THE PROCESSES OF APOPTOSIS IN THE BONE MARROW AT THE STAGES OF POSTNATAL ONTOGENESIS UNDER THE INFLUENCE OF SULFUR-CONTAINING GAS (EXPERIMENTAL RESEARCH)

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ABSTRACT — In the course of the experimental study, we evaluated the state of bone marrow apoptosis of the femur of white nonlinear male rats from 36 to 730 days of ontogenesis: intact and exposed to sub-toxic doses of sulfur-containing gas (90±3 mg/m^3). The industrial natural sulfur-containing gas of the Astrakhan gas condensate field was used as a toxic agent. Analysis of the results obtained in the course of the experiment allows us to assume that the conditions of the subtotal effect of the sulfur-containing gas activate pro-apoptogenic proteins, leading to a disruption in the regulation of apoptosis. When exogenous pollutants are exerted, the proliferative activity of bone marrow structures is inhibited against a background of relatively low intensity of regenerative processes.

KEYWORDS — apoptosis, sulfur-containing gas, rats, postnatal ontogeny.

The phenomenon of apoptosis is the result of the action of various factors leading to cell death. It is known that the development of various diseases (neoplasms, cardiovascular, acute and chronic inflammatory processes, diabetes mellitus, etc.) is associated with violations of mechanisms for the realization of apoptosis, leading to its excessive activation or inhibition [2, 9]. Along with this important link in the pathogenesis of these diseases is the imbalance of oxidative metabolism [4, 5].

Among hematological diseases, in which the apoptosis increases, anemia of different genesis predominates (refractory anemia, aplastic anemia, anemia associated with impaired blood formation, etc.), and inhibition of apoptosis is noted in the tumor transformation of hematopoietic cells [6, 7].

The production hazards of a gas producing enterprise, among which sulfur-containing gas occupies a leading position, adversely affect various systems of the human body [1, 3, 8]. Significant tension of adaptation mechanisms leads to premature aging and development of cardiovascular diseases, hematopoiesis diseases associated with this condition. According to the research of some authors [1], under the influence of production factors of a gas producing enterprise, a disorder of apoptosis regulation, imbalance in the system of oppositional cytokine pools and antioxidant protection is observed in persons without somatic pathology. Consequently, apoptosis plays an important role in regulating the state of stable equilibrium in a

rapidly renewing tissue system, which includes the erythroid population of red bone marrow.

MATERIALS AND METHODS OF RESEARCH

The experiment was performed on 75 white non-linear male rats. Two groups of species were formed: I. control; II. Impacted with sulfur-containing pollutants. Each species consisted of four groups of 10 individuals in each, the animals in which were at the same stages of individual development as people during postnatal ontogeny (Table 1). Group II were exposed to gaseous sulfur-containing pollutants at the time when the animals in them were of age: immature (young) — from 6 to 36 days, mature I — from 368 to 398 days, mature II — from 472 to 502 days and presenile age — from 700 to 730 days.

Table 1. *Correspondence of the terms of development of experimental animals in accordance with the periods of postnatal ontogenesis of man*

Men	Laboratory rats	
Period	Period	Ontogenesis day
Childhood	Immature (young)	6–36
Adult age, I period	Mature I	368–398
Adult age, II period	Mature II	472–502
Elderly age	Presenile	700–730

Toxicant was the natural stratal dehydrated gas from the Astrakhan gas condensate field. The experiments used the concentration of natural gas in the air-gas mixture of the chamber, which is 90±3 mg/m^3 when measured by hydrogen sulphide, which is 30 times greater than the maximum permissible concentration of hydrogen sulfide for working areas with simultaneous presence of hydrocarbons.

Inhalation with sulfur-containing gas was carried out for 4 hours in the autumn-winter seasons by a static method, with simultaneous presence in the chamber of 5 individuals daily for 30 days, except on Sundays, strictly from 10 to 14 hours, the temperature in the chamber was +22±2° C. Relative humidity during the experiment increased from 53±4% to 66±6%. The gas concentration and experimental conditions fully comply with the requirements set forth in the WHO publication "Principles and methods for assessing the toxicity of chemicals".

As a control, rats of similar experimental age groups were studied, which for 5 days were kept in a hermetically sealed primer chamber for the same time and under the same conditions as the experimental ones, but without the presence of a sulfur-containing gas.

In our work the immunohistochemical method was used to study the content of i-NOS in different age groups in normal and sub-toxic effects of sulfur-containing pollutants, antibodies and diluents from Spring Bioscience (USA) were used. All materials were subjected to statistical processing to establish the reliability of the data obtained when analyzing the results of the study. Materials were processed on a personal computer using programs "Microsoft Office Windows 2007", "Microsoft Office Excel 2007".

