**About of Mechanisms of Change in the Activity Acetylcholinesterase**

**of Erythrocyte by Some Pesticides and Antioxidants.**

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 **Abstract**

The kinetics and mechanisms of the effect of some pesticides: PCNB, TCU, Rohor, PCPNa, chlorophos, heptachlor, and photodynamic herbicides on the AChE activity and mechanical resistance of erythrocytes to ultrasound have studied. A changing degree of the ability of the treated erythrocytes to ultrasound has identified. Data on the violation of the structural and functional properties of erythrocytes correlate with the results of the toxic effects of these medications on various biological objects. The obtained data can be used for the selective search of drugs with the lowest toxicity in their further use in economic activity.

Keywords: pesticides,ultrasound,athecylcholine,RBC,hemolysis

**1.Introduction**

Pesticides are chemical compounds that have used to control pests. The global range of these drugs is more than 10,000 items and based on more than 600 chemical compounds of various classes. However, despite the advantages of the chemical method of plant protection, also its drawbacks should be noted. This, above all, the accumulation of drugs in the environment; the emergence of sustainable populations of pests, the emergence of new species of pests, the potential threat to beneficial inhabitants of the biosphere and human health, the violation of natural biosynthesis. According to many authors, a high incidence rate of various etiologies have associated with the effect of anthropogenic environmental pollution on human health. During the life of a person exposed to a huge number of various chemicals, the possibility of entry into the body in various ways is very high. Due to the development of man-made factors, the population of large cities is experiencing a strong impact of heavy metals such as lead, chromium, copper, cadmium, as well as aromatic hydrocarbons and pesticides. The compounds of these substances harm the functional state of the digestive organs, pancreatic cells, and also irritate the mucous membrane of the small intestine [1,2]. An increase in the frequency of chromosomal aberrations in peripheral blood lymphocytes was found in persons who had industrial contact with pesticides and also in persons with chronic lead intoxication. The presence of chromosome aberrations indicates a mutagenic effect of production factors on the organism of the examined. Thus, in humans and animals in contact with severe metals and pesticides have a mutational process in cells that can cause spontaneous abortions, congenital malformations [3]. In many countries, much work is being done to overcome these shortcomings. Toxic chemicals were replaced by less resistant and less toxic drugs that cannot accumulate in the environment and organisms. A bright example in this matter is the replacement of DDT with methoxychlor, dichlor, and other insecticides. When interacting with blood cells, chemicals contribute to changes in the composition of the blood, characterized by abnormal levels of protein in the plasma, which requires constant medical control. As shown by research by scientists of the National Cancer Institute / USA / on the example of donors, the effect of pesticides on the body in the age range of 30-94 years doubles the likelihood of developing blood diseases, the risk of multiple myeloma - cancer of the bone marrow. Research from oncologists has shown that agricultural workers are likely to be at high risk of hematological diseases leading to death than the general population.

The development of practical methods for analyzing the physicochemical properties of erythrocytes in various pathological conditions of the body, as well as negative environmental factors, is one of the important biomedical problems. The fundamental similarity of the structural and functional organization of the cell membranes of the body and the established general mechanisms of their changes in several pathological conditions allow the use of red blood cells as models. Therefore, it was important to develop a method for studying changes in red blood cells under various damaging effects and pathological processes in the body. The applications of ultrasound, which is widely used to target and selectively affect cells, could provide information on the mechanical stability of red blood cells based on the use of the hemolytic effect of ultrasound. Since it has known that pesticides have a damaging effect on cell membranes, it was possible to study the patterns of the effects of chlorine-containing pesticides using a model system — a suspension of erythrocytes. The study of ultrasound hemolytic resistance of erythrocytes is important since pesticides are widely used in economic activity and

 changes in the mechanical resistance of erythrocytes treated with pesticides, it is possible to characterize the quantitative patterns of their damaging effects[3].

 Under the action of ultrasound on cells and cell suspension, there are several different effects associated :

 - by heating the medium is being sounded (temperature factor);

 -chemical damage caused by the action of free radicals and products of their transformations and arising from cavitation;

 - the mechanical disturbance caused by shock waves and acoustic currents. (mechanical factor).

 Earlier it has shown that the damaging effect of insecticides in biological systems is directly related to their effect on the lipid phase of biomembranes [5]. However, the quantitative criteria for their effect on erythrocytes have not investigated yet. Relevant was the study of the quantitative patterns of the damaging effects of physiology aktivite connections(PAC)

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The purpose of this work is to study the kinetics of the action of chlorine-containing pesticides on the activity of membrane-bound acetylcholinesterase (AChE-ase), as well as the obtained quantitative criteria for the comparative assessment of the effectiveness of pesticides.

