NANOTECHNOLOGIES

Effects of Nanodispersed Iron on the Morphofunctional Parameters of the Blood System

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The effects of nanodispersed iron forms on the morphology and function of the blood system were studied. Maghemite and lepidocrocite caused a leukocytic shift towards segmented neutrophil forms, reduction of lymphocyte rigidity, and stimulated their compactization. In addition, the counts of small hyperchromatic erythrocytes with high rigidity increased in the blood flow. The results indicated that a single dose of nanodispersed iron-containing drugs improved the blood respiratory function and its microrheology.

Key Words: *nanodispersed iron forms; erythrocytes; lymphocytes; semicontact atomic force microscopy*

The biological and toxic characteristics of nanosized objects are determined by their dispersion, physicochemical and structural parameters [5]. Interactions between nanoparticles of different chemical composition and structure and biological objects have been studied [7]. Metal oxide nanoparticles are characterized by cytotoxic, embryotoxic, and teratogenic effects [6], they accumulate in lymphoid tissue and cause DNA aberrations [1], disorder the blood clotting system work [3]. The toxicity of nanomaterials depends on their physical nature, method of preparations, size, structure of nanoparticles, and on the biological model on which the trials are carried out. Importantly that the nanomaterials do not always exhibit toxic or destructive effects [4], and hence, the development of tests for evaluation of the nanomaterials toxicity is in progress.

We have studied the effects of nanodispersed iron forms on the morphofunctional parameters of erythrocytes and leukocytes.

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MATERIALS AND METHODS

Experimental studies were carried out at Laboratory of Adaptation Processes Physiology of Belgorod State National Research University. Iron-containing nanodispersed preparations were used: maghemite (g-Fe_2O_3), lepidocrocite (g-$FeOOH$), and solution containing iron ions. Nanodispersed maghemite form consisted of nanotubules 100 nm long and 10 nm in diameter, with 2-3-nm channels inside the tubules. Lepidocrocite nanoparticles were cylindrical nanorods 100-150 nm long and 5-8 nm in diameter. Iron ion-containing solution was prepared from Mohr's salt $(NH_4)2Fe(SO_4)_2{\cdot}6H_2O$, chemically pure. The concentration of iron ions in the solution was 0.25 mmol/liter.

Biological activity of iron-containing preparations was evaluated on adult outbred laboratory male rats (n=24). The study was carried out in accordance with the regulations of the Helsinki Declaration on Humane Handling of Animals and Directives of the EEC on the Protection of Animals Used with Experimental and Other Research Purposes. The animals were kept on

standard rations; nutrition, temperature, and humidity were the same for all animals. Group 1 (n=6) were controls. Animals of groups 2, 3, and 4 (6 per group) received a single intragastric (through a tube) dose (1 ml) of *g-*Fe_2O_3, *g-FeOOH* nanoparticles, or Mohr's salt, respectively. The concentrations of maghemite and lepidocrocite nanoparticles was 100 mg/ml, of iron ions in solution 0.25 mmol/liter. Before administration, the suspensions were processed in Sapfir USV-1.3 TTZ ultrasonic bath (10 min) for destruction of the nanoparticles agglomerates. Controls received 1 ml water.

Blood was collected by decapitation of narcotized animals 24 h after administration of the studied substances. Heparin (20 U/ml) served as the anticoagulant. Smears from whole blood were prepared for evaluation of the differential leukocytic count and stained after Romanowskii–Giemsa. Erythrocyte and leukocyte populations were isolated by blood centrifugation (10 min, 1500 rpm). The leukocyte ring was collected. Erythrocytes were washed and resuspended in isotonic buffer. Erythrocytes were counted in Goryaev's chamber for all animals, hemoglobin concentration was measured by photocolorimetry, and hematocrit was evaluated after whole blood centrifugation on a hematocrit centrifuge. Smears for atomic force microscopy were made from the resultant leukocyte and erythrocyte suspensions. Scanning was carried out in a humid box in the semicontact mode using silicon probes (lot NSG03 (NT-MDT), 1.1 H/m rigidity, rounding radius 10 nm, scanning resolution frequency 0.6-0.8 Hz). Cell diameter, height, and volume were measured in the resultant scans. The results were processed using Nova 1.0.26 Build 1397 software (NT-MDT Company). The effects of nanodispersed iron-containing preparations on the elastic properties of formed elements of the blood were studied by atomic force spectroscopy by load application to local sites of the cell surface.

Plasma iron content in control and two experimental groups (maghemite and soluble iron) was measured by X-ray spectrochemical analysis. The studies were carried out on an ARL Optim X X-ray fluorescent spectrometer at Center for Common Equipment

