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ABSTRACT

of the dissertation for the degree of Doctor of Science

MECHANISMS OF ACTION OF ULTRASOUND WAVES AND MACROLACTONE COMPOUNDS ON THE DURABILITY AND CONDUCTIVITY OF CELL AND LIPID MEMBRANES

Specialty: 2406.01– Biophysics

Field of science: Biology

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GENERAL DESCRIPTION OF WORK

The relevance of the dissertation topic. The study of the mechanism of action of ultrasonic (US) radiation on cells is an urgent task of physicochemical biology. In connection with the widespread use of ultrasonic methods in medicine, biology, economic activity and industry, the analysis of the patterns of biological effects of ultrasonic waves in relation to biological systems is of particular interest. The biological effects of ultrasonic exposure are determined by many factors, such as: time and intensity of exposure, temperature and pressure, structure of the sound field, choice of the object of study, etc. Due to the complexity of the analysis for all the specified parameters, it was simultaneously necessary to identify the required range of ultrasonic parameters to solve the tasks. For this purpose, the dependences of the stability of the state of cell membranes on the intensity, frequency, time and energy of ultrasonic exposure at the molecular, cellular, tissue, and organismic levels were considered. However, systematic studies of the state of cells under the combined effect of ultrasound and some macrolactone compounds have not been previously implemented.

It is known that the biological effects of ultrasound on cells are associated with physical, physicochemical, biophysical, biochemical and physiological factors. US has taken a leading place in the field of medical research in the study of the state and effects on biological structures. According to WHO, today more than 60 million people undergo ultrasound examinations annually. In this regard, in medical and economic practice, it is important to determine the optimal and safest modes of exposure to ultrasound both *"in vivo"* and *"in vitro"*.

Erythrocytes are the most accessible and informative element in biomedical research. The most revealing methods for studying the effect of external and internal factors on biological objects, in particular on blood cells, are spectrophotometrical, microscopic and hemolytic. An important task is to find effective methods for studying the physicochemical properties of erythrocytes.

There are various methods for studying the physicochemical characteristics of erythrocytes, such as irradiation, cryolysis, hemolysis, microscopy, EPR, NMR. The use of ultrasound at low therapeutic intensities could undoubtedly provide more information on the mechanical hemolytic stability of erythrocytes *"in vitro"*.

Literature data on the study of the behavior of blood cells and other cellular structures in the US field[1,2, 3, 4] have been published. In this regard, the purpose of this study is to develop the safest and most informative method for studying the combined effect of US waves and macrolactone compounds on cells with the identification quantitative criteria for assessing the degree of effectiveness of their biological action.

In pathological processes, the structural and functional properties of cell membranes are violated. In this connection, the practical aspects of the use of ultrasound and membranotropic compounds to detect violations of blood cell membranes in oncological, cardiological and hematological patients are of particular relevance. They are the leaders in a number of diseases leading to the greatest number of deaths.

As a model of plasma membranes for experimental studies, erythrocyte membranes and bilayer lipid membranes (BLMs) were chosen. Spectrophotometric methods for studying the effect of external and internal factors on erythrocytes are the most optimal for detecting their hemolytic activity. The introduction of drugs into the body leads to changes in the structure and function of cell

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[3]Горняк С.А. Физические механизмы взаимодействия ультразвука с биологическими структурами и их моделями. 2003, Автор. дис. канд. Физ-ат. наук Харьков, 26 с

[4] Хилл. Применение ультразвука в медицине. Физические основы. 2008. М:МИР (перевод с англ.)

membranes[5]. Of particular relevance is the identification of damage to blood cell membranes in various diseases, as well as under the influence of certain physical and chemical factors on the body. In this regard, it is important to study the mechanism of the combined action of various physicochemical factors on cell membranes and to find the optimal modes and concentrations of their effects[6][7].

Purpose and objectives of the research. The main goal of the dissertation is to analyze the biophysical mechanisms of the action of ultrasonic waves on the structural and functional properties of erythrocytes under various exogenous and endogenous influences and to study the physicochemical features of the interaction of a number of macrolactone compounds and antioxidants with erythrocyte membranes in the field of ultrasonic waves to identify their relationship with the chemical structure of compounds and mechanisms of their distribution inside the membrane.

This creates the possibility of obtaining information about the mechanism of action of membrane-active compounds and determining approaches to the synthesis of new drugs.

The main stages of the work to address the issues are identified as follows:

- to conduct a comparative analysis of the effectiveness of the structure-modifying action of polyene antibiotic derivatives (PA) and to study the hemolytic activity of antioxidants and macrolactone compounds of the polyene structure and to identify their optimal effective concentrations;
- to study the patterns of changes in the physicochemical

[5] Черницкий Е. А., СеньковичО.А., Розин В.В. Зависимость параметров гемолиза и везикуляции эритроцитов от концентрации Na-додецилсульфата: везикулярно-конкуретный гемолиз // Биологические мембраны. – 2000. – Т.17, №5. – С. 503–508.

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[7] Solodovnikov S. P., Koroteev M.P., Kaziev G. Z., etal Electron spin resonance study of the cavitatedlarch wood? Russian Journal of General Chemistry, издательство Maik Nauka /Interperiodica Publishing (Russian Federation), том 81, № 1, с. 158-159

properties of erythrocytes under various sonication modes, as well as under the influence of certain physical environmental factors, and to substantiate the possibility of using ultrasound in a physiotherapeutic mode to analyze the state of cells and diagnose damage to cell membranes;

- to conduct a comparative analysis and correlation of the action of various compounds (PA, ethanol, DMSO, anticarcinogenic, antioxidants) on the structural and functional properties of erythrocytes and bilayer lipid membranes (BLMs);
- to identify the contribution of erythrocytes of different ages to the hemolytic picture of red blood (erythron) in pathologies and establish the relationship between them;
- to study the effect of physical factors on the functional state of erythrocyte membranes in breast cancer.
- to identify the correlation of erythrocyte behavior in the field of ultrasound and develop a model of cell behavior in the physiotherapeutic mode of ultrasonication, identify the priority components of ultrasound waves in the mechanism of action on blood cells and develop a physical model of cell behavior in the ultrasound field.

Scientific novelty. The possibility of using ultrasound in the physiotherapeutic ultrasonication mode to analyze the state of biological membranes is substantiated. A spectrophotometric method for studying the kinetics of erythrocyte hemolysis in the field of ultrasound waves is proposed to analyze the state of blood cell membranes also under the influence of various physical and chemical factors. For the first time, a systematic study of the mechanical properties of the membranes of red blood cells of various age fractions *in vivo* in the bloodstream and *in vitro* in malignant tumors was carried out. The influence of a number of PAs with a known molecular structure on the mechanical properties and structural features of the erythrocyte membrane was studied. It has been found that polyenes induce morphological changes in erythrocytes, having a modifying effect on cell membranes. Differences in the kinetics and efficiency of the transforming action of PA derivatives with different structures and hydrophobic

properties were found.

The dependence of hemolytic activity on the concentration and structure of PA derivatives is shown. A change in the microviscosity of the surface (where AChE-ase is located) and deeper regions of the erythrocyte membrane was revealed.A comprehensive study of the effect of a number of compounds (antioxidants, polyene antibiotics, ethanol, DMSO, anticarcinogens) on the mechanical properties of red blood cell membranes was carried out. The effect of the selective action of some macrolactone compounds - amphotericin B, levorin, nystatin, filipin on the resistance and conductivity of plasma (erythrocytes) and bilayer membranes is shown. For the first time, the degree of change in the functional condition of erythrocytic membranes in patients suffering from malignant neoplasms was revealed under the combined action of ultrasound, gamma therapy, antioxidants, ethanol, anticarcinogens and PA. It was noted that under the action of certain membrane-active compounds-antioxidants and PA, three types of influence on the membrane characteristics of erythrocytes are observed: inhibition and activation of hemolysis and virtually no changes in the membrane characteristics of erythrocytes. The concentration dependence of the action of dimethyl sulfoxide (DMSO) and ethanol on blood erythrocytes was studied. It has been established that in the concentrations used in the preparation of solutions of PA and antioxidants, DMSO and ethanol do not have a negative effect on blood cells. The MTT-test method for the first time showed the effective effect of methyllevorin, as well as amphotericin B, on the proliferation and metabolism of *HeLa* and *C6* tumor cells. For the first time, a comparative model of the movement of erythrocytes in various parts of the bloodstream was proposed as an analogue of their behavior in the field of ultrasound in the physiotherapeutic mode of ultrasonication (f=0.88MHz,I=0.1-1.0 W/cm^2).

The practical significance of the work. The presented model of cell behavior in the field of ultrasonic waves and the resulting characteristics of the mechanical stability of erythrocytes (time and rate of hemolysis, half-life coefficient) can be recommended as a

test method for the control of the state of cells both in normal and pathological conditions. The hemolytic effects caused by the studied drugs (PA, antioxidants, anticarcinogen, DMSO and pesticides) make it possible to determine the effective concentrations of their use in medical practice and economic activity. The possibility of determining structural and functional changes in erythrocyte membranes as a diagnostic test for choosing the most effective drugs and their concentrations was determined. An express method has been developed for determining the biological activity of PA. A number of studied drugs can be recommended for their practical use in the fight against viral, bacterial and fungal diseases in humans and animals. The developed method of ultrasonic impact on cells in the physiotherapeutic mode can be used to monitor changes in the state of erythrocytes in oncological, hematological and viral diseases.

The main provisions of the dissertation submitted for defense

- The proposed method for studying the mechanical factors of the ultrasonic field on biological membranes allow assessing the latent damage to erythrocyte membranes and determine the most optimal concentrations of a number of drugs and modes of therapeutic effects. The proposed method makes it possible to assess the degree of impact of the studied drugs on the membranes within concentrations of 0.01-10% in the test suspension.
- The range of different conditions of exposure to ultrasound was studied depending on the intensity, frequency and time, as well as environmental factors on biological objects. This method allows us to assess the degree of change in the functional state of cell membranes depending on the age of cells in the bloodstream, tumor localization, concentration and chemical structure of the drug.
- Analysis of the action of drugs on biological cells allows us to get closer to understanding the mechanisms of their binding to the studied objects (erythrocytes and BLM) by forming new transport systems in them - ion channels without disturbing the membrane structure, as well as to protect it from mechanical injury by creating a protective shell of nano sizes around the cell

membrane. The dependence of the degree of modifying action of the studied antibiotics on their structure was revealed.

➢The division of red blood cells into age fractions that occur in the bloodstream of humans and animals *in vivo* makes it possible to identify the role of each of these groups on the hemolytic pattern of the studied blood groups in control and in pathologies. The relationship between the dynamics of the development of the oncological process in humans and animals with the physic-chemical characteristics of blood cells was revealed.

