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Research paper

The state of the red bone marrow in rats depending on age in case of poisoning with lead acetate and potassium dichromate

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Abstract

Introduction: The current ecological situation is described by a rapid increase in air pollution through various chemical emissions.

Aim: The study aims to define how the level of lead and chromium compounds affects the parameters of red bone marrow and blood in animals and how these processes depend on their age.

Material and methods: To study the toxic intraday effect of heavy metals, a metal probe was used for combined priming of rats with lead acetate and potassium dichromate. The indicators were analyzed with the use of a cytogram, and the indices of the ratio between young and mature forms were calculated. The obtained factual material was subjected to computer processing with the calculation of the Student's criterion and confidence intervals.

Results and discussion: It was established that in young rats there is an accumulation of polychromatophilic cells with a large amount of hemoglobin, which leads to a violation of their maturation – with that, the subsequent stage of division passes very quickly, when, upon transforming into orthochromatic normoblasts, they pass into the blood after 15–20 h, forming a variant of terminal division. In old animals, there was a decrease in total bone marrow cellularity and severe anemia.

Conclusions: After the experiments and profound analysis, the author of the study defined the difference of toxic effects on young and old animals.

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1. INTRODUCTION

Chemicals have become an integral part of human life, but at present, the rapid growth of the chemical environment is leading to significant damage to public health. According to the World Health Organisation (WHO), airborne particulates alone cause about 3.1 million premature deaths each year. The total contribution of air pollution by WHO experts is estimated at 3.2% of the global burden of disease. Rational, deliberate reduction of air pollution levels, including lead and chromium, reduces the incidence of blood diseases, respiratory infections, cardiovascular diseases (especially heart attacks and strokes), lung cancer, and other oncological diseases.[1–3]

The current environmental situation is described by the fact that urban air pollution from motor transport emissions has reached such a degree that industrial enterprises have become a secondary source of air pollution with lead, chromium, and other metals. Lead emissions into the atmospheric air cause its increased concentration in soil and vegetation, especially in places of heavy traffic.[4–6] In 1969, the WHO expert commission recognised the maximum concentration of lead in the ambient air at the level of 14–25 $\mu g/m^3$. In most of the commonwealth of independent states (CIS), lead in atmospheric precipitation usually contains 1–3 $\mu g/L$, less often up to 10 $\mu g/L$, in cities with a population of up to 0.1 million people, the concentration of lead in the air amounts to 1.5 $\mu g/m^3$, with a population of more than 2 million people – 2.9 $\mu g/m^3$.[7]

According to data from previous studies, in people living in areas remote from civilisation, the concentration of lead in the blood is only 0.02 $\mu mol/L$, while in people living in industrial areas, this indicator reaches 1.9 $\mu mol/L$, leading to the development of toxic anemia.[8] Workers of lead-hazardous and chrome production are exposed to a double 'exposure' of lead and chromium: on the one hand, the adverse effect from working at the enterprise, on the other hand – the negative effect of lead from atmospheric air and water.[5,9] By analogy with natural focal diseases, a relatively new and understudied phenomenon became relevant – the focal nature of human ecological pathology. An epicenter is distinguished, where the main sources of permanent environmental pollution are located, and peripheral zones with an indirect, more delayed and transformed, but no less harmful influence of technogenic factors.[4,10–12]

For example, unfavourable environmental conditions in Shymkent (Republic of Kazakhstan) significantly increase the risk of lead accumulation in the body in children of the third generation of the population living in contaminated areas, cause violations of the antioxidant defence in the respiratory system, significantly reduce the barrier-protective properties of the cellular systems of local immunity, violate the processes of hematopoiesis. According to the data provided in the article by Bokombaeva[13] from the Kyrgyz Republic, the average level of lead in the blood of people amounts to 10–25 μg per 100 mL (i.e., 0.10–0.25 $\mu g/mL$). These standards, as she believes, should not apply to children, they should be lower since lead is especially harmful to the growing body of a child. According to her, the lowest level of lead, corresponding to WHO standards, was observed only in the blood of children in a relatively clean area. In the technologically polluted zones of Ak-Tyuz and Orlovka of the Kyrgyz Republic, the lead level exceeds the norm 3.0–3.5 times.[12]

