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CLONAL HAEMATOPOIESIS AND IONIZING RADIATION: RISKS FOR HEMATOLOGICAL MALIGNANCIES AND SOMATIC DISEASES

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Introduction. The influence of radiation-induced genetic instability on the formation of clonal expansion is a relevant problem in health monitoring and preventive diagnostics of oncohematological and somatic pathology in individuals exposed to long-term low-dose anthropogenic irradiation, such as nuclear industry workers and radiation diagnostics doctors.

Objective. Identification of possible application points of preventive diagnostics of genome instability markers and clonal hematopoiesis in groups of individuals exposed to long-term low-dose anthropogenic irradiation.

Results and discussion. Genetic instability in genes of epigenetic regulation (*DNMT3A, TET2, ASXL1*), signaling pathways and cell proliferation (*JAK2, FLT3*), DNA repair regulators (*TP53, PPM1D*), RNA splicing factors (*SF3B1, SRSF2*) most often initiates clonal hematopoiesis, which is realized more frequently by myeloid and less frequently by lymphoid neoplasia. The influence of clonal hematopoiesis on the development of somatic diseases is mediated by the combined effect of carrying these mutations and the processes of chronic inflammation. Low-dose ionizing radiation is capable of initiating clonal expansion mainly due to mutations in *DNMT3A* and *TET2* genes. There is a lack of studies on the assessment of increased morbidity against the background of clonal hematopoiesis in groups of occupational risk of low-dose ionizing radiation exposure (workers in the nuclear industry and doctors of radiation diagnostics), which requires further study.

Conclusions. Studies aimed at identifying risk markers of morbidity growth in the setting of clonal hematopoiesis in groups of workers exposed to long-term anthropogenic action of low-dose ionizing radiation form the basis for developing cohort-oriented programs of disease prevention in these individuals.

Keywords: genetic instability; clonal hematopoiesis; clonal hematopoiesis of undetermined potential; low-dose ionizing radiation; somatic mutation

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КЛОНАЛЬНОЕ КРОВЕТВОРЕНИЕ И ИОНИЗИРУЮЩЕЕ ИЗЛУЧЕНИЕ: РИСКИ РАЗВИТИЯ ОНКОГЕМАТОЛОГИЧЕСКОЙ И СОМАТИЧЕСКОЙ ПАТОЛОГИИ

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Введение. Влияние радиационно-индуцированной генетической нестабильности на формирование клональной экспансии актуально для мониторинга здоровья и превентивной диагностики онкогематологической и соматической патологии у лиц, подвергающихся длительному воздействию техногенного облучения в малых дозах (работники атомной промышленности и врачи лучевой диагностики).

Цель. Выявление возможных точек приложения превентивной диагностики маркеров нестабильности генома и клонального кроветворения у групп лиц, подвергающихся длительному воздействию техногенного облучения в малых дозах.

Обсуждение. Генетическая нестабильность в генах эпигенетической регуляции (*DNMT3A, TET2, ASXL1*), сигнальных путей и клеточной пролиферации (*JAK2, FLT3*), регуляторов репарации ДНК (*TP53, PPM1D*), факторов сплайсинга РНК (*SF3B1, SRSF2*) наиболее часто инициирует клональное кроветворение, реализующееся чаще миелоидными и реже лимфоидными неоплазиями. Влияние клонального кроветворения на развитие соматических заболеваний опосредовано сочетанным действием носительства указанных мутаций и процессами хронического воспаления. Ионизирующее излучение в малых дозах способно инициировать клональную экспансию преимущественно за счет мутаций в генах *DNMT3A* и *TET2*. Исследований по оценке повышения заболеваемости на фоне развития клонального кроветворения в группах профессионального риска воздействия малых доз ионизирующего излучения (работники атомной промышленности и врачи лучевой диагностики) в настоящее время мало, что требует дальнейшего изучения.

Выводы. Исследования по выявлению маркеров риска роста заболеваемости на фоне развития клонального кроветворения в группах работников, подвергающихся длительному техногенному действию ионизирующего излучения в малых дозах, позволят сформировать когортно-ориентированную программу профилактики заболеваний у данных лиц.

Ключевые слова: генетическая нестабильность; клональное кроветворение; клональное кроветворение неустановленного потенциала; малые дозы ионизирующего излучения; соматическая мутация

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INTRODUCTION

Research into the radiation-induced genetic instability and its interrelation with clonal expansion is a relevant direction with regard to health monitoring of nuclear industry workers and radiologists. Identification of risk factors in the emergence of adverse consequences in these categories of individuals, along with dispensary monitoring and timely prophylaxis, may contribute to combating the development of related pathologies [1].

On a daily basis, human beings are exposed to low-dose ionizing radiation (IR) emitted both by natural (environmental radionuclides found in the atmosphere, soil, and water) and artificial or man-made sources. The cumulative effect of all types of natural radiation is defined as the natural radioactive background. The global average values of such radiation in different regions of the world range from 2.4 to 4.0 mSv/year, with the average level in the Russian Federation comprising 3.36 (from 2.10 to 8.60) mSv/year [1–3].

The impact of IR on humans is realized through the influence of the natural radioactive background, occupational exposure at the workplace, as well as when receiving medical care (medical exposure). The widespread use of IR in medical and other industries, primarily in the nuclear power industry, contributes to an increase in radiation exposure of workers. Thus, for a certain list of industries, where additional anthropogenic exposure to IR is possible, the limit of permissible effective IR dose reaches 5 mSv/year for Group B personnel and 20 mSv/year for Group A personnel [3].

At present, the immediate and delayed consequences of human exposure to high-dose IR in technogenic catastrophes, characterized by critical changes leading to defects in the functioning of organs and systems, have been studied and described in sufficient detail. At the same time, exposure to low-dose IR, although without clinical manifestations of radiation damage, may also induce damage at the genetic and epigenetic levels, with the effect depending on the radiation dose in a non-monotonic polymodal character. The recent data points to the effect of low-dose radiation on genome instability, modifying cellular and tissue processes. This may eventually contribute to changes in the body sensitivity to the action of additional non-radiation factors [4–6].

Over the past decade, research interest has been revolving around clonal hematopoiesis (CH), considering it as a biological condition predisposing to the development of malignant blood disorders (MBD), solitary tumors, cardiovascular pathology, autoimmune diseases, and other pathologies [7–9].

This work aims to identify the possibility of preventive diagnostics of genome instability markers and CH markers in work groups exposed to long-term low-dose technogenic irradiation.

RESULTS AND DISCUSSION

General understanding about clonal hematopoiesis

Hematopoietic cells of the bone marrow (BM) exhibit the highest proliferative potential. The processes of blood cell proliferation and differentiation in BM are carried out continuously. About one trillion blood cells are formed daily. The basis of hematopoiesis are hematopoietic stem cells (HSCs), which have the ability to differentiate into all mature cells of peripheral blood. It was first postulated by Prof. A.A. Maximov in 1909. The hematopoiesis model proposed by Maximov was confirmed by experimental data obtained in the era of active study of radiation effects on the human body [10].

Over the past decades, our understanding of the hematopoiesis system has been significantly extended [10–13]. Thus, numerous studies have advanced the idea of continuous differentiation landscapes having a few or no discrete differentiation stages and smooth cell state transitions. In this context, cells in a heterogeneous pool of progenitors differentiate along multiple potential trajectories that contain some branching points determining the fate of a particular cell. However, the notion that the progeny of a single HSC represents a clone of a particular HSC remains unchanged. The normal hematopoietic system is polyclonal that assumes production of “own” cell clones by different HSCs and maintenance of their number as well as the ratio of cells with varied degrees and directions of differentiation in BM within stable limits. Hematopoietic cells with a high proliferative activity are characterized by accumulation of genetic “breaks” with increasing number of divisions [14].

Upon human aging, physiological quantitative and qualitative changes occur in hematopoiesis, including a decrease in the total proliferative HSC activity with a relative increase in their total number, an increase in the erythroid precursors number, a decrease in the lymphoid precursors number with age-related changes in the immune system function. In general, hematopoiesis tends to oligoclonality [15]. Genetic and epigenetic processes most likely underlie the age-related changes in BM. The age-related decrease of HSC regenerative potential [16], specific changes in expression of transcription regulator genes, accumulation of somatic mutations [17], and pronounced shortening of telomeric chromosome parts [18] were noted. In addition, in elderly people, increased expression of genes involved in the regulation of apoptosis and inflammation in the hematopoiesis system was detected; however, in young people, the activity of genes regulating the processes of proliferation and metabolic activity of hematopoietic progenitor cells is high. All the described trends are manifestations of regular processes characterizing changes in hematopoiesis in the process of aging of the organism [19].

At the same time, the accumulation of mutations can lead to a CH with activated proliferation of one HSC or more differentiated hematopoietic progenitor cell and with formation of a progeny clone carrying gene mutations. While CH is not an independent nosology, it can be an intermediate stage between normal hematopoiesis and MBD [20]. It has been shown that CH can arise as a result of neutral drift or directed selection. In the case of neutral drift, all cell clones initially have equal chances of their contribution to the formation of the pool of self-renewing HSCs; the decisive influence is exerted by random processes, such as depletion of the stem cell pool. In the case of directed selection, somatic changes influence the selective growth advantage in

certain HSC clones relative to others, with the subsequent clonal expansion [21].

In 2014, two independent groups published the results of large-size studies conducted using next-generation sequencing (NGS). According to the data obtained, CH is associated with somatic mutations most frequently occurring in three genes of epigenetic regulation of transcription. These include *DNMT3A* — a gene encoding a protein that performs de novo methylation; *TET2* — encoding a protein that catalyzes the conversion of the modified DNA base methylcytosine into 5-hydroxymethylcytosine and participates in the transcription process regulation; and *ASXL1* — encoding a nuclear protein involved in the epigenetic regulation of gene expression and in the process of chromatin remodeling. The obtained results were confirmed in a study of groups of patients with different types of MBD and solid neoplasms [22, 23]. The work by M. Xie et al. convincingly demonstrated that mutations in *DNMT3A*, *TET2*, *ASXL1*, and *TP53* genes occurred most frequently and almost in all analyzed nosological subgroups [24].

In 2015, on the basis of the accumulated data, the term "clonal hematopoiesis of indeterminate potential" (CHIP) was coined. Thus, CHIP is defined in the presence of a hematopoietic cell clone with a gene mutation associated with the risk of MBD in the absence of cytopenia and criteria of other hematological diseases, according to the classification of the World Health Organization. The allele load level of the indicated gene should be not less than 2% (the value is accepted as the minimum clinically significant threshold level for the next-generation sequencing method — NGS), and for X-linked genes in men — not less than 4% [25, 26]. The frequency of mutation detection increases with age, and their presence is associated with an increased risk of MBD. It was determined that about 15–20% of people over 70 years of age without oncohematological diseases have somatic mutations associated with an increased risk of oncopathology [27]. Later, the term "age-related clonal haematopoiesis" was proposed for such changes, which is defined in elderly patients with a somatic mutation in genes regardless of its clinical significance and allele load level, as well as with no changes in the hemogram and MBD criteria [23, 27].

The term "clonal haematopoiesis of oncogenic potential" (CHOP) was introduced to designate the state of carrying mutations that can act as a direct driver of MBD [28, 29]. The allocation of such a category and the approach to the division of genes according to their direct role in the development of MBD (i.e., their oncogenic potential) is currently rather tentative and debatable, thus requiring data accumulation and analysis.

The term "clonal cytopenia of undetermined significance" can also be found in the scientific literature, which is characterized by the presence of somatic mutations in the gene(s) associated with MBD, with an allele burden of at least 2% (or 4% in men in the case of X-linked genes mutations); absence of criteria for MBD, other causes of cytopenia and molecular aberrations; persistent cytopenia in more than one hematopoietic growth (hemoglobin less than 100 g/L, neutrophils less than 1.8×10^9/L, platelets less than 100×10^9/L) for at least four months. The term "idiopathic cytopenia of undetermined significance" (idiopathic cytopenia of undetermined significance) is also proposed to be used in the presence of persistent cytopenia in more than one hematopoietic growth and in the absence of criteria for myeloid neoplasm and other blood system diseases [30].

Clonal hematopoiesis and oncohematological diseases

It has been proven that individuals with CH are more likely to show signs of genetic instability due to somatic mutations in epigenetic regulation genes (*DNMT3A, TET2, ASXL1*), RNA splicing genes (*SF3B1, SRSF2, ZRSR2*), signaling pathways and cell proliferation (*JAK2, FLT3*), genes related to metabolism and cell differentiation (*IDH1, IDH2*), and in DNA repair regulation genes (*TP53, PPM1D*). In comparison with the overall population, such individuals are also frequently diagnosed with cytopenia of unclear etiology. Mutations in *DNMT3A*, *TET2*, and *ASXL1* genes account for about 80% of CHIP cases [22, 23].

Mutations characteristic of myelodysplastic syndrome (MDS), myeloproliferative neoplasms, and acute myeloid leukemia are observed in *JAK2*, *PPM1D*, *TP53*, *SRSF2*, and *SF3B1* genes, being less frequent [26]. The frequency of detection of these mutations is much higher than the incidence of MBD; nevertheless, CH can be considered as an event preceding the development of hemoblastosis. Signs of CH are revealed in 50% of patients with aplastic anemia; CH-specific *DNMT3A* and *ASXL1* gene mutations are found in 15% of patients [31, 32]. Another example of CH with its transformation into a pathological process is paroxysmal nocturnal hemoglobinuria. In this disease, the clone arisen from HSCs with glycosyl-phosphatidylinositol deficiency is less susceptible to T-cell-mediated destruction in comparison with normal HSCs [30, 33].

In addition, CH with point mutations or structural variations, such as gene copy number changes, leads to a ten-fold increase in the risk of MBD. The role of antitumor immunity disorders in the transformation of CH into MBD requires further investigation [22]. At the same time, the presence of CHIP is associated with a 13-fold increase in the risk of hemoblastosis, with the frequency of its occurrence in 0.5–1% of patients per year [8].

In most cases, CHIP is benign, especially when the clone size is small and multiple, without driver mutations [20]. The main risk factors for the development of the disease (not only hematologic but also somatic) into CHIP include: a significant clone size (10% or more) and its growth acceleration, clonal changes in more than one cell line, multiple driver mutations, *TP53* gene mutations, and chromosomal aberrations [34].

Until recently, the accumulated information and expansion of detectable genetic mutations spectrum has not allowed differentiation of mutations that initiate the onset of CH from those that are an early event in the development of MBD, as well as to identification of second-order mutations that make the most significant contribution during the disease progression. Thus, the isolation of driver mutations, passenger mutations, and background and cooperating mutations has been proposed [20, 22]. The works published in 2024 indicate that methods have already been

developed to identify driver mutations, such as “a method for enriching nonsynonymous mutations over neutral synonymous mutations” or machine learning based algorithms [35, 36].

Thus, in accordance with modern concepts, mutations of genes associated with CHIP is a predisposing factor in MBD. Appearance of such mutations leads to transformation of normal HSC into “pre-leukemia” neoplastic HSC. Altered HSC gives rise to small subclones and, itself, does not practically differ from normal HSC. The frequency of mutation detection increases with age, being a natural biological process accompanying aging. However, “pre-leukemia” HSC has an increased risk for transformation into leukemia HSC at acquisition of additional molecular-genetic damages. Factors in such transformations require further study. Classification of genetic breakages according to their oncogenic potential is also an area of interest for current research. The CH model presented in Fig. 1 summarizes our ideas about driver, cooperating, passenger, and background mutations, as well as the causes of genetic instability that determine clonal evolution with the development of MBD.

Clonal hematopoiesis and somatic pathology

The development of CHIP is an independent risk factor concerning not only various hemoblastoses, but also cardiovascular diseases, Alzheimer’s disease, type 2 diabetes mellitus, thrombosis, autoimmune processes, and other pathologies [7]. The clonal expansion of HSC encounters no anatomical restrictions, with clone cells circulating in the bloodstream in large numbers [21]. Thus, CH is associated with an increased risk of developing both MBD (more often myeloid and less often lymphoid) and various somatic pathologies [21, 37].

A number of publications have convincingly demonstrated that CHIP correlates with the risk of acute leukemia, MDS, and other hemoblastoses, as well as with such adverse cardiovascular events, as acute cerebral circulatory disorder and acute myocardial infarction [38–40]. Two independent meta-analysis studies also showed the link of CHIP with a high risk of atherosclerosis and cardiovascular disease [40, 41].

The presence of CHIP contributes to the acceleration of atherosclerotic vascular lesions. This fact was confirmed in animal models, and similar conclusions were obtained for humans [41–43]. The mutations characteristic of CHIP accelerate the atherosclerotic process, which was noted even under a slight increase in their allele load [41]. An experimental model of CHIP in mice showed that an atherosclerotic lesion of the aorta was 60% more pronounced in the experimental animals compared to the control group [43]. Such modeling of CHIP showed its exceptional reproducibility in other animal experiments [43, 44]. Similar results were obtained when modeling CHIP associated with *JAK2V617F* mutation. The presence of this mutation in animals caused an increase in erythrophagocytosis in atherosclerotic plaques, which accelerated their destabilization [45]. In addition, this mutation was associated with a higher frequency of thrombosis in atherosclerotic plaques [45, 46].

Clonal hematopoiesis of indeterminate potential increases not only the risk of acute cardiovascular events, but also aggravates the course of heart failure (HF) [40, 45]. In a study by Sano S et al., mice were transplanted with 10% Tet2-/- BM cells, and the association of inactivated driver gene with high incidence of HF development was found [43]. A detailed transcriptomic analysis of heart tissues in animals demonstrated an increased expression of NLRP3-inflammasome-related genes, as well as IL1B gene. The use of NLRP3 inhibitor decreased the incidence of cardiac remodeling and the risk of developing HF in mice [44, 47, 48]. Thus, driver mutations of CHIP were demonstrated to intensify the proinflammatory response leading to the development of cardiovascular pathology [7, 45, 48].

At the same time, new data continues to emerge, stimulating further studies regarding the role of CHIP in the development of various variants of the course of type 2 diabetes [7], Alzheimer’s disease [7, 49], autoimmune diseases [7, 50], thrombosis [7, 46], and other pathologies. The decisive role in somatic pathology is attributed to the combination of effects of somatic mutations characteristic of CHIP with chronic inflammatory process, prolonged hyperactivation of the immune system, concomitant pathology and its therapy, lifestyle, as well as the impact of external factors [46].

Ionizing radiation as an inductor of genetic instability and clonal hematopoiesis

The action of IR leads to disruption of repair processes, interphase or mitotic reproductive cell death, and increasing deficit of differentiated cells with the formation of deterministic effects of irradiation. The latter consist in the direct damaging effect of IR on cells and tissues, manifesting themselves only after irradiation above the threshold dose with clinically significant consequences, both deterministic (tissue reactions) and stochastic. The degree of damage severity increases rapidly as the radiation dose accumulates. [51, 52].

When exposed to low-dose IR, stochastic effects develop. Low doses in this case are commonly understood as follows: a single equivalent dose of up to 0.1 Sv or 10 rem; an absorbed dose of up to 0.1 Gy or 10 rad; an effective equivalent dose of up to 0.1 Sv/year, which approximately corresponds to the exposure dose of 750 μR/h [1, 6]. In case of such exposure, when an insignificant damage to cells under the IR influence occurred but was completely eliminated by regenerative processes, the cells retain viability but acquire genetic mutations. The probability of such damage at a single exposure to low doses of II is minimal, although sharply increasing with increasing the effective equivalent dose and duration of exposure. Consequently, stochastic effects are characterized by the absence of a dose threshold and are assumed to occur with a probability linearly proportional to the influencing IR dose. Stochastic effects include the development of MBD and solid tumors, as well as hereditary pathologies [4, 6, 51].

Thus, the main reaction of the body to the chronic effect of low-dose IR is a disorder of genome stability and regulatory processes. In the setting of genetic instability, various reactions of the organism to the impact of external factors, up to death, are possible. An increase in the number of

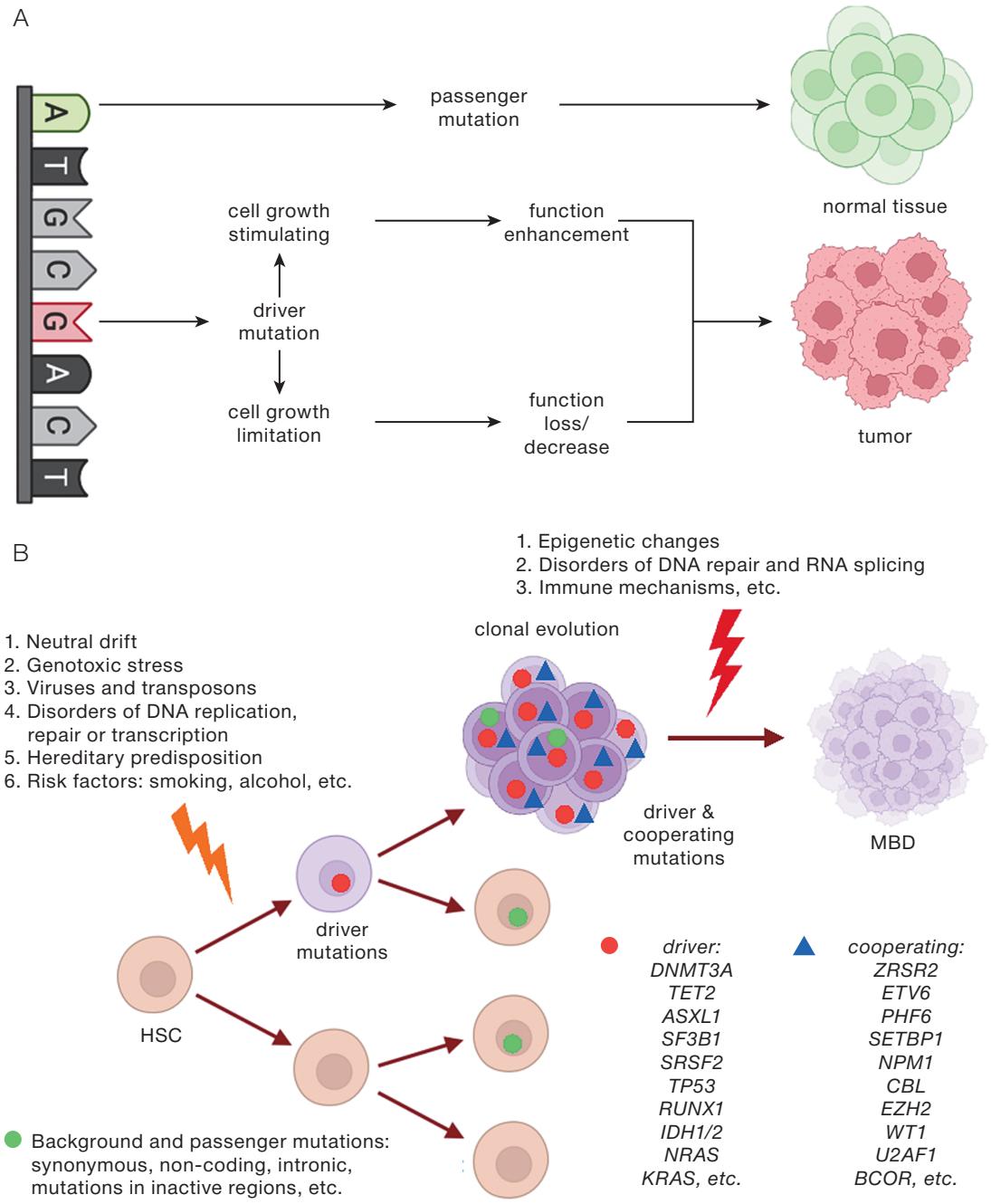

Figure prepared by the authors using data from [24]

Fig. 1. Model of clonal hematopoiesis: realization of passenger (A) and driver (B) mutations activity

chromosomal aberrations may precede the development of secondary immunodeficiency, premature aging, neoplasia, including MBD, as well as somatic pathologies. Low-dose IR is a stress factor for the organism, with the distant consequences of its long-term chronic exposure consisting in the depletion of compensatory capabilities of the organism [7, 52].

