Research Paper

Effect of Etoksidol Against Sulfur-Containing Gas Induced Reduction in Bone Marrow Microcirculation in Different Stages of Ontogenesis in Rats

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Abstract

Objective: To investigate the state of femoral red bone marrow microvasculature of nonlinear white male rats in the course of an acute experiment. **Materials and Methods:** The ontogenesis of experimental animals were between 36 and 730 days: Intact ones, those subjected to the action of subtoxic doses of sulfur-bearing gas, and those receiving a protector-drug "Etoksidol". The data on microcirculation were obtained using laser Doppler flowmetry. The industrial sulfur-containing natural gas of the Astrakhan gas condensate field was used as a toxic agent. It was revealed that the reducing intensity of blood microcirculation in the red bone marrow of intact rats is statistically more significance in the presenile period of ontogenesis compared with the earlier periods. **Results:** The use of "Etoksidol" along with the influence of sulfur-containing pollutant on the experimental animals of different age groups led to improvement of microcirculation in the bone marrow at all the studied stages of ontogeny. However, such improvement was statistically highly significant ($P < 0.01$) in the mature age I, very close to significant in the mature age II, and was not statistically significant in the young and presenile age. **Conclusions:** As a result, the following findings were obtained: The toxic effect greatly reduces the intensity of the microcirculation in the bone marrow, which is most pronounced in younger animals; the experimental results suggest that the drug "Etoksidol," with an antihypoxic and antioxidant action, has a positive effect on red bone marrow microvasculature.

Keywords: Etoksidol, hemomicrocirculation, postnatal ontogenesis, rats, sulfur-containing gas

Introduction

Experimental medicine is the basis for the development and testing of various preventive and treatment technologies at the organismal level.[1] Gaseous substances – pollutants contained in the atmosphere and in the air of industrial productions – are of greatest importance among numerous anthropogenic toxins. Permanent release of these pollutants into the environment leads to their accumulation, especially in case of continuous production at oil and gas processing plants, which ultimately leads to chronic ecotoxicity of the environment.[2] Modern medicine is undoubtedly in dire need of means capable of enhancing the energy-producing function of cells under conditions of their scarcity as well as withstanding the development of toxic stress successfully.[3]

It is known that the typological features of humans and animals are in close correlation with various physiological indicators, including the indicators of microcirculation,[4] which is an important part of metabolism. Homeostasis and adaptive capacity of the body are determined by microhemodynamics. Adequate functioning of proper microvasculature, especially in the red bone marrow, is a basic factor in maintaining the normal functioning of the erythron system.

The microcirculation pattern reflects many interrelated and interdependent processes, such as, in particular, blood flow patterns in the microvessels and the state of the endothelium, both in the normal course of different ontogenesis stages and under the influence of adverse environmental factors.[5]

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The purpose of this experiment was to study the characteristics of microcirculation in the bone marrow of rats at postnatal ontogeny stages using laser Doppler flowmetry (LDF) in the cases when rats were subjected to subtoxic doses of sulfur-bearing gas and when they received the drug "Etoksidol" (3-hydroxypyridine malate) as a protector. The drug was synthesized by the specialists of the All-Russian Scientific Center for Safety of Biologically Active Substances (Staraya Kupavna, Russia) under the leadership of Professor Skachilova and patented under the name "Etoksidol".[6] "Etoksidol" is an inhibitor of free-radical processes, has membrane protecting, antihypoxic, nootropic, anticonvulsant, anxiolytic effects, and increases the resistance of organisms to stress.

Materials and Methods

The study of microcirculation involved 124 white nonlinear male rats divided into three groups: (I) the control group (intact rats); (II) exposed to sulfur-containing pollutants; (III) exposed to sulfur-containing pollutants and received a preparation with a protective effect. Each group included four subgroups of ten animals each, whose stages of individual development were similar to the stages of human postnatal ontogenesis [Table 1]. Groups II and III were exposed to gaseous sulfur-containing pollutants when the animals were aged: immature - from 6 to 36 days, mature I - from 368 to 398 days, mature II - from 472 to 502 days, and senile - from 700 to 730 days.

The toxicant was natural dehydrated gas from the Astrakhan gas condensate deposit. The gas was obtained at the installation U-121 that received it from the well no. 17.

The concentration of natural gas in the air-gas mixture of the chamber was 90 ± 3 mg/m^3 if measured by hydrogen sulfide. The concentration of hydrogen sulfide in the exposure chamber (the type developed by Kurlyandsky) was measured by the indicating tubes produced by Auer (Germany). The exposure to sulfur-containing gas was held through the static method in the autumn and winter seasons. Five animals were simultaneously placed in the chamber for 4 h every day except Sundays, strictly from 10 am to 2 pm; the experiment duration was 30 days. The temperature in the chamber was +22 ± 2°C. During the experiment, the relative humidity was increased from 53% ± 4% to 66% ± 6%.