➢The study of the mechanical properties of erythrocytes by ultrasonic hemolysis allows determining the degree of influence of certain compounds - antioxidants based on oxypyridines, polyene antibiotics and some anticarcinogenic drugs within concentrations of 0.01-10% in the studied suspension on the structural and functional characteristics of body cells and offer optimal concentrations of their use in biomedical practice.

➢The study of the behavior of cells in an ultrasound field makes it possible to analyze the mechanisms of the impact of the components of ultrasound on the objects under study and to present a physical model of the behavior of cells under the influence of ultrasound of the physiotherapeutic regime. The generality of the impact on erythrocytes of mechanical factors of the cardiovascular system and ultrasound waves of the physiotherapeutic mode of sounding is shown, which made it possible to propose the mechanism of the action of ultrasound on blood cells as an analog of the model of cell behavior in the human bloodstream.

Objects of research The objects of study were erythrocytes and their shadows, BLM, tumor cells NKLY, S-37, HeLa and C6, macrolactone compounds, antioxidants from the group of oxypyridines and ultrasound energy.

Methods of research.

The studies were carried out using the methods of ultrasound, osmotic and acid hemolysis, determination of AChE-ase activity, separation of erythrocytes into age fractions in a density gradient, EPR, MTT test, statistical data processing, computer methods.

Approbation of work. The dissertation materials were

presented at Conference "Prospects for the Development of Experimental Biology" BSU 2002; XVII International Biological Congress in Adana (Turkey), 2004; XVII International Biological Congress in Adana (Turkey), 2004; IV International conference "Regulation of growth, development and productivity of plants", Minsk, 2005; IV International Conference "Molecular, Membrane and Cellular Basis for the Functioning of Biosystems", Minsk, 2006; International Scientific Conference "Genetic Resources of Medicinal Aromatic Plants". Moscow, 2006; International Scientific Conference "Molecular, Membrane and Cellular Basis for the Functioning of Biosystems" Minsk, 2006; International Symposium "Natural Disasters and Global Problems of Modern Civilization" Baku, 2007; VII International Symposium "New non-traditional plants and prospects for their use", Pushchino, 2007; Republican conference dedicated to the 70th anniversary of the Institute of Botany of ANAS, Baku, 2008; IV International symposium "Mechanisms of action of ultra-low doses", Moscow, 2008; IV International Scientific and Practical Conference "Science and Modernity-2010", Novosibirsk, 2010; XVIII International Conference "Bioantioxidant", Moscow, 2010; International scientific and practical conference "Actual problems of biological and chemical ecology", Moscow, 2012; 5th World Conference on Drug Absorption, Transport and Delivery (WCDATD), Sweden, Uppsala, 2013; First European West Conference of biology and medicine, Austria, 2014; Materials of the III International Mycological Forum, Moscow, 2015, 2016, 2017; Inter. Conf. "Innoative Approaches to Conceration of biodiersity, Baku, 2016; Collections of International conference dedicated to the 95th anniversary of academician V.Yu.Akhundov, Baku 2017; Russian Cancer Congress, Moscow, 2017;Russian Hematology Congress 2018, International Scientific Conference "Science-Medicine" Institute of Physiology, Minsk 2017, 2018, 2019; Int. conf. dedicated to 95th anniversary of acad. V.Yu. Akhundov, Baku 2017; Int. Conf. dedicated to the 90th anniversary of Acad. V.J. Hajiyeva, Baku, 2018;Symp. on mycology dedicated to the 120th anniversary of acad. V. I. Ulyanishcheva, Baku, 2018; VI Congress of

Biophysicists of Russia, Sochi 2019; Conference dedicated to COVID 19, Baku, 2020; Sevastopol "Topical issues of biological physics and chemistry" 2020; Congress "Modern Problems of Pharmacy» dedicated to the (90th) anniversary of AMU, Baku 2021,Conference "Karabakh's biodiversity, land and water resources, past, present and future" Baku 2021, 11th Intern. Conferen. arhiefement and challenges in biology devoted to 120thanniver. of professor Mirali Akhundov october 13-14, 2022 Baku State University, Conf. on Biomedical and Pharmaceutica August 27-28, 2022.

Publications. In total, the author published 90 scientific papers, of which the main results of the dissertation are presented in the form of 74 printed works in foreign and republican scientific publications. Of these, on the topic of the dissertation, 52 articles are in Western journals and conference proceedings, and 20 are theses.

The structure and scope of the dissertation. The dissertation work is presented on 285 pages of typewritten text and contains 60 figures and 24 tables. The dissertation work consists of an introduction, a general description of the work, a literature review, a methodological part, results and discussion, conclusions and a bibliographic list of references, including 420 titles. The work was carried out in the laboratory "Cell Biophysics" of the Institute of Botany of ANAS in 1990-2018, partly in the laboratory "Physico-chemical properties of nucleic acids" of the Institute of Chemical Physics of the BAS in 1991-1995 and in the laboratory "Cellular technologies" of the Institute of Physiology of the Russian Academy of Sciences in 2015, 2016 and 2017 within the framework of the agreement on scientific and technical cooperation between the Institute of Botany of ANAS and the Institute of Physiology of the National Academy of Sciences of Belarus and with the financial support of the Science Development Foundation under the President of the Republic of Azerbaijan **- Grant No. EIF-BGM-3-BRTFR-2+/2017-15/12/3.**

BRIEF REVIEW OF LITERATURE

The use of ultrasound and macrolactone compounds in fundamental research of plasma membranes

Interest in the use of ultrasound in medicine and biology arose in the 50s of the 20th century and was due to the possibility of obtaining information about tissues using ultrasound waves.

The use of ultrasound in medicine and biology enable to use it both as an influencing factor and as a factor in the study of biological structures in order to obtain information about them. These interactions depend not only on the properties of the studied tissue, but also on the characteristics of the ultrasound field, leading to the variation of cells and tissues. Our analysis of studies of various aspects of the use of therapeutic ultrasound in medical and biological practice suggests that the main factors of the destructive effect of ultrasound are cavitation, acoustic flow, microstream, shearing stresses, radiation pressure and cell lysis, which must be taken into account when choosing operating modes of ultrasonic devices, used in clinical medicine and the biomedical industry. The effectiveness of the action is characterized by minimal changes in parameters (for example, temperature, acoustic pressure, etc.) in the object under study during ultrasonic sounding.

A lot of works have been devoted to the study of the hemolytic activity of PA[8,9], however, the kinetic characteristics of the combined action of PA and US on the hemolysis of erythrocytes have not been previously studied. Some PAs, including amphotericin B, have nephrotoxicity and hemolytic activity[10]. Research on filipin in the initial stages was thought to have only

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detergent properties. Subsequently, the channel-forming properties of this antibiotic were discovered.Scanning electron microscopy detected revealed that filipin at high concentrations, interacting with the membrane, causes the formation of niches with a diameter of 25 nm[11]. By means of hemolytic testing, confirmation of the "sterol hypothesis" of the interaction of filipin and its components with cellular (erythrocytes) and model (BLM) membranes was obtained and it was shown that filipin components can be arranged in a row in order of decreasing their effectiveness: filipin II > filipin III ≥ filipin I > filipin IV, which corresponds to a number characterizing their biological activity.At the same time, among PA, the most active in relation to cell and lipid membranes are levorin and filipin.

Oncological diseases can be classified as the most severe and difficult to treat diseases. At the same time, the course of any disease is accompanied by changes in the structural and functional properties of blood corpuscle, such as mobility, deformability, aggregation activity, viscosity (concentration of proteins and lipids), blood osmolarity (glucose concentration).In oncological and cardiac patients, there is a decrease in the deformability and an increase in the viscosity of erythrocytes, which is apparently a consequence of an increase in glycated hemoglobin (Hb_{A1c}), and leads to difficulty in blood circulation in the capillaries and a decrease in the diffusion coefficient of oxygen delivery to tissues[12].

One of the indicative methods characterizing the state of red blood cell membranes is hemolysis. Types of hemolysis are distinguished by the nature of the course, localization and mechanism of occurrence.

The PAs (Amphotericin B and levorin) used in this work are poorly soluble in water, which reduces their therapeutic effect.

The highest biological efficiency of PAs is observed in a

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[12] Wilson P.W.F., Grandy S.M. The metabolic syndrome: practical guide to origins and treatment: part I. Circulation. 2003, 108: 1422-1425.DOI:10.1161/01.CIR.0000089505.34741.E5

solution of dimethyl sulfoxide (DMSO)[13]. DMSO is widely used to protect cells from the damaging effects of detergents.[14][15].Apparently, DMSO is able to integrate into the lipid monolayer of plasma membranes through hydrophilic protein aquaporin channels, interacting with their lipid and protein components and changing their structural properties[16].

In recent years, the method of cardiopulmonary bypass (CB) is one of the most commonly applied methods in cardiac surgery. Prolonged blood circulation in the CB device can be accompanied by trauma to its formed elements and the development of intravascular hemolysis[17]. Similar to the behavior of cells in the cavitation mode in the ultrasound field, in the aorta and arteries, the blood flow occurs at a high speed, further, decreasing in the veins, it reaches a minimum in the capillaries, approaching the pre-cavitation mode[18].In this sequence, pressure, blood velocity and vessel diameter decrease. At the same time, the behavior of erythrocytes in

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[17] Давыдова Е.В., Гордиенко О.И. Влияние температуры на проницаемость мембран эритроцитов для криопротекторов с различной степенью гидрофобности // Проблемы криобиологии. 2009. Т.19, №3. С. 261–272 http://nbuv.gov.ua/UJRN/KrioBiol_2009_19_3_5.

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the blood flow is determined by their mechanical resistance and deformability. Obviously, the insufficient ability of erythrocytes to change their shape at moderate blood flow rates close to physiological, which *in vivo* is realized mainly in the vessels of the microcirculatory bloodstream, and *in vitro* - in the oxygenator and filters, causes the destruction of red blood cells during AC. A model of mechanical destruction of cells in an ultrasonic field was proposed.

MATERIALS AND METHODS OF RESEARCH

In the work, the following were used as an object of study: erythrocytes isolated from whole blood of donors and animals in control, as well as in the development of lymphocytic leukemia; shadows of erythrocytes; clonogenic cells of ascitic tumor *NKY, S-37*, *HeLa* and solid tumor *S6*. As factors of influence, drugs applied in economic activities and medicine were used: macrolactone compounds - polyene antibiotics, anticarcinogenic drugs used in chemotherapy, antioxidants, as well as physical impact factors, such as ultrasound and ionizing radiation.

2.1. Method for isolating erythrocytes and obtaining their shadows, followed by isolation of the phospholipid fraction. Gravitational methods for the separation of blood cells based on the difference in the specific density of blood cells are most widely used. From the mass of donor blood, it is possible to obtain fractions of blood cells differing in volume (erythrocytes>neutrophils and eosinophils>lymphocytes and monocytes>thrombocytes).Erythrocytes were obtained in several stages by sequential centrifugation in a density gradient.