TABLE 1. Blood System Morphology and Function

Parameter	Control	Maghemite	Lepidocrocite
Leukocyte count, 10^9 liter^{-1}	3.6±0.6	3.00±0.35	2.80±0.15
Monocytes, %	1.8±0.6	1.7±0.3	–
Eosinophils, %	1.3±0.2	0.70±0.08*	–
Young neutrophils, %	2.5±0.3	0.90±0.01*	1.000±0.001*
Stab neutrophils, %	16.9±0.6	16.3±0.2	13.80±0.08*
Segmented neutrophils, %	17.4±0.3	24.4±1.5*	21.5±1.0*
Lymphocyte diameter, μ	7.50±0.08	7.30±0.08	6.90±0.07
Lymphocyte height, μ	1.40±0.04	1.40±0.01	1.60±0.02*
Lymphocyte surface area, μ^2	44.40±0.98	41.90±0.04	38.20±0.81*
Lymphocyte volume, μ^3	60.5±1.5	55.6±0.7	58.7±1.2
Lymphocyte rigidity, Pa	16.9±0.7	14.8±0.6*	14.3±0.5*
Erythrocyte count, 10^{12} liter^{-1}	7.50±0.24	6.800±0.005*	6.30±0.11*
Hemoglobin concentration, g/liter	145.3±3.7	230.1±8.0*	235.2±2.6*
Hematocrit, %	36.00±0.01	37.00±0.01	35.00±0.01
Mean hemoglobin concentration in erythrocyte, pg	20.1±3.6	33.54±0.90*	37.5±2.5*
Erythrocyte diameter, μ	5.80±0.07	5.70±0.04	5.60±0.06
Erythrocyte height, μ	0.50±0.01	0.40±0.01*	0.50±0.01
Erythrocyte volume, μ^3	30.5±1.4	23.5±1.1*	30.6±1.5
Erythrocyte surface area, μ^2	52.2±8.7	40.3±6.5*	52.2±2.7
Erythrocyte rigidity, Pa	17.6±2.5	22.5±1.2*	19.9±1.0*

Note. *p<0.05 in comparison with the control.

Exploitation of the University. The results were statistically processed, the significance of differences was evaluated by Student's *t* test.

RESULTS

A single oral dose of iron-containing drugs led to an increase of plasma concentration of iron atoms, by 86% ($p<0.05$) in response to Mohr's salt and by 43% ($p<0.05$) in response to maghemite.

The differential leukocytic count in experimental animals was shifted towards mature segmented neutrophils, the counts of eosinophils decreased. The levels of segmented neutrophils increased by 40 and 23.5% ($p<0.05$) in response to maghemite and lepidocrocite, respectively, while the levels of young neutrophils decreased by 36 and 40% ($p<0.05$) in comparison with the control (Table 1). Nanodispersed iron-containing drugs caused no changes in the total leukocyte count. The lymphocyte morphometric parameters changed under the effects of nanodispersed iron forms. Lymphocyte height increased by 10% ($p<0.05$) and their surface area decreased by 14% ($p<0.05$) in the lepidocrocite group in comparison with the control. Soluble iron (Mohr's salt) led to a reduction of the lymphocyte diameter and surface area by 13 and 28%, respectively ($p<0.05$), while the cell height increased by 11% ($p<0.05$) in comparison with the control. Lymphocyte rigidity decreased by 14.8 and 18.18% ($p<0.05$) in response to iron nanoparticles and soluble iron, respectively, in comparison with the control.

Total erythrocyte counts decreased in response to maghemite and lepidocrocite by 10 and 19%, respectively ($p<0.05$), while hemoglobin concentration increased by 58.4 and 62% ($p<0.05$) in comparison with the control. Maghemite caused a decrease in the erythrocyte height, volume, and surface area by 12, 23, and 23% ($p<0.05$), respectively, in comparison with the control (Table 1). Lepidocrocite exhibited no appreciable effect on the erythrocyte morphometric parameters. On the whole, the reaction of the blood erythroid component to iron nanoparticles was aimed at cell shrinkage and was paralleled by changes in the functional characteristics of erythrocytes. This manifested by an increase of hemoglobin concentration in the erythrocyte to 33.5±0.9 pg in the maghemite group and to 37.5±2.5 pg in the lepidocrocite group, this involving an increase of oxygen capacity of the blood by 58.4 and 50% ($p<0.05$), respectively, in comparison with the control. Interestingly, erythrocyte diameter and hematocrit virtually did not differ in experimental and control groups. Erythrocyte elasticity increased under the effect of iron nanoparticles by 28% ($p<0.05$) in response to maghemite and by 13% ($p<0.05$) in response to lepidocrocite.

Hence, a single dose of nanodispersed iron-containing drugs enhanced phagocytosis by increasing the counts of mature segmented forms and reducing the percentage of young neutrophils, presumably by accelerating their maturing. In addition, reduction of lymphocyte rigidity under the effects of nanoparticles and soluble iron forms improved the blood rheology. Pathomorphologic findings indicated that a single dose of nanosized iron of about 250 mg/kg was nontoxic for organs and tissues; on the other hand, this substance led to stimulation of immune reactions [2].

Nanodispersed iron forms improved the respiratory function of the blood, modulating the geometrical profile of erythrocytes and their hemoglobin saturation. Presumably, the appearance of hyperchromatic erythrocytes of small size was explained by triggering of redistribution reactions in the system, aimed at the maintenance of oxygen homeostasis. Specifically, the geometrical profile and hemoglobin content in each erythrocyte play the key role in oxygenation processes: the smaller the erythrocyte, the more rapid is oxygen consumption by hemoglobin in the lungs. Hyperchromatic erythrocytes of experimental rats maintained the oxygen-transporting function of the blood under conditions of reduced erythrocyte counts, thus improving blood rheology. On the other hand, the increase of erythrocyte rigidity in experimental groups indicated adsorption and transport of nanodispersed iron forms on erythrocyte surface.

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