**2.Material and Methods**

 Investigated suspensions of erythrocytes isolated by sedimentation from 3 ml of heparinized or citrated fresh blood of donors (0.5 ml of citrate + 2.5 ml of whole blood) and washed twice from plasma with isotonic 0.9% NaCl. Centrifugation was carried out at 6000 rpm for 10 minutes (3 times). The stained cells have suspended in 8 ml of saline. In the study of ultrasound hemolysis, the suspension of erythrocytes has diluted with a physiological solution in a ratio of 0.5 ml + 23.5 ml of 0.9% NaCl (50 times). The cell concentration in the test suspension was 30x106 cells/ml.

Simultaneously, spectrophotometric evaluation of the kinetics of the action of mechanical and physical factors, including ultrasonic fields, on biological membranes was carried out, which made it possible to estimate latent damage to erythrocyte membranes. The erythrocyte stability was analyzed based on the photoelectric method we acquired for hemolysis of red blood cells under the influence of constant ultrasound at a frequency of 0.88 MHz within the intensity range of 0.1–1.0 W / cm2 at a constant temperature.

The membrane-bound AChE activity was determined by a potentiometric method: acetylcholine chloride (АХСI) served as a substrate with an initial concentration in the measuring cell of 2.5 mm. To study the kinetics of enzymatic hydrolysis, an incubation mixture consisting of a 0.9% NaCl solution containing 2.5 mm, Tris-HCl, a suspension of red blood cells (5-8x104 cells/ml), ethanol (or an alcohol solution of MAS of not more than 2% by volume was used. The experimental kinetic curves obtained as a result of a change in the pH of the incubated mixture during the enzymatic hydrolysis of ACh determined the reaction rate in the control and after treatment with the inhibitor drug (Vk and V1), respectively. The ratio of these parameters in the sample 30-minute incubation of this mixture. The measurements were carried out at 37 ° C characterized the relative activity in the sample. The initial rate (V) of ACH hydrolysis at various concentrations of the substrate from 10-2 to 10-3 M was determined. The incubation time of erythrocytes with drugs 2 min. A kinetic analysis of the results of determining the cache activity of erythrocytes was determined before and after treatment with preparations by calculating the enzymatic activity process by changing the relative activity (A) (by the slope of the kinetic howling) under the action of the drug, the slope of the kinetic curves of changes in the pH of the incubated mixture and the concentration of PAM, causing inactivation of the enzyme by 50% (CA50), inhibition inhibition constants-K1, Michaelis constants-Km and V max were calculated directly from the experimental curves. Also studied specific AChE-aznye activity in the presence of an alcohol solution of the drug and ethanol, respectively.Parallel to the determination of AChase activity in erythrocyte suspensions, we measured the kinetics of erythrocyte destruction during ultrasound lysis (f = 0.88 MHz and, I = 0.3W / cm2) using the previously developed ultrasound hemolysis technique[ 4 ]. The speed of mechanical destruction of cells in the process of sounding before and after Drug treatments (V0 ′ and V1 ′), determined directly from the kinetic curves of ultrasonic disintegration, were characteristics of the mechanical resistance of erythrocyte membranes.

**3.Results and discussion**

As chlorine-containing pesticides, herbicides TClA and sodium pentachlorophenol (PClFNa), chlorophos, fungoid pentachloronitrobenzene (PClNB ), Heptachlor, whose structural formulas are shown in Table 1, as well as rohor, were used.

          Table 1.  Efficiency of pesticides action on acheasic erythrocytes actiity

|  |  |  |
| --- | --- | --- |
|  Title  |  Structural formul |  СА50 (mМ) |
|  TClA (trichloroacetic acid) |   CCL3 COONa |  120 |
|  PCLFNa(pentachlorophenalate sodium) |  Cl ClCl ONa Cl Cl |   5х10ˉ1 |
|  PClNB(pentachloronitrobenzene) |  Cl ClCl NO2 Cl Cl |   2х10ˉ2 |
|  Chlorofos  | CH3O P CH CCl3CH3O  O OH  |  5х10ˉ3 |
|  Rohor | CH3O P S CH2CNHCH3CH3O  S O |  --- |

 The detected inactivation of the external enzyme AChE-ase suggests that the inhibitory effect of chlorine-containing pesticides caused by damage to erythrocyte membranes with which AChE-ase associated.