Erythrocytes precipitated from 3 ml of blood with the addition of citrate or heparin (0.5 ml of citrate + 2.5 ml of whole blood) and twice washed from plasma with isotonic 0.9% NaCl. Centrifugation was carried out at 6000 rpm within 10 minutes (3 times). The washed cells were suspended in 8 ml of physiological solution. In the study of ultrasound hemolysis, the suspension of erythrocytes was diluted with physiological solution in the ratio of 0,5 ml + 23,5

ml of physiological solution (50 times). The cell concentration in the studied suspension was $30x10^6$cells/ml.

2.2. Separation of erythrocytes in a density gradient by sequential centrifugation methods. To separate the erythrocyte mass into age fractions of young and old erythrocytes, a method was used to obtain various fractions of erythrocyte in a density gradient.

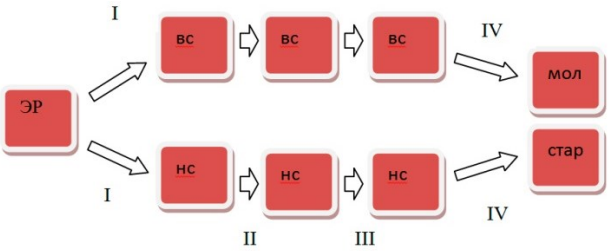

Fig. 1. Scheme of division of erythrocytes in the density gradient into age fractions

In summary, fractions containing 10-12% of highly resistant erythrocytes - reticulocytes, that is, the most resistant young erythrocytes, are obtained from the initial total mass of erythrocytes.

2.3. Determination of resistance of erythrocytes by osmotic and acid hemolysis. *Performing osmotic hemolysis of erythrocytes*. To determine the percentage of hemolysis, 0.4 ml of 5% erythrocyte suspension was added to 2 ml of a hypoosmotic solution of sodium chloride. For each sample, hemolysis of erythrocytes was evaluated in 0.3%, 0.4% and 0.5% NaCl solutions. Then, the suspension was centrifuged within 5 minutes at 3000 rpm and the optical density of the supernatant was measured at a hemoglobin absorption wavelength of 535 nm on a T92+ spectrophotometer. From the resulting hemolysis curves, the percentage of hemolyzed erythrocytes was calculated. The change in the optical density of the cell suspension (cell volume) was also registered using a FEK photoelectrocalorimeter on a red light filter. The results were recorded by recording the output signal. The optical density of the suspension was calculated on the degree of transmission (%)

according to the formula D=lg (100/T)

2.4. Kinetic method of ultrasonic hemolysis proposed to characterize the mechanical properties of erythrocyte membranes. The proposed method is based on the principle of spectrophotometric assessment of erythrocyte membrane damage under the action of mechanical and physical factors of ultrasound. The resistance of erythrocytes was studied on the basis of our developed method of photoelectric automatic registration of the process of hemolysis of red blood cells under the action of continuous ultrasound at a frequency of 0.88 mHz within the intensity range of 0.1 - 1.0 W/cm^2 at a constant temperature.

It turned out that in the studied mode of ultrasonic treatment, erythrocytes are mainly affected by mechanical factors, that is, the obtained indicators of the resistance of erythrocytes to ultrasonic characterize their mechanical resistance. Under the influence of a uninterrupted ultrasonic field on biological membranes, the most optimal concentrations of a number of drugs and modes of therapeutic effects (intensity, frequency and time) that reveal hidden structural defects of the membrane were determined. The study of the behavior of cells in the ultrasound field allowed to analyze the possible impact mechanisms of the components of the ultrasound wave on the objects under study and to present in the physico-therapeutic mode of sounding a physical model of the behavior of cells in the field of ultrasound.

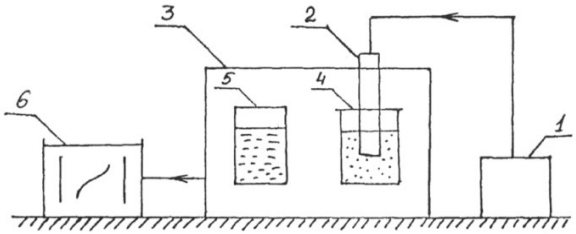

Fig.2. Installation for kinetic ultrasonic hemolysis of blood erythrocytes consists of: 1-therapeutic generator of ultrasonic waves T-5, 2-emitter 3-photocalorimeter, 4 and 5 - quartz cells with samples, 6- potentiometer

2.5. Method for determining the biological activity of macrolactone compounds and antioxidants. The main solvent for PA is dimethyl sulfoxide (DMSO). The biological activity and mechanisms of functioning of PA are determined by the method of studying the conductivity of bilayer lipid membranes (BLM).

The biological activity of the compounds used was studied by determining their hemolytic activity in the following sequence:

1. *Obtaining erythrocytes* was carried out according to the method 2.1. To prepare for research, the obtained erythrocyte mass was diluted with 0.9% NaCl solution containing 0.15 M sodium chloride and 0.01M phosphate-buffer saline at pH 7.4. to obtain 5% suspension by volume.

2. *Incubation of erythrocytes with drugs.* Preparations were added to 5 ml of a 5% suspension of erythrocytes and incubated in closed test tubes at a temperature of 37°C. An equal volume of physiological solution was used as a control solution.

3. *Hemolytic activity* of PA was determined in two methods.

Method I: 5 mM Tris HCI buffer, pH 7.5, containing 150 mM NaCl was used as the main medium. The isolated erythrocytes were washed three times and the hematocrit was determined by centrifugation at 8000 rpm, 6 min. After incubation of a 2% suspension of erythrocytes with PA for a certain time, they were centrifuged for 5 minutes at 5000 rpm. Optical absorption was determined on a spectrophotometer at $\lambda = 540$ nm

Method II The ultrasonication of a suspension of erythrocytes in the same dilution as in the kinetic method was carried out through an intermediate medium (distilled water) using a piezoquartz head with an area of $4cm^2$ as an emitter (Fig. 2.1b). A plastic cylinder was placed on the head, on which a vessel with the examined erythrocyte suspension was fixed.

Samples after ultrasonication were centrifuged for 10 minutes at 6000 rpm, and the concentration of hemoglobin in the supernatant liquid was determined by measuring the optical density at a wavelength of 540 nm.

2.6. Determination of AChE activity of erythrocyte membranes. Membrane-bound AChE activity was determined by

the potentiometric method: the substrate was acetylcholine chloride (AChCl) with an initial concentration in the measuring cell of 2.5 mm. To study the kinetics of enzymatic hydrolysis, an incubation mixture was used consisting of a 0.9% solution of NACL containing 2.5 mm Tris-HCI, a suspension of erythrocytes (5-8x10^4cells/ml), ethanol (or alcohol solution of MAC not more than 2%), not more than 2% by volume). The reaction was started by introducing AChE after a 30-minute incubation of this mixture. Measurements were carried out at 37°C.

From the obtained kinetic curves as a result of changing the pH of the incubated mixture during the enzymatic hydrolysis of ACh, we calculated changes in the relative activity (A) (according to the slope of the kinetic curve) under the action of the drug causing enzyme inactivation by 50% (CA_{50}).

2.7. Method for assessing the proliferation and metabolism (MTT) of tumor cells *in vitro*. To assess the effect of PA on the growth and metabolism of HeLa and S6 tumor cells, levorin and its derivatives, namely, methylated levorin, butylated levorin, integral levorin, and amphotericin B, were used.

All antibiotics, including antibiotic levorin A_2 derivatives, dissolve up to 10 mg per 1 ml of DMSO, but the most effective concentrations are 0, 1-1%.

1)The final concentration of the presented antibiotics was 2 mg/ml. The concentration in the wells of a 96-well plate was doses of 20 and 40 μg per well, respectively.

2)*Preparation of cell culture* HeLa (human cervical cancer) and C6 (rat glioma) cell lines were cultured on DMEM growth medium (Sigma, USA) with 10% ECT (Sigma, USA).

Determination of cytotoxicity by MTT test. The MTT test was used in experimental studies. Formazan was transferred into solution using DMSO. Subsequent photometry of the samples was carried out on an enzyme immunoassay analyzer in 96-well plates, which makes it possible to accurately compare the change in the optical density of the solution relative to the control with the change in the number of viable cells, and in cytotoxic studies to evaluate the specific death of cells induced by polyene antibiotics. The optical density of the

resulting formazan solution was measured by ELISA at λ= 505 nm (Chem-Well, USA). 100% cell vitality was taken as the colour intensity in the wells with cells not treated with PA.

2.8. Statistical processing of the results was carried out in the program STATISTICA 6.0 with the calculation of the arithmetic mean and standard error. Validity of differences between groups was assessed using Student's t-test. The differences were considered valid at $p<0.05$.

ANALYSISOF MEMBRANE CHARACTERISTICS OF ERYTHROCYTES IN THE FIELD OF ULTRASOUND

3.1. Comparison of the mechanisms of action of various hemolytic factors for the analysis of the state of cell membranes using the kinetic method of ultrasonic hemolysis.

One of the reasons for the appearance of damaging factors of red blood cells in pathology is a change in the osmolarity of blood plasma. Endogenous intoxication is characterized by a change in the permeability and sorption capacity of erythrocyte membranes. At the same time, differences in the resistance of cells of different rheology were revealed[19].

Osmotic and chemical resistance of erythrocytes are determined by the common method of acid hemolysis. Analysis of some types of erythrocyte lysis revealed differences in their mechanisms. Acid hemolysis can be represented as a multistage process, where on contact with an acidic medium, conformational changes in integral membrane and membrane proteins are observed. Further, hemoglobin denaturation occurs, which leads to a violation of protein-lipid, lipid-lipid membrane interactions. The mechanism of osmotic hemolysis is assumed to be a one-stage process leading to a change in the shape and deformability of cells with the release of hemoglobin into the environment surrounding the cell.

[19]ЧерницкийЕ.А., СеньковичО.А., РозинВ.В. Зависимость параметров г емолиза и везикуляции эритроцитов отконцентрацииNa-додецилсульфата: везикулярно- конкуретныйгемолиз // Биологическиемембраны. – 2000. – Т.17, №5. – С. 503–508.

Otherwise, the transport of the solution surrounding the cell into it is carried out according to the concentration gradient and precedes the process of cell disruption.

Differences with data of US hemolysis seem to be due to the fact that osmotic and acid resistance, on the one hand, and US resistance, on the other hand, are possibly due to the different nature of the influencing factors. The action of mechanical forces on erythrocytes occurs at sufficiently high values of shear stress (10I dyn/sm^2), at which, apparently, differences in the properties of erythrocyte membranes in different age groups level out.

3.2. Identification of the optimal mode of sonication of erythrocyte suspension for subsequent analysis of the mechanisms of the impact of ultrasound on cell membranes

A number of authors[20] have shown that, under certain conditions, ultrasonic exposure can lead to a modification of those cells and tissues and organs through which ultrasonic waves propagate.