The number of publications on occupational exposure effects, including low-dose IR in a certain professional contingent (workers in the nuclear industry, radiologists, etc.) is currently growing. In this regard, a study by Jasra S et al published in 2022 seems noteworthy. The researchers analyzed the effects of chemical substances and dust particulate matter on 481 rescue workers involved in combating the disaster in the World Trade Center on September 11, 2001. A significant increase in the CH risk in these individuals was determined, with the most frequent mutations affecting the *DNMT3A* and *TET2* genes. The frequency of their detection increased with age in comparison with 255 employees from the comparison group [53].

Another large-scale study, conducted in 2019 among atomic bombing survivors without an MBD diagnosis, found the radiation influence to result in blood cell clonal expansion. This led to a long-term increase in circulating monocytes in the group of people older than 60 years [54]. Also in 2022, the results of a study analyzing the presence of CH driver genes mutations in NASA astronauts were published. The study identified 34 nonsynonymous

mutations in 17 driver genes, with the highest frequency of occurrence in the *TP53* and *DNMT3A* genes [55].

A recent research focus has been the occupational IR impact on oncologic and cardiovascular pathologies in employees of the nuclear industry and radiologists. Thus, a large meta-analysis found the absorbed IR dose, above which the mortality from circulatory system diseases in these categories of workers increases, to be equal to 0.5 Gy [56].

A meta-analysis study (data from 15 countries) on the total mortality and mortality from all malignant neoplasms for workers in the nuclear industry, as well as for workers in contact with the most toxic heavy metals and chemical compounds, revealed no obvious increase in mortality from all malignant neoplasms compared to the population [57]. In a meta-analysis of data on the risks of cardiovascular pathologies, the authors also noted no significant differences with the population [58]. However, in the context of the effect of low-dose IR on the CH in these categories of individuals, no information is currently presented in large-scale works on the identification of mutations associated with CH. The majority of domestic studies are centered around the characteristics of IR and a retrospective analysis of morbidity, as well as the survival of employees exposed to long-term low-dose IR. Consequently, the possibility of identifying mutations associated with CH is of greatest interest for current research.

CONCLUSION

To date, the association of CH with natural aging processes has been well established. The influence of CHIP on the increased risk of hemoblastosis, somatic pathology, and overall mortality has been documented. Genetic instability in the *DNMT3A*, *TET2*, *ASXL1*. *JAK2*, *TP53*, *PPM1D*, *SF3B1*, and *SRSF2* genes, which most often initiate CH in combination with chronic inflammation and increased immune activation, is associated with these diseases.

At the same time, there is a lack of comprehensive information on the risks of transition of clinically silent CHIP into disease. Timely screening aimed at identification of factors correlating with unfavorable outcomes can facilitate timely identification of people with an increased risk of pathology development.

Research into the influence of radiation-induced genetic instability on the formation of clonal expansion is relevant for health monitoring and preventive diagnosis of oncohematological and somatic pathologies in individuals with long-term low-dose anthropogenic exposure (nuclear industry workers and radiologists).

Further studies should elucidate the role of CH and CHOP in various pathologies, determining the possibilities of therapeutic effects aimed at preventing unfavorable course of the disease and increasing life expectancy.

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THREATS AND RISKS TO HEALTH IN EMERGENCY SITUATIONS: BIOMEDICAL, PREDICTIVE ANALYTICAL, AND MATHEMATICAL ASPECTS

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Introduction. The methodology for assessing health threats and risks is becoming increasingly in demand in the public management of the sanitary and epidemiological welfare of the population. New biomedical, predictive analytical, and mathematical approaches are being developed to assess and analyze health threats and risks in emergencies, including within biological risk monitoring. These issues require a scientifically based comprehensive consideration drawing on various scientific fields, including medicine, biology, management, prediction, sociology, and mathematics (probability theory, set theory, measure theory, etc.). To solve this problem, the authors adopted a convergent approach, paying special attention to the role of effective threat and risk management, which has a significant impact on the quality of life of people exposed to adverse factors in emergency situations.

Objective. To improve the technology for analyzing and predicting threats and risks to human health in emergencies using a convergent multidisciplinary approach.

Materials and methods. The authors searched electronic bibliographic databases in the Russian (eLibrary and CyberLeninka) and English (Web of Science, Scopus, PubMed, Google Scholar, and Cochrane Library) languages. The database "Regulatory Legal Acts on Radiation, Chemical, and Biological Monitoring" created at the Centre for Strategic Planning of the Federal Medical and Biological Agency served as the basis for analyzing regulatory documents. As the information platform in this study, the authors used the information system of the Federal Information and Analytical Center for Monitoring Biological Risks, which aggregates data on the monitoring of biological risks falling within the competence of the Federal Medical and Biological Agency, The predictive and analytical part of the study was scientifically justified using the database "Methods of Scientific Prediction" created at the Centre for Strategic Planning, which contains systematized methodological prognostic information. The theoretical methods used in the study include logical methods (analysis and synthesis of knowledge; analogy method), mathematical methods (modeling, probability theory, measure theory, graph theory, and set theory), and the method of theoretical generalization.

Results. In the study, the existing approaches to assessing health threats and risks arising in emergency situations are summarized and systematized; their main characteristics and key parameters are considered. The phases of the process involving the emergence of threats and risks to health and the specifics of their management are analyzed. The scientific medical and biological point of view on the essence and general characteristics of health threats and risks is presented. The predictive analytical and mathematical aspects of the problem under consideration are outlined. An example algorithm for predictive and analytical calculation of indicators characterizing the resource capability and readiness of the healthcare system to adequately respond to a biological threat is described in detail. The required bed capacity of medical organizations is assessed, as well as the need for artificial lung ventilation devices in case of an epidemic; the final values are calculated. The analysis of the specified issues using a comprehensive convergent approach creates the prerequisites for effective management of health threats and risks in emergency situations.

Conclusions. Predictive and analytical approaches are based on advanced ideas and mechanisms, including risk-based technologies, digital certification of territories and objects, active use of geoinformation developments, assessment procedures drawing on the combination of estimated and field data, situation modeling under changing or specified conditions, consideration of combined impact factors, etc. Characterizing the risk through a health hazard measure that combines the probability of health threats occurring in an emergency and the consequences of adverse effects for life and health, the authors define the value of risk as the mathematical expectation of the product of a function for assessing damage (consequences) to the health of an organism/population and the probability of combined impact of adverse factors in an emergency.

Keywords: health threats and risks; emergency situation; risk management; prediction of threats and risks; threat probability; epidemics; ALV; bed capacity

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УГРОЗЫ И РИСКИ ЗДОРОВЬЮ ПРИ ЧРЕЗВЫЧАЙНЫХ СИТУАЦИЯХ: МЕДИКО-БИОЛОГИЧЕСКИЕ, ПРОГНОЗНО-АНАЛИТИЧЕСКИЕ И МАТЕМАТИЧЕСКИЕ АСПЕКТЫ

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Введение. Методология оценки угроз и рисков здоровью становится все более востребованной в решении задач государственного управления санитарно-эпидемиологическим благополучием населения. Развиваются новые медико-биологические, прогнозно-аналитические и математические подходы к оценке и анализу угроз и рисков здоровью при чрезвычайных ситуациях (ЧС), в том числе в рамках мониторинга биологических рисков. Появляется необходимость научно обоснованного рассмотрения указанной проблематики, используя в едином комплексе знания из различных научных областей, включая медицину, биологию, управление, прогнозирование, социологию, математику (теорию вероятностей, теорию множеств, теорию меры и др.). Для решения этой задачи авторы в процессе исследования основывались на принципе конвергентного подхода, уделяя особое внимание роли эффективного управления угрозами и рисками, которое оказывает существенное влияние на качество жизни людей, попадающих под воздействие неблагоприятных факторов при ЧС.

Цель. Совершенствование технологии анализа и прогнозирования угроз и рисков здоровью человека при чрезвычайных ситуациях на основе конвергентного мультидисциплинарного подхода.

Материалы и методы. В качестве основы для анализа нормативно-правовых материалов использовалась созданная в ФГБУ «ЦСП» ФМБА России база данных «Нормативные правовые акты радиационного, химического и биологического мониторинга». Информационной платформой для исследовательской работы послужила информационная система Федерального информационно-аналитического центра мониторинга биологических рисков ФМБА России, агрегирующая данные мониторинга биологических рисков, относящихся к компетенции ФМБА России. Для научного обоснования прогнозно-аналитической части исследования использовалась созданная в ФГБУ «ЦСП» ФМБА России база данных «Методы научного прогнозирования», содержащая систематизированную методологическую прогностическую информацию. К методам теоретического уровня, использованным в исследовании, относятся логические методы (анализ и синтез знаний, метод аналогий), математические методы (моделирования, теории вероятностей, теории меры, графов, множеств) и метод теоретического обобщения.

Результаты. В ходе исследования обобщены и систематизированы существующие подходы к оценке угроз и рисков здоровью, возникающие при чрезвычайных ситуациях, рассмотрены их основные характеристики и ключевые параметры. Проанализированы фазы процесса, связанного с возникновением угроз и рисков здоровью, и особенности управления ими. Изложены прогнозно-аналитические и математические аспекты рассматриваемой проблематики на примере алгоритма прогнозно-аналитического расчета показателей, характеризующих ресурсную возможность и готовность системы здравоохранения к адекватному ответу на угрозу биологического характера. Произведен расчет потребности коечного фонда медицинских организаций и аппаратов искусственной вентиляции легких при эпидемии, вычислены конечные значения. Констатировано, что рассмотрение вышеперечисленных вопросов на основе комплексного конвергентного подхода формирует предпосылки для реализации эффективного управления угрозами и рисками здоровью при чрезвычайных ситуациях.

Выводы. Прогнозно-аналитические подходы базируются на передовых идеях и механизмах, включая риск-ориентированные технологии, цифровую паспортизацию территорий и объектов, активное использование геоинформационных разработок, методики оценки на базе сопряжения расчетных и натурных данных, ситуационное моделирование при изменяющихся или задаваемых условиях, учет факторов сочетанного воздействия и т. д. Характеризуя риск через меру опасности здоровью, сочетающую вероятность реализации угроз здоровью при ЧС и последствия поражающих воздействий для жизни и здоровья, авторы определяют значение риска как математическое ожидание произведения функции оценки ущерба (последствий) здоровью организма/населения и вероятности совокупного воздействия поражающих факторов ЧС.

Ключевые слова: угрозы и риски здоровью; чрезвычайная ситуация; управление рисками; прогнозирование угроз и рисков; вероятность угроз; эпидемии; аппарат ИВЛ; коечный фонд

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Финансирование: работа выполнена без спонсорской поддержки.

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INTRODUCTION

The methodology for assessing health threats and risks is currently becoming increasingly in demand in the public management of sanitary and epidemiological welfare of the population [4]. The relevance and significance of this issue can be primarily attributed to the fact that the health of the Russian population is considered to be the main value of the country and one of the most important national security criteria. This makes it important to study the essence of threats and risks to human health.

Within the general methodology, new biomedical, predictive analytical, and mathematical approaches are being developed both in the system for biological risk monitoring of the Federal Medical and Biological Agency (FMBA) of Russia and in the state system for socio-hygienic monitoring to assess and analyze health threats and risks in emergencies. These issues require a scientifically based comprehensive consideration drawing on various scientific fields, including medicine, biology, management, prediction, sociology, and mathematics (probability theory, set theory, measure theory, etc.). To solve this problem, the authors used a convergent approach, paying special attention to the role of effective threat and risk management, which has a significant impact on the quality of life of people exposed to adverse factors in emergency situations.

The present work aims to improve the technology for analyzing and predicting threats and risks to human health in emergency situations using a convergent multidisciplinary approach.

MATERIALS AND METHODS

The database “Regulatory Legal Acts on Radiation, Chemical, and Biological Monitoring” created at the Centre for Strategic Planning (FMBA) served as a basis for analyzing regulatory documents [7]. As the information platform in this study, we used the information system of the Federal Information and Analytical Center for Monitoring Biological Risks, which aggregates data on the monitoring of biological risks falling within the FMBA competence. The predictive and analytical part of the study was scientifically justified using the database “Methods of Scientific Prediction” created at the Centre for Strategic Planning (FMBA), which contains systematized prognostic methodological information [8].

The theoretical methods used in the study include logical methods (analysis and synthesis of knowledge; analogy method), mathematical methods (modeling, probability theory, measure theory, graph theory, and set theory), and the method of theoretical generalization.

RESULTS

Primary source and evolution of the health risk concept

The primary source of the risk concept is considered to be the medieval work "The Salerno Code of Health" by the alchemist Arnold de Villa Nova (Arnoldus de Villa Nova, 1235–1311) from the Salerno Medical School near Naples, which is claimed to be the oldest medical school in Europe. In his work, the scientist presented data on various factors leading to diseases, considering, among other things, their combined effect, thus laying the foundation for a systematic approach to diseases [17].

Subsequently, the understanding of the essence and assessment of health risk factors underwent repeated evolutionary changes resulting from the adoption of various foreign (USA and Europe) and Russian approaches (in the late 20th century and at the dawn of the 21st century). Empirical research has long used attributive risk assessment to determine what part of the current disease burden is due to the accumulated effect of all previous exposures [1].

It is currently relevant to address issues related to the probability and severity of biomedical consequences that occur following the emergence of physical, chemical, and biological factors and exposure to them.

The article considers physical, chemical, and biological threats and risks to the health of citizens in natural or man-made emergencies. The concept of an emergency situation is defined in GOST R 22.0.02-2016 as a situation in a territory that has developed as a result of an accident, a dangerous natural phenomenon, a catastrophe, a natural or other disaster that may or have led to human casualties, damage to human health or the environment, significant material losses, and disruption of people's livelihoods. The risk of an emergency is defined as a measure of danger in an emergency situation, combining the probability of an emergency and its consequences [3].

Definitions of threats and risks: analysis of modern legislation of the Russian Federation

We analyzed the modern legislation of the Russian Federation, including the main normative legal acts and standards defining the concepts of threats and risks. The following documents were identified from the list: the Law of the Russian Federation No. 2446-1 as of 03/05/1992 "On Security"; Federal Law No. 492 as of 12/30/2020 "On Biological Safety in the Russian Federation"; Decree of the President of the Russian Federation No. 97 as of 03/11/2019 "On the Fundamentals of State Policy of the Russian Federation in the Field of Chemical and Biological Safety for the Period up to 2025 and Beyond"; "Guidelines for Assessing the Risk to Public Health when Exposed to Chemicals Polluting the Environment" (R 2.1.10.1920-04); Federal Law No. 184 as of 12/27/2002 "On Technical Regulation"; "Guidelines for Assessing the Risk to Public Health when Exposed to Chemicals Polluting the Environment" (R 2.1.10.3968-23); Federal Law No. 7 as of 01/10/2002 "On Environmental Protection"; GOST R 22.0.02-2016; GOST R 70620-2022; GOST ISO 12100-2013, etc.

The conducted study shows that the conceptual framework related to the categories of "threats and risks" in the Russian legislation can be found in a single information space; exhibits equally structured logical semantic (essential) relationships between these concepts, which represent the risks of consequences depending on the types and nature of threats; exhibits no contradictions; differs logically depending on the application areas.

Here are some definitions. In general, the "safety threat" is defined in the Law of the Russian Federation "On Security" as a set of conditions and factors that pose a danger to the vital interests of the individual, society, and the state [5].

In the Federal Law "On Biological Safety," a biological threat (danger) is the presence of potentially dangerous biological objects, as well as the presence of internal (located on the territory of the Russian Federation) and external (located outside its territory) dangerous biological factors, that can lead to the emergence and (or) spread of diseases with the development of epidemics, epizootics, epiphytotics, and mass poisoning, exceeding the permissible level of biological risk. Biological risk is defined as the probability of harm (taking into account its severity) to human health, animals, plants, and (or) the environment as a result of exposure to dangerous biological factors [16].

In R 2.1.10.3968-23, danger is a set of properties of environmental factors (or a specific situation) that determine the ability to cause adverse health effects under certain exposure conditions. In this case, risk is considered a characteristic of danger depending on the level of exposure to a chemical factor and the specifics of its actual or potential effects under specific conditions. Risk is the probability of harm to the life and health of citizens, property of individuals and legal entities, state or municipal property, as well as habitat, life, or health of animals and plants, taking into account the severity of this harm. Health risk is the probability of harm to human life and health or a threat to the life or health of future generations, taking into account the severity of this harm, due to the impact of environmental factors [14]. In other words, health risk is defined as a combination (product) of the damage probability and the severity of this damage [9].

R 2.1.10.1920-04 clarifies that the risk, unlike danger, is the result of actual or potential exposure to a chemical compound and depends on exposure and the specifics of particular exposure conditions [13].

World Health Organization (WHO): health hazards and risks

In the WHO practical guidelines for biological safety under laboratory conditions, a dangerous factor is defined as an object or situation that can lead to negative consequences when an organism, system, or group (subgroup) of the population is affected by it. The concept of risk is defined as a combination of the incident probability and the severity of harm (consequences) if this incident occurs. It is emphasized that a dangerous factor does not become a "risk" until the probability and consequences

of this dangerous factor causing harm are taken into account [19].

The fourth edition of WHO guidelines presents the results of research on how the likelihood and consequences of danger affect health risk [11]. For example, the likelihood of exposure to cigarette smoke, which is a common hazard, depends on the situation. The impact will be greatest for a smoker, moderate for passive smokers, and smallest for a person using respiratory protection or staying in smoke-free areas. The effects of exposure to cigarette smoke can vary from mild nausea and irritation of the respiratory tract to various heart and lung diseases, cancer, and even a fatal outcome, depending on cigarette toxicity, the frequency and duration of exposure, as well as other factors related to human sensitivity.

Phases of threats and risks to health in emergencies

The study logic required the identification of three main phases of the process involving the emergence of health threats and risks and their management; the relevant data are presented in Figure 1.

The phase involving the emergence of the threat and potential risks (I) includes the following stages:

1) occurrence and development of emergency situations (as a source of threats);

2) occurrence of adverse factors of physical (including radiation), chemical, and biological nature and their manifestations;

3) existence of circumstances and creation of conditions under which the contact of a dangerous chemical, physical, or biological agent with the human body is possible.

The phase of threat realization and occurrence of real risks (II) is characterized by the adverse effects of physical, chemical, and biological factors on the body. The conceptual apparatus of this phase (for example, with chemical exposure) includes “exposure, dose/concentration, effect.”

The phase of managing real risks and eliminating the consequences of an emergency (III) involves the provision of medical and sanitary assistance, which is intended to prevent and eliminate damage to health. For example, when the body is exposed to chemicals, the conceptual apparatus of the phase includes “response, disease, and outcome.”

Biomedical view of physical, chemical, and biological threats and risks in emergencies

Depending on the type of emergency, the phases have clear or blurred boundaries since the emergent adverse effect may be of a short-term (sometimes instantaneous) or prolonged temporary nature. Short-term effects occur, for example, when lightning strikes a group of people standing under a tree (lightning discharges of natural origin). Adverse factors can manifest themselves in various forms: electromagnetic pulses, light radiation, high-temperature effects, and shock waves. All systems of the body can be affected: musculoskeletal, respiratory, cardiovascular, genitourinary, endocrine, nervous, sensory, visual, etc. Aside from vision loss, seizures, paralysis, stroke, and heart attack, this can sometimes cause chronic headaches and memory problems.

It is generally believed that natural emergencies are hard to predict. However, it is possible to predict the occurrence of adverse factors and take preventive measures against them, thus reducing the likelihood of threats and risks, as well as the level of their impact on health, that is, to manage health threats and risks.

