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Lipid Peroxidation in the Liver and Lungs in SiO_2-Induced Granulomatosis

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LPO activity in the liver and lungs of Wistar rats during the development of granulomatous inflammation caused by intravenous injection of SiO_2 microparticles was evaluated by the content of conjugated dienes, ketodienes, conjugated trienes, and products of interaction of LPO intermediates with TBA. In the lungs, changes in LPO activity manifested in increased content of ketodienes and conjugated trienes (by 1.6 and 1.5 times on days 3 and 14 after injection of SiO_2 microparticles, respectively) and conjugated dienes (by 1.2 times on day 21) compared to the control levels, but the content of TBA-reactive substances remained within the normal range. In the liver, the content of conjugated dienes increased by 1.6 times and that of ketodienes and conjugated trienes by 2.3 times; the content of TBA-reactive substances increased by 1.7 times compared to the control. The content of TBA-reactive substances in the liver gradually increased and by day 14 this parameter surpassed the control level by 3.9 times, but on day 21 it returned to normal. Thus, LPO processes in the liver after injection of SiO_2 microparticles were more intensive than in the lungs and their activity underwent phasic changes.

Key Words: *SiO_2 granulomatosis; lipid peroxidation; liver; lungs*

Nano- and microparticles (0.1-3.0 μ) daily enter the gastrointestinal tract and lungs of humans; their volume is estimated as 10^{12}-10^{14} particles per day [9] and they are primarily presented by carbon-, silicon-, and titanium-containing particles. Translocation of mircoparticles from the lungs [13] and gastrointestinal tract [12] to systemic circulation was experimentally demonstrated. The risk of systemic granulomatous inflammation development in response to exposure to abiogenic granulomagenic factors is associated with considerable disturbances in structural homeostasis and primarily involves the liver and the lungs, the vital organs characterized by high content of resident macrophages [7]. Activation of LPO processes in organs associated with production of reactive oxygen and nitrogen metabolites by phagocytizing cells is a mechanism underlying the cytotoxic effect of silicon-containing particles [3,4]. These LPO products accumulated in organs can induce damage followed by fibrosis processes [14].

Here we studied LPO processes in the lungs and liver during the development of granulomatous inflammation induced by intravenous injection of SiO_2 microparticles.

MATERIALS AND METHODS

Experiments were carried out on 3-month-old male Wistar rats weighing 180-200 g (n=33) maintained on

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standard ration with free access to food and water. All tests were performed during morning hours. Granulomatous inflammation was induced by single injection of SiO_2 suspension (S-563 grade, 1-5-μ particles, Sigma) in a dose of 100 mg/kg body weight in 0.2 ml 0.85% NaCl sterile aqueous solution into the caudal vein [8]. Control rats received an equivalent volume of 0.85% NaCl into the caudal vein. The material for the study was obtained on days 1, 3, 14, and 21 after infection. Specimens of the right liver lobe and right lung (200 mg) were homogenized on cold in 1 ml 0.85% NaCl with 0.1% EDTA in a Potter homogenizer and then centrifuged at 4000 rpm (15 min); the supernatants were frozen at -18°C. On the next day, LPO activity in homogenates was evaluated by the relative content of lipoperoxides in a heptane–isopropanol system and concentration of products interacting with TBA [1]. The content of lipoperoxides was measured on an SF-2000 spectrophotometer in the heptane phase of the lipid extract at λ=220, 232, and 278 nm. The results were expressed in oxidation index units. Oxidation indexes reflected the level of conjugated dienes ($E_{232/220}$) and ketodienes and conjugated trienes ($E_{278/220}$). The concentration of TBA-reactive products was measured spectrophotometrically at λ=532 nm and expressed in μmol/kg (molar extinction coefficient was taken as 1.56×10^{-5} mol^{-1} cm^{-1}). The data were processed by methods of variation statistics using nonparametric Mann—Whitney test [6].