According to the data[21], there is a clearly defined threshold intensity of ultrasonic exposure, the excess of which leads to cell lysis.

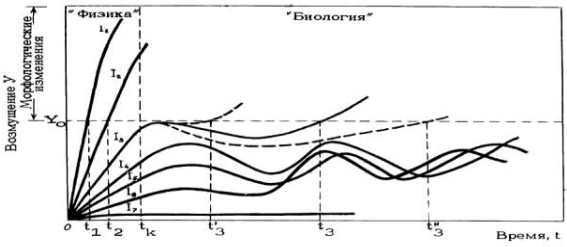

Fig.3. The interdependence of the course of processes in biological objects located in the field of US action on the intensity and time of exposure to US.

It appeared that that under the action of external factors on

[20] Margulis M.A., Margulis I.M. Regarding of mechanism of biological action of ionizing radiation (in comparison with ultrasonic cavitation).J. of Physical Chemistry, 2005 , v. 79, №6, pp. 1142-1151

[21]Ter Haar G. Ultrasound bioeffects and safety. Proc Inst Mechanical Eng H 2010;224,363–373 /DOI: 10.1243/09544119JEIM613

erythrocytes, the mechanical properties of their stroma change. The dependence of these parameters on the properties of the erythrocytes themselves (age, shape, rheology), on the conditions of sounding, the factor of influence and the presence of pathology in the body was obtained.

Thus, it can be expected that the mechanisms of morphological and functional damage to biological cells by ultrasound are based on mechanical factors - shock waves, microflows and acoustic currents, which is essential to know for its effective use for medical purposes and the microbiological industry[22].

INFLUENCE OF MACROLACTONE COMPOUNDS WITH KNOWN CHEMICAL STRUCTURE ON SOME CHARACTERISTICS OF CELL AND LIPID MEMBRANES

4.1. Modification of the hemolytic process under the influence of amphotericin B and its derivatives.

It turned out that under the action of external factors on erythrocytes, the mechanical properties of their stroma change. The dependence of these parameters on the properties of the erythrocytes themselves (age, shape, rheology), on the conditions of ultrasonication, the impact factor and the presence of pathology in the body.

As a result of the study of the hemolytic characteristics of a number of polyene antibiotics PA - amphotericin B, nystatin, mycoheptin and levorin, it was shown that they selectively increase the permeability of cell and bilayer lipid membranes (BLM) for ions and organic compounds by forming sterol-containing channels of molecular sizes[23][24]. Figure 5 presents the results of studying the

[22] *Г.Г. Султанова, Х.М. Касумов*Физико-хиические свойства мембран эритроцитов при взаиодействии с полиеновыи антибиотиками в поле дейсвия ультразвуковых волн. Ж.Биофизика, т. 66, №2, с.302-311 DOI: 10.31857/S0006302921020113

[23] Kasumov Kh., Bolard J. Transient permeability induced by cationic derivatives of amphotericin B in lipid membranes.Pol. J. Chem.2004, 78, 1057-1065.

[24] Garcia-Chaumont C., Seksek O., Jolles B., Bolard J. A cationic derivative of amphotericin B as a novel delivery system for antisense oligonucleotides. - Antisense Nucleic Acid Drag Dev 2000 b. 10,3, 177-184.doi: 10.1089/oli.1.2000.10.177.

lytic action of amphotericin B.

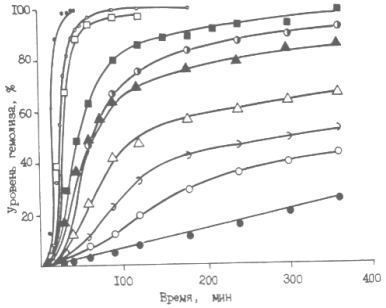

Fig. 4. Kinetics of erythrocyte lysis under the influence of various concentrations of amphotericin B (μM), here: ● – 1.5 μM; ○ – 2.0 μM; ↄ – 2.5 μM;– 3.0; ▲– 4.0 μM;– 6.0 μM; ■ –8.0 μM; □ – 10.0 μM; 14.0; ● – 20.0 μM;

The kinetic curves of the release of Hb are S-shaped.Percent-hemolysis curves for osmotic, thermal and acid hemolysis, as well as photohemolysis curves have analogical form. Some authors have shown a linear relationship between the number of decayed erythrocytes and the time of hemolysis in the interval of 10-90%.

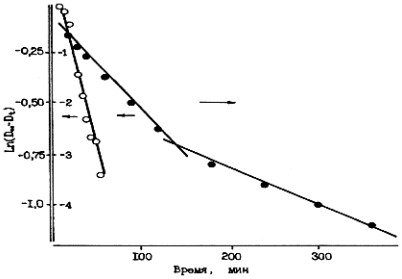

Fig.5. Kinetics of hemolysis induced by amphotericin B in semi–logarithmic coordinates, where on the abscissa axis the hemolysis time in minutes; on the ordinate axis - the value of Ln optical density at different concentrations of the antibiotic (o–1–3 microns; ● –2–14 microns).

From Fig. 5. it can be seen that the growth phase of the hemolysis level is described by the sum of two processes in semi-logarithmic coordinates and is formed by two segments at small and large concentrations.

The analysis of the conducted studies has shown that the biological activity of antibiotics is directly dependent on the chemical structure of the molecules. The availability of antibiotic molecules to chemical modification by functional amine and carboxyl groups creates a condition for obtaining new drugs with improved physico-chemical properties for targeted use in the clinic.Analysis of the literature data suggests that the biological activity of PA depends on the intermolecular interactions of the antibiotic and phospholipids with the formation of a hydrogen bond between them. In some cases, such bonds are formed on the outside of the membrane, even in the absence of sterols. It is noted that, despite the high activity of PA in relation to pathogenic fungal cells, they are characterized by a certain toxicity to the human body, determined by a change in hemolytic activity in the presence of antibiotics. The main role in these processes is played by the interaction of polyenes with the lipid component of membranes. High concentrations of PA lead to the destruction of membranes, and low concentrations lead to structural changes of membranes.Our experiments at J=0.2-1/0 W/cm^2 also confirmed the presence of a straight-line portion of curve in the range of 10-70%, which seems due to the specificity of the action of ultrasound of different intensities.

Control experiments showed that with an increase in the intensity of ultrasound to 1 W/cm^2, straight-line portion of the hemolysis curve increases significantly (5-95%).

By their chemical nature, PAs belong to the group of macrolide antibiotics[25][26]. Figure 6 shows the absorption spectra of 1375:43–51. DOI: 10.1016/s0005-2736(98)00134-5

[25] Cotero, B. V., S. Rebolledo-Antu´nez, and I. Ortega-Blake. . On the role of sterol in the formation of the amphotericin B channel. Biochim. Biophys. Acta.1998,

[26] Cybulska B., Gadomska I., MazerskiJ.et.al. N-Methyl-N-D-fructosyl amphotericin B methyl ester (MF-AME), a novel antifungal agent of low toxicity: monomer/micelle control over selective toxicity.Acta Bioch. Pol.2000 ;47 3666366(1):121-31.

amphotericin B and levorin, which are characterized by three main maxima in the UV region associated with the presence of conjugated double bonds in the chromophorous part of the molecule. The analysis of the conducted studies showed that the biological activity of antibiotics is directly dependent on the chemical structure of the molecules. The availability of antibiotic molecules for chemical modifications by functional amine and carboxyl groups creates a condition for obtaining new drugs with improved physicochemical properties for targeted use in the clinic.

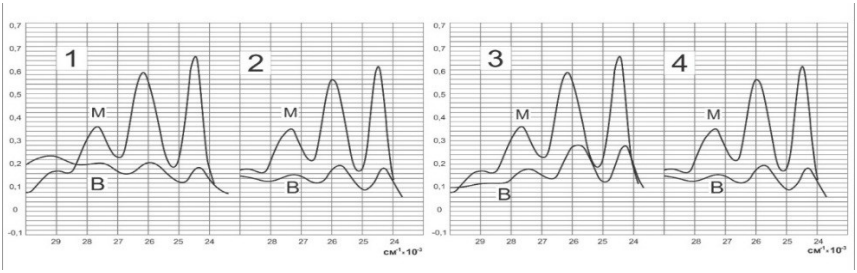

Fig.6. UV spectra of the initial amphotericin B (1) and its alkyl derivatives: methyl - (2), ethyl - (3), propyl - (4) at concentrations of $3 \cdot 10^{-5}$ M in methanol (M) and in water (B).

Many organic compounds dissolve well in DMSO, allowing it to be used in pharmacology, molecular biology, and medicine[27][28]. It is also known that the AMP channel is formed by sterol and antibiotic molecules in a ratio of 1:1 (in the case of amphotericin B- in a ratio of 8:8). For various PA stoichiometric coefficient vary within 3-17.

High concentrations of PA lead to destruction and low concentrations lead to structural changes in

[27] Yu Z., Quinn P. The modulation of membrane structure and stability by dimethyl sulphoxide (Review) Mol. Membrane Biology, 1998, v.15,p.59-68.DOI: 10.3109/09687689809027519 To link to this article: https://doi.org/10.3109/09687689809027519

[28]Verheyden P., Franco W., Pepermans H., Vanbinst G. Conformational study of a somotostatin analog in DMSO/water by 2D NMR.Biopolym,1990,v.30, p.855-860. DOI: 10.1002/bip.360300720

membrane[29]. Amphotericin derivative B-MS-8209 recommended by some authors for the treatment of purulent infections due to lower toxicity[30]. At the same time, despite the creation of new forms of amphotericin B, in terms of effectiveness action on systemic fungal infections surpasses them. PA remain the most effective compounds in the fight against fungal infections to date.

4.2. Determination of the hemolytic activity of PAs similar in structure to amphotericin B - filipin, levorin and its derivatives

Despite the positive effects of its action, many of the studied antibiotics of varying degrees have hemolytic properties. Differences in the hemolytic activity of PA may be based on the properties of the channels formed by them, the ability to solubility, the possibility of binding to the membrane surface, etc.

One of the important characteristics of the interaction of PA withthe membrane is the size of the pair formed by them, that is, the radiusof the channel, cation-anion selectivity. It has been shown that amphotericin and its alkyl derivatives form anion-selective ion channels in lipid membranes. The results of comparing the effects of PA on lipid membranes by studying the cation-anion selectivity of channels and comparing the parameters of these channels in terms of conductivity, voltage (Vm) and fixation time (T) are presented in table1. Table 1 shows that with the growth of the alkyl chain of the substituent, an increase in the anionic selectivity of polyene-induced channels is observed, and in addition to levorin and derivatives forming cation-selective channels, all the studied preparations form anion-selective ion channels in BLM

[29] Konopka K., Guo L.S., Dusgunes N. Anti-HIV activity of amphotericine B – cholesteryl sulfate colloidal dispersion in vitro. Antiviral Res. 1999, 42:197-209.