Table 1: Periods of human postnatal ontogenesis and their correlation with the developmental periods of the experimental animals

Humans (period)	Laboratory rats	
	Period	Age of gas exposure (days of ontogenesis)
Childhood	Immature	6-36
Adulthood		
Period I	Mature I	368-398
Period II	Mature II	472-502
Advanced age	Presenile	700-730

Table 1 is based on the data given in.[7-9]

Animals suspected of having spontaneous pathologies were culled. The test records concerning animal selection and maintenance, pathological processes modeling and removing animals from the experiment were done in accordance with the principles of bioethics and good laboratory practice; they correspond to the ethical standards set out in the Geneva Convention, (1981) "International Guiding Principles for Biomedical Research Involving Animals" (1985), and in accordance with the order No. 267 issued by the Ministry of Health of the Russian Federation on June 19, 2003.

The control rats were of similar age groups. To level the effects of stress, their conditions were similar to those of the experimental animals. Six rats from each age group were also placed into a hermetically closed exposure chamber for the same 4 h a day but sulfur-containing gas was absent in the chamber. Microcirculation was measured in the conditions of an acute experiment. after anesthesia by intraperitoneal administration of pentobarbital solution (4 mg per 100 g of animal body weight), the researchers accessed the proximal metaphysis of the femur – the part located in a dorsolateral direction between the femoral greater and lesser trochanter. To evaluate the bone marrow microcirculation, the metaphysis bone tissue was gently fenestrated with the formation of the hole diameter of 1.5 mm. The experiments were conducted at the ambient temperature of 21–23°C. The data on microcirculation were obtained using the equipment LAKK-02 of scientific productive enterprise "Lazma" (Russia). The results of LDF testing were registered in relative perfusion units (PU), which reflect the degree of perfusion (mainly by blood erythrocyte fraction), units of tissue volume per unit of time, and allow tracing its dynamics at different conditions.[10,11]

Results

The values of the indicators of microcirculation active mechanisms for the ontogenesis stages under the study are presented in Table 2 and illustrated by Figure 1 - intact animals, Figure 2 - after the exposure to gaseous sulfur-containing pollutants.

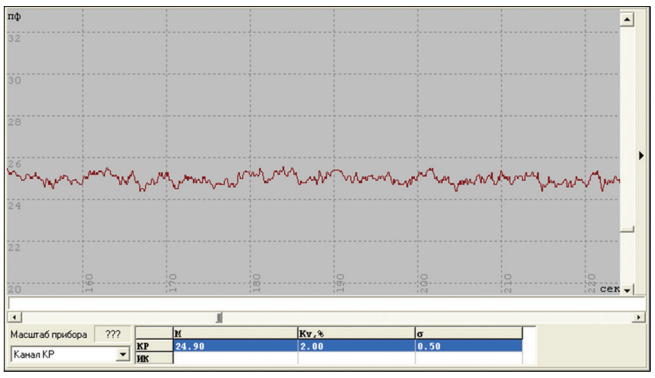

Figure 1: Laser Doppler flow of microcirculation in the red bone marrow of an intact rat of immature age

In the control subgroup of young animals, the index of microcirculation was 24.84 ± 0.75 PU. The response to the experimental exposure to natural gas was a decrease in this parameter to the value of 13.18 ± 0.35 PU, which means a reduction of blood flow in the studied area.

In the experimental animals of the control subgroup of the ontogenesis mature period I, the registered microcirculation index was 25.73 ± 1.24 PU, the mature period II - 24.86 ± 0.62 PU, and the presenile period - 20.38 ± 0.58 PU.

After inhalation of sulfur gas, there was a drastic decrease of the microcirculation parameter in the ontogenesis mature period I down to 13.82 ± 0.28 PU, the maturity period II - 13.72 ± 0.22 PU, and the presenile period - 13.53 ± 0.30 PU.

The use of the protector "Etoksidol" together with exposure to the sulfur pollutant led to an improvement of the state of microcirculation in the bone marrow in all the studied stages of ontogenesis. However, the improvement was statistically highly significant ($P < 0.01$) only in the mature period I. In the mature period II, it was on the verge of significance; and in the young and presenile age, it was not statistically significant.

