0.062*	7.650±0.075*	13.24±0.96^{*+}
	20	8.980±0.053*	8.450±0.048^{*+}	20.14±1.32^{*+}
Fibrous tissue V_v in granuloma, %	3	5.57±0.45	7.12±0.61	8.25±0.42^{+}
	10	4.95±0.27	7.75±0.45	16.75±0.85^{*+}
	20	8.02±0.31*	8.20±0.58	22.84±1.26^{*+}
Fibroplastic "activity" of fibroblasts	3	1.07±0.10	1.03±0.04	1.12±0.08
	10	0.90±0.07	1.010±0.015	1.17±0.10
	20	0.89±0.05	0.970±0.045	1.130±0.075
Fibrous tissue concentration coefficient in organ	3	2.90±0.09	3.20±0.10	2.46±0.07^{+o}
	10	3.22±0.17	3.86±0.22*	13.40±0.34^{*+}
	20	5.77±0.36*	5.17±0.43*	16.18±0.59^{*+}

Note. $p<0.05$ compared to: *previous term, $^{+}$group 1, ^{o}group 2.

granulomas consisted mainly of macrophages, few lymphocytes and fibroblasts (Table 1). After 10 days of balneotherapy (day 23 after SiO_2 injection), the granuloma N_{ai} in the liver increased in animals of all groups (Table 1). In group 3, this value was by 23% higher than in group 1 and 60% higher than in group 2. After 20 days of balneotherapy (day 33 after SiO_2 injection), N_{ai} decreased by 10% only in group 3 rats and virtually did not differ from the value in animals of other groups (Table 1).

Granulomas increased in size on day 10 of balneotherapy in animals of all groups. In comparison with the previous term (day 3), enlargement of granulomas in groups 1 and 2 was negligible, while in group 3 this parameter increased 2-fold and was 1.4 times higher than in other groups (Table 1). On day 20, granulomas in the livers of group 3 rats decreased by 30% in comparison with the previous term and their size virtually did not differ from that in rats of other groups (Table 1).

On day 10 of balneotherapy, the number and size of granulomas in the livers of group 3 rats were significantly higher than in other groups, presumably due to the prooxidant effect of radon [3, 10] and activation of free radical oxidative processes. It is known that less than 1% radon present in radon water penetrates into the body after radon baths, while the greater part (60%) of radon diffuses into the derma [7]. Radon present in thermal waters releases α-particles, which stimulate the formation of superoxide radicals and peroxide compounds in the derma [7]; in addition, radiogenic radicals accumulate with subsequent oxidation of a variety of biosubstrates at the molecular level [10]. This leads to modification of the serum pro- and antioxidant activities [6,8,10], which serves as an indicator of the modulatory effect of radon, primarily on macrophages. Serum prooxidant activity increases during the early period of balneotherapy (day 3) [6,10], which under conditions of a granulomatous inflammation can be caused by macrophage activation and enhanced production of activated oxygen metabolites, proinflammatory cytokines chemoattractants [4,6,10]. This leads to intense migration of mononuclears and other immunocompetent cells (including lymphocytes) into the focus of inflammation, which presumably explains enlargement of granulomas on day 10. On day 20 of balneotherapy (group 3), the increase in serum antioxidant activity [6,10] was paralleled by macrophage activation with increased secretion of anti-inflammatory cytokines (IL-1, IL-10) [8] and attenuation of the inflammatory process [6,10].

On day 20 of balneotherapy, the specific cellular reaction under the effect of radon degradation products in liver granulomas in group 3 rats manifested by a 3-fold reduction in lymphocyte count in comparison with the previous term (day 10), which was paralleled by a 50% increase in fibroblast number (Table 1) and formation of fibrosing granulomas. Fibrous tissue V_v in liver granulomas of groups 1 and 2 rats was 2.35 times lower than in group 3, while fibroplastic "activity" of fibroblasts was virtually the same in all groups during all periods of observation (Table 1). The coefficient of fibrous tissue concentration in the organ in the radon therapy group was significantly higher on day 10 (4-fold higher than in groups 1 and 2) and on day 20 (3-fold higher than in groups 1 and 2). According to the results of an analogous experiment, radon baths modify connective tissue metabolism, which can be seen from a reduction of fibronectin level and increased concentration of glycosaminoglycans in the sera of animals with SiO_2 granulomatosis [5]. Interleukin-4 and IL-13 stimulate macrophage secretion of arginase 1, responsible for the synthesis of polyamines and proline, essential for fibroblast proliferation and collagen production [4]. The results indicate that balneotherapy therapy with radon-containing water reduces the severity of acute inflammation presumably due to improvement of the antioxidant potential at the expense of a sort of a "training" effect of antioxidant systems [10], and stimulate the processes of reparative regeneration of the connective tissue, manifesting in increased proliferative, but not synthetic activity of fibroblasts. This can be useful in such diseases as osteochondrosis, vertebral instability in distortions of different genesis.

These data necessitate more strict definition of indications for radon therapy in other diseases, as its negative effect in chronic inflammatory processes, for example, in the lungs cannot be ruled out.

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