[30] Beringue V.,AdjouK.,T.,Lamoury F.et al. 2000 Opposite effects of dextran sulfate 500, the polyene antibiotic MS-8209, and Congo red accumulation of the protease – resistant isoform of PrP in the spleens of mice inoculated intraperitoneally with scrapie agent. J. Virol., 74, :5432-5440. m.org/DDOI: 10.1128/jvi.74.12.5432-5440.2000

Table 1

Cation-anionic selectivity of channels induced in lipid membranes by some PA

№	substance	Vm, mV	T, min
1	Amphotericin B	-20,4±1.8	0.10±0.04
2	Metamphocin	-20.6 ± 0.8	0.09±0.02
3	Etamphocin	-22.3 ± 2.0	0.06±0.04
4	Propamphocin	-21.4 ± 1.4	0.08±0.03
5	Butamphocin	-23.2 ± 0.3	0.04±0.01
6	Karbamphocin	-21.2 ± 0.6	0.08±0.01
7	Nistatin	-18.2 ± 1.3	0.14 ± 0.02
8	Mikoheptin	-12.6 ± 0.8	0.25 ± 0.02
9	Pozeofunqin	-16.2 ± 0.5	0.18 ± 0.01
10	Levorin	23.7 ± 0.6	0.97 ± 0.01
11	Metlevorin	23.3±1.2	0.96 ±0.02
12	Butlevorin	20 ± 1.0	0.9 ±0.01
13	Karblevorin	23.8 ±0.4	0.97 ± 0.01
14	Levoridon	24.7±0.6	0.99 ±0.01
15	Izolevoridon	25.5± 0.5	1.02 ± 0.01

It is of interest to study the hemolytic activity of some PA, such as nystatin, mycoheptin, levorin and filipin, which are chemically similar to AmP. Filipin, as a neutral polyene, differs from other PA. The ability of some antibiotics to cause lysis of human erythrocytes has been investigated. When comparing the lytic action of PA, a different degree of their membranotropic activity was revealed. Characterizing the effectiveness of the action of Pa depending on the structure of their molecules, it can be noted that mycoheptin, like AmP, is a heptaene, levorin also belongs to the heptaene group, but differs in that it in turn belongs to the group of aromatic macrolides and contains a π-aminophenyl radical. Nystatin, unlike AmP, is a tetraene, since it has a break in the chain of conjugated double bonds. Roseofungin is a pentaene, that is, the structure of the molecule contains five conjugated double bonds. To compare the relative effectiveness of the studied

compounds, equally effective concentrations of hemolytics causing 50% erythrocyte lysis were investigated.

The ability of PA to hemolyze erythrocytes in an isotonic medium was studied. It has been shown that amphotericin B and levorin, as well as their derivatives –methamphocin, ethamphocin, carboamphocin, levoridone, isolevoridone and carbolevorin, when incubated with erythrocytes in suspension, exhibit hemolytic activity in an isotonic medium at concentrations of 10^{-5} – 10^{-4}M. However, treating erythrocytes with a pure solution of DMSO (CH_3) 2SO in an isotonic medium at a concentration of 0.1% -10%, as well as with propamphocin, and butamphocin does not lead to hemolysis. Differences in hemolytic activity of the PA may be based on the properties of the channels formed, the solubility, the possibility of binding to the surface of the membrane, etc. One of the important characteristics of the interaction of PA with the membrane is the size of the pore they form, that is, the channel radius, cation anion selectivity. Amphotericin and its alkyl derivatives have been shown to form anion-selective ion channels in lipid membranes[31]. In the process of comparing the efficiency of the studied compounds, equally effective concentrations of hemolytics causing 50% erythrocyte lysis were studied.

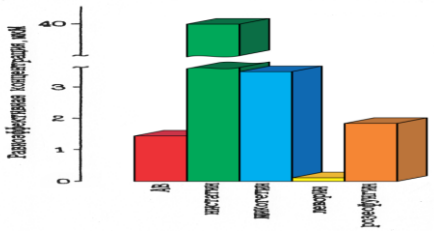

Fig.7. Comparison of hemolytic activity of equally effective concentrations of some PAs.

As shown in Figure 6, levorin is the most effective hemolytic, as when incubating with erythrocytes, lysis is observed at the lowest

[31] Касумов Х.М. Открытие одиночных полиеновых каналов и изучение их свойств в мембранах// LambertAcademic Publishing, 2020

concentration of the drug.

According to some authors, the most likely mechanism underlying the lytic effects of PA is a colloidal osmotic mechanism, which suggests that lysis is a consequence of the imbalance in ion fluxes through the cell membrane. The two proposed mechanisms of PA-induced hemolysis (colloidal osmotic and chemical degradation) do not exclude each other, as the development of peroxide processes in membranes makes the membranes more sensitive to osmotic shock.A characteristic feature of the colloid osmotic process is the S-shaped curve of erythrocyte lysis induced by PA.

4.3.Determination of the resistance of erythrocyte membranes under the combined action of US and PA with a known molecular structure.

The combined action of alkyl derivatives of amphotericin B and a range of levorin derivatives modified by amine and carboxyl groups and US on erythrocyte hemolysis were studied.

From Table 2 it can be seen that PA has different effects on the hemolytic resistance of erythrocytes in the field of ultrasound action: some activate, others slow down, and others stabilize the mechanical destruction of erythrocytes. These types of changes in the mechanical strength of erythrocytes under the influence of the polyenes appear to be related to the violation of the microviscosity of the protein-lipid system[32].

Experimental evidence suggests that some drugs stabilize membrane structure at low concentrations, and contribute to its destruction at high concentrations, apparently adsorbing on the membrane, drugs protect it from destruction, i.e. prevent oxidative free-radical transformation of lipid and protein components of the membrane[33,34]

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[32]Kasumov Kh., Bolard J. Transient permeability induced by cationic derivatives of amphotericin B in lipid membranes.Pol. J. Chem.2004, 78, 1057-1065.

[33] Eneida A. Romero Elizabeth Valdivieso, B. Eleazar Cohen Formation of Two Different Types of Ion Channels by Amphotericin B in Human Erythrocyte Membranes Jour. of Membr. Biology, 2009,230, 2: 69 22(1):64-73.doi: 10.1096/fj.07-9097 com

Table2

Influence of a number of PAs with a known molecular structure on the hemolytic activity of erythrocytes

Name of PA	Chemical composition	Drug concentration	t -hem (US)	Stabil.	Accel.	Spont. hemol	Kinetic hemol
AmphotericinB	$C_{47}H_{73}NO_{17}$	10^{-6}-$5\cdot10^{-6}$ 10^{-5}-$5\cdot10^{-5}$ 10^{-4}	500±22 350±15 250±18	- - -	30 50	- 10 5	 20 10
Methamphocin	$C_{49}H_{79}NO_{17}$	10^{-6} $5\cdot10^{-6}$ 10^{-5} -$5\cdot10^{-5}$ 10^{-4}	575±24 450±12 400±15 300±9	15- -	- 10 20 40	- - - 5	- - - 20
Etamphocin	$C_{51}H_{83}NO_{17}$	10^{-6} $5\cdot10^{-6}$-10^{-5} $5\cdot10^{-5}$-10^{-4}	850±25 750±60 400±12	70 50 -	- - 20	- - 70	- - 100
Propamphocin	$C_{53}H_{87}NO_{17}$	10^{-6} $5\cdot10^{-6}$ 10^{-5} $5\cdot10^{-5}$-10^{-4}	350±20 470±68 625±25 250±12	- - 25 -	30 - - 50	does not cause	does not cause
Butamphocin	$C_{55}H_{91}NO_{17}$	10^{-6}-$5\cdot10^{-6}$ 10^{-5}-$5\cdot10^{-5}$ 10^{-4}	600±23 350±11 275±16	20 - -	- 30 45	- - -	- - -
Levoridon	$C_{65}H_{104}N_3O_{24}$	10^{-6}-$5\cdot10^{-6}$ 10^{-5}	475±19 75±20	- -	5 25	- 5	- 10
Isolevoridon	$C_{65}H_{104}N_3O_{24}$ Transizomer levorinun	10^{-6}-$5\cdot10^{-6}$ 10^{-5} $5\cdot10^{-5}$ 10^{-4}	500±25 600±45 525±27 425±19	- 20 5 -	- - - 15	- - - 20	- - -
Karbolevorin	$C_{61}H_{94}N_2O_{24}$	10^{-6}-10^{-5} $5\cdot10^{-5}$ 10^{-4}	550±27 450±30 90±17	10 - -	- 104	- 30	- 80

[34]Doménech-Carbó A[1], Martini M, de Carvalho LM, Viana C, Doménech-Carbó MT, Silva M.Screening of pharmacologic adulterant classes in herbal formulations using voltammetry of microparticles .JP harm Biomed Anal. 2013 Feb 23; 74:194-204. DOI: 10.1016/j.jpba.2012.10.031

Control – DMSO	$CH_3SOS=O$	0,1 %	500±15	-	-	-	-
		0,25%	500±17	-	-	-	-
		0,5 %	500±14	-	-	-	-
		1%	500±12	-	-	-	-
		10%	650±25	30	-	-	-

Apparently PA, when penetrated into the lipoprotein region of the membrane, are able to interact with hydrophobic areas of the erythrocyte membrane or can themselves form structural channels therein, while changing the ion permeability of the membranes.

The biological activity of PA depends on the content of sterols of a certain structure in the membranes, upon interaction with which antibiotics form ion channels in membranes that are selectively permeable to ions and organic compounds[35]. It has been shown that amphotericin B derivatives - methamphocin, ethamphocin, carboamphocin and levorin derivatives - levoridon, isolevoridon, carbolevoridon in erythrocyte suspension have hemolytic activity in an isotonic medium at concentrations of 10^{-5}–10^{-4}M. However, treating erythrocytes with a pure DMSO solution at a concentration of 0.1 - 1%, as well as with propamphocin and butamphocin, does not lead to hemolysis.

As can be seen from the Table 1, the hemolytic effect of PA is of a concentration nature. It is shown that according to the degree of change in the permeability of lipid membranes, PA can be arranged in the following order in terms of increasing efficiency: filipin > amphotericin > levorin > nystatin[36]. This indicates a correlation between PA action on cell and lipid membranes, allowing the use of quantitative parameters of US hemolysis of erythrocytes to assess the biological effectiveness of PA. The results obtained are of great practical importance in connection with the use of PA in medicine as the main fungicidal, antiviral and antifungal drugs, which is relevant to the spread of infectious diseases.

[35] Miller M.W., Sherman T.A. Brayman A.A. Comparative sensitivity of human and bovine erythrocytes to sonolysis by 1-MHz ultrasound, Ultrasound Med Biol 2000, 26(8), 1317–1326.DOI: 10.1016/s0301-5629(00)00254-4

[36]Sultanova G.G., KasumovKh.M. Physical and chemical properties of erythrocyte membranes in interaction with polyene antibiotics in the field of ultrasonic waves 2021 J Biofizika V. 66. 2. P.

