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ISOLATED AND COMPLEX EFFECTS OF LEAD, CADMIUM, AND ZINC IONS ON THE ACID RESISTANCE OF ERYTHROCYTES

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ABSTRACT

The acid resistance of erythrocytes of laboratory rats was studied under isolated and complex exposure to lead, cadmium, and zinc ions. It was shown that when exposed to heavy metal ions, there is a shift in erythrograms, an increase in the proportion of low-resistant erythrocytes, and a reduction in hemolysis time. The most significant changes in erythrocyte membranes are observed with the chronic action of Pb^{2+}, Cd^{2+}, Zn^{2+} ions, and a mixture of heavy metals. It was found that when Pb^{2+}, Zn^{2+}, and Cd^{2+} ions were exposed to laboratory rats for 30 days, the peak of erythrograms was 0.5, 1.0, and 1.5 minutes, respectively. The proportion of erythrocytes subjected to hemolysis at the peaks of erythrograms was significant and about 3 times higher than the control at prolonged exposure to Pb^{2+} and Zn^{2+} ions and almost corresponds to the control (36.0%) when exposed to Cd^{2+} ions. The hemolysis time was significantly reduced: 2.5 minutes at Pb^{2+} and Zn^{2+} ions, and 4.5 minutes at Cd^{2+} ions. It should be noted that by the 30th day of the experiment, all rats that received water with heavy metals had died. Thus, the results obtained indicate significant qualitative changes in the composition of the erythrocyte population of rats exposed to chronic exposure to heavy metal salts.

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Introduction

The blood system reacts not only with quantitative but also with qualitative changes in its composition to any exogenous and endogenous effects to maintain homeostasis [1, 2]. Under the influence of toxicants, the oxygen content in the blood and tissue fluids decreases, which entails morphological and functional disorders at the level of erythrocyte membranes [3, 4]. Such toxic substances cause the intensive production of free radical oxidation products, leading to the development of oxidative stress and, consequently, to the oxidative destruction of cell membranes [5-7].

The functional usefulness of erythrocytes as an integral indicator of the integrity of cell membranes is important [8-10]. Such studies contribute to understanding the mechanisms of adaptation and compensatory processes occurring in the blood system under chronic exposure to heavy metal ions, which is important not only for assessing the qualitative composition of the erythrocyte population of peripheral blood in the acute period of intoxication but also for monitoring the blood system in the dynamics of compensatory mechanisms [11-15]. For instance, Cd^{2+} can directly initiate lipid peroxidation, and displace iron from heme and hemoproteins, while the mechanism of action of Pb^{2+} is the binding of SH groups of protein and non-protein thiols [16, 17]. According to Hosseini *et al*. [18], pre-administered tryptophan partially limits the increase in spontaneous hemolysis of erythrocytes caused by the addition of zinc sulfate. At the same time, the absence of such an effect in the case of

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lead sulfate injection into the body indicates the presence of another mechanism, apparently associated with the high affinity of Pb^{2+} or Cd^{2+} ions for the groups of membrane proteins [19, 20]. In this regard, to assess the qualitative composition of the erythrocyte population, it is important to study the state of acid resistance of peripheral blood erythrocytes.

Thus, this work aimed to study the isolated and complex effect of drinking water pollution with cadmium, lead, and zinc ions on the resistance of erythrocytes in laboratory rats.

Materials and Methods

The work was performed based on the Laboratory of Anatomy, Physiology, and Histology of Chechen State University (Grozny, Russia). The experiment used white rats weighing 100-150 g, grown in the vivarium of Chechen State University (Grozny, Russia).

The following pollutants were tested in chronic laboratory experiments:

1. experimental group 1: 0.25 mg/L cadmium chloride (MPC is 0.005 mg/L) [21];
2. experimental group 2: 0.5 mg/L lead acetate (MPC is 0.1 mg/L) [22];
3. experimental group 3: 0.1 mg/L zinc sulfate (MPC is 0.01 mg/L) [23];
4. experimental group 4: 1.0 mg/L lead acetate, 0.05 mg/L cadmium chloride and 0.1 mg/L zinc sulfate.

Rats kept under normal vivarium conditions served as controls. Each group included 20 rats. Acid resistance of erythrocytes was studied in the peripheral blood of rats at different periods of exposure of rats to drinking water with lead, cadmium, and zinc ions (5, 15, 30, and 40 days of the experiment) [24]. The obtained results are subject to variational statistical processing using STATISTICA 12.0 software.

Results and Discussion

The results of the research are presented in **Figures 1-4**. It follows from the data obtained that heavy metal ions, both when isolated and combined, cause significant changes in the erythrocyte membranes of rats.