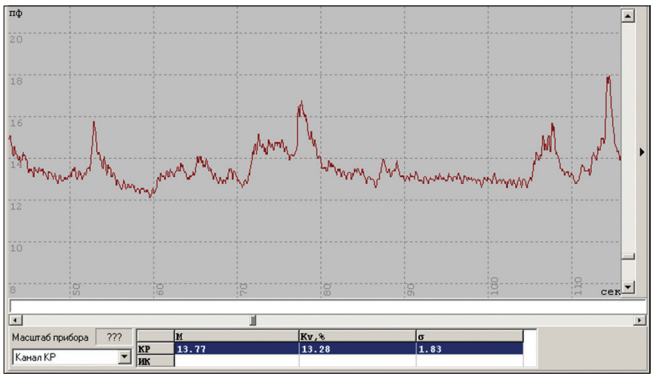

Figure 2: Laser Doppler flow of microcirculation in the red bone marrow of a rat of ontogenesis mature period I after the influence of sulfur-containing gas

Discussion

An analysis of the results shows that studying microcirculation in intact animals, the best indicators were recorded in mature I and mature II subgroups. Most likely, this is due to the full maturity of the cardiovascular system and regulatory systems. The study has shown that age-related fluctuations of the intensity of blood microcirculation in the red bone marrow of intact rats were not statistically significant over the time of 225 to 487 days of postnatal ontogenesis.

With increasing age of the animals up to 630 days, we observed a highly significant (P<0.01) fall in the microcirculation intensity - by 18 ± 2%. Perhaps this was due to the age-related pressor effects on the vascular system and the reduction of components of the microcirculatory bed. The impact of natural gas leads to a reduction in blood flow in the study areas.

It is known that initial changes in the action of natural gas are accompanied by an increase in the tone of the arterioles of the microcirculatory bed and the increase in the production of vasoconstrictor compounds. The younger animals and the animals of the pre-senile group showed the lowest possible index of microcirculation after the influence of sulfur-containing pollutants ($P<0.05$).

A similar trend with changes in the studied parameters of microcirculation was observed in all groups of animals. However, the most pronounced changes were in the young and presenile group. In the first due to a violation of the balance between the cardiovascular system and regulatory

Table 2: The indicators of microcirculation after exposure to sulfur-bearing gas with the hydrogen sulfide concentration of 90 mg/m³

Indicator	Group			
	Immature	Mature I	Mature II	Presenile
MI				
Control	24.84±0.75	25.73±1.24	24.86±0.62	20.38±0.58*
Gas	13.18±0.35*	13.82±0.28*	13.72±0.22*	13.53±0.30*
Gas + protector	16.54±0.89*	20.82±0.58*	17.31±0.54*	16.79±0.49*
A_N				
Control	0.94±0.03	0.88±0.07	0.90±0.1	0.98±0.11
Gas	1.58±0.08*	1.36±0.12*	1.45±0.14*	1.62±0.15*
Gas + protector	1.35±0.09*	1.24±0.08*	1.27±0.11*	1.51±0.12*
A_M				
Control	2.46±0.12	2.84±0.14*	2.73±0.11*	2.04±0.12*
Gas	1.35±0.14*	1.74±0.12*	1.68±0.12*	1.20±0.09*
Gas + protector	1.64±0.16*	2.58±0.17	2.43±0.13	1.52±0.11*

*The difference with the control is significant at $P<0.05$. MI=Index of microcirculation (M±m, PU), A_N=The amplitude of neurogenic oscillations (M±m, PU), A_M=The amplitude of myogenic oscillations (M±m, PU), PU=Perfusion units. To establish the reliability of data obtained during the analysis of results of study, all materials were subjected to statistical processing on a personal computer using the program "Microsoft Office 2010". Mean arithmetic meanings (M), mean errors (m), and probability of difference (p) from the Student's *t*-test were determined

systems, and in the second due to a decrease in adaptive and regenerative abilities.

In the mature period I of ontogenesis, the animals exposed to gas showed a statistically significant ($P<0.05$) increase in the microcirculation level in comparison with the previous period 5 ± 1%. With further increase in the age of the animals, the microcirculation intensity fluctuations were not statistically significant.

We used a drug of the Russian production with the protective action "Ethoxidol" for reducing of the damaging effect of natural gas on the microcirculatory bed of the red bone marrow and its function.

In our study, the use of "Ethoxidol" under the influence of pollutant led to an improvement in the state of microcirculation in the red bone marrow in all the stages of researching ontogenesis. Improvement with the use of the protective agent "Ethoxidol" was statistically highly significant ($P<0.01$) only in the mature period I. In the mature period II, it was on the verge of significance; and in the young and pre-senile age, it was not statistically significant. However, in these two groups there was a tendency to improve the studied parameter to control values.

Conclusions

1. The toxic effect after the influence of sulfur-containing pollutants greatly reduces the intensity of the microcirculation in the bone marrow, which is most pronounced in younger animals and the animals of the presenile group.
2. The drug "Etoksidol" has a positive impact on the bone marrow microvasculature. The mentioned observations reveal a possibility of increasing the body's resistance to the chemical components of gas by the Russian drug "Etoksidol" having an antihypoxic and antioxidant action.
3. The results of the investigation will help discover the mechanisms and ways of nonspecific protection increase in case of chronic exposure to hazardous and harmful environmental factors at the stages of postnatal ontogenesis.

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Conflicts of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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