4.4. Evaluation of the effect of membrane-active channel-forming antibiotics levorin and amphotericin B and their derivatives on the proliferation and metabolism of clonogenic tumor cells HeLa and C6

This chapter presents the results of a study of the cytotoxic effect of PA - levorin and its derivatives, as well as amphotericin B on erythrocytes and *HeLa* and *C6* tumor cells *in vitro* and their survival.

An important parameter determining the toxicity of an antibiotic is the residence time of the antibiotic in the membrane and the degree of its binding to the membrane components. The use of alkyl derivatives of levorin allows reducing the antibiotic's residence time in the membrane and thereby reduce the degree of its toxicity.

In contrast to non-aromatic antibiotics, PA with an aromatic group, in particular, levorin A and its derivatives, dramatically increase the permeability of lipid and cell membranes for alkali metal cations, that is, levorin is an antibiotic with a wide antifungal spectrum.

Table 3.

Results of the study of the cytotoxic effect of levorin and its derivatives, as well as amphotericin B on the proliferation and metabolism of tumor cells.

№	Compound	Dose, mKg	Survival of tumor cells in relation to control, %	
			C6(rat glioma)	HeLa(cervical carcinoma)
1	Levorin A_2	20	124,16±1,14 p<0,01	101,22±2,42 p>0,05
		40	118,0±1,56 p<0,01	106,69±2,41 p<0,01
		200	94,76±1,62 p<0,01	64,12±1,57 p<0,01
2	Levorin A_2 (methylated)	20	87,30±2,01 p<0,01	91,44±2,51 p<0,01
		40	38,35±2,85 p<0,01	31,40±1,23 p<0,01
3	Levorin A_2 (integral)	20	97,91±0,99 p<0,01	105,68±1,39 p<0,01
		40	123,34±1,66 p<0,01	117,44±3,76 p<0,01
4	Amphotericin B	20	158,33±3,56 p<0,01	137,4±3,94 p<0,01
		40	106,04±4,33 p>0,05	76,11±0,7 p<0,01

Under the action of levorin and alkyl derivatives of amphotericin B - methamphocin and butamphocin on experimental

tumors, there is an increase in inhibition of the growth of ascitic and solid tumor cells by 46.3 - 79.0% compared to control animals. The PA in combination with DMSO also have anticarcinogenic properties, which was revealed when animals were treated with the carcinogen diethylnitrosamine (DENA).

It was shown that at doses of 20 and 40 μg, the parent molecule of levorin A_2 has no inhibitory effect on the survival of C6 and HeLa tumor cells. A highly pronounced antitumor effect of levorin A_2 is observed at a dose of 200 μg against tumor cells of the HeLa line. It has been established that methylated levorin A_2 at doses of 20 and 40 μg, when exposed to tumor cells of C6 and HeLa lines, has a significant antitumor effect, which manifests itself in the suppression of cell survival and amounts to IC70.

The analysis of the conducted studies showed that the biological activity of antibiotics is directly dependent on the chemical structure of the molecules. The availability of antibiotic molecules for chemical modification by functional amine and carboxyl groups creates a condition for obtaining new drugs with improved physical and chemical properties for targeted use in the clinic. Through chemical modification and genetic engineering methods, it is possible to create a new generation of less toxic and more effective PAs[37,38.]

STUDY OF THE MEMBRANOTROPIC EFFECT OF CYTOSTATS AND ANTIOXIDANTS ON THE STRUCTURAL AND FUNCTIONAL PROPERTIES OF ERYTHROCYTES

[37]Страчунский Л.С., Козлов С.Н. Современная антимикробная химиотерапия. Медицинское информационное агентство. 2009

[38] Preobrazhenskaya M.N., Olsufyeva E.N., Solovyeva S.E. Chemical modification and biological evaluation of new semisynthetic derivatives of 28-29 Didehydronystatin A1(S44HP), a genetically engineered antifungal polyene macrolide antibiotic.//J. MedChem., 2009; 52(1), 189- 196. DOI: 10.1021/jm 800695k

5.1. Possible mechanisms of action of antioxidants from the group of oxypyridines on the physicochemical properties of erythrocyte membranes

The search for effective pharmacological preparations that protect cellular structures from oxidative damage is a topical problem of biophysics for many years. It is shown that the increase in the concentration of active oxygen forms in the body leads to premature aging of the body and the development of various diseases, including oncological.

Currently, the search and development of antioxidant pharmacotherapy is conducted in two directions: firstly, the search for effective drugs among natural antioxidants and, secondly, obtaining more advanced analogues and their derivatives. One of the modern approaches in scientific research may be the combined application of physical factors and drugs with antioxidant properties.

It is shown that under the action of antioxidants *in vivo*, erythrocyte membranes are stabilized and their resistance to various hemolytic factors increases[39]. The mechanical effect of ultrasound on the membranes in the physiotherapeutic mode was modelled by adding mexidol and emoxipin preparations to the erythrocyte suspension. Analysis of the content of low-resistant forms of erythrocytes in suspensions showed that during the incubation of erythrocytes in oxypyridine-containing solutions, the content of low-resistant erythrocytes was lower than the number of highly resistant erythrocytes compared with the control, indicating an increase in the overall resistance of erythrocytes.

The cytoprotective effect of mexidol and emoxipin may indicate a protective effect of the drug on erythrocyte membranes in experimental pathology both *in vitro* and *in vivo*. When modeling *in vitro* pathological processes occurring *in vivo*, one should take into account the reactions of hematopoietic systems and blood destruction. From the literature data it is known that in the process of blood circulation in CPBdevices, traumatization of its uniform elements takes

[39] Клебанов Г.И., ЛюбицкийО.Б., Васильева О.В. и др..Антиоксидантные свойства производных 3-оксипиридина: мексидола,эмоксипина и проксипина.//Вопросы медицинской химии, 2001, 47,3, с. 288-300

place[40][41]. At the same time, the behavior of erythrocytes in the blood flow is determined by their mechanical resistance and deformability.

It has been shown that the drugs used - mexidol and emoxipin at certain concentrations significantly increase the resistance of blood cells *in vitro* and *in vivo* in case of hypotonic hemolysis and mechanical trauma in artificial blood-circulation apparatus.Thus, compounds from the class of oxypyridines - mexidol and emoxipine have a protective effect against the lytic action of US. An increase in the intensity of ultrasound leads to an increase in the action of its mechanical factors and, accordingly, to a decrease in the protective effect of these compounds.

At the same time, mexidol has the greatest protective effect. It was found that in the presence of antioxidants, the ability of the blood to bind sufficient amounts of oxygen is preserved. Analyzing the above and taking into account the fact that all the compounds studied in this work are antioxidants, and some of them arealso radioprotectors, it can be assumed that their stabilizing effect is associated with the ability to resist oxidative free radical transformations of the lipid and protein components of the membrane[42].

5.2. Evaluation of the effect of PA solvents DMSO and ethanol on the structural and functional properties of erythrocyte membranes

The kinetics of erythrocyte hemolysis under the influence of various concentrations of DMSO and ethanol was studied. It was found that the hemolytic response in the presence of DMSO has a staged character. Cells not exposed to DMSO were controls. Curves of hemolysis of erythrocytes were recorded by changing the optical

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[40] Vercaemst L. Hemolysis in cardiac surgery patients under-going cardiopulmonary bypass: A review in search of a treatment algorithm // The J. of Extra Corporeal Technology. 2008. V. 40, № 4. P. 257—267.PMCID:PMC4680715

[41]Атауллаханов Ф.И., Корунова Н.О., Спиридонов И.С. Как регулируется объем эритроцита, или что могут или не могут математические модели в биологии // Биологические мембраны,- 2009. Т. 26, № 3. - с. 163–179.

[42] Бурлакова Е.Б. Биоантиоксиданты. // Российский химический журнал. 2007. Вып. 51, №1. с.3-12

density of the samples, at a wavelength of λ=670 nm and with continuous stirring[43]. The rate constants of hemolysis were determined from the tangent of the angle of the corresponding segments of the kinetic curves at their half-heights.

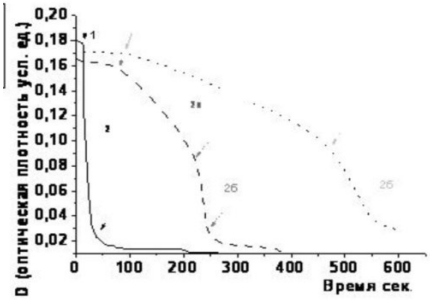


Fig.8. Curves of hemolysis of erythrocytes under the influence of levorin at different concentrations of DMSO: 1-control, 2-15% DMSO, 3-20% DMSO, respectively.

As can be seen, the process of hemolysis of erythrocyte samples, both control and modified with DMSO, is a 2-stage character. The greatest protective effect is observed when erythrocytes are treated with a 20% DMSO solution.

Thus, the use of DMSO as a solvent for PA increases the resistance of erythrocytes to hemolysis under the action of macrolactone compounds. PA and DMSO appear to be competitors in interaction with the same structural components of the cell membrane, primarily lipids. Therefore, pre-treatment of erythrocytes with DMSO should reduce the degree of PA binding to membrane lipids. There may also be a direct mechanism to increase the structural stability of membranes by DMSO treatment by forming hydrogen bonds with structural water molecules interacting with macromolecules of membranes, resulting in their stabilization.

Thus, it can be concluded that pre-treatment of DMSO erythrocytes increases their resistance to hemolysis under the action

[43] Хакл Е.В.,Берест В. П. Изменение подвижности липидов мембраны при взаимодействие грамицидина S с эритроцитами человека// Биофизический вестник 2008,.2, 21. 56-63

of membrane-active macro-laminated compounds from the polyene group. When preparing solutions of some drugs, ethanol is used as a solvent. In this regard, the effect of ethanol on cell membranes has been studied. According to the statistics of many years of research, the highest rate of deaths from acute poisoning is due to the toxic effect of low-quality alcohol[44,45].

The effect of ethanol in the field of ultrasonic action on the plasma membranes of erythrocytes during hemolysis was studied. The results of the studies showed that the concentration of ethanol in the test sample ≤ 0.1% does not affect the strength of erythrocytes to ultrasonic exposure. Alcohol concentration ≥ 0.1% by volume has a stabilizing effect on erythrocyte cells. An analysis of the state of cell membranes under the action of various concentrations of ethanol in the blood showed that low doses of alcohol in 50% of the subjects can lead to the activation of protective mechanisms to preserve the integrity of the membranes and the half-life of erythrocytes increases in them. With an increase in alcohol concentration, the stabilization of the erythrocyte membrane decreases. It appears that, under the action of low concentrations of ethanol, the protective mechanisms of cell membranes are triggered, and with an increase in the level of alcohol, the half-life of membranes decreases, which is confirmed by data on cell membranes under the action of high doses of alcohol.