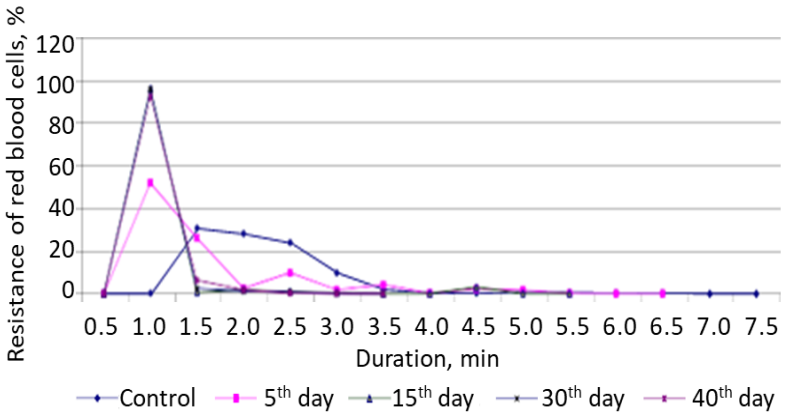

Figure 1. Changes in the acid resistance of rat's erythrocytes depending on the duration of intoxication of drinking water with lead acetate

We compared the acid erythrograms in the experimental groups based on the duration of erythrocyte hemolysis, the peak of erythrocyte hemolysis, and the nature of the erythrogram [25]. The first minute notes a left shift of the erythrogram on the 5th day of exposure to Pb2+ and Cd2+ ions **(Figures 1 and 3)**. A mixture of heavy metals (Pb2+, Cd2+, and Zn2+) causes a curve shift of 0.5 minutes **(Figure 4)**. Over time, more and more erythrocytes were breaking down at the peak of the erythrograms: 51.9% of erythrocytes broke down when Pb2+ ions were present, 57.1% when Zn2+ ions were present **(Figure 2)**, 36.0% when Cd2+ ions were present, and 76.0% when both types of metal ions were present. Notably, the duration of hemolysis also changed. Thus, during Cd2+ ion poisoning, the hemolysis time was 4.5 minutes. The experimental groups treated with Pb2+ ions and a mixture of metals experienced the smallest reduction in hemolysis time, up to 5.5 minutes.

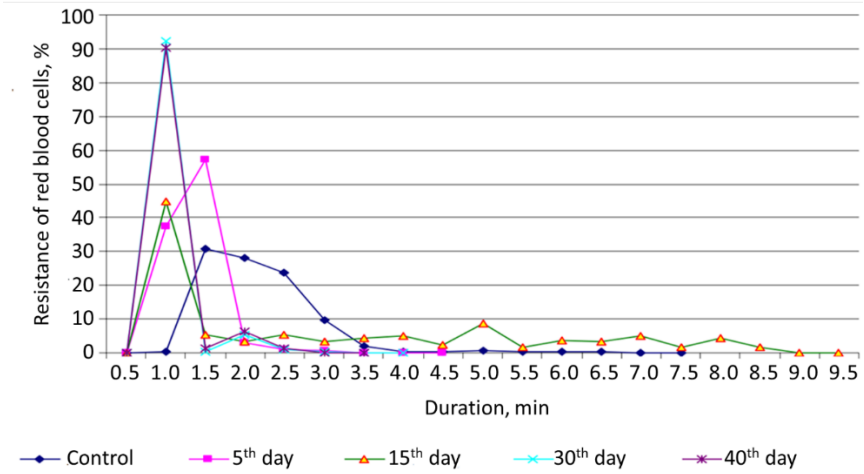

Figure 2. Changes in the resistance of red blood cells of rats to the action of zinc sulfate depending on the duration of intoxication of drinking water

On the 15th day of heavy metal salts exposure, erythrograms were characterized by a left shift. There was also a shift of the erythrogram to the 1st minute and under the action of Zn^{2+} ions. In parallel, Cd^{2+} ions caused a shift to the right by 2 minutes. A further increase in the number of erythrocytes subjected to hemolysis at the peak of the erythrogram was revealed.

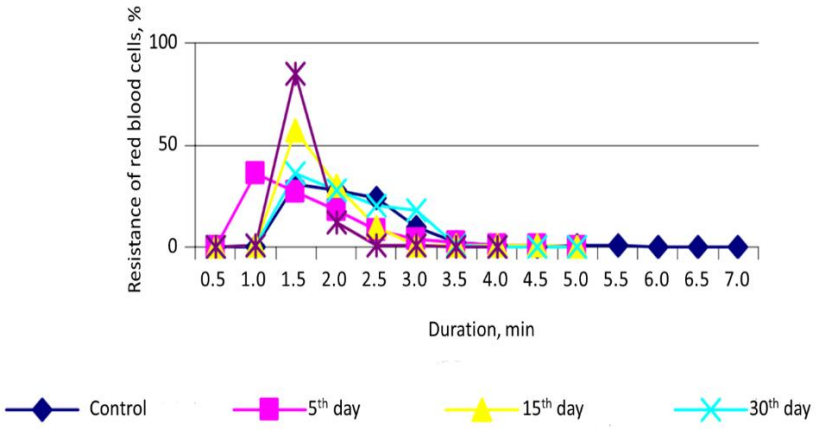

Figure 3. Changes in the resistance of rat's erythrocytes to the action of cadmium chloride depending on the duration of intoxication of drinking water