For example, the typical natural emergencies of Yakutia are spring and summer floods. The flood changes the structure and functional connections of natural foci and leads to a wide spread of pathogens of bacterial, viral, and rickettsiosis infections, thus significantly increasing the intensity of contacts between the population and natural foci. During a flood, the risk of infectious diseases (viral hepatitis A, dysentery, and typhoid fever) rises. In flooded areas, water supply is disrupted, and the risk increases of the river being polluted from sewage, cattle burial grounds, and cesspools; from warehouses by pesticides and petroleum products, etc. As a result, the probability of epizootics rises, and the risk of people contracting infectious and parasitic diseases increases (leptospirosis, tularemia, hemorrhagic fever with renal syndrome, yersiniosis, pseudotuberculosis, toxoplasmosis, etc.). The burden on infectious hospitals grows. Due to overcrowding, the airborne transmission of

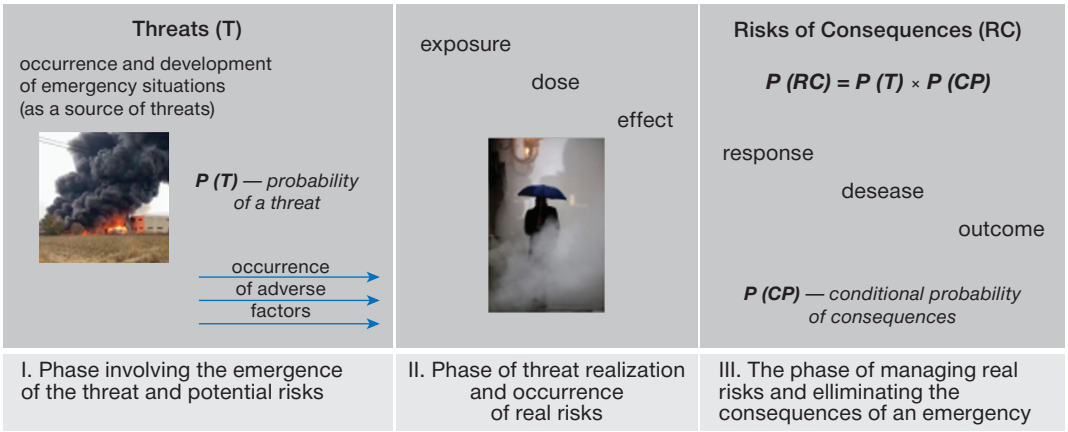

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Fig. 1. Phases of threats and risks in case of emergency

ARVI (acute respiratory virus infection) and other pathogens increases.

Yakut specialists analyzed factors associated with the maximum water levels during the spring flood; the combination of these factors often leads to catastrophic floods. In order to make effective decisions aimed at minimizing risks, a mathematical solution using Bayesian networks was proposed. In particular, the Bayesian network was used to analyze the probability of certain conditions with various combinations of selected factors and investment amounts in the form of preventive measures [15].

All emergencies that result in chemical pollution are associated with long-term exposure: for example, industrial waste incineration at a landfill, accompanied by emissions into the atmosphere and the spread of toxic combustion products toward the residential area. Threat prevention can be accomplished through proactive measures: from landfill remediation to the evacuation of population from the predicted spread area. According to experts, despite the extensive regulatory framework developed in the Russian Federation for the permissible content of chemicals in the atmospheric air (1300 maximum permissible concentrations and 450 approximately safe impact levels), most examined sources of pollutants entering the atmosphere are not currently regulated by legally approved hygienic standards [12]. This fact complicates the early and short-term planning and management of health measures to eliminate the consequences; in other words, risk planning and management.

Recent years have seen an increase in the number of diseases associated with new pathogenic viruses, with the exacerbation of diseases caused by them and the involvement of new regions previously untouched by these diseases. Experience shows that infectious diseases caused by new strains of viruses, which had high virulence and the potential for multiple mutations, had a rather severe course in the early stages, with a high mortality rate per the total number of infected people, and were also difficult to treat with the use of chemotherapy drugs [2]. About 20% of patients needed artificial lung ventilation. During this period, planning and risk management were objectively insufficient, which affected the epidemiology of morbidity [10]. According to the authors, the lack of immunization among doctors and nursing staff had a negative impact on the situation since a sharp reduction in the number of available qualified staff increased the risk of a severe course and outcome of diseases in patients. The most popular ways to prevent threats caused by epidemics and pandemics are scientifically based sanitary and anti-epidemic (preventive) measures: preventive vaccination, emergency prevention, disinfection, and restrictive measures.

Scientific and practical assessment of health threats and risks and their main characteristics

Health risk assessment constitutes one of the components of risk analysis, which includes risk assessment, risk management, and risk communication. From the scientific point of view, risk assessment is a consistent systematic consideration of all aspects of the impact that the analyzed factor has on human health, including the justification of acceptable exposure levels. In scientific and practical applications, the rationale for the risk assessment task consists in obtaining and summarizing information about the possible environmental health impacts; in the hygienic justification of the optimal management decision to eliminate and mitigate risk; in optimizing the control of exposure levels and risks [14].

Proceeding from the above, the threat to health in an emergency is defined as a set of phenomena, processes, and factors that contribute (in the context of the occurrence and development of natural or man-made emergencies) to the emergence of adverse effects of harmful physical, chemical, and biological factors on the body. The health risk in an emergency is also characterized as a measure of danger to health, combining both the likelihood of health threats in an emergency and the consequences of adverse effects for human life and health and future generations.

Predictive and analytical aspects

The basis of information and analytical support for the development and implementation of management decisions consists in the monitoring of physical, chemical, and biological risks, as well as social and hygienic monitoring.

The stages and components of analytical and practical monitoring are as follows: data collection, identification of threats and risks, their verification and analysis, situation modeling, prediction of how the situation is going to develop in the present and future periods, and development of appropriate solutions. The analysis and prediction results serve as a reliable basis for developing medical, sanitary-epidemiological, hygienic, socioeconomic, organizational, and technical measures for effective management aimed at eliminating and localizing threats and risks of emergencies.

In the modern theory and practice of prediction, a significant number of different methods are available [8], as well as approaches to their application. These approaches are not limited to using a single method. It is common to use a combination of various prediction methods: for example, information and computer modeling in combination with probabilistic and statistical methods, etc. The combined approach in prediction should be considered the most promising. To increase the reliability and accuracy of predictions, a scheme is used to compare the results of various prediction methods that validate and complement each other or demonstrate any discrepancies in the obtained predictive estimates for their correction.

Predictive and analytical approaches are currently based on advanced ideas and mechanisms, including risk-based technologies, digital certification of territories and objects, active use of geoinformation developments, assessment procedures drawing on the combination of estimated and field data, situation modeling under changing or specified conditions, consideration of combined impact factors, etc. [20].

Mathematical aspects of assessing and predicting health threats and risks in emergencies

Characterizing the risk through a measure of health hazard that combines the probability of health threats occurring in an emergency and the consequences of adverse effects for life and health, we define the risk value as the mathematical expectation of the product of a function for assessing damage (consequences) to the health of an organism/population and the probability of combined impact of adverse factors in an emergency (Fig. 1).

$$R(x) = \int F(x) \times P(x)\, dx, \quad (1)$$

Note: R — risk;
x — adverse effects;
$R(x)$ — integral risk measure;
$F(x)$ — function for assessing damage (consequences) to health with exposure to an adverse factor;
$P(x)$ — the probability of an adverse effect occurring in an emergency.

If a probabilistic approach and, accordingly, a probabilistic function are used to determine the measure, then the laws of probability theory and mathematical statistics should be applied in the calculation method. In this case, the function $F(x)$ is probabilistic in character. The resulting integral function $R(x)$ is also probabilistic, and its values $R(x)$ are always less than or equal to one.

Let us denote the threat by T; the probability of the threat (more precisely, the occurrence probability of an adverse effect) is P(T). The risk of health consequences is denoted by CR. Then, the probability of a consequence risk — P(CR) — will be expressed through the product formula:

$$P(CR) = P(T) \times P(CP), \quad (2)$$

where P(CP) — conditional probability of consequences (given the occurrence of a probabilistic event, i.e., the impact of an adverse factor).

It follows from Eq. (2) that the lower the probability of a threat, the lower the risk. In the absence of a threat, the risk is zero. Similarly, the lower the conditional probability of consequences that an adverse effect can have, the lower the risk of these consequences. In the absence of consequences, there is no risk.

The additional probability of a disease associated with the combined effect of climatic and chemical factors is calculated from the modeling of cause-and-effect relationships using multiple regression analysis. The construction of mathematical models uses data on morbidity in the context of classes of diseases or nosologic forms, which are affected by both climatic factors and chemicals, with the latter, in turn, being influenced by climatic factors [9, 18].

Hygienic approaches and calculations distinguish between a priori (predictive) risk based on dose-effective hygienic-normalized effects and a posteriori (real) risk based on a statistical assessment of actual events.

An example algorithm for predictive and analytical calculation of indicators characterizing the resource capacity of the healthcare system to adequately respond to a biological threat

Let us consider a specific example and calculate the final values. Problem statement: in the city of Z with a population of ten thousand people, during the period (T) 1000 people contracted an infectious disease “X” during an epidemic, 200 people were hospitalized, and 20 people were placed on a ventilator due to the severe course of the disease — (E). During the same period, T depends on the diseases characteristic of this city and time of year: 100 patients were reported (N), ten people were admitted to hospital (C), and two patients required a ventilator (F). It is necessary to perform a predictive calculation of the hospital bed capacity and artificial lung ventilation (ALV) units for the city R with a population of twelve thousand people and the impending epidemic of “X” for the same period (T) if it is known that socioeconomic and sanitary characteristics in cities are similar. All events that involve contracting different diseases are incompatible.

To solve this problem, we will construct a predictive graph, a tree of elementary events, using a group of logical and a class of formalized prediction methods [6]. First, we will calculate an a posteriori estimate for the city of Z using a graph in which elementary events are represented by the vertices of chains running from the original vertex Z to the final vertices. The corresponding data are shown in Figure 2.

The probability of the disease spreading through the city of Z during an epidemic is calculated as follows: P_{ZM} = M/Z = 1000/10,000 = 0.1. By analogy: the probability of contracting a characteristic nosologic disease is P_{ZN} = 0.01; the probability of hospitalization during an epidemic is P_{MA} = 0.2; the probability of hospitalization with characteristic diseases is P_{NC} = 0.1; the probability of a medical organization using ALV during an epidemic is P_{AE} = 0.1; the probability of ALV use in patients with characteristic diseases is P_{CF} = 0.2.

Let us transform the event tree into a probability graph, where the edges of the tree are the probabilities of chain events (Fig. 3).

In order to find the probability of an elementary event, that is, a chain, it is necessary to multiply the conditional probabilities along this chain. So, the probability of hospitalization due to an infectious disease “X” is equal to $P_{ZMA} = P_{ZM} \times P_{MA} = 0.1 \times 0.2 = 0.02$, where P_{ZM} is the probability of disease caused by an epidemic; P_{MA} is the conditional probability of hospitalization due to the disease “X.”

The probability of hospitalization in the city of Z is equal to the total probability of elementary events: $P_{HOSP} = P_{ZMA} + P_{ZNC} = P_{ZM} \times P_{MA} + P_{ZN} \times P_{NC} = 0.021$. By analogy, the probability of ALV use in the city of Z amounts to $R_{ALV} = P_{ZMAE} + P_{ZNCF} = 0.0022$.

For the city of R with a population of twelve thousand people and the impending epidemic of “X,” the predictive a priori calculation of hospital bed capacity and ventilators for the period of T is performed as follows:

- Hospital bed capacity: $R \times P_{HOSP} = 12000 \times 0.021 = 252$.

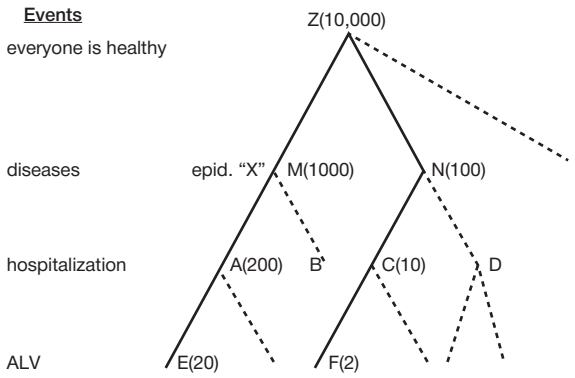

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Fig. 2. Graph showing the tree of elementary events
Note: the number in parentheses indicates the number of people involved in elementary events: those who are ill, hospitalized, and placed on ALV; the dashed line represents a transition to alternative and/or insignificant events to be considered.

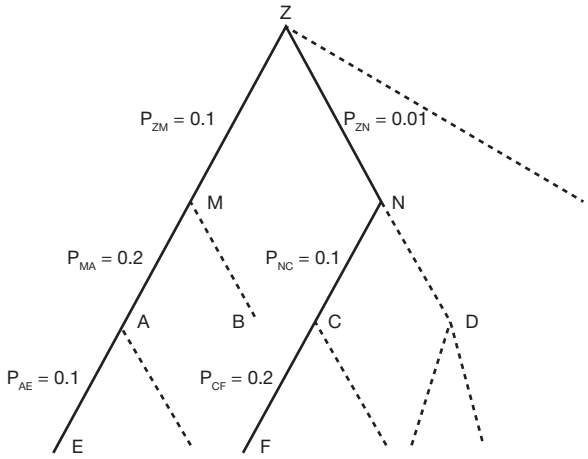

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Fig. 3. Probability tree graph
Note: the dashed line represents a transition to alternative and/or insignificant events to be considered.

- Reserve of ALV devices: $R \times P_{ALV} = 12000 \times 0.0022 =$ $= 26.4$; that is, at least 27 devices.

Another prediction method acceptable for calculations under epidemic conditions is modeling using compartment models (in particular SIR models) that describe the spread of the disease and divide the population into groups called compartments. SIR (Susceptible–Infected–Recovered) models are based on a system of differential equations that express the dynamics between different epidemiological conditions of the population, with recovery providing relatively long-term resistance.

CONCLUSION

The analysis of the specified issues using a comprehensive approach creates favorable prerequisites for effective management of health threats and risks in emergency situations. In the course of the study, we drew on various scientific fields adopting a convergent approach. The implementation of this principle will provide a means to develop measures aimed at reducing and eliminating threats and risks to health in order to ensure the sanitary and epidemiological welfare of the Russian population and its future generations.

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SUBSTANCE P AND STRESS ARE ASSOCIATED WITH THE DEVELOPMENT OF CHRONIC URTICARIA

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Introduction. Allergic diseases are a pressing challenge in practical healthcare, attracting increased attention of various medical specialists. The pathogenesis of stress-induced urticaria is driven by neurogenic immune inflammation, accompanied by an increase in the level of neuropeptide substance P (SP).

Objective. Assessment of the relationship between stress factors and substance P levels with the purpose of justifying the use of SP as a biomarker for assessing the clinical course and prognosis of the disease in patients with chronic urticaria.

Materials and methods. The study was involved 165 adults aged 18–68 years. The main group included 97 patients with the confirmed diagnosis of chronic urticaria (CU) who were treated in a hospital setting in the period from 2018 to 2023. The comparison group included 68 practically healthy individuals, comparable in gender and age with the study group of patients. The level of substance P in the blood serum was estimated by immunoenzymatic techniques (Infinite F50 Tecan, Austria), using a CEA393Hu test system. Statistical processing of the results was performed using the STATA 18 software package (StataCorp LLC).

Results. An increase in the production of substance P to 220.62 pg/mL in CU patients, compared to 96.57 pg/mL in the reference group ($p < 0.001$), was observed. The logistic regression revealed an association between stress and substance P levels in CU patients. Thus, an increase in the concentration of substance P by 1 pg/mL led to a 1.02-fold increase in the CU risk. The CU risk increased by 3 times in the presence of a stress situation as a trigger.

Conclusions. The constructed multivariant logistic regression model produced positive values of the model parameters ($p \leq 0.01$). This indicates the correlation between the increased blood levels of substance P under the impact of stress factors and the risk of chronic urticaria development. The data obtained suggests that the concentration of substance P in the blood of CU patients can be considered as a potential diagnostic biomarker. This biomarker can be recommended for extending panel screening tests to clarify the pathogenesis of the disease, thus improving the differential diagnosis of the disease and facilitating early detection of patients with stress-induced urticaria.

Keywords: substance P; stress; chronic urticaria; mast cell

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СУБСТАНЦИЯ Р И СТРЕСС АССОЦИИРОВАНЫ С РАЗВИТИЕМ ХРОНИЧЕСКОЙ КРАПИВНИЦЫ

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Введение. Аллергические заболевания являются актуальной проблемой практического здравоохранения и в последнее десятилетие привлекают все более пристальное внимание врачей различных специальностей. Ведущим звеном в патогенезе стресс-индуцированной крапивницы является нейрогенное иммунное воспаление, сопровождающееся повышением уровня нейропептида субстанции Р (SP).

Цель. Анализ взаимосвязи между стрессорным фактором и уровнем субстанции Р с последующим обоснованием показателя в качестве биомаркера для оценки клинического течения и прогноза заболевания у пациентов с хронической крапивницей.

Материалы и методы. Исследование проведено с участием 165 взрослых в возрасте от 18 до 68 лет. В основную группу были включены 97 пациентов с подтвержденным диагнозом хронической крапивницы (ХК), проходивших лечение в клинике в период с 2018 по 2023 г. Группу сравнения составили 68 практически здоровых лиц, сопоставимых по полу и возрасту с исследуемой группой пациентов. Уровень субстанции Р в сыворотке крови определяли методом иммуноферментного анализа (Infinite F50 Tecan, Австрия) с использованием тест-системы CEA393Hu. Статистическую обработку результатов осуществляли с использованием программного комплекса STATA 18 (StataCorp LLC).

Результаты. Отмечалось повышение продукции субстанции Р у пациентов с ХК 220,62 пг/мл по отношению к группе сравнения — 96,57 пг/мл, $p < 0,001$. При анализе логистической регрессии выявлена ассоциация между стрессом, уровнем субстанции Р у пациентов с ХК и установлено, что при увеличении концентрации субстанции Р на 1 пг/мл шанс возникновения ХК увеличивался в 1,02 раза, при наличии стрессовой ситуации в качестве триггера риск развития ХК повышался в 3 раза.

Выводы. С помощью построения мультивариантной модели логистической регрессии получены положительные значения параметров модели (с уровнем значимости $p \leq 0,01$), указывающие на то, что именно воздействие стресс-фактора и повышение концентрации субстанции Р в крови ассоциировано с увеличением шанса возникновения хронической крапивницы. На основании полученных данных концентрация субстанции Р в крови пациентов с ХК может рассматриваться в качестве потенциального диагностического биомаркера, который можно рекомендовать для расширения панели скрининговых тестов, уточняющих патогенез возникновения заболевания, что позволит улучшить дифференциальную диагностику нозологии и обеспечить раннее выявление пациентов со стресс-индуцированной крапивницей.

Ключевые слова: субстанция Р; стресс; хроническая крапивница; тучная клетка

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INTRODUCTION

Allergic diseases as an urgent problem of practical healthcare have been attracting increased attention of physicians of various specialties. At present, the interrelation of immunologic and neurogenic links is considered as the leading mechanism in the formation of inflammatory response in allergic diseases.

Urticaria is a serious problem in modern allergology, ranking third after allergic rhinitis and bronchial asthma in terms of incidence [1]. Stress is a umbrella term denoting a number of physiological reactions of the organism in response to adverse environmental factors [2]. Such a response encompasses three interrelated concepts: stimuli (both internal and external) that cause stress; physiological and behavioral reactions activated in response to these stimuli; and pathological consequences of excessive stimulation. Psychogenic factors act as a link in the sequence of immunologic events and lead to disease exacerbation, being in close connection with the main factors of pathogenesis [3].

At present, immunologists adhere to the concept of neurogenic inflammation as the main link in the formation and exacerbation of chronic urticaria (CU), which is caused by excessive release of hypothalamic neuropeptides conjugated to skin neuropeptides produced in keratinocytes, endothelial cells, etc. Most researchers agree that mental factors may potentiate the course of allergic diseases. Some authors also emphasize the possibility of urticaria aggravation by psychoemotional stress under the influence of negative emotions [4]. In this case, activation of cortical areas due to stress leads to changes in the production of substance P (SP) by the adrenal glands and descending autonomic fibers [5].

Recent studies confirm the importance of stress in the development of CU [6, 7]. A high incidence of post-traumatic stress disorders under the action of anxiety among patients with this nosology was established [8, 9]. A significant proportion of CU patients have a history of stressful situations long before the onset of symptoms and signs of this disease [10]. Psychogenic factors stimulate the hypothalamus-pituitary axis, associated with activation of autonomic nervous system centers and release of neurotransmitters (neuropeptides and hormones) affecting the effector systems of the body (immune, cardiovascular, and skin) [5, 11]. Chronic stressors lead to an increase in the density of cutaneous nerve fibers, increased production of mast cells, nerve growth factor (NGF), and certain neuropeptides [12]. Wang et al. [13] demonstrated the activation of mast cells (MCs) of both brain and skin under exposure to stress factors in animal experiments.

Mast cells express a variety of receptors that allow them to recognize and respond to a wide range of infectious pathogens and endogenous molecules produced by damaged tissues. For example, the high-affinity immunoglobulin E receptor (FcεRI) and the tyrosine kinase receptor (KIT) are expressed on MCs, which play an important role in the development of allergic reactions and in the immune response during worm invasion [14]. At the same time, mast cells express microbial pattern recognition receptors, i.e., the Toll-like receptor (TLR) and the NOD-like receptor [14]. MCs participate in Th2-type immune inflammation through alarmin receptors of the interleukin family, such as for IL-33 and receptor for thymic stromal lymphopoietin (TSLP) [15]. Mas-related G-protein coupled receptor X2 (MRGPRX2) recognizes neuropeptides, antimicrobial peptides, and insect venom peptides. In addition, these receptors are also present on other effector cells of the immune system, such as basophils and eosinophils [16].

Membrane protein of the adhesion G protein-coupled receptor E2 (ADGRE2) group is a mechanical sensor in mast cells. It induces degranulation in response to vibration exposure in people suffering from vibration urticaria, being a signal of helminth penetration and migration through the skin [17]. Thus, mast cells play an important role in human defense against helminths, bacteria, as well as animal and insect venoms. The release of corticotropin-releasing factor from eosinophils and sensory neurons (under stress) leads to MC degranulation via corticotropin-releasing hormone receptors [12].

The development or relapse of urticaria is frequently caused by stress factors. The leading link in its pathogenesis is neurogenic immune inflammation, accompanied by an increase in the level of neuropeptide substance P (SP) [12]. SP is a neuropeptide of the peripheral endings of sensitive C fibers of the skin, having a wide range of direct and indirect biological effects leading to pathophysiologic responses such as edema, vasodilation, and pruritus [17]. SP is expressed in the central and peripheral nervous system and immune cells. It is a neuropeptide whose biological activity is manifested through two receptors on the cell membrane — neurokinin G-protein coupled receptor (NKR) and MRGPRX2. It is known that MCs in patients with chronic urticaria express MRGPRX2 in greater amounts [18], and the neurotransmitter SP is the most informative diagnostic marker in patients with chronic urticaria [19]. NKR activation

mediates neural signaling pathways associated with the occurrence of sensations and various emotional responses.