As a result, about 0.6% of the global disease burden of modern humans is attributable to lead exposure. It was proven that the pre-existing signal level of 10 $\mu g/dL$ of blood causes a decrease in the intelligence index (IQ) in children. At present, the signal level is recognised as 1 $\mu g/dL$ of blood, upon reaching which in Western countries, where a system of free compulsory determination of lead in the blood of newborns is established and when a child changes the place of residence, the necessary preventive and therapeutic measures are taken.[8,14] It is of interest how the level of lead and chromium compounds affects the parameters of red bone marrow and blood in animals, depending on their age.

2. AIM

The study aims to define how the level of lead and chromium compounds affects the parameters of red bone marrow and blood in animals and how these processes depend on their age.

3. MATERIAL AND METHODS

The experiments were carried out on 59 male white non-inbred rats: young rats aged 2.5–6.0 months with a bodyweight of 180 g and old animals aged 1.7–2.0 years with a bodyweight of 250 g. All animals were divided into 4 groups. Young animals: intact group ($n = 11$); experimental group – lead acetate and potassium dichromate ($n = 19$). Old animals: intact group ($n = 15$); experimental group – lead acetate and potassium dichromate ($n = 14$). To study the toxic effects of heavy metals for 21 days per. os., a metal probe was used to inoculate rats with lead acetate at a dose of 15 mg per 1 kg of body weight and potassium dichromate 3 mg per 1 kg of body weight, according to the method described in the articles of Udartseva,[15] Akanov et al.[16]

The indicators of the bone marrow cytogram were studied: indicators of the red line in bone marrow smears. In the study of bone marrow punctate, apart from the number of counted cells, the indices of the ratio between young and mature forms were calculated; bone marrow index. Upon the analysis of the data obtained, authors took into consideration that hematopoiesis is a complex, multi-stage process of cell division and differentiation, as a result of which mature, functionally complete blood cells are formed. The modern scheme of hematopoiesis describes the sequence of differentiation in the hematopoietic tissue, starting from the initial cellular links and ending with forms that are incapable of proliferation. The main hematopoietic organ is the bone marrow, where 5 classes of blood cells are conventionally distinguished. In order of priority, at the first stage, myelokar-

yocytes were counted in a diluted bone marrow punctate in a counting chamber, followed by recalculation per 1 μL of punctate. The reagent used was 3%–5% acetic acid solution.

In a test tube with 4 mL of the acetic acid solution, 0.02 mL of bone marrow punctate was added (diluted 200 times). The contents of the test tube were thoroughly mixed and the counting chamber was filled. After the sedimentation of the formed elements, 1–2 minutes later, myelokaryocytes were counted, i.e., all nuclear elements in 100 large squares. The number of myelokaryocytes in 1 μL of punctate was calculated according to the equation (1):

$$X = \frac{n \cdot 200 \cdot 250}{100} = n \cdot 500 \quad (1)$$

where X is the number of myelokaryocytes (in 1 μL) of punctate, n is the number of myelokaryocytes in 100 large squares; 200 – breeding bone marrow punctate; 250 – a multiplier for converting to 1 μL, since the volume of one large square is 1/250 μL; 100 is the number of calculated large squares.

Next, the counting of megakaryocytes in the diluted bone marrow punctate was carried out in the counting chamber, followed by recalculation per 1 μL of punctate. In a test tube with 0.4 mL of the acetic acid solution, 0.02 mL of bone marrow punctate was added (diluted 20 times). The counting of megakaryocytes (giant cells) was performed in the entire grid and was calculated according to the equation (2):

$$X = \frac{n \cdot 20}{3.2} \quad (2)$$

where X is the number of megakaryocytes (in 1 μL) of bone marrow punctate; n is the number of megakaryocytes in the entire chamber; 3.2 – chamber volume (in μL); 20 – the degree of dilution of bone marrow punctate.