Thus, when exposed to Pb^{2+} ions and a mixture of metals at this stage of rat exposure, about 96.0% of erythrocytes were destroyed. Exposure to Zn^{2+} and Cd^{2+} ions leads to hemolysis of 45.0 and 55.0% of erythrocytes, respectively. The hemolysis time was significantly reduced (up to 1.5 minutes) when exposed to a mixture of heavy metals. Interestingly, in the case of intoxication with Zn^{2+} ions, on the contrary, there was an extension of the hemolysis time to 8.5 minutes. The action of lead and cadmium salts reduced the hemolysis time to 4.5 and 4.0 minutes, respectively.

At 30 days of exposition of Pb^{2+}, Zn^{2+}, and Cd^{2+} ions in laboratory rats, the peak of erythrograms was 0.5, 1.0, and 1.5 minutes, respectively. The proportion of erythrocytes subjected to hemolysis at the peaks of erythrograms was significant and about 3 times higher than the control with prolonged exposure to Pb^{2+} and Zn^{2+} ions. Surprisingly, the data obtained in the group treated with Cd^{2+} ions were relatively comparable with the control group (36.0%). The hemolysis time was significantly reduced: 2.5 minutes for Pb^{2+} and Zn^{2+} ions treatment and 4.5 minutes for Cd^{2+} ions. It should be noted with sorrow that by the 30th day of the experiment, all rats that received water with heavy metals had died.

Thus, the results obtained indicate significant qualitative changes in the composition of the erythrocyte population of rats exposed to chronic exposure to heavy metal salts. The predominance of erythrocytes with low acid resistance in the population indicates its significant aging, which may be associated with destructive processes developing in erythrocyte membranes under conditions of chronic intoxication of the body with lead, cadmium, and zinc ions, as well as a mixture of these metals [26-28]. The aging of erythrocytes is a pathological process that can be explained by the inhibition of erythropoiesis and a violation of the stability and permeability of erythrocyte membranes, which in turn may be associated with the activation of lipid peroxidation, a decrease in the activity of antioxidant enzymes [29, 30].

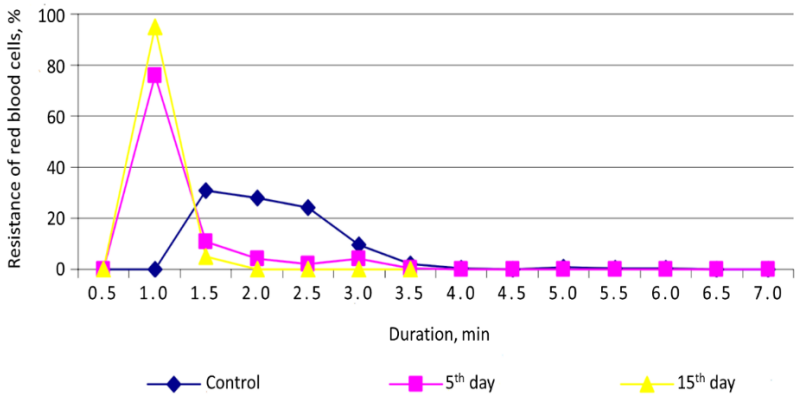

Figure 4. Changes in the stability of rat blood erythrocytes under the complex influence of heavy metals (cadmium, lead, manganese) depending on the duration of intoxication of the aquatic environment

Conclusion

The conducted studies have shown that the study of the kinetics of acid hemolysis during intoxication with heavy metal salts makes it possible to simultaneously assess violations of the structure of erythrocyte membranes and the functional activity of hematopoietic organs. Notably, at 30 days of exposition of Pb^{2+}, Zn^{2+}, and Cd^{2+} ions in laboratory rats, the peak of erythrograms was 0.5, 1.0, and 1.5 minutes, respectively. The proportion of erythrocytes subjected to hemolysis at the peaks of erythrograms was significant and about 3 times higher than the control with prolonged exposure to Pb^{2+} and Zn^{2+} ions. Surprisingly, the data obtained in the group treated with Cd^{2+} ions were relatively comparable with the control group (36.0%). The hemolysis time was significantly reduced: 2.5 minutes for Pb^{2+} and Zn^{2+} ions treatment and 4.5 minutes for Cd^{2+} ions. It should be noted with sorrow that by the 30th day of the experiment, all rats that received water with heavy metals had died. Thus, the results obtained indicate significant qualitative changes in the composition of the erythrocyte population of rats exposed to chronic exposure to heavy metal salts.

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Conflict of interest: None

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Ethics statement: The protocol for experiments with laboratory animals complied with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

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