SP plays an important role in the feeling of pain due to its function of transmitting information about tissue damage from peripheral receptors to the central nervous system, where it is converted into pain sensations. While pain signals are transmitted along the axons of the somatosensory region of the brain, sensory neurons also release SP in the area of damaged tissue [20]. This subsequently leads to mast cell degranulation, vascular dilation due to relaxation of vascular smooth muscle, and chemotaxis of immune system cells. This interaction between the immune and nervous systems is referred to as neurogenic inflammation [21]. In addition, SP was shown to increase the expression of endothelial and leukocyte adhesion molecules on microvascular endothelial cells, leading to leukocyte diapedesis [22]. There are examples of synergistic action of various mast cell triggers. Thus, Tarakanova et al. showed that SP increases the expression of IL-33 ST2 receptor, and IL-33 increases the expression of NKR on human mast cells [23]. This leads to an increase in the secretion of IL-1β, which is a key proinflammatory cytokine.

Despite the achieved progress, the mechanism of neurogenic inflammation in CU remains to be elucidated. This link of pathogenesis continues to be underestimated in the modern clinical protocols for the management of CU patients.

In this research, we set out to analyze the relationship between stress factors and substance P levels in order to justify the possibility of using SP as a biomarker for assessing the clinical course and prognosis of the disease in CU patients.

MATERIALS AND METHODS

The study was carried out using the facilities of the Nikiforov Russian Center of Emergency and Radiation Medicine. The study involved 165 adults aged 18–68 years, with 89 men and 76 women. The main group included 97 patients (45 men and 52 women) with a confirmed diagnosis of chronic urticaria, treated in a hospital setting in the 2018–2023 period. The comparison group consisted of 68 practically healthy individuals, comparable in gender and age with the main group, without urticaria symptoms and other allergic/somatic pathology [24].

The criterion for inclusion of patients in the main group was the presence of recurrent urticarial rashes and/or angioedema for more than six weeks. The diagnosis of chronic urticaria was established in accordance with the Federal Clinical Guidelines for the Diagnosis and Treatment of Urticaria [25].

In the course of the study, the patients' medical and life history was collected. The questions that formed the basis for the formation of a group of risk factors for the development or exacerbation of chronic urticaria were clarified during the interview. Special attention was paid to stress factors as potential triggers for the onset or exacerbation of CU.

The serum level of SP was determined in patients with chronic urticaria and in the comparison group by enzyme immunoassay (Infinite F50 Tecan, Austria) using a CEA393Hu test system (Cloud-Clone Corp., China). Blood samples for serum preparation were collected into a vacutainer with a clotting activator and centrifuged at 3000 rpm for 10 min. Serum samples were stored in Eppendorf type tubes at –80 °C. All kit components and serum samples were kept at room temperature +22 °C prior to testing.

Statistical processing of data was performed using the STATA 18 software package (StataCorp LLC). Data samples were compared using the Mann–Whitney U-criterion. The critical level of significance was taken as $p \leq 0.05$.

RESULTS AND DISCUSSION

An examination of the CU patients revealed all of them to be in the stage of disease exacerbation at the time of the study. Both study groups were comparable in terms of descriptive characteristics (gender and age).

In the course of the study, statistically significant differences in SP serum levels of patients with CU were obtained. Thus, the SP level in CU patients was more than 56% higher than in the comparison group. The descriptive characteristics of the patients and SP neuropeptide concentrations are presented in Table 1.

In order to independently assess biomarkers and the probability of developing chronic urticaria, a multivariate regression model with direct sequential inclusion of statistically significant variables of prognostic factors (SP serum concentrations, presence of a stressful situation, age, and gender) was constructed. The characterization of multiple logistic regression is presented in Table 2. The positive values of model parameters (with a significance level of $p \leq 0.01$) indicate that exposure to a stressor and an increased concentration of SP in the blood are associated with an increased risk of chronic urticaria. The influence of other factors was not significant.

According to the regression analysis data presented in Table 2, a 1 pg/mL increase in the concentration of SP increased the chance of CU by 1.02 times. In the presence of a stressful situation as a trigger, the risk of CU increased by 2.873 times. The data on the increase of SP levels in patients with urticaria relapse as a result of a stressful situation indicated the possibility of using the quantitative level of neurotransmitter SP as a biomarker to identify a trigger of CU.

In order to determine the effectiveness and quality of the model for calculating the risk of CU, a ROC curve (Receiver Operator Characteristic) was constructed and the AUC (area under the curve) was calculated. The corresponding data are presented in Fig. 1.

The multivariate logistic regression model can be used to predict the probability of a relationship between the development of chronic urticaria and exposure to risk factors (stress, gender, age, increased concentration of SP). In the logistic regression model, the cut-off point allows us to suggest the association or absence of the disease. Thus, if the predicted probability exceeds the cut-off threshold, the disease is present; otherwise we conclude that it is absent. The quality of correct diagnosis of the presence or absence of the disease is assessed by the sensitivity of the model Se (the proportion of correctly predicted cases of

Table 1. Descriptive characteristics and SP levels in patients with chronic urticaria and in the comparison group

Indicator/characteristic	Comparison group, *n* = 68	Patients with chronic urticaria, *n* = 97
Age, years	41.10±12.52	38.59±8.96
Gender m/f	44/24	45/52
Substance P, pg/mL	96.57 [78.04–138.16]	220.62 [127.30–302.65] *

Table prepared by the authors using their own data

Note: data are presented as Me [Q1–Q3].
* — statistical significance of the differences between the main and comparison groups, $p < 0.05$.

Table 2. Multivariate logistic regression model including age, gender, trigger stress, substance P level

Chronic urticaria	Odds ratio	*p*-value	[95% confidence interval]
Stress	2.873	0.014	0.15–0.81
Substance P	1.015	0.000	1.01–1.02
Gender	0.611	0.210	1.01–1.02
Age	1.0309	0.100	0.99–1.07

Table prepared by the authors using their own data

the disease) and specificity Sp (the proportion of correctly predicted cases of the disease absence).

The overall sensitivity of the model was evaluated using the ROC curve showing the dependence of sensitivity on the value of 1 — specificity. The overall sensitivity of the model was defined as the area of the AUC figure under the ROC curve (its value was 0.8326). This value exceeds 0.8, which allows us to conclude that the model has a high prognostic power. The optimal cutoff threshold was determined as a balance point between sensitivity and specificity of the model. The most optimal was the cutoff criterion of 0.7, since it showed the most optimal ratio of specificity and sensitivity of the risk model of CU development depending on prognostic risk factors.

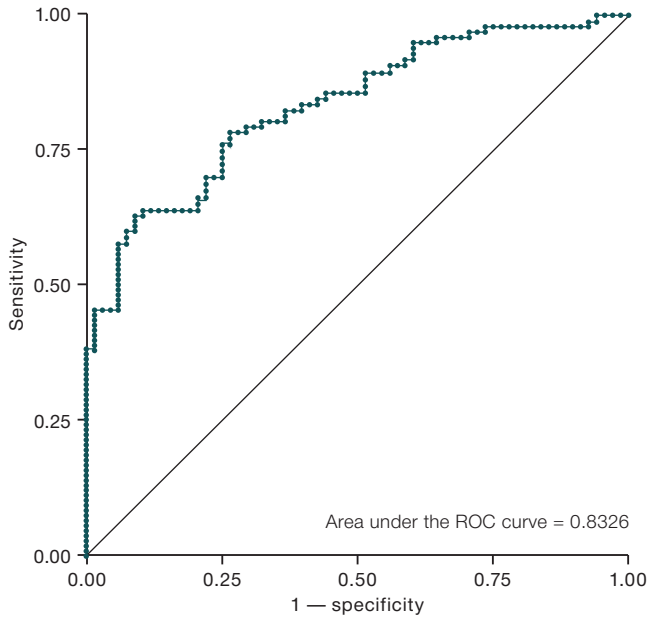

Figure prepared by the authors using their own data

Fig. 1. ROC curve constructed to evaluate the association model of stress, substance P, gender, and age with chronic urticaria

The greatest value for the diagnosis of urticaria was found for stress factors and SP concentrations. The data on the increase of SP concentration in patients with urticaria exacerbation as a result of a stressful situation indicate the possibility of using the neurotransmitter SP as a biomarker to identify the trigger of CU. The presence of clinical symptoms and elevation of SP in the bloodstream indicate the disease, which makes it possible to adjust therapy to achieve a more sustainable effect.

SP is a proinflammatory stress-related neuropeptide released from sensory nerve endings, being a key mediator in CNS-skin communication. It causes mast cell degranulation and the appearance of blisters and/or angioedema in CU. SP is one of the key neurotransmitters that is the first to respond to a stressor, thus contributing to the ongoing inflammation in the skin with a neurogenic component [19].

The increase in the level of SP in patients suffering from CU in relation to the comparison group was statistically significant, which is consistent with the data of other studies [26, 19, 27, 28]. Some studies [29, 30] investigated the relationship between the level of SP and the severity of depression in patients with CU. This data confirmed the causal relationship between the development of CU and depression. However, the identified relationships require further in-depth research.

In our study, according to the developed statistical model, elevated blood levels of SP and stress exposure were predictive risk factors for the development of chronic urticaria. Stress-induced CU may accompany various somatic diseases, aggravating their course. An analysis of SP dynamics in other pathologies may improve the current understanding of the relationship between stress and the disease in terms of immune interactions, thus forming the basis for targeted interventions and improved approaches to disease diagnosis and treatment.

It is important to assess the impact of stress in patients with CU for their timely referral to a psychotherapist for psychological or psychopharmacological support to reduce the severity of urticaria manifestations.

CONCLUSION

Thus, the study revealed a statistically significant increase in the serum neuropeptide substance P in patients with CU. By constructing a multivariate logistic regression model, positive values of the model parameters (with a significance level of $p \leq 0.01$) were obtained, indicating that it is the effect of the stress factor and an increase in the substance P blood level that is associated with an increase in the patients with chronic urticaria. The constructed logistic model had a high overall sensitivity, which allows us to conclude about its high predictive power. Based on the data obtained, the substance P blood level in patients with CU can be considered as a potential diagnostic biomarker that can be recommended for expanding the panel of screening tests clarifying the CU pathogenesis, which will improve the differential diagnosis and ensure early detection of patients with stress-induced urticaria for the pathogenetic therapy.

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PROSPECTS FOR THE USE OF INTRANASAL NANOSCALE POLYMER DELIVERY SYSTEMS FOR DRUGS AND ANTIDOTES IN EXTREME MEDICINE

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Introduction. The development of improved formulations of antidotes and remedies, which can be used not only by qualified medical personnel, but also in self- and mutual assistance, is an urgent task for extreme medicine.

Objective. Evaluation of the possibility of using nanoscale polymer delivery systems for medicines and antidotes intended for intranasal administration (into the nasal cavity) in extreme medicine.

Discussion. The main submicron-sized polymer carriers which are promising as the basis for the creation of an intranasal form of antidotes are identified. The bioavailability of the substance delivered is dependent on the physico-chemical properties of the carrier, the conditions for its production, as well as physiological and anatomical factors. Data is presented regarding possible ways of correcting these factors in order to increase bioavailability. Examples of the use of polymer nanocarriers in the treatment of poisoning with heavy metals and rocket fuel components, as well as lesions caused by radioactive substances, are presented. It is shown that carriers (dendrimers, cyclodextrins) can act as antidotes in certain cases. The study presents a list of antidotes approved for use within the territory of the Russian Federation, for which the development of intranasal forms is possible, taking their physico-chemical and pharmacokinetic properties into account.

Conclusions. Following a review of literature sources, the most promising submicron-sized polymer carriers for the intensification of intranasal delivery of drugs and antidotes are herein proposed: dendrimers, liposomes, nanocapsules, nanoparticles, and cyclodextrins. Using the list of antidotes approved for use in the Russian Federation as an example, promising drugs that can be potentially developed on the basis of these carriers are proposed.

Keywords: antidote; dendrimer; nanocapsule; liposome; delivery system; intranasal delivery

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ПЕРСПЕКТИВЫ ПРИМЕНЕНИЯ ИНТРАНАЗАЛЬНЫХ НАНОРАЗМЕРНЫХ ПОЛИМЕРНЫХ СИСТЕМ ДОСТАВКИ ЛЕКАРСТВЕННЫХ ПРЕПАРАТОВ И АНТИДОТОВ В МЕДИЦИНЕ ЭКСТРЕМАЛЬНЫХ СИТУАЦИЙ

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Введение. Создание усовершенствованных лекарственных форм антидотов и средств терапии, применение которых возможно не только квалифицированным медицинским персоналом, но и в порядке само- и взаимопомощи, является актуальной задачей медицины экстремальных ситуаций.

Цель. Оценка возможности применения наноразмерных полимерных систем доставки лекарственных средств и антидотов, предназначенных для введения в носовую полость (интраназально), в медицине экстремальных ситуаций.

Обсуждение. В ходе исследования были выделены основные полимерные носители субмикронного размера, которые являются перспективными для дальнейшего возможного создания интраназальной формы антидотов. На биодоступность доставляемого вещества влияют физико-химические характеристики самого носителя, условия его получения, а также физиологические и анатомические факторы. Представлены данные о возможных способах коррекции указанных факторов с целью повышения биодоступности. Вторая часть работы посвящена примерам применения полимерных наноносителей в терапии отравлений тяжелыми металлами, компонентами ракетного топлива и поражений, вызванных радиоактивными веществами. Показано, что в некоторых случаях носители (дендримеры, циклодекстрины) могут сами выступать в качестве антидотов. В исследовании представлен перечень антидотов, разрешенных к применению на территории Российской Федерации, для которых возможна с учетом их физико-химических и фармакокинетических свойств разработка интраназальных форм.

Выводы. На основании анализа данных литературы предложены наиболее перспективные полимерные носители субмикронного размера для интенсификации назальной доставки лекарственных средств и антидотов: дендримеры, липосомы, нанокапсулы, наночастицы и циклодекстрины. На примере перечня антидотов, разрешенных к применению на территории Российской Федерации, предложен список препаратов, для которых применение данных носителей является перспективным.

Ключевые слова: антидот; дендример; нанокапсула; липосома; система доставки; интраназальная доставка

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INTRODUCTION

In pharmacology/toxicology, an antidote is usually any drug which can neutralize a toxicant and eliminate its pathological effect. For speed of action, such drugs are usually developed and produced in formulations of injection solutions. Despite the successes and achievements of the modern pharmaceutical industry, for objective pharmacoeconomical reasons, there is now more than ever a shortage in both the global pharmaceutical market and in Russia of a number of medicines used in the treatment of acute and chronic poisoning with heavy metals, narcotic and organophosphorus compounds (OPCs), cyanides and hydrazine.

Antidote therapy is part of the comprehensive treatment of acute poisoning of both a domestic and technogenic nature. It is used by specialists in toxicological teams at the pre-hospital stage, physicians of medical institutions at the hospital stage, as well as in the order of self- and mutual assistance in peacetime and wartime.

Over the past decade [1], the growing need for convenient and effective medicines for the treatment and relief of intoxication effects has led to significant progress in pharmaceutical technologies. The number of improved formulations of antidotes and remedies has increased significantly. They can be used not only by qualified medical personnel, but also in self- and mutual assistance. A growing trend is the use of modern delivery systems to modify the detoxification properties of antidotes. When developing new formulations of known antidotes, differences need to be taking into account regarding the pharmacokinetic parameters relative to traditional, mainly injectable drugs. The greatest interest in this aspect is linked to the use of intranasal forms of medicines. They allow the neutralization of the differences in speed between invasive and non-invasive dosage forms of administration, albeit not proving adequate bioavailability and therapeutic effect in all cases. One of the possible solutions for the development of intranasal medicines is the use of polymer micro- and nanocapsules as carriers for antidote delivery. This formulation is one of the current trends in the treatment of metal poisoning, as well as the prevention of severe poisoning with narcotic substances and alcohol [2, 3].

The development of non-invasive dosage forms of antidotes, in particular intranasal forms using polymer nanocarriers, may not only increase the effectiveness of known drugs, but also expand the scope of their medical use. However, the drug delivery system, including nanoscale delivery, should not change the initial properties of the antidote/drug substance or lead to side effects [4]. For example, this could be respiratory depression caused by the undesirable delivery of loperamide to the central nervous system (CNS) resulting from co-administration with P-glycoprotein inhibitors [5]. On the contrary, the most promising application of the intranasal form of drug administration in the treatment of acute poisoning with neurotoxicants is due to its potential to intensify the transport of the active substance into brain tissue, bypassing the blood-brain barrier (BBB) and the metabolism phase in internal organs. For example, in the case of poisoning with OPCs, the low ability of pralidoxime to penetrate through the BBB as an antidote has little effect on centrally mediated respiratory depression caused by the OPC action. However, when administered intranasally in the form of cationic liposomes, pralidoxime chloride (2-pyridine aldoxime methyl chloride or 2-PAM) as a carrier is able to reduce brain damage and mortality in rats with their poisoning with paraoxone [6].

Intranasal forms of antidotes may be promising in the choice of therapy for poisoning caused by pulmonotoxicants, vesicants and nerve agents, as well as in acute radiation injuries [7, 8]. Therefore, for the relief of nausea and vomiting syndrome, the nasal form of antiemetics significantly simplifies the provision of assistance to the victim, when the administration of traditional remedies in the form of dispersible tablets or buccal films would be impossible.

Modern nanocarriers proposed for drug modification include: nanoparticles such as carbon nanoparticles (nanotubes, graphenes); non-carbon nanoparticles (iron, gold particles); nanoparticles made of biopolymers (capsules, liposomes), nanorobots and nanochips [9–12]; dendrimers — three-dimensional branched monodisperse polymers; and clathrates — beta cyclodextrin complexes. Liposomes are biocompatible and biodegradable bilayer lipid vesicles up to 0.5 microns in size, capable of encapsulating both polar and nonpolar compounds [13, 14].

The main distinguishing characteristic of these nanomaterials is their size and composition. They initially determine the primary physical and chemical properties of the future carrier: solubility in water and biological fluids, surface charge, sorption, aggregation and adhesion abilities, intermolecular interactions, interaction with cell membranes and proteins, cytotoxicity [15].

Drugs based on polymer micro- and nanocarriers can be administered in various ways: orally, buccally, transdermally, nasally, parenterally, etc.

In the case of intranasal administration, the absorption of substances occurs mainly in the nasal cavity [16, 17] and depends on the various factors shown in Figure 1, the features of which and ways to correct them will be discussed further.

A characteristic feature of intranasal administration is the absence of the effect of the first passage through the liver. Due to the anatomical features of the nasal mucosa, substances enter directly into the arterial bloodstream. High bioavailability, ease of use and high rate of effect development allow this route of administration to be considered as promising for the delivery of drugs based on polymer micro- and nanocarriers.

This work is aimed at evaluating the possibility of using nanoscale polymer delivery systems for medicines and antidotes intended for injection into the nasal cavity (intranasally) in extreme medicine.

RESULTS AND DISCUSSION

Physical and chemical factors of substance absorption in the intranasal administration route and methods of their correction

The bioavailability of substances in the intranasal administration route depends of the physical and chemical

properties of nanocarriers, such as surface-to-mass ratio, strength, conductivity, solubility, stability, and reactivity [17].

The development of synthetic carriers capable of capturing target biomacromolecules is one of the directions for creating a new generation of antidotes. In nature, interactions between biomacromolecules are realized due to weak electrostatic, hydrophobic, hydrogen, and Van der Waals forces. When creating antidote carriers capable of capturing target molecules, multipoint electrostatic interactions are simulated by including functional monomers (chelating ligands). For example, nanoparticles made of poly(ethylene-co-glycidylmethacrylate) functionalized with triethylenetetramine, N,N-di(2-pyridylmethyl)amine, 8-hydroxyquinoline or 8-hydroxyquinoline-2-sulfonic acid can be used as antidotes for copper poisoning [18].

Polymer capsules can include hydrophilic and hydrophobic antidotes, forming both covalent and non-covalent bonds with them. Polymer nanocapsules are able to protect the antidote from its degradation caused by blood proteins or enzymes (peptidases, phospholipases) of the blood-brain barrier. The release of the antidote from micro- and nanocapsules depends on the physico-chemical characteristics of the drug (particle size, concentration and solubility) and the polymer itself (structure, molecular weight, porosity and mechanical strength).

Anatomical and physiological factors of substance absorption in the intranasal administration route and methods for their correction

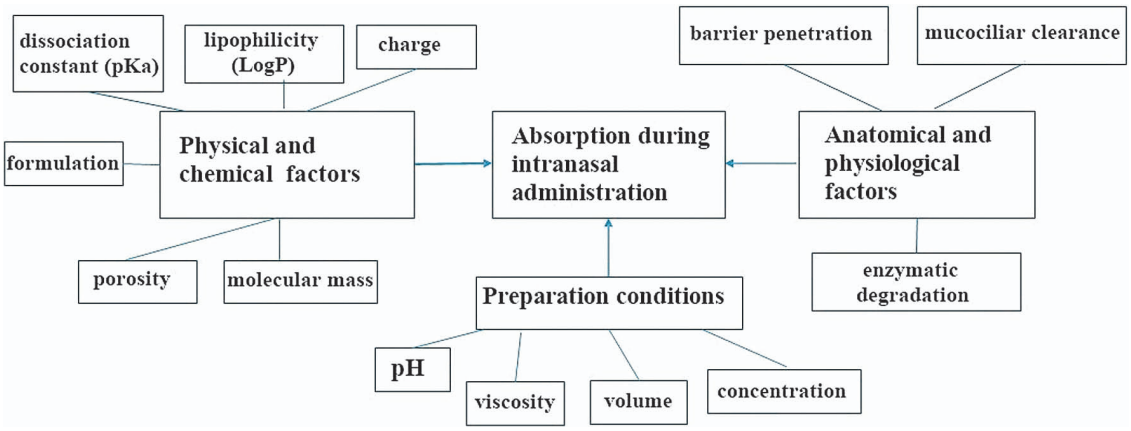
The physical chemical characteristics have a significant impact on the further pathways of the drug after intranasal administration. If the drug is more than 1 kDa in size, does not dissolve in the nasal mucosa, or has a pronounced negative charge (repels from a negatively charged mucous membrane), then it will not be able to penetrate the mucous membrane of the nasal sinuses. The penetration of substances through the mucous membrane can occur in several ways, for example, transcellular penetration and paracellular (between cells). Lipophilic substances carry out transcytosis by passive diffusion and are rapidly absorbed by vesicular transport mechanisms. Therefore, the use of lipophilic delivery systems for non-lipophilic antidotes can significantly increase their effectiveness. Polar antidote delivery systems pass through the epithelium paracellularly. The latter is less effective for large molecules (more than 1000 Da) [16].