   As a criterion for evaluating the effect of the studied pesticides on the AChE - ase of erythrocytes, the drug concentration parameter was used, causing the enzyme inactivation by 50% (CA50). It was found that in the studied concentrations, these pesticides have a certain anti-AChE-aznoe activity (Table 1). From the data table. 1 can see that the studied compounds are not specific inhibitors of AChase, as they decrease the activity of the enzyme in significantly higher concentrations (CA50s are 120, 10 - 1 - 10 ̄3 mmM) than the known anti-AChE agents fosdrin, amiton, etc. . (CA50 ~ 10 ̄6 - 10 ̄8 mM) [6].

    The detected inactivation of the external enzyme AChE - ase suggests that the inhibitory effect of chlorine-containing pesticides is due to damage to erythrocyte membranes, which are associated with ACh-ase [4].

 These changes based on either the interactions of the studied pesticides with erythrocytes by distributing them on the surface hydrophobic membrane sites where the ache-ase inactivated by the pesticide located, or by introducing them into the lipoprotein structure of the erythrocyte membrane ( )

One of the extreme manifestations of the modifying action of chemical compounds on erythrocyte membranes, leading to disruption of cell integrity, is hemolysis. It has been established that PCP-Na, chlorophos, and Rogor have their hemolytic activity in an isotonic environment, and the treatment of erythrocytes with TCU and PCNB does not lead to their hemolysis.

It can be seen that the hemolytic effect naturally depends on the concentration of the pesticide: with an increase in the concentration of the drug in the incubation medium, the time of hemolysis is –them and the half-life time50 decreases, and the rate of hemolysis –V increases accordingly. The studied method of studying the quantitative characteristics of hemolysis of erythrocytes with different effects allows in each case to establish the lowest concentration of the drug, which has hemolytic activity.

      It is seen that the kinetic curves are S-shaped in nature with a certain induction period, and the rate of hemolysis is concentration-dependent. With increasing concentration of the pesticide in the incubation medium, the rate of hemolysis increases, and the induction period decreases. The findings suggest a strong

the modifying effect of PCPNa a and chlorophos on the structure of the erythrocyte membrane, leading to hemolysis of erythrocytes.

     We also studied the resistance of erythrocytes treated with pesticides to mechanical hemolysis under the action of ultrasound using the automatic registration of the kinetics of erythrocyte destruction in the cell of a spectrophotometer [3]. In control experiments, it was found that ethanol used as a solvent for pesticides at a concentration of <0.1% by volume does not have a noticeable hemolytic effect on erythrocyte cells.

    For some pesticides and bactericidal drugs causing hemolysis of erythrocytes in an isotonic medium, it was also shown that, along with this, they stabilize erythrocyte membranes to hemolysis in a hypotonic medium at concentrations (9 μM - 150 μM). It could be expected that the modifying effect of pesticides on erythrocyte membranes also leads to a change in their mechanical resistance to the action of ultrasound. We obtained the kinetic curves of ultrasound hemolysis of erythrocytes treated with various concentrations of pesticides (PCP Na, PCNB, heptachlor, chlorophos ,and THU).

    That agrees with the data of Braginsky [3] on the effect of surfactants on the mechanical stability of red blood cells.

 Table 2 presents the quantitative parameters characterizing the change in the resistance of erythrocytes to ultrasonic action under the influence of various concentrations of pesticides, indicating a change in the mechanical resistance of cells in the presence of these chemical compounds.

It is known that pesticides can affect the structure and biological activity of blood cells . Erythrocytes can be a convenient model for studying the damaging effects of various factors, including pesticides on cell membranes, as well as changes in other plasmatic membranes [ 6]. Ache-ase is an external enzyme of the erythrocyte membrane surface, brain cells, nervous tissue, etc., and is responsible for transmitting nerve impulses as a result of acetylcholine hydrolysis. Achease activity in the blood and its components can serve as an additional diagnostic criterion for analyzing some pathological conditions caused by toxic compounds. Several studies have studied the dependence of the hemolytic activity of some pesticides depending on the level of the initial activity of cellular enzymes and on the ability of the drug to reduce membrane fluidity [7,8]. However, the kinetic characteristics of the action of several pesticides on the hemolysis of erythrocytes and their resistance to the action of mechanical factors that characterize certain patterns The physiological activity of these compounds are poorly investigated. In this connection, the study of the kinetics and mechanisms of the effect of pesticides on ache is relevant. knowing the activity of red blood cells. The method of kinetic analysis seems to be most effective for finding out the mechanisms of the effect of drugs on erythrocyte achease when conducting enzymatic reactions in erythrocyte suspensions, as well as in an enzyme solution. Studying the damaging effects based on the kinetic constants of the enzymatic reaction without the research and in their presence, it can be informative for analyzing the mechanisms of their influence on the cholinesterase properties of red blood cells.