[44]Василевич Н. В., Платошкин Э. Н.,Запольский Д. В. Острые отравления алкоголем и суррогатами алкоголя в клинической практике врача на стационарном этапе лечения. Проблемы здоровья и экологии,Клиническая медицина,2012, с.38-44

[45]Василевич Н .В., Платошкин Э. Н., Запольский Д.В. Острые отравления алкоголем и суррогатами алкоголя в клинической практике врача на стационарном этапе лечения. Проблемы здоровья и экологии,Клиническая медицина,2012, с.38-44

5.3. Model of the impact of ultrasonic waves on erythrocytes in suspension *in vitro*, as an analogue of their behavior *in vivo* in the body.

When comparing the results of studying the resistance of erythrocytes in the bloodstream *in viv*o and in the field of ultrasonic waves *in vitro*, we made an attempt to draw an analogy between these processes. It is known that the cardiovascular system is formed by vessels of various sizes and physico-chemical characteristics that affect blood flow and the composition of red blood.

At the same time, the blood flow in different parts of the circulatory system passes with variable speeds and pressure, since erythrocytes during the flow in the bloodstream undergo various influences - mechanical, hydrodynamic, biochemical.

We have developed a spectrophotometric method for studying the permeability of biological membranes in the physiotherapeutic mode of ultrasonication, and proposed to solve the set tasks[46,47].

Table 4.

Correspondence of the parameters of the ultrasound field to the mechanical indicators of the cardiovascular system

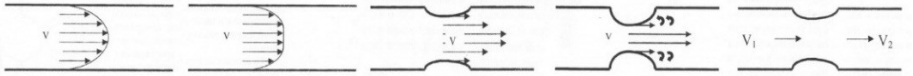
ULTRASOUND system		Heart-vascular
Pre-cavitation mode	Cavitation mode	Heart
0.01-0.2W/cm^2	$\geq$ 0,3	Aorta, arteries
0.88MHz	> 1 MHz	Veins, capillaries

Figure 9 shows a diagram of the turbulent flow of blood in the

[46]Нагорнов Ю.С., Пахомова Р.А., Жиляев И.В. и др. Моделирование морфологии эритроцита и расчет внутриклеточного давления по данным атомно-силовой микроскопии // Российский журнал биомеханики. 2015. Т. 19, № 4: 398-408

[47] Султанова Г.Г., Багирова А.А., Рагимов Н.Р., Николаевич Л.Н. Действие некоторых макролидных антибиотиков invitro на стойкость эритроцитов и пролиферацию и метаболизм злокачественных клеток // Ж.Гематология и трансфузиология (Мат. IVКонгресса Гематологов России), 2018, 63.1, 184-185

aorta and arteries.



abcde

Fig. 9. Scheme of turbulent blood flow in the aorta and arteries.

As can be seen from the Figure 8 in the aorta and arteries (as well as with stenosis), in the vessels there is a turbulent flow of blood at a high speed corresponding to the cavitation regime, further the blood flow velocity decreases (a, c, e) in the veins and reaches a minimum in the capillaries - the pre-cavitation regime. In this sequence, pressure, blood flow velocity and the diameter of vessels change. At the same time, when comparing the size of the aorta and the total length of all capillaries in the body, it is quite logical that it is in the capillaries that erythrocytes, performing their main functions, are at the greatest risk of destruction than in the aorta and arteries. The action of vortex flows, shear stresses, microflows occuring in the ultrasound field can be extrapolated and compared with friction, collisions, vortex movements, pulsation when cells move in blood vessels, that is, there is also the effect of mechanical factors on the cells in the vascular bed. At the same time, the rheological parameters of cells in different parts of the bloodstream correlate with data on the behavior of erythrocytes under various ultrasonication conditions. The above analysis suggests that there is a correlation between the resistance of cells under different modes of ultrasonic exposure and their behavior in the bloodstream, which can be represented as a model of the behavior of erythrocytes in different parts of the bloodstream.

PRACTICAL ASPECTS OF THE USE OF ULTRASOUND AND MACROLACTONE COMPOUNDS IN BIOLOGY AND MEDICINE.

6.1. The study of the rheological properties of erythrocytes by the optical method and the determination of the quantitative characteristics of the mechanical properties of their stroma with a preliminary division into age fractions. Hemolytic stability is a function of the membrane properties associated with the physicochemical characteristics of the membrane lipids. The stability of the membrane also depends on the cholesterol/phospholipid ratio. Phospholipids increase the osmotic stability of erythrocytes, and cholesterol prevents the erythrocytes from becoming spherical, which is the first sign of hemolysis. Erythrocytes of different ages can be divided by density and, accordingly, by age, and reticulocytes are the lightest, containing 4-5 times more total lipids and phospholipids than mature and old cells. At the same time, with aging of erythrocytes, the functional properties of cells change significantly. It has been shown that young erythrocytes are characterized by a higher resistance than old ones, which in a certain way is related to the properties and enzymatic composition of their stroma, leading in turn to a change in the resistance of red blood cells. A number of authors have shown that the development of experimental leukemias and tumors is accompanied by a violation of the molecular organization of membranes, a change in the physicochemical properties of lipids, an increase in lipid peroxidation in the membranes, both of the cells themselves and of cell organelles[48]

In this regard, it is relevant to assess the morphofunctional state of erythrocytes and the features of its changes in clinically healthy donors and people with various diseases, to determine the dependence of these parameters at the growth of experimental tumors and to conduct a correlation analysis of these results with changes in the physicochemical characteristics of erythrocytes in

[48]Корман Д.Б Альтернативная терапия рака М.: Изд. Практическая медицина, 2016, 216 С

control and in a number of diseases.

6.2. Changes in the structural and functional state of erythrocyte membranes in onco- and hematological diseases. Due to the fact that oncological pathologies are practically not detected at the initial stages of the development of the process, it is important to use biological “models” to study the structural and functional properties of blood cells in cancer patients at various stages of the disease. Presumably, the experimental “model” of leukemia can reflect similar changes in some blood parameters in the conditions of the development of the oncological process in humans[49].The work was carried out in two stages: the study of the general patterns of changes in the parameters of blood cells in animals with experimental leukemia and the extrapolation of the results obtained in the analysis of the study of blood cells in pathologies of various localization and stages of the disease. Work with animals was carried out in accordance with the principles of the Declaration of Helsinki on the Humane Treatment of Animals[50]. On the model system, it was found that young erythrocytes have an increased resistance to the effects of mechanical factors of ultrasound. The most pronounced changes in red blood parameters during tumor growth, both solid and ascitic, are observed at the middle stage of the process, and closer to death, an imaginary improvement in blood parameters is observed. This, apparently, is the reaction of the body to the development of the oncological process, leading to an intensive removal of old erythrocytes from the bloodstream and to a relative increase in the total resistance of erythrocytes. It should be noted that it is the increase in the number of aging erythrocytes that can be an indicator in the diagnosis and prediction of the tumor carrier organism and give a number of important recommendations for complex treatment in chemotherapy.

[49]Н.Забиняков. Атомно-силовая микроскопия.Оценка морфометрических параметров клеток крови позвоночных животных (Russian) Paperback – 24 Nov. 2010 Publisher:LAP LAMBERT Academic Publishing (24 Nov. 2010)

[50] Горняк С.А. Физические механизмы взаимодействия ультразвука с биологическими структурами и их моделями. 2003, Автор. дис. канд. Физ-ат. наук Харьков, 26 с.

The developed method can be used to monitor changes in the quantitative characteristics of the state of erythrocytes during the development of pathological processes in the body. In the differential study of cell suspensions in the ultrasound field, it became possible to monitor the development of the state of the hematopoietic system in pathological conditions of the body. The stability of mouse erythrocytes in the field of ultrasound action was studied after transplantation of lymphocytic leukemia and with the introduction of cyclophosphamide (CP). From the results presented in Figure 9 and 10, it can be seen that during the period of tumor development, there is a dynamics of changes in the resistance of erythrocytes.

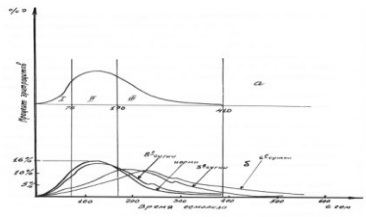


Fig. 10. Curves of normal distribution of cells (erythrograms) in control (1) and in infected animals on days 2, 3, 6 and 8(curves 2.3.4.5, respectively) in the field of US action.

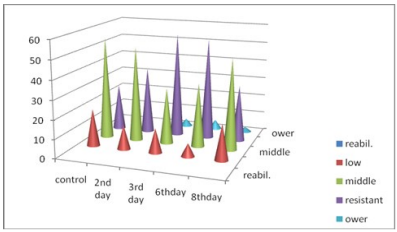


Fig. 11. Diagram of the distribution of erythrocytes by resistance groups at different times after ascites inoculation

Presented in Table 4 data indicate that in most cancer patients with tumors of different localization, there is an increase in the microviscosity of blood cell membranes, depending on the localization of the tumor, the stage of the disease, histology, gradation, etc. Analysis of the results obtained allows us to conclude that the quantitative composition of the blood does not always reflect the changes taking place in the body, especially in the presence of such a powerful factor as the cancer process. The kinetic method of ultrasound hemolysis allows diagnosing the pathological process before and without the manifestation of clinical signs of the disease. The results of the percentage distribution of various groups of erythrocytes by resistance are shown in Figure 9.

The results obtained on model systems were confirmed by the

analysis of the state of erythrocyte membranes in tumor pathologies. Thus, in the field of ultrasound, it is possible to analyze the physicochemical characteristics of cell and lipid membranes under various influences on the body (cancer processes, MAC, natural aging of blood cells in the bloodstream).

Table 5

Physico-chemical characteristics of erythrocytes in some pathologies (clinic)

Disease	Mean volume of erythrocytes km	Relative refractive index at λ =805 nm	The average concentration of dry substances in erythrocytes % per 100 ml	Mean quantity of dry matters of erythrocytes	The number of erythrocytes mln in 1 ml.
BC (breast cancer)	83,6±5.0*	1.060±0.002*	34.4±1.0*	29.3±0,9*	2,8±0.4*
SC (stomach cancer)	77,2±4,0*	1.062±0.002*	35.2±0 ,7*	27.6±0,4*	2,8±0.1*
LC (Lung cancer)	82,6±5,4*	1.058±0.002*	33,9±0 ,88*	27.9±0,8**	2,8±0.3*
Pneum.	65,4±3,0	1.066±0.002*	38,2±0 ,5	25.2±0,6	3,8±0.2*
Control	63,5±0,2	1.071±0.001	41,6±0 ,2	26.4±0,2	4,8±0.2

It was found that during pathological processes, an increased number of young erythrocytes accumulate in the peripheral blood and the maximum death of old worn-out erythrocytes is observed. It was found that young erythrocytes have increased resistance to the effects of mechanical factors of ultrasound.