The possibility of axonal transport, which enables bypassing of the BBB, is important for the intranasal delivery of many analgesics and antispasmodics, for example, as well as for the relief of various syndromes caused by the action of toxicants. However, with this pathway, the carrier must be capable of retrograde and anterograde movement in axons and dendrites [19]. Important physical chemical characteristics of nanocarriers in this penetration pathway are their size and chemical composition of the surface. Nanoparticles of 20–50 nm in size are able to penetrate directly into the central nervous system by axonal transport and can enhance bioavailability [20]. Negatively charged nanocarriers are attracted by the membranes and synaptic slits of neurons. Therefore, such carriers may be applicable for the intraneural delivery of drugs. Positively and neutrally charged nanocarriers are characterized by slow axonal transfer, while negatively charged carriers are characterized by rapid transfer [21].

The effect of the production conditions of dosage forms on the substance absorption in the intranasal administration route

Substance absorption during the intranasal administration route depends on the production conditions of a nanoscale delivery system. The development of each separately selected system also requires an individual approach and a study of its toxicity. The use of toxic organic solvents (as in the case of the use of ethylene glycol in the formation of some nanocapsules) the desired size reduction to be achieved. However, in certain cases, it can lead to increased toxicity if not completely removed. The method for obtaining a nanocarrier is based on the physical and chemical properties of the drug included. The selected method of preparation should not negatively affect the substance carried (contribute to its degradation or modify its properties).

A competent choice of the nanocarrier polymer material includes the following conditions: polymers should



The figure was prepared by the authors based on own data [15]

Figure 1. Factors affecting the absorption of drug delivery systems during intranasal drug administration

not be toxic, carcinogenic or mutagenic, and should be biocompatible. Some branched polymers (polypropylenimine dendrimers (PPI), polyamidamine (PAMAM) and polylysine (PLL)) are known to have significant cytotoxicity due to the high content of terminal amino groups [22]. Therefore, the most often used carriers are no higher than the 4th generation. In most cases, the toxic effects caused by polymer nanocarriers are associated with an increase in their cytotoxicity, namely: a decrease in cell viability, an increase in apoptosis, DNA destruction, rupture of the cell membrane and activation of lipid peroxidation [23].

One of the significant disadvantages of intranasal administration of most drugs is the relatively low permeability of the nasal mucosa to large macromolecules and intensive mucociliary clearance. The use of bioadhesive polymers (such as chitosan, carbopol, cyclodextrin and pluronic), which are part of the nanocarrier, will increase the residence time of the drug in the nasal cavity, thereby improving absorption. The nature of the polymer affects its bioadhesive qualities. In addition, the bioadhesive polymer must be polar and have sufficient viscosity. Polymer mucoadhesion is significantly dependent on the flexibility of the polymer chain (rigid crosslinking of polymer chains significantly limits their diffusion through membranes and interaction with mucin), molecular weight (the lower the molecular weight, the easier the permeability through the mucosa), the degree of swelling, and the ability to form hydrogen bonds.

Since the nasal mucosa is lipophilic, the degree of absorption of lipophilic carriers can be assumed to be more effective. In order to ensure adequate pharmacokinetics of the polymer necessary for the realization of the therapeutic effect during intranasal administration, special solvents and penetrant substances which increase the ability to penetrate biological membranes are used, as well as various systems of carrier particles of various nature, shape, and size. For example, chitosan supplementation can increase the paracellular transport of substances with intranasal administration of 0.5% chitosan and 1% atropine sulfate drops in the treatment of organophosphorus poisoning [24]. Cyclodextrin (dimethyl-beta-cyclodextrin) may also be of interest as a carrier due to its ability not only to transfer medicinal substances, but also, like chitosan, to increase their paracellular transport [25].

Despite a number of significant differences in the pharmacokinetics of parenteral and intranasal methods of administration, the latter has a significant advantage, namely, noninvasiveness of the antidote administration procedure, the possibility of dose reduction due to better bioavailability, simplification of production technology, and the possibility of self-administration [18, 20].

At the same time, promising carriers can simultaneously deliver various groups of drugs (for example, antiemetics and analgesics). The use of carriers for medicines enables their physical and chemical properties (increase hydrophilicity, permeability of biological barriers) to be modified, and bioavailability increased, thereby optimizing the pharmacokinetic parameters.

From the entire variety, we selected what we consider to be four of the most promising submicron carriers for the nasal administration route: liposomes, dendrimers, nanocapsules, and cyclodextrins.

Use of nanocarriers to intensify the penetration of drugs and antidotes through the blood-brain barrier

Many substances dangerous to humans (narcotic substances, organophosphorus pesticides, nerve agents, etc.) easily overcome the blood-brain barrier (BBB) and penetrate into the tissues of the central nervous system. At the same time, only a small part of the medicines used in medicine are capable of effectively overcoming the BBB, which makes it difficult for antidotes for CNS-active substances to be developed. In most cases, only hydrophilic compounds with a molecular weight of less than 150 Da and hydrophobic compounds with a mass of less than 600 Da are able to penetrate the BBB by passive diffusion [26–28]. Thus, many of the drugs used are not able to provide effective protection of the central nervous system in cases of intoxication of various origins.

The penetration rate of drugs through the BBB determines the adequacy, timeliness, and effectiveness of therapeutic action [28]. For example, the experimentally established rate of naloxone intake into the brain is 8–10 times higher than the rate of morphine intake [29], which explains its pronounced antidote effect. However, in the case of poisoning by centrally active toxicants rapidly penetrating through the BBB, the antidote activity of naloxone is significantly reduced [30]. At the same time, the combination of effective delivery systems and nasal administration can significantly enhance the effect of traditional antidotes and ensure their use not only by qualified medical personnel, but also in self- and mutual assistance [28].

Nanocarrier-based delivery systems can provide drugs with improved penetration efficiency through the BBB in four ways: by accumulating on the walls of brain blood capillaries, thus increasing the concentration gradient of the antidote between the bloodstream and central nervous system tissues; by passing in free form or together with the carrier due to the disclosure of dense connections between brain endothelial cells; by endocytosis of endothelial cells and the subsequent release of the antidote in the endothelial layer and diffusion into the brain tissue; and through transcytosis through a layer of endothelial cells into the brain [31]. Nanoparticles made of biodegradable polymers such as polylactide glycolide (PLGA), human serum albumin (HSA), and chitosan are most often used as carriers for drug delivery to the brain [32]. For example, quercetin, used as an antidote to arsenic, encapsulated in PLGA nanoparticles, is capable of crossing the BBB, which enables the depletion of brain cells in poisoning [33] to be neutralized.

Dendrimers (three-dimensional branched monodisperse polymers) capable of passing through the BBB have proved to be promising polymer nanocarriers for antidotes of neurotoxicants [32]. Thus, conjugates of the low molecular weight antidote of the OPC pyridine-aldoxime with polyamidamine dendrimer (PAMAM) of the 5th generation have shown their effectiveness in a mice model of paraxone intoxication [34]. Polyester dendrimers 2,2-bis(hydroxymethyl)propanoic acid (bis-MPA) are

capable of binding organophosphorus compounds, in particular dichlorvos, allowing such a polymer nanocarrier to be considered as an independent antidote to OPC [35].

The use of polymer nanocarriers for heavy metals poisoning

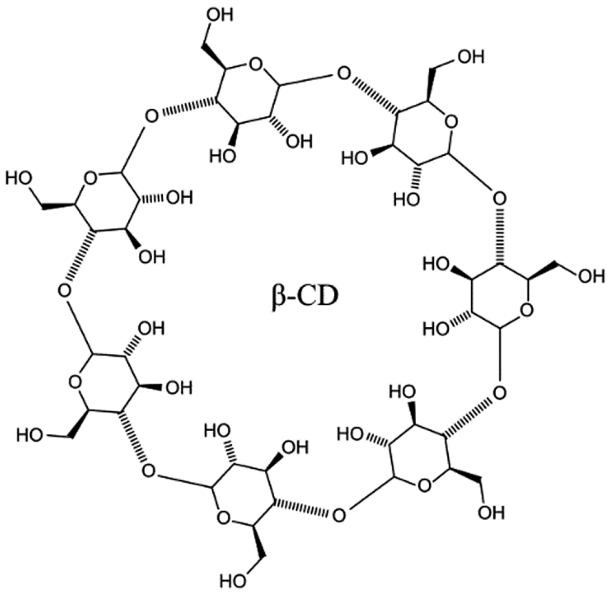
The group of heavy metals includes mercury, lead, cadmium, arsenic, chromium, cobalt, molybdenum, nickel, antimony, zinc, scandium, manganese, vanadium, strontium, barium, and tungsten. Their organic and inorganic compounds are found in many industries and are actively used in agriculture and everyday life. The mechanism of action of many heavy metals is based on the blocking of sulfhydryl, amine, and carboxyl groups of proteins-enzymes and structural proteins. As a result, protein, carbohydrate, and fat metabolism in the body is disrupted. The development of new antidotes and chelates for the treatment of heavy metal intoxication remains an important and pressing task [36].

Chelation therapy is one of the ways to treat heavy metal poisoning. It is based on the formation of an insoluble, less toxic metal complex easily excreted from the body. Therefore, a promising direction in the treatment of heavy metal poisoning is the use of polymer nanoparticles both as delivery carriers and independently, taking the chelating properties of these compounds into account.

Cyclodextrins are natural polymer delivery systems. They are representatives of a separate class of macrocyclic ligands in supramolecular chemistry [36], characterized as non-toxic substances capable of forming complexes with many toxicants (Fig. 2).

The use of cyclodextrin as an independent antidote became known for the first time in 2002, when its ability to neutralize the effects caused by muscle relaxants was demonstrated [38]. This allowed the development of a drug for the reversal of neuromuscular blockade based on modified gamma-cyclodextrin administered intravenously at doses from 1 to 16 mg/kg. The administration route and dosage allow the possibility of an intranasal route of cyclodextrin administration to be considered as an alternative, particularly given that these compounds have the ability to bind to many xenobiotics, in particular opioid analgesics. Therefore they can be used as detoxifying substances in poisoning with long-acting opiates such as methadone, for which there are no available antidotes [39]. Since the outer surface of natural cyclodextrins contains primary and secondary hydroxyl groups which can covalently bind to heavy metal ions, they can also act as promising antidotes for poisoning with heavy metals such as copper, lead (the strongest affinity) and cadmium [40]. Since certain cyclodextrins are also allowed for use in the food industry in the production of specialized food products, they can be recommended as a therapeutic and preventive nutrition for employees of enterprises in contact with heavy metals.

Chelation therapy with dimercaptosuccinic acid (DMSA), ethylenediaminetetraacetic acid (EDTA), 2,3-dimercaptopropanol (BAL), and D-penicillamine is proposed for the treatment of chronic poisoning with heavy metals (in particular lead). However, their main disadvantages include weak solubility in water, low bioavailability when ingested



The figure was prepared using PubChem data [37]

Figure 2. Structural formula of beta-cyclodextrin

(orally), and a short half-life, which significantly limits their clinical use [41]. At the same time, heavy metal adsorbents used in industry, such as mesoporous silicon nanoparticles (MSN) [10], can potentially be used as a drug adsorbent in the body, for example, in the treatment of cases of poisoning with thiol poisons. Thus, MSN nanoparticles modified with EDTA chelator showed a good effect on iron intoxication [42]. Curcumin encapsulated in chitosan nanocapsules with a diameter of 50 nm is also being considered for the treatment of heavy metal poisoning. The chitosan shell protects the compound from absorption by the reticuloendothelial system (RES), increases its bioavailability, and allows longer circulation in the blood. This approach permits the dose of curcumin to be significantly reduced when administered orally for the effective removal of heavy metals from the body [43]. Similarly, encapsulation of selenomethionine in PLGA nanocapsules resulted in a 7-fold increase in its detoxifying efficacy against mercury-containing substances compared with the traditional method of administration [44].

The use of dendrimers and dendrigrafts as carriers enables certain hydrophobic substances to be rendered hydrophilic. Their use in complexation with water-soluble chelators such as DMSA allows hydrophilic complexes with optimal bioavailability [41] to be obtained. Water-soluble complexes of dendrimers with polyphenol quercetin provide the ability to penetrate the BBB, thus being promising for combating oxidative stress in arsenic poisoning [33, 45].

Use of polymer nanocarriers for the delivery of antidotes and radioprotectors

Pyridoxine (vitamin B_6) is considered the only effective antidote for poisoning with unsymmetrical dimethylhydrazine (UDMH) [46]. This drug relieves convulsive syndrome and reduces the toxic effect of UDMH and its metabolites on the central nervous system. The neurotoxic effect of

asymmetric dimethylhydrazine is manifested in a decrease in the content of pyridoxal phosphate due to its interaction with pyridoxal contained in brain tissue cells. As a result, toxic pyridoxal hydrazones are formed which inhibit the activity of pyridoxal kinase and thereby block the synthesis of pyridoxal phosphate in the cell [46].

The structure of vitamin B_6 contains a hydroxyl group, suggesting the possibility of electrostatic interaction with polymer carriers having positively charged functional groups, for example, dendrimers. The polyphenol curcumin, enclosed in a nanoliposomal form (NLC), has also proven itself as a therapy for dimethylhydrazine poisoning in mice. The administration of nanoliposomal curcumin at a dose of 150 mg/kg significantly reduced serum alanine aminotransferase (ALT) and lactate dehydrogenase (LDH). This increased under the action of dimethylhydrazine, at the same time as significantly increasing the level of gamma-aminobutyric acid (GABA) in the hippocampus [47].

Salts of hydrocyanic acid are highly dangerous to humans. At the same time, liposomal methemoglobin (MetHb@Lipo) has shown its effectiveness as a new antidote for cyanide poisoning [48], significantly increasing the survival of animals after contact with hydrogen sulfide. This is achieved by maintaining the activity of cytochrome C oxidase and suppressing metabolic acidosis [13].

Damage by radioactive substances can occur only in non-standard emergency situations (waste transportation, testing and work on machine with radioactive elements). The development of a non-invasive form of radioprotectors is also an important task. For example, Amifostin, the most well-known and effective radioprotector, can currently be administered only parenterally, but with this method of administration it is quickly eliminated from the body. Therefore, researchers around the world are actively developing a new form of antidote which could provide more effective dosing of the drug and reduce its toxicity. *Mandal TK* et al. developed hybrid microcapsules of PLGA and chitosan, enabling Amifostine to be effectively capsulated. This composition of the microcapsule ensured a 45% reduction in the drug release rate. In addition, the introduction of chitosan into the shell is believed to increase the absorption of the drug and increase its bioavailability [49].

Use of polymer nanocarriers for the development of symptomatic therapy

A number of chemicals cause poisoning by a wide range of pathophysiological life-threatening processes. In such cases, the simultaneous use of both etiotropic therapy and pathogenetic, symptomatic therapy is required, in order to eliminate individual symptoms of intoxication. The improvement of therapeutic drugs for symptomatic therapy through the development of delivery systems can presumably not only significantly facilitate the procedure of their administration, but also increase the effectiveness of treatment. For example, modified antiemetic drugs can be used to inhibit the gag reflex in acute radiation injuries and OPC poisoning. Currently, the classic routes of administration of this group of drugs are oral and parenteral. The intranasal administration of antiemetics can significantly simplify the procedure of drug administration and neutralize the effect of the first passage through the liver [50]. In the study by *Ozsoy Y,* and *Gungör S*, nasal administration of metoclopramide *in-situ* in the formulation of poloxamer 405 gels increased its bioavailability by 19% compared with oral administration [51]. The high level of efficiency with intranasal administration of ondancetron and granisetrone was obtained due to their inclusion in chitosan microparticles crosslinked with glutaraldehyde [52]. Granisetron preparation encapsulated in microparticles based on cyclodextrin and carboxymethylcellulose showed high antiemetic activity during intranasal administration [53].

In the case of poisoning with irritating substances, burns, and in dental and surgical practice, analgesics (opioid and non-opioid analgesics of central action, adjuvant analgesics, nonsteroidal anti-inflammatory drugs) are used to treat pain. However, many analgesics have a short period of action, leading to an increase in the administration frequency to the patient. In this case, the use of a delivery system may allow for a gradual low-dose controlled intake of the drug into the victim's body while maintaining effectiveness and reducing side effects. For example, the inclusion of Benzocaine® in polymer nanoparticles (PLGA, PLA, PCL) makes it possible to prolong the analgesic effect when compared with a conventional drug [54].

No less important in acute poisoning with many toxicants is the relief of convulsive syndrome. The use of anticonvulsants, such as carbamazepine in the formulation of carboxymethyl chitosan nanoparticles, with intranasal administration makes it possible to ensure the necessary concentration of the drug in the brain and, accordingly, increase the effectiveness of treatment [55].

Proposals for the modification of antidotes approved for use in the Russian Federation

Based on the review of domestic and foreign literature, as well as taking the structural and physical and chemical characteristics of drugs recommended for use as antidotes into account, we have proposed possible polymer carriers for modifying some antidotes currently allowed for use in the Russian Federation (Table 1).

Obviously, there is no a need to develop all antidotes need in an intranasal form. For example, drugs such as ethyl alcohol or calcium gluconate were initially excluded from our analysis. Moreover, the development of intranasal forms for some antidotes is initially undesirable, since it can enhance the effect of the drug and lead to possible dangerous side effects. For example, in the case of the anticholinesterase drug proserin, it is undesirable to increase its effect on the central nervous system. Some of the antidotes used in medicine (such as atropine sulfate, Cuprenyl® and pyridoxine hydrochloride) with good bioavailability and efficacy in conventional forms do not need significant modifications, except for convenience of use. Nevertheless, the development of an intranasal form of pyridoxine, for example, may prove promising as a possible preventive antidote for workers who have professional contact with rocket fuel components. The structure of both pyridoxine and atropine technically allows them to bind electrostatically to the end

groups of the dendrimer and, based on our assumptions, such a delivery system can demonstrate greater efficiency due to the intensification of absorption and penetration through the BBB. This will allow the antidote to be administered in emergency situations by means of self- and mutual assistance.

In some cases, a less invasive intranasal form may be an alternative to parenteral and oral administration and find its application, for example, in pediatrics. A well-known antidote such as Vikasol is used for overdose with vitamin K antagonist drugs (warfarin, fenindione, acenocumarol). The development of intranasal dosage forms of synthetic vitamin K may have potential in pediatrics for the prevention of vitamin K-dependent hemorrhagic syndrome in newborns. Parenteral administration of the antidote to children with this syndrome leads to excessive traumatization, while the liposomal intranasal formulation of vitamin K will not only resolve the problem of simplifying the administration of the drug to patients, but also ensure its effective delivery to the body.

The expediency of developing non-invasive dosage forms currently approved for use in the Russian Federation [56], taking into account their physical and chemical and pharmacokinetic properties, is considered in Table 1.

CONCLUSION

The conducted research allows us to conclude that the most promising nanoscale polymer delivery systems for drugs and antidotes intended for intranasal administration are dendrimers, liposomes, cyclodextrins, and nanocapsules.

The absorption of the drug and antidote delivery system during intranasal delivery depends on the physical and chemical characteristics of the carrier (surface-to-mass ratio, strength, conductivity, solubility, stability, and reactivity), the physiological and anatomical factors (ability to penetrate the BBB), as well as the conditions for obtaining carriers (presence or absence of impurities in the form of solvents and penetrant substances).

It was shown that some carriers can act as antidotes. Thus, dendrimers are capable of reacting with OPC (as in the case of the bis-MPA polyester dendrimer and dichlorvos), while cyclodextrins are antidotes for muscle relaxants.

At the moment, not only modified chelating substances (such as mesoporous silicon nanoparticles modified with EDTA), but also chitosan nanoparticles containing curcumin and selenomethionine encapsulated in PLGA nanocapsules have shown a high level of efficiency in heavy metal poisoning.

The intranasal method of administration may be convenient for radiation injuries, when oral administration of the antidote is difficult (due to vomiting). Increasing the effectiveness of known radioprotectors and reducing their toxicity while simultaneously administering them non-invasively is also a promising and still unresolved area in this field.

The possibility of using polymer nanocarriers in order to improve therapeutic drugs for symptomatic therapy (antiemetics, anti-inflammatory, anticonvulsants, painkillers and antihistamines) was evaluated. The possibility of prolonging the analgesic effect of the drug is demonstrated by the example of inclusion of benzocaine in PLGA nanoparticles.

This review provides suggestions for modifying the reserve of antidotes used in the Russian Federation. For many of them, it is shown that the intranasal form of administration is the most promising due to its non-invasiveness (parenteral administration causes additional traumatization of the affected tissues and can serve as an entrance gate for secondary infection), ease of use, as well as the rapid speed of drug delivery. This work also notes that microcapsulation allows, if necessary, programmable release of the drug to be achieved. Most nanocarriers have both undeniable advantages and limitations. In this regard, the issues of searching for the most effective intranasal carriers remain open today.

Table 1. Assessment of the prospects for the development of non-invasive formulation of certain antidotes approved for use in the Russian Federation, taking into account their physical and chemical and pharmacokinetic properties

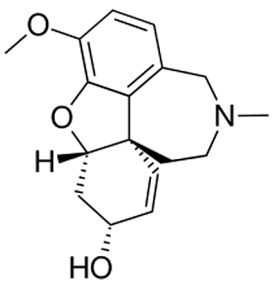
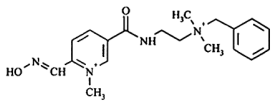
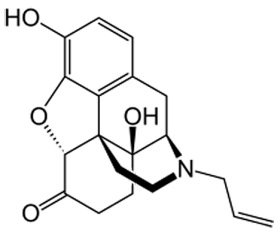
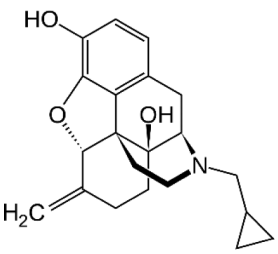
Antidote name and indications	Formulation, dose	Physical and chemical properties	Absorption	Feasibility of developing non-invasive dosage forms
Galantamine  Cholinolytic poisoning	Sol. for intravenous and subcutaneous administration 1 mg/mL	M = 368.27 g/mol Slightly soluble in water and alcohol It is soluble in chloroform pKa 8.32	Galantamine relieves cerebral cholinergic syndrome. The absolute oral bioavailability is about 90%. Half-life: 7 h	Liposomes and dendrimers with a particle size of 3–5 microns effectively deliver drugs to the brain. Based on the physical and chemical properties of the drug, the development of its liposomal formulation by hydration/rehydration of a thin film is promising due to its weak solubility in water and alcohol, but good solubility in chloroform. The reactive hydroxyl group of galantamine can bind electrostatically to dendrimers having positively charged amino groups, in particular to a third-generation polylysine dendrimer capable of penetrating the BBB. The resulting electrostatic bond is rather fragile and will allow for a smooth release of the drug from the carrier molecule. Effective delivery of the drug to the central nervous system is expected with intranasal administration, which may make it possible to provide the necessary therapeutic dose of galantamine.