The purpose of this study is a comparative study of the kinetics and mechanisms of action of these pesticides and herbicides of the photodynamic action of α, α-dipyridyl, o-phenanthroline and acepox and pesticides on mechanical resistance to the action of ultrasound (USE) and on the activity of membrane-bound AChase, as well as quantitative evaluation of the effectiveness of drugs.

What has been said above has shown the urgency of finding new methods for identifying the negative effects of drugs on the human body and animals and proposing new, more harmless drugs for use in agriculture. In tab. 2. The quantitative parameters characterizing the change in erythrocyte resistance to ultrasound exposure in the presence of various concentrations of pesticides are presented.

 Table 2.The effect of pesticides of different concentration on the parameters of the ultrasound hemolysis and AChE activity in the estimated suspension of erythrocytes (the volume mode contineous, I = 0.4W / cm2, V=0.88 Mgs)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Samples (м) | Contentration [M] | t гem, c | V гem  сˉ1 | СА 50(мМ) |
|  Testing | 0 | 500 +̄ 10 | 1,09 +̄ 0,03 |  |
| PXNB | 10ˉ410̄ˉ3 | 700 ± 221200 ± 150 | 0,6 ± 0,020,3 ± 0,09 | 2х10ˉ2 |
| TClA | (3-6)х10ˉ3(2-3)х10ˉ2 | 420 ± 17360± 19 | 1,12 ±0,041,30 ± 0,05 | 120 |
| PXF Na | 10̄ -6 -10ˉ510ˉ4-10̄ˉ310ˉ2 | 450 ± 27415 ± 46200± 3 | 1,22 ± 0,091,30± 0,054,0 ± 0,07 | 5х10ˉ1 |
| Heptaxlor | 10ˉ510ˉ4 | 565 ± 47830 ± 43 | 0,97 ± 0,040,42± 0,12 |  |
| Хlorofoc | 10ˉ35х10ˉ310ˉ2 | 1550± 120700 ± 80250 ± 27 | 0,2 ± 0,180,6± 0,141,3 ± 0,08 | 5х10ˉ3 |

**4. Conclusion**

The magnitude of hemolysis and the protective effect of some surfactants correlate with the composition of phospholipids in the erythrocyte membrane; these changes may also reflect violations of the ultrastructure of erythrocyte components containing hydrolytic enzymes.

 Thus, the quantitative characteristics of the effect of chlorine-containing pesticides on the structural and functional imitation of red blood cellhenia were obtained. From the presented results, it can be seen that the studied chloro-containing ones lower the functional (AChE) activity of erythrocytes and change their mechanical hemolytic resistance.

The data obtained indicate that PCP Na, PCNB, heptachlor, ragor, chlorophos are relatively weak inhibitors of the AChE-basics of the erythrocyte membrane, but have a pronounced structural-determining effect, namely: they can cause hemolysis in an isotonic medium (PCP Na, rohor, chlorophos) and accelerate (PCP Na, THC, chlorophos) or slow down (PCNB, heptachlor) the speed of ultrasound hemolysis (see table2). Therefore, the quantitative parameters characterizing ultrasound hemolysis of erythrocytes (them, V hem) can be used as criteria for evaluating the membranotropic action of pesticides. It can be assumed that it is possible to judge the reaction to the preparations of other biological cells by the revealed disturbances in the erythrocyte membranes.

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**О механизмах изменения активности АХЭ-азы эритроцитов некоторыми пестицидами и антиоксидантами.**

Султанова Г.Г.

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Изучены кинетика и механизмы влияния некоторых пестицидов: ПХНБ, ТХУ, рогор, ПХФNa, хлорофос, гептахлор, а также фотодинамических гербицидов на АХЭ-азную активность и механическую резистентность эритроцитов к ультразвуку.Обнаружена различная степень повреждающей способности рассмотренных в работе препаратов в отношении ебран эритроцитов.Данные по нарушению структурно-функциональных свойств эритроцитов коррелируют с результатами токсического действия этих препаратов на различные биологические объекты. Полученные данные могут быть использованы для избирательного поиска препаратов, обладающих наименьшей токсичностью при их дальнейшем применении в хозяйственной деятельности.