From the results presented above, presented in Figs. 8 and 9 and in Table 4, the proposed method for detecting changes in optical density under the influence of physicochemical factors allows to estimate the number of erythrocytes at any time in suspension; the rate constant of a decrease in the number of erythrocytes for any given period of time; the average rate constant of hemolysis; the proportion of non-hemolyzed erythrocytes as a

result of a given exposure; characteristics of mechanical properties of erythrocyte membranes.

Effect of ionizing radiation on the functional state of erythrocyte membranes in breast cancer

In connection with the use of ionizing radiation in radiology and oncology, it is important to study its effect on the state of cell membranes.

The results of the study of the state of erythrocyte membranes of women - donors, as well as those diagnosed with breast cancer at the age of 20-70 years before and after radiation therapy are shown in the Table 5.

Table.6

The half-life of erythrocyte membranes T_{50} in different age groups of cancer patients (BC)

Age	Healthy T_{50}, min	Cancer patients before irradiation	*p*	Cancer patients after irradiation	*P*
20-30 age	23,4±1,8	-		-	
31-50 age	26,7±2,3	19,9±2,0	<0,05	30,2±2,7	<0,001
51-70 age	27,5±2,4	23,7±2,1	<0,05	26,8±2,3	<0,05
> 70 age	29,9±3,3	27,2±2,3		20,2±1,9	<0,01

An increase in T_{50} in the control group indicates the presence of a larger number of non-destroyed erythrocytes after exposure to X-ray therapy.

This indicates that the applied dose of radiation contributes to the “strengthening” of erythrocyte membranes due to the inclusion of reserve capabilities by the body to preserve erythrocyte membranes from the effects of negative factors, which prevents the development of hemolysis.

It can be seen that in persons over 60 years of age, the reserves for the preservation of erythrocyte membranes from the action of external adverse factors are exhausted, which indicates that in persons of mature age, internal compensatory reactions of the

body turn on under radiation exposure, but not for people over 70. Therefore, under the background of radiation, there is a false improvement (remission) that does not lead to a final recovery.

The study of various effects on the erythrocytic membrane in the whole blood of the patient and on the isolated suspension of erythrocytes using the ultrasound method makes it possible, firstly, to evaluate the direct effect of preparations on the structure of the cell membrane i.e. to evaluate the protective properties of membrane structures (proteins) and other blood chemical compounds during the action of the studied drugs, and, secondly, to identify the most optimal doses of the studied drugs.

SUMMARY

To solve the problems set in the thesis the main stages of work were determined. As a result, the hemolytic activity of antioxidants and macrolactone compounds of the polyene structure was studied and the optimal doses of their active concentrations were identified; a comparative analysis of the effectiveness of the structure-modifying effect of PA derivatives with side substituents different in structure and hydrophobic properties on cell membranes was carried out; the possibility of using ultrasonic waves in the physiotherapeutic mode for the analysis of cell condition and the diagnosis of damage to cell membranes is substantiated; in the ultrasound field of damage to biological membranes caused by the action of a number of biologically active drugs is determined; the patterns of changes in the physicochemical properties of erythrocytes under various modes of ultrasonication, as well as under the action of some physical environmental factors, were studied and analyzed; the priority components of ultrasonic waves in the mechanism of action on blood cells were identified and a physical model of cell behavior in an ultrasonic field was developed; the mechanisms of action of various concentrations of a number of macrolactone compounds (PA, ethanol, DMSO, anticarcinogens, antioxidants) on the structural and functional properties of erythrocytes were analyzed and a correlation of their

action on biological and bilayer lipid membranes (BLM) was established; the contribution of erythrocytes of different ages to the hemolytic picture of red blood (erythron) in pathologies was identified and the relationship between them was established; the general regularities of the influence of physicochemical characteristics on the membranes of blood cells were revealed; the effect of physical factors on the functional state of erythrocyte membranes in experimental leukemia was studied and a correlation in the behavior of erythrocytes in the field of action of ultrasonic waves on cell and lipid membranes was found, and as well as a model of cell behavior in the physiotherapeutic mode of ultrasonication was developed.

Analysis of the action of PACon biological cells allows us to get closer to understanding the mechanisms of their binding to the studied objects (erythrocytes and BLM) by forming new transport systems of ion channels in them without disturbing the membrane structure, as well as to protect it from mechanical injury by creating an invisible shell around the cell membrane. The dependence of the degree of modifying action of the studied antibiotics on their structure was revealed. The generality of the impact on erythrocytes of the mechanical factors of the cardiovascular system and ultrasonic waves of the physiotherapeutic regime of ultrasonication is shown.

The division of red blood cells into age fractions that occur in the bloodstream of humans and animals *in vivo* makes it possible to identify the role of each of these groups on the hemolytic picture of the blood of cancer patients.

The relationship between the dynamics of the development of the oncological process in experimental animals with physicochemical and rheological characteristics of blood cells was revealed.

The study of the mechanical properties of erythrocytes by the method of ultrasonic hemolysis makes it possible to determine the degree of influence of certain PAC (antioxidants, pesticides and some anticarcinogenic drugs) on the structural and functional characteristics of body cells and to propose optimal concentrations

for their use in biomedical practice.

The results of the study of various modes of ultrasonic ultrasonication of blood cells allows proposing an analogue of the model of cell behavior in the human bloodstream.

Thus, the presented method allows to evaluate changes in the functional state of cell membranes depending on the age of erythrocyte composition, tumor localization, drug concentration, chemical structure, as well as the effects of environmental factors on biological objects.This gives wide opportunities for using the kinetic method of ultrasound research as a screening method in various areas of biomedical research.

CONCLUSION

1.For the first time, quantitative characteristics of changes in the mechanical resistance of erythrocytes depending on a number of physicochemical parameters of the medium were determined: Frequency and intensity of ultrasound, number of erythrocytes, hemoglobin content, total protein content in cells and erythrocyte sedimentation rate. The most optimal mode of ultrasonic action on cell membranes was determined (at frequency f=0.88 MHz, intensity J=0.2-0.4 W/cm^2).

2.The degree of protective action of a number of membrane-active compounds was determined using the kinetic method of ultrasonic hemolysis of erythrocytes. An analysis was made of changes in the structural characteristics of erythrocytes modified with various polyenes: amphotericin B, levorin and their derivatives, nystatin, etruscomycin, mycoheptin, filipin.

3.It has been shown that antioxidants - emoxipine and mexidol derivatives of oxypyridines at concentrations of $1\cdot10^{-}5$-$1\cdot10^{-}6$ M in the studied suspensions increase the resistance of erythrocyte membranes to the mechanical action of ultrasound by 35-45%.

4.It was revealed that polyenes, when interacting with erythrocytes, cause structural and concentration changes in the physicochemical characteristics of their membranes. At low

concentrations of the antibiotic 10^{-6}M, the effect of membrane stabilization by amphotericin B and levorin is observed, and at high concentrations, the studied antibiotics, with the exception of levorin, are weak hemolytic agents. It has been shown that antibiotics, penetrating into erythrocyte cells, chemically modify hemoglobin molecules, leading to its denaturation. The nature of the change in the resistance of erythrocytes is determined by the chemical nature of the active modifier, its concentration and the time of interaction with the cell. It was found that propamphocin and levoridone at concentrations of $5 \cdot 10^{-6}$ mol/l – $5 \cdot 10^{-5}$ mol/l lead to irreversible changes in erythrocyte membranes.

5.It is shown that the combined action of a number of compounds in the field of ultrasonic waves has protective properties. It was shown that the antioxidants emoxipin and mexidol at concentrations of $1 \cdot 10^{-4}$ - $1 \cdot 10^{-5}$M in the studied suspensions increase the resistance of erythrocyte membranes to the mechanical action of ultrasound by 35-45%.

6.A method for monitoring the functional state of erythrocyte membranes in the presence of dimethyl sulfoxide and ethanol was developed. At low concentrations of ethanol, membrane strengthening is noted, and with an increase in ethanol concentration, a deterioration in the condition of erythrocyte membranes is observed.

7.For the first time, the percentage distribution of blood erythrocytes according to their resistance to ultrasonic hemolysis in normal and pathological conditions was studied. It has been established that young erythrocytes in the bloodstream have the highest resistance to ultrasound and acid hemolysis. The relationship between an increase in the mechanical resistance of erythrocytes to US and the accumulation of an increased number of young erythrocytes in the peripheral blood and an increase in their resistance during the development of lymphocytic leukemia in the ascitic form was revealed. A two-phase nature of the change in the mechanical resistance of erythrocytes corresponding to certain stages of the kinetics of the tumor process was found. It has been shown that cyclophosphamide inhibits the development of a tumor

without affecting the resistance parameters - the time and rate of hemolysis. The kinetic characteristics of red blood hemolysis can be presented as additional criteria that determine the development of an experimental tumor and will allow diagnosing changes in erythrocyte membranes.

8.It was found for the first time that amphotericin B, levorin and their alkyl derivatives significantly inhibit the development of the tumor process. At the same time, a change in the distribution of erythrocytes according to the resistance group is shown in the direction of an increase in low-resistant erythrocytes. The kinetic characteristics of red blood hemolysis can be presented as additional criteria that determine the development of an experimental tumor. It has been established that methylated levorin at doses of 20–40 μg/ml, when exposed to C6 and HeLa tumor cells, has an antitumor effect, which manifests itself in the suppression of cell survival. Amphotericin B at a concentration of 40 μg/ml slightly reduces the survival of HeLa tumor cells compared to a dose of 20 μg/ml, and at doses of 20 and 40 μg/ml does not affect the survival of C6 tumor cells.

9.A model of mechanical destruction of cells in an ultrasonic field *in vitro* is proposed as an analog of their destruction in the bloodstream *in vivo*. It has been shown that both in the ultrasonic field (vortex flows, shear stresses, microflows) and when moving in blood vessels (friction, collisions, vortex motions, pulsation), mechanical factors act on cells. It seems that there is a correlation between the resistance of cells under different modes of ultrasonic exposure and their behavior in the bloodstream, which can be represented as a model of the behavior of erythrocytes in different parts of the bloodstream.

PRACTICAL RECOMMENDATIONS

1.The proposed kinetic method for studying the state of red blood cells can be applied in clinical conditions to determine the functional capacity of cell and lipid membranes.

2.This method makes it possible to predict the effect of a

number of macrolactone compounds on erythrocyte membranes, gives access to the search for the most effective drugs needed in various fields of medicine and the national economy.

3.The proposed method can be used as a screening method for studying the population to identify the most common pathological processes (oncological, cardiovascular) in the body.

4.When exposed to ultrasonic waves, the behavior of erythrocytes in the sound field of the physiotherapeutic regime is modelled as an analogue of their behavior in various parts of the bloodstream.

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