Table 1 (continued)

Antidote name and indications	Formulation, dose	Physical and chemical properties	Absorption	Feasibility of developing non-invasive dosage forms
Glucagon* NH_2-His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-Ser- Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr-COOH Overdose with calcium channel blockers	Lyophilisate for injection. 1 mg	M = 3485 g/mol Hydrophobic The solutions of the drug are stable for 48 h at a 5°C pKa 7,1	With i/v glucagon 1 mg/mL, the maximum blood level 7.9 ng/mL is reached after 20 min. With i/m administration, the maximum level 6.9 ng/mL is reached after 13 min. With intranasally glucagon 3 mg, the maximum level 6130 pg/mL is reached after 15 min.	Consideration of the structure, physical and chemical properties of glucagon allows us to expect the inclusion of this peptide in the hydrophobic part of the liposome, which can provide its protection from the effects of enzymes and degradation. The charged reactional end groups of the peptide (NH2 and COOH) suggest the possibility of formation of electrostatic interactions with charged dendrimers. When administered intranasally, such liposome-based or dendrimer-based drugs can provide the required concentration of glucagon necessary to ensure therapeutic action.
Carboxim*  OPC poisoning	Solution for injection 15% — 1 mL (150 mg)	M = 413.35 g/mol Hydrophilic	Central nervous system cholinesterase reactivator. Restores neuromuscular conduction.	Due to its structure, Carboxim does not pass through the BBB well. A large dose is required for intramuscular delivery of this drug. The use of a properly selected drug carrier can significantly reduce its high dose while maintaining the therapeutic effect. At the same time, ensuring optimal bioavailability with intranasal administration of Carboxim may make it possible to create an antidote convenient for use within the framework of self- and mutual assistance. Some functionalized cyclodextrins are stoichiometrically capable of absorbing OPC. The intranasal delivery system «acetylcholinesterase reactivator — cyclodextrin» will ensure the paracellular transport of the drug and, probably, will make it possible to increase its effectiveness. Since Carboxim and other acetylcholinesterase reactivators are hydrophilic drugs, they can be included in both nanocapsules and liposomes. However, the most promising solution is the formation of a dendrimer-carboxim complex due to charged fragments of the molecule. This can increase the efficiency of the passage of carboxim through the BBB. In addition, the dendrimer is able to react independently with some OPC, which will also increase the effectiveness of the antidote.
Naloxone  Narcotic analgesics poisoning	Injection for solution, 0.4 mg/mL, 1 mL, ampoules	M = 327.4 g/mol Hydrophilic pKa 7.9	μ-opioid receptors antagonist Half-life: 1-1.5 h Oral bioavailability is up to 20%	Liposomal and dendrimeric formulations of opioid receptor antagonists with intranasal administration make it possible to maximize the bioavailability of active substances by increasing their absorption by simple diffusion, necessary for a reliable therapeutic effect in cases of poisoning with narcotic analgesics. At the same time, the lack of bioavailability with this method of administration is compensated by the direct entry of antagonists into the tissues of the central nervous system which provides a therapeutic effect no worse than that of their injectable drugs. On the contrary, in the treatment of socially significant diseases (various types of addictions), microcapsulation in biodegradable polymers such as PLGA or chitosan is the most promising, which usually provides a slow prolonged effect of such drugs when administered in depot form and provides their protection from the effects of RES.
Nalmefene  Alcohol addiction treatment	Film-coated tablets, 18 mg	M = 339.4 g/mol Hydrophilic pKa 7.6	A long-acting opioid antagonist with affinity for the k-opioid receptor and the m-opioid receptor. It is not subjected to presystemic metabolism.	

The table was prepared by the authors using PubChem data [37]

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EXPERIMENTAL MODEL OF CONVULSIVE SYNDROME BASED ON PHENYLCARBAMATE

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Introduction. Carbamates are widely used in the pharmaceutical industry, agriculture, and household chemicals. Being reversible cholinesterase inhibitors, carbamates can cause the development of generalized convulsive syndrome. Untimely treatment contributes to the emergence of persistent neurological disorders. In order to develop and adequately assess in preclinical studies the specific activity of new drugs for the relief of convulsive syndrome in acute intoxication with this group of substances, an easily reproducible experimental model of convulsive carbamate-induced syndrome is required.

Objective. Development of an experimental model of generalized convulsive syndrome in rats using phenylcarbamate as a model toxicant for testing in preclinical studies of therapies for poisoning with cholinesterase inhibitors.

Materials and methods. The study was performed using mongrel sexually mature male rats, aged 3 months (80 animals), divided into 4 groups (3 experimental and 1 control). At the first stage, the parameters of convulsive syndrome caused by model toxicants were compared: phenylcarbamate 1 mg/kg bw, corazol 65 mg/kg bw, and thiosemicarbazide 8 mg/kg bw. The following parameters were studied: motor activity (open field test), neuromotor functions (grip strength test), cognitive functions (conditioned avoidance responses, CAR), and cardiovascular indicators (ECG and cardiac rhythmogram assessment). The severity of the convulsive syndrome was identified by Racine stages. Additionally, the structure of brain tissues was evaluated by histological methods. second stage, biochemical parameters were studied in three experimental (with toxicants) and control groups. Some biochemical parameters were studied in the blood serum, assessing the function of the liver, kidneys, prooxidant and antioxidant systems. At the third stage, the activity of cholinesterase in the blood and brain was studied in 30 control and 30 experimental rats after phenylcarbamate exposure. Statistical processing of the results was carried out using Statistica v.10.

Results. When modeling convulsive syndrome in rats, phenylcarbamate is comparable to corazol in terms of the onset of the latency period, duration and intensity of seizures. When implementing the model, a significant decrease in heart rate was recorded 48 h after administration. The CAR test found that the introduced substance increases the time of the first entry into the dark compartment before training. Significant changes in markers of liver function (ALT, bilirubin, cholesterol, triglycerides), lipid peroxidation and the antioxidant system (MDA, GPx) confirm the complexity of mechanisms responsible for the development of seizures and neurological disorders. The results of histological examination of brain tissues indicate that phenylcarbamate induces pronounced disorders of the brain structure in an experiment on rats.

Conclusions. The developed experimental model of phenylcarbamate-based convulsive syndrome in rats is easy to reproduce, thus being recommended for preclinical studies of new drugs for the relief of convulsive syndrome in poisoning with cholinesterase inhibitors.

Keywords: carbamate; convulsive syndrome; cholinesterase inhibitor; experimental model; preclinical studies

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ЭКСПЕРИМЕНТАЛЬНАЯ МОДЕЛЬ СУДОРОЖНОГО СИНДРОМА НА ОСНОВЕ ФЕНИЛКАРБАМАТА

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Введение. Карбаматы широко используются в фармацевтической промышленности, сельском хозяйстве и бытовой химии. Являясь обратимыми ингибиторами холинэстераз, карбаматы могут вызывать развитие генерализованного судорожного синдрома. Несвоевременное лечение способствует формированию стойких неврологических нарушений. Для разработки и адекватной оценки специфической активности в доклинических исследованиях новых средств купирования судорожного синдрома при острых интоксикациях данной группой веществ необходима легко воспроизводимая экспериментальная модель судорожного синдрома на основе карбаматов.

Цель. Разработка экспериментальной модели генерализованного судорожного синдрома на крысах с применением фенилкарбамата как модельного токсиканта для тестирования в доклинических исследованиях средств терапии при отравлении ингибиторами холинэстераз.

Материалы и методы. Исследование проведено на беспородных половозрелых крысах-самцах возрастом 3 месяца (80 животных), распределенных на 4 группы (3 опытные и 1 контрольная). На первом этапе сравнивали параметры судорожного синдрома, вызываемого модельными токсикантами: фенилкарбаматом в дозе 1 мг/кг м.т., коразолом в дозе 65 мг/кг м.т. и тиосемикарбазидом в дозе 8 мг/кг м.т. Изучены: двигательная активность (в тесте «Открытое поле»), нейромоторные функции (тест на силу хвата), когнитивные функции (по условной реакции пассивного избегания болевого раздражения — УРПИ) и показатели сердечно-сосудистой системы (оценка ЭКГ и ритмограммы сердца). Выраженность судорожного синдрома определяли по шкале Racine. Дополнительно оценивали структуру тканей мозга гистологическими методами. На втором этапе изучали биохимические показатели в 3-х опытных (с токсикантами) и контрольной группах. В сыворотке крови изучены некоторые биохимические показатели, оценивающие функцию печени, почек, прооксидантной и антиоксидантной систем. На третьем этапе изучали активность холинэсте-

разы в крови и головном мозге у 30 контрольных и 30 опытных крыс после воздействия фенилкарбамата. Статистическая обработка результатов осуществлялась с помощью Statistica v.10.

Результаты. При моделировании судорожного синдрома у крыс по времени наступления латентного периода, продолжительности и интенсивности судорог фенилкарбамат сопоставим с коразолом. При реализации модели зафиксировано достоверное снижение частоты сердечных сокращений через 48 ч после введения. В тесте УРПИ установлено, что введение увеличивает время первого захода в темный отсек до обучения. Достоверные изменения маркеров функции печени (АЛТ, билирубин, холестерин, триглицериды), перекисного окисления липидов и антиоксидантной системы (МДА, ГП) подтверждают наличие комплексных механизмов развития судорог и неврологических нарушений. Результаты гистологического исследования тканей мозга свидетельствуют, что фенилкарбамат провоцирует выраженные нарушения структуры мозга в эксперименте на крысах.

Выводы. Разработанная экспериментальная модель судорожного синдрома у крыс на основе фенилкарбамата проста в воспроизведении и может эффективно применяться в доклинических исследованиях новых средств купирования судорожного синдрома при отравлении ингибиторами холинэстераз.

Ключевые слова: карбамат; судорожный синдром; ингибитор холинэстеразы; экспериментальная модель; доклинические исследования

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Соответствие принципам этики: исследование выполнено с соблюдением правил биоэтики, утвержденных Европейской конвенцией о защите позвоночных животных, используемых для экспериментальных и других целей. Проведение исследований одобрено на заседании биоэтического комитета ФГБУ НКЦТ им. С.Н. Голикова ФМБА России (протокол № 2/23 от 06.04.2023).

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INTRODUCTION

Carbamates are derivatives of carbamic acid, the amino and carboxyl endings of which are replaced by structurally diverse alkyl, aryl, or alkyl-aryl groups [1]. Carbamates are structurally similar to amides and esters. Due to their chemical and conformational stability, resistance to proteolysis, as well as the ability of many representatives of the group to pass through the cell membrane and the blood-brain barrier, carbamates are increasingly used in the pharmaceutical industry both as an active component of dosage forms and as carriers replacing peptides in finished dosage forms [2]. In addition to the pharmaceutical industry, carbamates are actively used in agriculture and as part of household chemicals, which often causes accidental or even intentional poisoning.

A number of carbamic acid derivatives belong to highly toxic compounds, reversible (unlike organophosphorus compounds, OPs) cholinesterase inhibitors, leading to the formation of the so-called “cholinergic crisis” associated with the development of generalized convulsive syndrome, in severe cases ending in coma and death. Under the action of carbamates during acute intoxication, due to the rapid hydrolysis of the C = O bond (decarbomylation of the enzyme), the activity of cholinesterases in survivors is restored within several hours; complete restoration of cholinesterase function is observed after 24–48 h [3]. At the same time, poisoning with cholinesterase inhibitors results in inflammatory reactions in the tissues of the nervous system, apoptosis of nerve cells, and neurodegenerative changes that potentiate the development of persistent neurological disorders in survivors.

It should be noted that the levels of mortality and disability in acute carbamate poisoning are rather high. Thus, according to WHO data from 2019, more than 1600 people died globally as a result of deliberate suicide using carbamates alone, the number of non-fatal poisonings ranged from several hundred thousand to several million per year (according to statistics in different countries). As a result of ineffective or untimely treatment, a significant proportion of survivors subsequently experience tremors, dizziness, headaches, partial memory loss, emotional lability, confusion, cognitive impairment, peripheral neuropathy, and autonomic dysfunction [4].

It should be noted that the treatment of acute carbamate poisoning relies on the methods similar to those used in OP poisoning (atropinization, administration of benzodiazepine-type drugs to relieve convulsive syndrome in the initial 10–20 min after exposure) and methods of treatment of acute attacks of true epilepsy with the development of seizures refractory to benzodiazepines (administration of barbiturates, anesthetics) [5]. The effectiveness of antidotes is high only provided they are used at the earliest stages of poisoning [6].

Recently, an increasing amount of evidence indicates that the pathogenesis of carbamate and OP poisoning includes, among other things, manifestations of noncholinergic toxicity through the induction of reactive oxygen species (ROS) and the formation of carbonylated proteins. OPs and carbamates also interact with mitochondrial translocation proteins, with androgen, estrogen, and glucocorticoid

receptors involved in cholesterol metabolism, negatively affecting their functions. At the same time, the mechanisms and severity of OP and carbamate effects on the body differ [7, 8]. This justifies the need to develop improved therapies for carbamate poisoning. However, this task is impossible to achieve without adequate experimental models capable of assessing the specific activity of new drugs in preclinical studies. To date, the creation of anticonvulsants has been carried out mainly for the treatment of true epilepsy; maximum electric shock, corazol administration, etc., are used as experimental models [9]. Organophosphorus compounds (diazinon, malaoxon, chlorfenvinphos, and dichlorvos) are commonly used as toxicants–pesticides affecting cholinesterase activity in experiments to simulate generalized convulsive syndrome in animals [10]. Experimental models of generalized convulsive syndrome using carbamates, which impose significantly lower safety requirements during preclinical studies, have not been so far described in scientific publications.

Thus, in the present work, we set out to develop an experimental model of generalized convulsive syndrome in rats using phenylcarbamate as a model toxicant for testing of therapies for poisoning with cholinesterase inhibitors in preclinical studies.

MATERIALS AND METHODS

The study was conducted on white mongrel 3-month-old male rats, weighing 150–250 g, provided by PLZH "Rappolovo" of the National Research Center "Kurchatov Institute," Leningrad Oblast, Russia. The animals were kept in standard vivarium conditions [11]. A 12 h lighting cycle was maintained; food and water were provided *ad libitum*.

In a series of preliminary experiments, the median lethal dose (LD_{50}) of phenylcarbamate (PC) was determined for intraperitoneal and intragastric routes of administration to rats, amounting to 1.43 ± 0.12 mg/kg of body weight (bw) and 10.0 ± 0.77 mg/kg bw, respectively [12]. For higher reproducibility of the experimental model of convulsive syndrome in preclinical studies, the intraperitoneal route of administration of phenylcarbamate was selected. This route is an alternative to the intravenous route of administration, enabling 100% bioavailability of the drug. In our previous work, a convulsive dose of phenylcarbamate of 1 mg/kg bw was also experimentally selected, which ensures the induction of convulsive syndrome in 100% of animals with a minimum percentage of mortality.

The experiment consisted of three consecutive stages.

At the first stage, the nature of the convulsive syndrome was assessed. To that end, laboratory animals, depending on the convulsive agent used, were divided into four groups and a control group with the introduction of 0.9% sodium chloride solution. Each group consisted of 20 heads. The simulation of convulsive syndrome in the first group was carried out using a reversible acetylcholinesterase inhibitor from the carbamate group, namely phenyl ether of carbamic acid (hereinafter phenylcarbamate) 1 mg/kg bw. The original compound was synthesized at the Golikov Research Center of Toxicology under the leadership of A.Ya. Bespalov. This compound is protected by the patent of the Russian Federation [13]. In the second and third groups, the following were used as model toxicants, respectively: corazol (Pentylentetrazol, 6,7,8,9-tetrahydro-5H-tetrazolo(1,5-a)azepine, $C_6H_{10}N_4$), produced by Sigma-Aldrich, at a dose of 65 mg/kg bw and Thiosemicarbazide (Hydrazinecarbothioamide, CH_5N_3S) at a dose of 8 mg/kg bw, resynthesized in the synthesis laboratory of the Golikov Research Center of Toxicology [14]. In each experimental group, 10 animals were used to study motor activity, neuromotor functions, and the cardiovascular system; 10 animals were used to assess cognitive functions.

The severity of convulsive syndrome after administration of toxicants was determined visually using the upgraded Racine scale [15].

Motor activity and anxiety were assessed using a computerized Open Field test developed by C.S. Hall (1936) [16] using the VideoMot2 system, TSE, Germany. The behavior was evaluated after 24 and 48 h. Seven components of behavior were recorded during 2 min of observation: horizontal and vertical (rack) activity, grooming, the speed of movement of animals, and the distance traveled by the animal during the experiment, total motor activity, the number of movements in the center of the site and on the periphery.

The study of neuromotor functions, clinically expressed by general weakness and asthenia, was carried out after 24, 48 h using a Bioseb GS3 grip analyzer, which automatically registers the gripping force of the lattice with the front paws of the rat and the exact moment of its release.

Cognitive functions were assessed by recording the parameters of the conditioned avoidance responses (CAR) 2, 24, and 48 h after training in a two-compartment PACS-30 installation (Columbus Instruments, USA). The following parameters were recorded: the time of the first entry into the dark compartment (before training to assess the presence of a mink reflex in an animal), the latent period of entering a dark punishable compartment, the time spent in the light and dark compartments. The total duration of the experiment for each animal was 120 seconds. Along with the time parameters of the CAR, in each group, during repeated tests, the number of trained animals was recorded, in which the latent period of entering the dark compartment was more than 120 seconds (the duration of animal observation).

To assess the activity of the cardiovascular system, an electrocardiographic examination (ECG) was performed in the II lead on a Poly-Spectrum-8B electrocardiograph veterinary device (Neurosoft, Russia) after 24 and 48 h. The measured parameters included heart rate (HR), the value of the R wave, the intervals PQ and QT, calculated according to the II standard lead. The heart rhythmogram was evaluated using the Baevsky cardiointervalography (KIG) on the same device [17].

In animals subjected to planned euthanasia and necropsy, a histological analysis of the brain was performed at the first stage of the experiment. The organ sections were dehydrated, impregnated with paraffin and stained with hematoxylin and eosin, followed by light microscopy on a Leica DM1000 microscope, Leica Microsystems Wetzlar GmbH (Germany) at 400x magnification. CO_2 inhalation using Open Science equipment (Russia) was used for euthanasia.

At the second stage of the experiment, some biochemical parameters were evaluated, as well as the ionic composition of animal blood. In the phenylcarbamate group, the antioxidant status was additionally assessed in comparison with the intact group. To that end, a new sample was formed in which the convulsive syndrome was modeled with phenylcarbamate at a dose of 1 mg/kg bw, corazol at a dose of 65 mg/kg bw, thiosemicarbazide at a dose of 8 mg/kg bw Depending on the time of blood collection (24 and 48 h; on days 7 and 14), laboratory animals were divided into four groups for the control of the studied parameters, including an intact group of 24 heads each.

Blood from animals for biochemical analysis was collected in a dry container after 24 and 48 h, on days 7 and 14. Next, the selected biological material was centrifuged (centrifuge Z 326 K, manufactured in Germany, series 66110159) at 3000 rpm, at 4°C for 10 min. For further studies, the infusion fluid, serum, was selected. A transparent serum without signs of hemolysis was examined. Biochemical parameters (triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (alkaline phosphatase), total cholesterol, urea, total bilirubin) were determined using a biochemical analyzer A-25 from BioSystems (Spain) using kits from Vector-Best JSC (Russia). The analyzer calibration and internal quality control of the studies were carried out on calibration and control materials of Vector-Best JSC (Russia).

To study the antioxidant system, blood plasma and erythrocyte suspension were used, obtained by centrifugation of whole blood at 3000 g for 3 min, followed by triple washing of erythrocytes with a saline solution and step-by-step centrifugation at the specified parameters. Hemolysate was prepared from the washed erythrocytes in an appropriate manner for each technique. In erythrocyte hemolysate, indicators of the antioxidant protection system were determined using the Habig WH and Jakoby WB method [19]: superoxide dismutase (SOD) [18], glutathione peroxidase (GP), glutathione reductase (GR). To assess the processes of lipid peroxidation (LPO), the concentration of reduced glutathione (RG) was determined [20], as well as the stable end product of LPO — malondialdehyde (MDA) [21]. The study was conducted on an A-25 biochemical analyzer (BioSystems SA).

At the third stage, to study the mechanism of action of phenylcarbamate, the activity of cholinesterase in the blood and brain was evaluated. Acetylcholinesterase activity was determined by the Ellman method [22]. The experimental animals were divided into two groups: the control in the number of 30 heads and the experimental in the number of 30 heads. Male rats from the experimental group were intraperitoneally injected with phenylcarbamate at a dose of 1 mg/kg bw. Animals from the control group were injected intraperitoneally with 0.9% sodium chloride solution. After decapitation, blood and brain tissues were taken from the studied groups at certain time intervals: 10, 30, 60 min, 6 and 24 h. Six animals were involved at one time point.

Statistical processing of the experimental data obtained was carried out using the Statistica v.10 statistical analysis software. A parametric analysis of variance (ANOVA) was used to assess the reliability of differences between the groups in functional and biochemical studies. The nonparametric Mann–Whitney criterion was used to assess the reliability of differences in the dynamics of changes in acetylcholinesterase activity in the brain and in the blood of rats.

RESULTS

The main characteristics of the convulsive syndrome observed with intraperitoneal administration of phenylcarbamate at a dose of 1 mg/kg bw, in comparison with the effects observed following administration of corazol and thiosemicarbazide (TSC), are presented in Table 1.