RESULTS

The development of granulomatous inflammation after intravenous injection of SiO_2 microparticles was accompanied by considerable activation of LPO processes in the liver, which was seen from accumulation of early intermediates (conjugated dienes, ketodienes, and conjugated trienes) preceding the accumulation of secondary TBA-reactive LPO products [4]. On the next day after injection of SiO_2 microparticles, the content of conjugated dienes in the liver increased by 1.6 times and that of ketodienes and conjugated trienes by 2.3 times (Table 1); the content of TBA-reactive substances in liver homogenates increased by 1.7 times compared to the corresponding parameter in the control group (Fig. 1). On day 3, the content of conjugated dienes, ketodienes, and conjugated trienes in the liver decreased by 2.6 and 4.2 times compared to the previous term and by 1.6 and 1.8 times compared to the control, respectively, which was probably related to their active transformation into secondary LPO products. At this term (day 3), the concentration of TBA-reactive substances in the liver of experimental animals continued to increase and surpassed the corresponding parameter on day 1 by 2.1 times and the

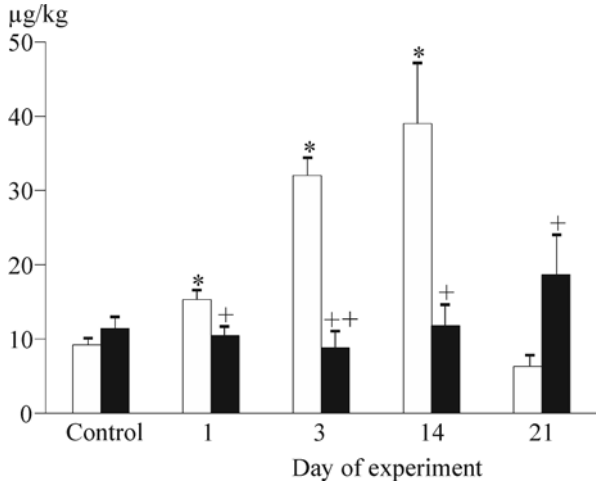

Fig. 1. Content of TBA-reactive products in the liver (open bars) and lungs (dark bars) of Wistar rats during the development of granulomatous inflammation after injection of SiO_2 microparticles. $^*p<0.05$ compared to the control, $^+p<0.05$, $^{++}p<0.01$ compared to the corresponding parameter in the liver at the same term of the experiment.

control level by 3.5 times. By day 14, the content of conjugated dienes, ketodienes, and conjugated trienes in the liver started to increase again compared to the level observed on day 3. At this term (day 14), the concentration of TBA-reactive LPO products in the liver of experimental animals attained the maximum and surpassed the control level by 3.9 times. By day 21 of the experiment, LPO activity sharply decreased: the content of primary LPO products in the liver did not differ from the control level, the level of TBA-reactive products in the liver no longer increased, but returned to the control value.

Changes in the content of primary LPO products in the lungs of experimental animals after injection of SiO_2 microparticles depended on their type. The level of conjugated dienes on day 1 decreased by 1.2 times compared to the control, but then this parameter increased and returned to normal on days 3 and 14, and even 1.2-fold surpassed the control level by day 21 (Table 1). The content of ketodienes and conjugated trienes on day 1 after injection of SiO_2 microparticles remained unchanged; on days 3 and 14 this parameter increased by 1.6 and 1.5 times, respectively, compared to the control, but on day 21 it decreased by 1.5 times. However, no accumulation of TBA-reactive products in the lungs was observed throughout the experiment (Fig. 1).

When comparing changes in the content of primary and secondary LPO products in the studied organs, we found some peculiarities of LPO processes after induction of SiO_2-induced granulomatosis. In the control, the contents of primary and secondary LPO products in the liver did not differ from the cor-

responding values in the lungs. However, on the next day after injection of SiO_2 microparticles, the contents of conjugated dienes and ketodienes with conjugated trienes in the lungs were by 2.5 and 4 times lower than in the liver, respectively, but after 3 days they were higher by 1.8 and 2.2 times, and by day 14 the differences disappeared (Table 1). By day 21 of the experiment, the content of ketodienes and conjugated trienes in the lungs was again 3-fold lower than in the liver. The content of TBA-reactive LPO products in the lungs was lower than in the liver over 2 weeks after injection of microparticles: by 1.5, 3.6, and 3.3 times on days 1, 3, and 14, respectively (Fig. 1). By day 21 of the experiment, the content of ketodienes and conjugated trienes in the lungs was again 3-fold lower than in the liver.