In the study of bone marrow punctate, apart from the number of counted cells, the indices of the ratio between young and mature forms were calculated. Bone marrow index of neutrophil maturation (BMIN) was calculated as:

$$\text{BMIN} = \frac{\text{promyelocytes + myelocytes + metamyelocytes}}{\text{stab + segmented}} = 0.6\text{–}0.8.$$

Maturation index of erythroblasts (MIE) was calculated as:

$$\text{MIE} = \frac{\text{polychromatophilic and oxyphilic enormocytes}}{\text{erythroblasts}} + \text{+ normocytes (basal, poly-, oxyphilic)} = 0.8\text{–}0.9.$$

The ratio of the number of leukopoiesis elements to the number of nuclear elements of erythropoiesis (K) was calculated as:

$$\text{K} = \frac{\text{granulocytes + monocytes + lymphocytes}}{\text{erythroblasts pronormocytes + normocytes}} = 2.1\text{–}4.5.$$

Normally, the leuko-erythroblast ratio is 4(3) : 1. The animals were determined by the generally accepted parameters of peripheral blood.

Animal studies were carried out per the *European Convention for the Protection of Vertebrate Animals used for Experimental and Scientific Purposes*[17] and the recommendations set out in the *Guidelines for Experimental (Preclinical) Study of New Pharmaceutical Substances*.[18] The sacrification of animals was performed in a humane way – euthanasia with chloroform. In a blood smear, morphological changes in erythrocytes associated with regenerative processes – polychromatophilia and reticulocytosis – were studied. Also, the resistance of the erythrocyte membrane was determined with the use of haepotanic solutions of different concentrations.[19,20]

The number of reticulocytes, a short-term phase of erythrocyte development, was counted in a smear after a special – supravital (intravital) staining. In a smear under a microscope with an immersion system, the number of reticulocytes was counted per 1000 erythrocytes. The colour index was calculated with the use of the formula for the ratio of hemoglobin to the number of erythrocytes. Along with the above parameters of peripheral blood, hematocrit and erythrocyte resistance were determined. Determination of the osmotic resistance of erythrocytes in animals was performed with the use of hypotonic solutions of different concentrations. The obtained factual material was subjected to computer processing with the use of the Microsoft Excel application package with the calculation of the Student's criterion and confidence intervals.

4. RESULTS AND DISCUSSION

When young animals are inoculated with toxicants lead acetate and potassium dichromate, blast cells are recorded in the white sprout of the bone marrow. The indicator of the granulocyte germ increases almost 2 times (Table 1).

In young rats, in the course of the study of randomly selected areas of the bone marrow in case of heavy metal poisoning, erythroblasts were not detecte. At the same time, the level of pronormoblasts decreased by half, and the number of normocytes with basophilic puncture increased. The level of

Table 1. Bone marrow parameters in young animals inoculated with lead acetate and potassium dichromate.

Indicators	Intact group $n = 11$	Lead acetate and potassium dichromate $n = 19$
Cell type		
Erythroblasts	0.6 ± 0.08	0
Pronormoblasts	1.1 ± 0.05	0.5 ± 0.2
Basophilic normocytes	3.8 ± 0.2	7.5 ± 0.7*
Polychromatophilic normocytes	5.1 ± 0.3	18.1 ± 1.1*
Oxyphilous normocytes	4.0 ± 0.2	9.5 ± 2.0*
Erythroid lineage	9.3 ± 3.9	35.6 ± 2.4*
Bone marrow index of neutrophils	0.4 ± 0.07	0.5 ± 0.1
Leukoerythroblastic ratio	3.0 ± 0.1	1.9 ± 0.2*
Red blood maturation index	0.7 ± 0.02	0.8 ± 0.03

Comments: all numbers are given in percentages as mean ± SD; * Statistically significant ($P < 0.05$) in relation to the intact group.

polychromatophilic normocytes increased threefold. The accumulation of polychromatophilic cells with a large amount of haemoglobin leads to a violation of their maturation, while the subsequent stage of division passes very quickly, when they, upon transforming into orthochromic normoblasts after 15–20 h, pass into the blood. These cells cannot free themselves from the tetroploid nucleus and die – a variant of terminal division. The index of the erythroid lineage decreased by almost 3 times, but, in comparison with the control group, with a wide spread of the mean deviation. Summarising these data in the form of indices, it becomes clear that a tendency is formed to disrupt the maturation of red blood.