The time of onset of the latent period of seizures in animals from the group receiving phenylcarbamate at a dose of 1 mg/kg bw is comparable to a similar parameter in male rats receiving corazol at a dose of 65 mg/kg bw (Table 1). A longer latent period of seizure appearance, manifested in the form of both clonic and tonic seizures, as well as extensions, was noted only in animals from the group that received thiosemicarbazide. In the group of animals using phenylcarbamate, the intensity of Racine level 5 convulsive syndrome was recorded in 60% of animals comparable to animals from the comparison groups (the use of corazol and thiosemicarbazide). At the same time, the intensity of Racine level 6 convulsive syndrome was recorded in animals from the groups with corazol and thiosemicarbazide models to a greater extent. It should be noted that the duration of convulsive syndrome at Racine levels 5 and 6 in animals from the group with the phenylcarbamate model was comparable to the data in animals from the group with the corazol model of convulsive syndrome. At the same time, in animals from the group with the thiosemicarbazide model, the duration of convulsive syndrome at both Racine levels 5 and 6 was much longer. In addition, the mortality rate in the phenylcarbamate and corazol groups was 20%, compared to 33% in the thiosemicarbazide group.

When evaluating behavior and motor activity using a multi-purpose open field system, it was found that animals treated with phenylcarbamate had a statistically significant

Table 1. Comparative assessment of the latency period and duration of convulsive syndrome of the studied convulsants

№	Parameter	PC, 1 mg/kg bw $n = 20$	Corazol, 65 mg/kg bw $n = 20$	TSC, 8 mg/kg bw $n = 20$
1	The latent period of seizures, min	5.33 ± 0.33	8.11 ± 0.6	106.8 ± 7.34
2	Duration of convulsive syndrome at level 5 according to Racine, min	30 ± 0.6	35 ± 1.9	129 ± 9.0
3	Duration of convulsive syndrome at Racine level 6, min	13 ± 1.5	12 ± 1.0	150 ± 7.1

Table prepared by the authors based on their own data

Note: the data is presented as the average value and the standard error of the average ($M \pm m$).

two-fold inhibition of motor activity parameters 24 h after administration compared to the control group of animals. In addition, when corazol was administered, a statistically significant increase in the total motor activity by 2.4 times, the number of horizontal movements by 4.3 times, and grooming acts by 2 times was observed compared to the control. In rats treated with thiosemicarbazide, a statistically significant increase in the total motor activity was revealed by 1.2 times, the number of horizontal movements by 1.9 times, the number of racks by 3.1 times, grooming acts by 1.9 times, average distance by 2.5 times, average speed by 2.6 times, and motor activity on the periphery by 6.6 times.

No statistically significant differences were revealed between grip strength indicators in the animals receiving convulsive doses of phenylcarbamate or other toxicants compared to the control group.

An assessment of cognitive impairment in a CAR test found that animals receiving phenylcarbamate in a convulsive dose registered an increase in the time of the first entry into the dark compartment before training. Thiosemicarbazide showed no statistically significant effect on learning both when the toxicant was administered a day before training and when administered a day after training. Corazole, administered 24 h before training, according to the results obtained, caused a dose-dependent violation of short-term memory by affecting the process of information fixation (memory trace).

When studying the function of the cardiovascular system, a significant decrease in heart rate (HR) was recorded 48 h after administration of phenylcarbamate (403.0 ± 18.8 versus 484.5 ± 8.7 beats per minute in the control group). When assessing the intensity index according to cardiointervalography data after 24 h, significant differences were noted between the groups receiving phenylcarbamate (47715.2 ± 10714.0 vs. 13889.6 ± 4623.0 CU) and thiosemicarbazide (14814.5 ± 6278.8 vs. 73743 ± 16103.0 CU).

Histological examination of brain tissue samples in animals treated with phenylcarbamate revealed a large number of elongated dark cortical neurons without a clear core boundary 24 h after administration; the nucleolus was not visualized compared to the control group (Fig. 1).

Focal rarefaction of the neuropile was observed in the white matter, mainly due to cell processes (astrocytes, oligodendrocytes, or neurons). The veins in the white matter are stretched, filled with red blood cells that are not stained with eosin well, without clear boundaries. Most neurons of the stem are dark and wrinkled; however, against this background there are individual cells in a state of acute swelling (Fig. 2). In the tissues of the cerebellum, Purkinje cells are dark; the nucleus and nucleolus are not defined. Foci of parenchymal hemorrhage, loss of cells, foci of elective necrosis are observed. The respective data is shown in Fig. 3.

It is noteworthy that phenylcarbamate, which does not lead to the development of pronounced disorders of motor activity and cognitive functions in the first 24 h after administration (unlike the toxicants of the comparison groups, i.e., corazol and thiosemicarbazide), when evaluated in open field and CAR tests, also negatively affects a number of biochemical parameters after two weeks after exposure. At the same time, after exposure to corazole, individual and less pronounced similar changes were observed.

The data on the biochemical composition of the blood of experimental animals for 24 h and for a longer period are presented in Table 2. After exposure to phenylcarbamate, an increase in triglycerides was recorded in the first days, whose level normalized by the end of the experimental observation period. Some of the changes occurred according to the type of decompensation: an excessive increase in the concentration of a biochemical indicator and its subsequent sharp decrease in response to exposure to a toxicant. At the same time, on the 14th day, a decrease in the average values was revealed for such indicators as ALT, LDH. In addition, on the 14th, day an increase in alkaline phosphatase, urea, and bilirubin, as well as a decrease in cholesterol levels were observed.

The evaluation of the ionic composition of blood (Table 3), characterizing, among other things, kidney function, found that the changes caused by phenylcarbamate on the first day (a decrease in the concentration of sodium and magnesium ions) are similar to those observed after exposure to thiosemicarbazide. This may indicate excessive ion consumption at the time of convulsive syndrome.

Since phenylcarbamate is an acetylcholinesterase (AChE) inhibitor, it was necessary to assess the level of its effect on the activity of AChE in the developed model of convulsive syndrome. Table 4 shows the results of

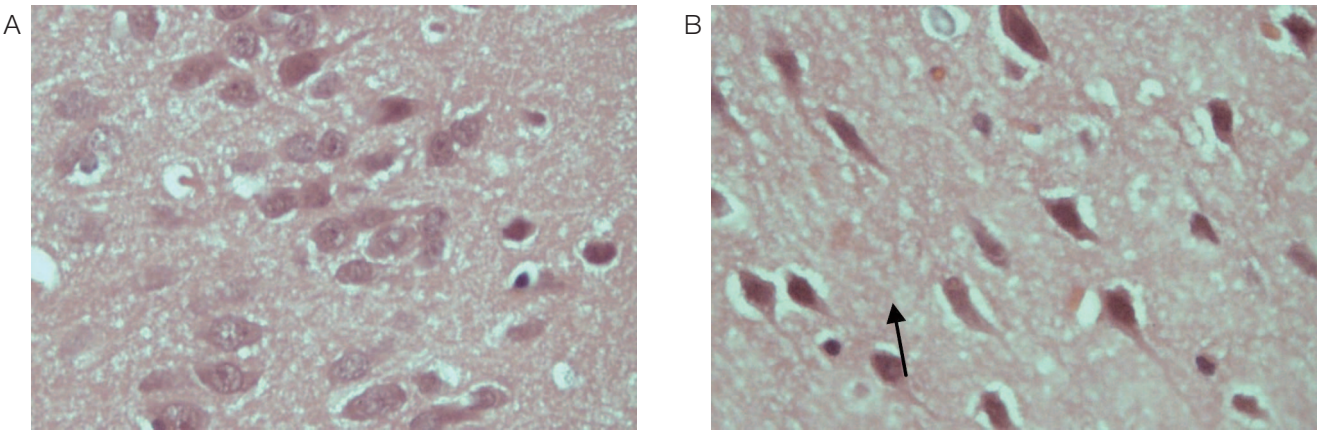

Figure prepared by the authors using their own data

Fig. 1. The cortex of the frontal lobe of the rat brain (magn. ×400)
A is a control animal; B — 24 h after administration of phenylcarbamate.
Note: the arrow indicates a dark elongated neuron without a clear core boundary.

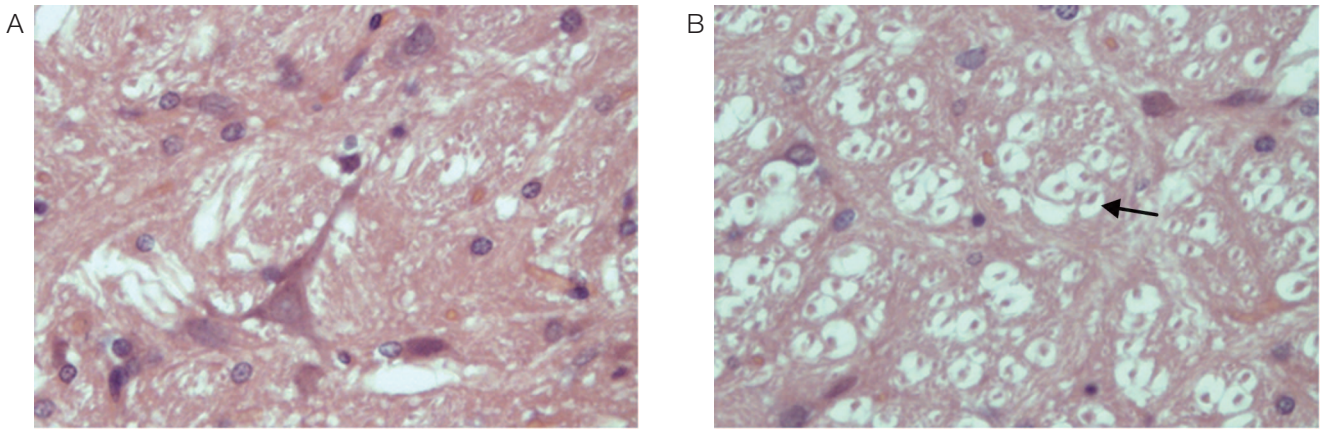

Figure prepared by the authors using their own data

Fig. 2. Rat brain stem (magn. ×400)
A is a control animal; B — 24 h after administration of phenylcarbamate.
Note: the arrow indicates cells in a state of acute swelling.

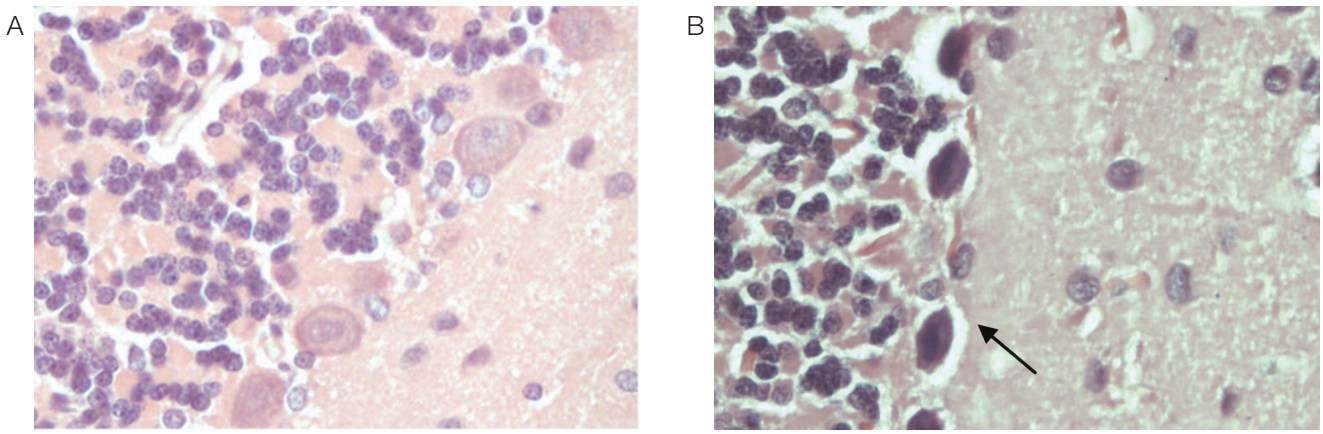

Figure prepared by the authors using their own data

Fig. 3. Rat cerebellum (magn. ×400)
A is a control animal; B — 24 h after administration of phenylcarbamate.
Note: the arrow indicates dark Purkinje cells, the nucleus and nucleolus are not defined.

determining the AChE level in whole blood and the percentage of inhibited AChE in the blood and brain after administration of phenylcarbamate in a convulsive dose.

An analysis of the data presented in Table 4 revealed significant inhibition of AChE in the brain and in the whole blood of white rats within 6 h after administration of phenylcarbamate. After 24 h, the enzyme activity was completely restored. It is important to note that 6 h after administration of phenylcarbamate, with continued inhibition of AChE by 42.3% and 30.5% in the central nervous system and peripheral blood, respectively, seizures in rats completely stopped.

Additionally, the state of individual components of the antioxidant system and markers of lipid peroxidation in different time periods after exposure to phenylcarbamate at a dose of 1 mg/kg bw was studied.

It was found that on the first day after administration of phenylcarbamate to animals, three of the four studied parameters changed significantly. At the same time, the level of malondialdehyde in the phenylcarbamate group on the first day of observation was reduced by almost twofold compared to the control group. On the 14th day of observation, its level significantly exceeded the control indicators; at the same time, the activity of glutathione peroxidase showed the opposite direction of changes compared to the control (Table 5).

DISCUSSION

Due to their specific characteristics, models using OP-based cholinesterase inhibitors show a greater promise for the development of antidotes than models for testing antiepileptic drugs [10]. According to our findings, the action of phenylcarbamate on the body of experimental animals have a number of specific features that should be considered when both studying the pathogenesis of poisoning and developing respective treatment approaches. The study of the key characteristics of convulsive syndrome in acute poisoning for 2 h demonstrated that, in general, the phenylcarbamate-based model (intraperitoneal administration) is comparable to the corazole-based screening convulsive model common in preclinical research practice. When using phenylcarbamate as a model toxicant, the intensity of seizures was slightly less pronounced (level 5 in >60% of animals) than that in the comparison groups (level 6 intensity with the administration of corazol and thiosemicarbazide). It is important to note that the severity of seizures of level 5 on the Racine scale corresponds to

Table 2. Effect of the studied convulsive toxicants on biochemical parameters in the blood of animals in different time periods

Test characteristic	Observation period	Intact group *n* = 24	PC, 1 mg/kg bw *n* = 24	Corazol, 65 mg/kg bw *n* = 24	TSC, 8 mg/kg bw *n* = 24
Triglycerides, mmol/L	24 h	0.32 ± 0.03	0.52 ± 0.05*	0.45 ± 0.06	0.62 ± 0.04*
	48 h	1.07 ± 0.16	1.24 ± 0.17	0.68 ± 0.06*	0.96 ± 0.08
	7 days	1.43 ± 0.26	0.87 ± 0.08	1.12 ± 0.10	1.32 ± 0.20
	14 days	0.83 ± 0.07	1.09 ± 0.14	1.32 ± 0.13*	0.76 ± 0.09
Lactate dehydrogenase, U/L	24 h	1001.3 ± 114.9	916.4 ± 112.6	870.8 ± 89	754.6 ± 32.3
	48 h	909.2 ± 78.6	894.2 ± 55.2	792.4 ± 52.5	867 ± 62.4
	7 days	894.4 ± 61.2	816.1 ± 83.3	1046.9 ± 79.9	929.9 ± 71.5
	14 days	935.7 ± 67.3	686.8 ± 63.2*	759.4 ± 81.3	783.7 ± 67.4
Alanine-aminotransferase, U/L	24 h	52.8 ± 2.2	56.4 ± 5.4	56.4 ± 2.3	62.5 ± 5.4
	48 h	60.7 ± 3.1	62.1 ± 2.7	65.0 ± 4.4	58.4 ± 2.7
	7 days	71.4 ± 2.5	69.2 ± 3.9	79.4 ± 7.1	70.8 ± 4.1
	14 days	55.5 ± 3.2	43.6 ± 2.1*	48.3 ± 1.6	53.3 ± 6.2
Aspartate aminotransferase, U/L	24 h	132.8 ± 7.6	156.6 ± 12.7	146.0 ± 5.1	146.5 ± 7.7
	48 h	143.5 ± 8.3	153.4 ± 5.2	150.7 ± 7.9	155.2 ± 7.4
	7 days	169.7 ± 7.9	160.9 ± 12.2	169.1 ± 10.9	170,9 ± 10.7
	14 days	140.3 ± 7.4	142.7 ± 6.7	125.7 ± 6.9	150.3 ± 14.4
Alkaline phosphatase, U/L	24 h	215.8 ± 12.5	179.6 ± 11*	198.1 ± 15.3	277.3 ± 20.4*
	48 h	292.0 ± 22.8	328.1 ± 15.9	256.2 ± 25.4	258 ± 29.3
	7 days	302.7 ± 27.9	269.7 ± 23.2	305.3 ± 34.3	343 ± 25.8
	14 days	210.4 ± 18.7	306.5 ± 24.7*	301.9 ± 22*	295 ± 46.1
Cholesterol, mmol/L	24 h	1.61 ± 0.05	1.40 ± 0.08*	1.22 ± 0.08*	1.48 ± 0.08
	48 h	1.14 ± 0.05	1.15 ± 0.06	1.15 ± 0.05	1.24 ± 0.05
	7 days	1.10 ± 0.08	1.03 ± 0.04	1.24 ± 0.07	1.30 ± 0.08
	14 days	1.61 ± 0.07	1.41 ± 0.08*	1.30 ± 0.07*	1.45 ± 0.08
Total bilirubin, μmol/L	24 h	10.2 ± 0.4	12.0 ± 1.0	12.1 ± 1.3	14.1 ± 1.5*
	48 h	18.2 ± 2.8	20.2 ± 1.8	10.5 ± 1.6*	17.6 ± 1.3
	7 days	13.8 ± 2.8	14.4 ± 2.3	19.6 ± 2.1	17.9 ± 2.0
	14 days	12.2 ± 1	17.6 ± 1.8*	18.2 ± 2.7*	14.3 ± 1.3
Urea, mmol/L	24 h	4.4 ± 0.2	3.6 ± 0.3*	4.0 ± 0.3	4.7 ± 0.3
	48 h	4.5 ± 0.3	5.9 ± 0.3*	4.7 ± 0.6	4.2 ± 0.1
	7 days	5.2 ± 0.5	5.4 ± 0.2	5.4 ± 0.2	6.4 ± 0.4
	14 days	4.2 ± 0.2	5.6 ± 0.3*	4.9 ± 0.2*	3.9 ± 0.3

Table compiled by the authors using their own data

Note: the data is presented as the mean and the standard error of the mean ($M \pm m$).
* — confidence probability of differences relative to the control group ($p < 0.05$).

generalized convulsive syndrome in humans, which allows us to recommend phenylcarbamate as an effective toxicant for modeling severe convulsive syndrome in the development of therapeutic treatments.

The revealed changes in heart rate indicate that developers of carbamate poisoning treatments should pay attention to drugs that affect the functions of the heart and blood vessels. At the same time, the developed model based on the administration of phenylcarbamate can serve as a basis for testing appropriate treatment approaches. In general, the recorded changes in ECG parameters agree with the literature data that described the pronounced effect of carbamates and OP on cardiovascular activity [23].

Standard tests for assessing motor activity and cognitive impairment are low-informative when used on the first day after exposure to phenylcarbamate. Therefore, it can

Table 3. The effect of the studied convulsive toxicants on the ionic composition of animal blood in various time periods of convulsive syndrome

Test characteristic	Observation period	An intact group *n* = 24	Corazol, 65 mg/kg bw *n* = 24	TSC, 8 mg/kg bw *n* = 24	PC, 1 mg/kg bw *n* = 24
K^+, mmol/L	24 h	4.7 ± 0.1	4.5 ± 0.1	4.6 ± 0.1	4.9 ± 0.1
	48 h	4.6 ± 0.2	4.6 ± 0.1	4.6 ± 0.1	4.8 ± 0.1
	7 days	4.2 ± 0.1	4.1 ± 0.1	4.0 ± 0.1	4.2 ± 0.1
	14 days	5.2 ± 0.2	5.1 ± 0.1	5.1 ± 0.2	5.7 ± 0.2
Na^+, mmol/L	24 h	160 ± 0.9	153.6 ± 1.5*	157.6 ± 1.4	155.4 ± 1.2*
	48 h	155.3 ± 1.3	157.7 ± 1.7	158.6 ± 1.2	159.0 ± 1.0*
	7 days	162.1 ± 1.3	161.1 ± 1.5	163.5 ± 1.0	162.7 ± 1.2
	14 days	145.2 ± 1.6	142.9 ± 1.9	143 ± 1.7	142.9 ± 1.9
Cl^-, mmol/L	24 h	96.6 ± 1.5	95.1 ± 1.1	99 ± 1.6	99.2 ± 1.1
	48 h	96.9 ± 1.0	94.8 ± 1.7	96.6 ± 0.7	99.2 ± 1.2
	7 days	93.6 ± 1.5	92.6 ± 1.6	92.9 ± 1.2	94.4 ± 1.1
	14 days	93.8 ± 0.7	92.5 ± 1.4	92.3 ± 1.7	96.5 ± 1.1*
Phosphorus (P), mmol/L	24 h	2.89 ± 0.07	2.82 ± 0.08	2.64 ± 0.07*	2.85 ± 0.08
	48 h	2.65 ± 0.05	2.69 ± 0.10	2.49 ± 0.03*	2.94 ± 0.06*
	7 days	2.14 ± 0.07	2.12 ± 0.05	2.14 ± 0.06	2.29 ± 0.07
	14 days	2.85 ± 0.08	2.68 ± 0.06	2.65 ± 0.11	2.63 ± 0.04*
Magnesium (Mg), mmol/L	24 h	1.37 ± 0.25	0.35 ± 0.07*	0.94 ± 0.01	0.27 ± 0.02*
	48 h	1.23 ± 0.19	0.89 ± 0.02	0.96 ± 0,02	0.90 ± 0.02
	7 days	1.27 ± 0.19	0.88 ± 0.03	1.19 ± 0.18	0.90 ± 0.02
	14 days	0.56 ± 0.03	0.63 ± 0.02	0.57 ± 0.05	0.67 ± 0.02*

Table compiled by the authors using their own data

Note: the data is presented as the mean and the standard error of the mean ($M \pm m$).
* — confidence probability of differences relative to the control group ($p < 0.05$).