Thus, the development of granulomatous inflammation after intravenous injection of SiO_2 microparticles with a size of 1-5 μ was characterized by more pronounced and prolonged LPO processes in the liver and less marked LPO activation in the lungs. At all terms of the experiment, the increase in LPO intensity in the lungs was broken at the stage of primary product formation and did not lead to accumulation of secondary products, whereas in the liver, which turned out to be less resistant to free-radical oxidation, considerable amounts of TBA-reactive products were accumulated. Comparison of the content of primary and secondary LPO products in the liver and lungs at different terms of the experiments showed that their levels were normally similar, but some differences appeared after injection of SiO_2 microparticles. The differences between these organs can be explained by the fact that alveolar macrophages are adapted to oxygen conditions and exhibit low capacity to generation of oxygen radicals, LPO initiators, in response to stimulation with bacteria or inorganic particles, compared to mononuclear phagocytes in other organs [4]. Second, liver macrophages constitute 80-90% all tissue macrophages [3], which considerably affects the total production of active oxygen metabolites. Similar results were obtained in experiments with intratracheal administration of microparticles with a diameter of 1-5 μ (polymetallic dust) to outbred male rats [2]. LPO processes in the liver can be initiated and intensified by macrophages producing oxygen and nitrogen metabolites and neutrophils secreting myeloperoxidase. Previous morphological studies showed that within 2 weeks after intravenous injection of SiO_2 microparticles of the same size, granulomas consisting primarily of macrophages and low numbers of neutrophils, lymphocytes, and fibroblasts are actively formed in the liver; by day 20, the relative content of neutrophilic granulocytes in granulomas decreased and the concentration of fibrous tissue in the liver increased [5]. Three weeks after intravenous injection of SiO_2 microparticles, activity of LPO processes in the liver decreased, which coincided with the previously described decrease in neutrophil content in granulomas and accumulation of fibrous tissue in the liver [5]. Production of growth factors, in particular, transforming growth factor-β, is an important element of fibrogenesis in the liver; this factor induces transformation of lipid-accumulating cells into fibroblasts producing collagen III [11]. It was previously shown that transforming growth factors-β1, -β2, and -β3 suppress the production of activated nitrogen forms by macrophages [10], which can decrease LPO activity in the liver.

TABLE 1. Relative Content of Primary LPO Products in Heptane Phase of Lipid Extracts from the Liver and Lungs during the Development of SiO_2-Induced Granulomatous Inflammation ($M \pm m$)

Group, term of experiment		Oxidation indexes			
		liver		lungs	
		conjugated dienes	ketodienes and conjugated trienes	conjugated dienes	ketodienes and conjugated trienes
Control (n=12)		0.565±0.090	0.276±0.080	0.513±0.023	0.118±0.010
Experiment	day 1 (n=6)	1.080±0.032*	0.418±0.013*	0.427±0.029*	0.104±0.008^{+}
	day 3 (n=5)	0.311±0.011	0.078±0.006*	0.569±0.018^{++}	0.171±0.010*$^{++}$
	day 14 (n=5)	0.473±0.027	0.103±0.007	0.556±0.024	0.158±0.007*
	day 21 (n=5)	0.588±0.015	0.215±0.017	0.606±0.013*	0.071±0.004*$^{++}$

Note. *p<0.05 compared to the control, ^{+}p<0.05, ^{++}p<0.01 compared to the corresponding parameter in the liver at the same term of the experiment.

Thus, the development of granulomatous inflammation after intravenous injection of SiO_2 microparticles manifested in enhanced production of only primarily LPO products in the lung and enhanced generation of both primary and secondary products in the liver; it should be noted that the level of TBA-reactive products underwent phasic changes, which was largely determined by differences in the cellular composition of these organs and their adaptation to different oxygen conditions.

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