RESULTS OF A RESEARCH

A positive reaction to the i-NOS protein was not detected in our study in both mature subgroups and subgroup of young animals, which allows us to state that there is no exogenous and endogenous stimulation of endothelial cells (Fig. 1). In the presenile subgroup of animals, a weak positive reaction to i-NOS was observed predominantly in the inner part of the compact substance, in the spongy substance of the bone beams of the positive reaction was not noted, as in the other subgroups.

Against the background of sub-toxic effects of sulfur-containing pollutants, positive-colored areas of the red bone marrow are revealed. The weakest reaction is determined in the 1st adult subgroup of animals, i-NOS-positive endotheliocytes are detected in the vessels of the bone trabeculae. In the second mature subgroup, the i-NOS-positive reaction is produced not only by endothelial cells of a compact substance, but also by single cells in a spongy substance. The most pronounced reaction is observed in a young subgroup of laboratory animals. I-NOS-positive endotheliocytes are defined in the sinusoidal capillaries of the bone marrow. This may be the result of insufficient maturity of enzyme systems producing inducible NO-synthase in response to exogenous stimulation with sulfur-containing pollutants. Nitric oxide formed in excess through true pores enters the hematopoietic islets, where it causes apoptosis of the hematopoietic elements, acting as an active radical.

In our work, we studied the expression of one of the proapoptogenic proteins — Bax. In a young subgroup of animals, a weak positive reaction to this protein is determined, mainly in a compact substance of bone tissue. Spongy substance contains single cells with signs of the protein being studied.

As the animals age, the activity of proapoptotic proteins increases. A positive reaction to Bax in all structural components of bone tissue is determined: in both compact and spongy substances.

After exposure to sub-toxic doses of sulfur-containing pollutants, pro-apoptogenic proteins are activated in all subgroups under study (Fig. 2).

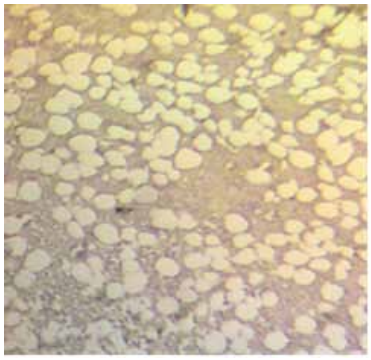

Fig. 1. *Negative immunohistochemical reaction to i-NOS in red bone marrow structures in a young subgroup of animals*

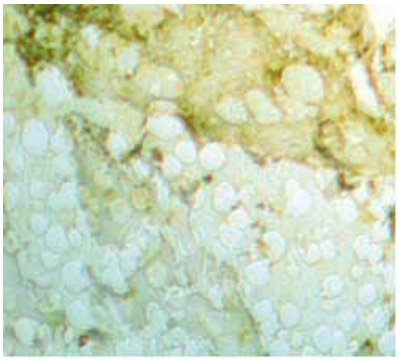

Fig. 2. *Strongly positive reaction to Bax in bone marrow structures in a young subgroup of animals against a background of sub-toxic effects of sulfur-containing pollutants*

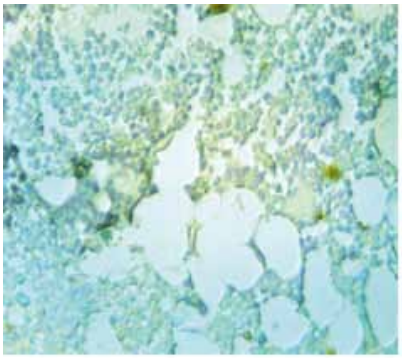

Fig. 3. *Positive Ki-67 reaction in red marrow cells in a young subgroup of animals*

The proliferation index of Ki-67 (%) was calculated by counting the number of immunopositive nuclei to the total number of nuclei. Counting was performed in at least 10 fields of vision. In the study of histological preparations, a high proliferation index was observed in all experimental subgroups. The largest was recorded in a young subgroup. Ki-67-positive cells were determined in all studied fields of vision (Fig. 3).

After exposure to natural gas, the proliferation index decreased, the severity of the immunohistochemical reaction decreased, especially in the presenile subset of animals. This suggests that the effect of exogenous pollutants inhibits the proliferative activity of bone marrow structures against a background of relatively low intensity of regenerative processes.

Thus, in the course of the experimental research, we found that, under the conditions of a sub-toxic sulfur-containing gas, one of the important links in pathogenesis is the activation of proapoptogenic proteins, which leads to a disruption in the regulation of apoptosis. The effect of exogenous pollutants inhibits the proliferative activity of bone marrow structures against a background of relatively low intensity regenerative processes.

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