When old rats were poisoned with heavy metals, there was a decrease in the cellularity of the red blood shoot in the studied areas of the bone marrow – no erythroblasts and pronormoblasts were detected (Table 2). There was a tendency towards a decrease in normocytes of the basophilic series and a significant decrease in normocytes of the polychromatophilic and oxyphilic series ($P < 0.05$). As a result, the erythroid lineage was two times lower in old animals compared to the intact group.

Exposure of young animals to compounds of lead acetate and potassium dichromate caused a statistically significant decrease in the level of haemoglobin and erythrocytes. The latter decreased due to the layering of terminal division (pathological process) on the normal erythropoiesis of polychromatophilic normoblasts, their destruction in the blood, which was confirmed by a decrease in the indicator of osmotic resistance of erythrocytes.[21,22] Exposure of old animals to compounds of lead acetate and potassium dichromate was accompanied by a decrease in the level of erythrocytes and haemoglobin.[23,24] In contrast to young animals, the drop in haemoglobin in them was more significant – 104.7 versus 114.6 ($P < 0.05$).

In the body, lead is found in an exchangeable and stable fraction, and the exchangeable part of 95% of lead is associated with erythrocytes, cells of the liver, kidneys, and other organs. The non-exchangeable part accumulates in the skeleton and belongs to the stable fraction. The quantitative content of lead in the blood, as a rule, indicates the current exposure, while its content in the bones indicates the past and, as a rule, long-term poison intake.[25]

Table 2. Bone marrow parameters in old animals inoculated with lead acetate and potassium dichromate.

Indicators	Intact group, % $n = 15$	Lead acetate and potassium dichromate, % $n=14$
Monocytes	0.9 ± 0.2	0
Erythroblasts	0.3 ± 0.2	0
Pronormoblasts	0.8 ± 0.3	0
Basophilic normocytes	6.6 ± 1.8	5.6 ± 1.1
Polychromatophilic normocytes	31.8 ± 0.6	9.5 ± 1.4*
Oxyphilous normocytes	14.6 ± 0.7	9.2 ± 1.5*
Erythroid lineage	54.2 ± 2.6	24.2 ± 3.7
Bone marrow index of neutrophils	0.5 ± 0.1	0.5 ± 0.2
Leukoerythroblastic ratio	1.8 ± 0.1	3.2 ± 0.6
Red blood maturation index	0.8 ± 0.04	0.8 ± 0.03

Comments: * Statistically significant ($P < 0.05$) in relation to the intact group.

5. CONCLUSIONS

By interacting with sulfhydryl groups and inhibiting key enzymes involved in haeme synthesis, lead has a significant effect on haeme synthesis. The state of erythrocyte membranes is disrupted when exposed to low doses of lead, while the electrophoretic mobility of erythrocytes decreases, lipid peroxidation processes are stimulated, antioxidant activity decreases, which leads to an increase in the cholesterol/phospholipid ratio and an increase in membrane microviscosity. Thus, the priming of experimental animals with lead and chromium compounds is manifested in young animals by irritation of the red blood shoot of the bone marrow and haematoxic anaemia, in old animals – by a decrease in the total cellularity of the bone marrow and severe anaemia.

Conflict of interests

None declared.

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None declared.

Ethics

All procedures performed in studies involving animal participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. A study was approved by the National Ethics Commission of the Ministry of Health of the Republic of Kazakhstan on April 23, 2020, No. 184-A.

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