Table 4. Dynamics of changes in acetylcholinesterase activity in the brain and blood of white rats after administration of phenylcarbamate

Time	PC (*n* = 6)		Control (*n* = 6)		% inhibition, blood	% inhibition, brain
	blood AChE, U/mL	brain AChE, U/mg	blood AChE, U/mL	brain AChE, U/mg		
10 min	463.1 [365.4; 480.5]*	35.1 [32.7; 38.5]*	757.4 [606.4; 796.4]	78 [70.7; 80.4]	38.9 [36,6; 51,8]	54.9 [50.6; 58.1]
30 min	370.9 [328.8; 396.2]*	66.3 [59.9; 79.7]*	563.7 [556.9; 634.9]	88.4 [87; 92.7]	34.2 [29.7; 41.7]	25.1 [9.9; 32.3]
60 min	441.1 [292.1; 494.7]	63.4 [51.3; 70.6] *	690.1 [469.2; 700.0]	92 [90.6; 104.5]	36.1 [28.3; 57.7]	31.0 [23.2; 44.2]
6 h	492.4 [418.8; 543.8]	68.1 [55.0; 86.2]	698.7 [686.8; 777.6)	120.3 [108; 144.6]	29.5 [22.2; 56.9]	43.4 [28.3; 54.3]
24 h	946.3 [398.6; 996.6]	123.9 [111.8; 134.6]	763.6 [629.8; 798.3]	117.3 [109.9; 126.3]	-23.9 [-30.5; 47.8]	-5.6 [-14.7; 4.8]

Table compiled by the authors using their own data

Note: the data is presented as median (Me), upper (UQ), and lower (LQ) quartiles.
* — confidence probability of differences relative to the control group ($p < 0.05$).

Table 5. Effect of phenylcarbamate on the level of malondialdehyde and indicators of the antioxidant system in the blood of laboratory animals

Experimental groups	Observation period	Test characteristic			
		RG, μmol/L	MDA, nmol/mL	SOD, U/g Hb	GP, U/g Hb
Control	24 h	0.28 ± 0.02	142.3 ± 8.3	1131.0 ± 51.3	34.7 ± 1.3
PC		0.36 ± 0.02*	79.6 ± 10.9*	1331.1 ± 70.8	42.5 ± 2.7*
Control	48 h	0.13 ± 0.04	188.3 ± 11.8	1274.9 ± 141.2	27.7 ± 2.4
PC		0.21 ± 0.03	151.3 ± 4.2*	1494.5 ± 194.1	33.7 ± 3.9
Control	7 days	0.41 ± 0.06	122.6 ± 3.9	1297.9 ± 76.6	35.8 ± 1.0
PC		0.40 ± 0.04	153.1 ± 9.7*	1096.7 ± 81.0	32.3 ± 1.6
Control	14 days	0.39 ± 0.03	164.0 ± 12.2	1702.8 ± 66.9	35.6 ± 1.1
PC		0.34 ± 0.07	197.6 ± 8.2*	1504.8 ± 59.7	31.6 ± 1.3*

Table compiled by the authors using their own data

Note: the data is presented as the mean and the standard error of the mean ($M \pm m$).
* — confidence probability of differences relative to the control group ($p < 0.05$).

Table 6. Algorithm for the development of an experimental model of convulsive syndrome with a reversible acetylcholinesterase inhibitor

Type of animals	White mongrel male rats aged 3 months, weighing 150–250 g
Number of animals in the group	At least 6
Model toxicant	Phenyl ether of carbamic acid [13]
Minimum convulsive dose	1 mg/kg bw (0.9% sodium chloride solution as a solvent)
Method for introducing the toxicant into the body	Intraperitoneal
Significant characteristics of convulsive syndrome	Clonic seizures in 100% of cases; latent period of seizures 5–6 min; the intensity of seizures is at least 5 points on the Racine scale for 30 min
When studying the effectiveness of convulsive syndrome therapy (up to 24 h), additional indicators may be taken into account	Elevated levels of triglycerides, reduced glutathione, and glutathione peroxidase activity Reduced concentrations of cholesterol, alkaline phosphatase, urea, Na^+, Mg^{2+}, malondialdehyde; Decrease in heart rate
When studying the effectiveness of therapies for the long-term effects of convulsive syndrome (up to 14 days), additional indicators may be taken into account	Increase in alkaline phosphatase, urea, bilirubin, malondialdehyde Reduction of cholesterol, lactate dehydrogenase, ALT, glutathione peroxidase

Table compiled by the authors using their own data

be assumed that disorders of the nervous system developing after such poisoning have a long and complex pathogenesis, requiring either longer observations or the use of alternative tests in experimental modeling of poisoning with carbamate compounds.

The conducted histological examination of brain tissues confirm that, despite the absence of pronounced deviations in tests for assessing cognitive impairment in experimental animals in the first 24 h after exposure, the introduction of carbamates lead to disorders in the structure of the brain. This information is relevant both to studies into the pathogenesis of poisoning and to the practice of preclinical research in the development of treatment approaches to poisoning.

Some of the revealed changes in biochemical parameters may be associated not only with a set of pathogenetic transformations during intoxication, but also with the individual sensitivity of subjects. In our study, the groups were limited in number, which might have affected the average values of indicators. However, the results of our study are confirmed by scientific data obtained when evaluating the effect of other cholinesterase inhibitors on humans. Thus, a study by Senarathne R. et al. proposed to use AST and ALT as alternative markers of carbamate and OP poisoning [24]. The revealed changes in the activity of liver enzymes, cholesterol, and triglycerides 14 days after exposure to phenylcarbamate indicate a violation of liver function. An increase in the concentration of urea in the blood can be associated with both impaired liver function and damage to muscle tissues. In addition, in the process of massive protein breakdown accompanied by hyperammonemia, toxic ammonia can cause the development of a number of neurological disorders.

When exposed to phenylcarbamate, along with magnesium deficiency, the parameters of heart rate changed. Therefore, it can be assumed that these changes contribute to convulsive activity independent of interaction with AChE, given that hypomagnesemia is frequently associated with myocardial hyperexcitability, tremor, and fasciculations.

The results of studying the content and function of AChE with the administration of phenylcarbamate are quite expectable, confirming the effectiveness of using this compound as a model toxicant in experimental modeling of convulsive syndrome in the setting of poisoning with cholinesterase inhibitors.

The studied markers of lipid peroxidation and those of the antioxidant protection system confirmed the data on the complex mechanism of the development of seizures and subsequent neurological disorders after carbamate poisoning. The previously detected change in the level of malondialdehyde, formed during lipid peroxidation and associated with changes in the lipid profile [25], correlates with the changes in triglyceride and cholesterol concentrations were established in our study. There were differences in the level of MDA for the first day in the phenylcarbamate group compared to the control group. However, starting from the second day and up to the 14th day, an increase in this indicator was observed compared to the control group. An increase in the levels of GP and RG on the 1st and 2nd days of observation was noted in the group of animals treated with phenylcarbamate, compared to the control group. This confirms impaired liver function and cardiovascular system, as well as the development of an inflammatory reaction in the setting of carbamate poisoning, accompanied by activation of antioxidant mechanisms.

Generalizing the results of our study into the development of an experimental model of convulsive syndrome based on the introduction of phenylcarbamate into the body, the following algorithm of its use can be proposed (see Table 6).

CONCLUSION

The developed experimental model of convulsive syndrome based on phenylcarbamate at a dose of 1 mg/kg bw has demonstrated acceptable characteristics compared to existing models. Therefore, this model can be recommended for preclinical studies when studying the pathogenesis of carbamate poisoning, developing means of relieving convulsive syndrome in acute poisoning, and developing approaches to prevention and treatment of long-term consequences of poisoning in survivors.

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GENERAL TOXIC EFFECT OF A CHITOSAN-BASED HEMOSTATIC AGENT IMPLANTED IN RATS

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Introduction. Hemostasis of ongoing bleeding during cavitary surgical interventions is an urgent problem both in civil and military healthcare. The development of new effective and affordable agents for hemostasis of internal bleeding and their introduction into clinical practice may contribute to increasing the survival of injured patients.

Objective. Study of general and local toxic effects of a local hemostatic agent (LHA) for intracavitary application.

Materials and methods. The study was performed on 20 mongrel rats (10 males and 10 females) weighing 180–220 g. The experimental group of animals was implanted with the LHA into the abdominal cavity at a dose of 512 mg/kg body weight (bw). The animals of the control group underwent surgery without LHA implantation. Data analysis was performed using the Microsoft Excel 2013 and Statistica 10.0 software applications.

Results. The health status, body weight, food and water consumption, and mass coefficients of internal organs in the experimental animals did not differ from those in the control group. The hematological and biochemical blood parameters showed values within the reference norm. The macro- and microscopic examination of the internal organs revealed a local irritant effect of the agent under study.

Conclusion. The laboratory animals tolerated the intraperitoneal implantation of the tested local hemostatic agent at a dose of 512 mg/kg bw well. A further study of its toxic properties and effectiveness is validated.

Keywords: local hemostatic agent; safety; general toxic effects; chitosan; implantation

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ИЗУЧЕНИЕ ОБЩЕТОКСИЧЕСКОГО ДЕЙСТВИЯ У КРЫС ПРИ ИМПЛАНТАЦИИ ГЕМОСТАТИЧЕСКОГО СРЕДСТВА НА ОСНОВЕ ХИТОЗАНА

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Введение. Остановка продолжающегося кровотечения при выполнении полостных оперативных вмешательств является актуальной проблемой хирургии как гражданского, так и военного здравоохранения. Разработка и внедрение в клиническую практику нового эффективного и доступного средства для остановки внутреннего кровотечения будет способствовать повышению выживания пострадавших.

Цель. Изучение общетоксического и местного токсического действия местного гемостатического средства (МГС) для внутриполостного применения.

Материалы и методы. Исследование проведено на 20 беспородных крысах (10 самцов и 10 самок) массой 180–220 г. Опытной группе животных местное гемостатическое средство имплантировали в брюшную полость в дозе 512 мг/кг массы тела (м.т.). Животным контрольной группы проводили операцию без имплантации МГС. Анализ данных осуществляли с помощью программ Microsoft Excel 2013 и Statistica 10.0.

Результаты. Результаты оценки состояния опытных животных, их массы тела, кормо- и водопотребления и массовые коэффициенты внутренних органов не отличались от результатов контрольных групп. Оценка гематологических и биохимических показателей крови крыс показала отсутствие выхода значений за пределы референтной нормы. При макро- и микроскопическом изучении внутренних органов животных зафиксировано наличие местнораздражающего действия изучаемого образца.

Заключение. Таким образом, лабораторные животные хорошо перенесли внутрибрюшинную имплантацию МГС в дозе 512 мг/кг м.т.; соответственно дальнейшее изучение его токсических свойств и эффективности является перспективным.

Ключевые слова: местное гемостатическое средство; безопасность; общетоксическое действие; хитозан; имплантация

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INTRODUCTION

Meticulous hemostasis during surgical interventions remains a pressing surgical issue in the 21st century. This problem is particularly significant for wounded and polytrauma patients, in whom timely hemostasis affects not only the timing of treatment, but also determines the overall outcome. The statistics of such injuries is contributed by road traffic accidents, negative crime situation, terrorist attacks, and military conflicts. Thus, a study by Trukhan et al. [1] found that during terrorist attacks in St Petersburg and Minsk, fatal injuries as the immediate cause of death were registered only in 33.3% of cases, while irreversible blood loss was the immediate cause of death in 66.7% of the deceased. The most frequent (almost 87.5%) cause of death in the victims was internal bleeding. In the structure of deaths from injuries received during armed conflicts, 78.1% of cases accounted for ongoing hemorrhage, with more than a half of the cases resulting from internal bleeding [2–6].

Although internal bleeding is almost impossible to stop at the pre-hospital stage, even timely patient transport to the operating room requires immediate control of bleeding. In addition to traumatic shock and hemorrhage, a cascade of pathophysiological reactions develops, including hypothermia, hypocoagulation, and acidosis (lethal triad). Early stopping of bleeding is a prerequisite for successful treatment of the wounded not only in the acute period of traumatic shock, but also in the third period of traumatic disease (development of infectious complications) [1]. At the same time, technical difficulties in intraoperative stopping of bleeding arise not only in urgent surgery (trauma and wounds), but also in elective surgery, particularly in patients with concomitant coagulopathy or those taking antiplatelet drugs and anticoagulants [7–10].

Various methods are currently used to stop hemorrhages occurring during cavitary surgeries, including wound closure, tight tamponade, electrocoagulation of various types, and local hemostatic agents in some cases (LHA) [11]. For intracavitary application, LHAs based on fibrinogen, cellulose, collagen, fibrin, thrombin, plant extracts, mucopolysaccharides (starches), etc., can be used [12].

Due to its wide availability and biocompatibility, chitosan is a promising substance for the preparation of LHAs. Materials based on chitosan and/or modified chitosan (suspensions, sponges, bandages, etc.) were found to be effective for stopping bleeding *in vitro*, promoting coagulation by activating platelets and/or agglutinating erythrocytes [13–17]. Previously, we demonstrated the high efficacy of a chitosan-based LHA in a model of intensive intra-abdominal bleeding in large biological objects [18].

To date, the action of chitosan and its derivatives has been extensively studied. However, before introduction into clinical practice, each newly developed polymer should be studied not only in terms of its efficacy, but also with regard to its safety for the potential patient due to the wide variability of formulation components. In this context, safety refers to both biocompatibility with the morphological structures in the area of direct contact and general toxicity to the organism. Such studies are also important due to the possible residue of the material in the human body.

In this study, we set out to investigate the general and local toxic effects of a hemostatic agent based on 90% lactic acid chitosan, 5% glycerol, and 5% calcium chloride for intracavitary application.

MATERIALS AND METHODS

The study was carried out using the facilities of Golikov Research Center of Toxicology (Saint Petersburg, Russia). In total, 20 mongrel rats (10 males and 10 females) weighing 180–220 g provided by the Rappolovo Laboratory for Animal Nursery of the National Research Center "Kurchatov Institute" were divided into experimental and control groups. The experimental group included male and female animals which were implanted with the local hemostatic agent (LHA) under study. The control group included male and female falsely operated animals. Animals were assigned to groups by sex and using the body weight as the main criterion such that the individual body weight value did not deviate from the mean value within the same sex by more than 10%. Prior to the study, all animals were adapted to the housing conditions for five days. During this period, the general condition of the rats was monitored daily by visual inspection.

The dose of LHA to be administered to animals was calculated based on the dose used in an efficacy study, 90 mg/kg (per finished dosage form, FDF) [18]. Considering the metabolic coefficient, the value of LHA dose for rats was: 90×37 (metabolic coefficient for a 60 kg human)/6.5 (metabolic coefficient for a 200 g rat) ≈ 512 mg/kg [19]. Thus, the investigated dose of the study sample administered to rats was 512 mg/kg bw. The mass of implanted LHA and, accordingly, the drug dose for each animal was calculated based on the value of its weight. Sterile samples

(radiation sterilization) of LHA were used in the study. LHA were implanted into the abdominal cavity of rats in native form. LHA are plates of light-yellow color with a relief surface and a porous structure, 4–6 mm thick. The appearance of the product is presented in Fig. 1.

The animals in the control group underwent surgical intervention without implantation of the test sample (falsely operated animals).

Injectable general anesthetic tiletamine/zolazepam (Zoletil® 100) was used as anesthesia during LHA implantation. This medication has a good analgesic and sedative effect. The medication was administered intraperitoneally at a dose of 50 mg/kg bw; complete relaxation and absence of pain sensitivity occurred 10–15 min after drug administration. The average duration of anesthesia was 60–90 min.

The surgical field was prepared according to the generally accepted rules of aseptic and antiseptic surgery. Lidocaine solution for injection 20 mg/mL in the volume of 0.2 mL was injected subcutaneously into the incision area for anesthesia. During anesthesia, the eyes of the animals remained open and a Vidisik® eye gel was applied to prevent the development of dry keratoconjunctivitis.

Surgical procedure. To perform a midline laparotomy, the animal was in the dorsal position. The skin incision was performed along the midline from the umbilicus in caudal direction with the length of about 1 cm, then blunt dissection of tissues up to visualization of the white line of the abdomen was performed. The peritoneum was dissected with a scalpel, the edges of the abdominal wall were grasped with tweezers and lifted upwards for convenient visualization of the abdominal organs of the rat. It should be noted that LHA was applied in a dry form; the studied material was freely placed in the abdominal cavity (Fig. 2).

Then the abdominal cavity was stitched layer by layer (Fig. 3). The experimental animals were placed in a heated cage with a warm floor for recovery after anesthesia. Full awakening of the animals occurred 2–3 h after induction of anesthesia.

During the study, we evaluated the animal survival rate, clinical picture of intoxication, body weight dynamics, water and feed consumption, hematological and biochemical parameters. In addition, the macro- and microscopic examination of internal organs was carried out along with calculation of the mass ratios of organs and evaluation of local reactions after LHA implantation [20].

The general condition of the rats was evaluated daily; deviations in the consumption of food and water by animals in separate cages were noted. Body weight was monitored one day before LHA implantation and then weekly after introduction into the experiment. The body weight of the starved animal immediately before necropsy was taken to calculate the percentage of internal organ weight to animal body weight. Animals were deprived of food the night before necropsy. Access to water was not restricted.

Blood sampling for studying hematological and biochemical parameters was performed on the 29th day of the study by exsanguination of animals after inhalation of carbon dioxide (CO_2).

Biological material was collected from animals after 14–15 h of fasting. Blood from rats in the amount of 0.5 ml was collected in blood collection tubes with ethylenediaminetetraacetic acid (EDTA). The blood sample was thoroughly mixed and placed in a refrigerator.

The sample was analyzed 120 min after collecting the biological material. For general clinical blood analysis, an automatic hematological analyzer Advia 2120 by Siemens (Germany) was used. The hematological analyzer is fully automated for counting blood cells and erythrocyte indices [21].

To assess biochemical parameters, blood was collected in a dry vacutainer. To obtain blood serum, whole blood samples were centrifuged at 3000 rpm at 4°C for 10 min followed by collection of the supernatant fluid (serum). Biochemical parameters (total protein, urea, creatinine, glucose, cholesterol, total bilirubin, alanine aminotransferase ALT, aspartate aminotransferase AST, alkaline phosphatase ALP) were determined on an A-25 biochemical

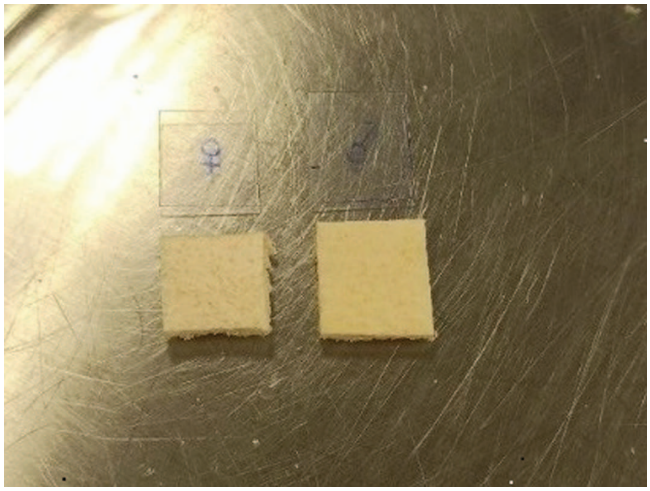

Figure prepared by the authors

Fig. 1. Appearance of the implemented local hemostatic agent (LHA)

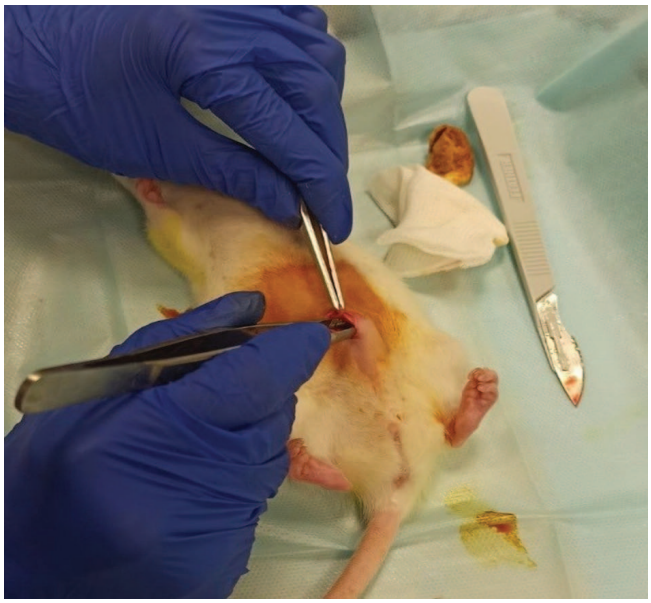

Figure prepared by the authors

Fig. 2. Implantation of the local hemostatic agent (LHA) into the abdominal cavity of a rat

analyzer from BioSystems (Spain) using Vector-Best JSC kits (Russia). The internal quality control of the studies was performed using control materials from Vector-Best JSC (Russia) [22].

Inhalation (CO_2) followed by exsanguination was used as the euthanasia method. An Open Science euthanasia unit (Russia) was used for euthanasia [23].

On day 29 of the study, the animals were subjected to a complete necropsy, which included an examination of the external surface of the body, all passages, cranial, thoracic, abdominal cavities, and their contents. During the planned necropsy, the brain, heart, lungs (pair), liver, adrenal glands (pair), kidneys (pair), spleen, ovaries/testes (pair), and thymus were weighed in all the studied animals. Paired organs were weighed together. In addition to the absolute weight of the organs, the ratio of the organ weight to the body weight of the animal was calculated, presented in percentiles (‰). A histological analysis of the isolated organs and tissues was performed in the animals with an additional assessment of the local effect of LHA after its implantation (peritoneal histology). Tissue samples were collected, which were dehydrated and impregnated with paraffin. The prepared sections were stained with haematoxylin and eosin. Histological preparations were examined by light microscopy using a Leica DM1000 microscope (Germany) under ×100 magnification.

A mathematical and statistical analysis of the results was performed using the Microsoft Excel 2013 and Statistica 10.0 software packages. The Mann–Whitney test (U-test) was used to assess the differences between the two groups [24]. Descriptive statistics were presented as a measure of central tendency and dispersion indicators. The following descriptive statistics of quantitative indicators were calculated: median (Me), upper (UQ), and lower (LQ) quartiles. Differences in the indicators of the experimental animals in relation to the control were considered statistically significant at $p \leq 0.05$.

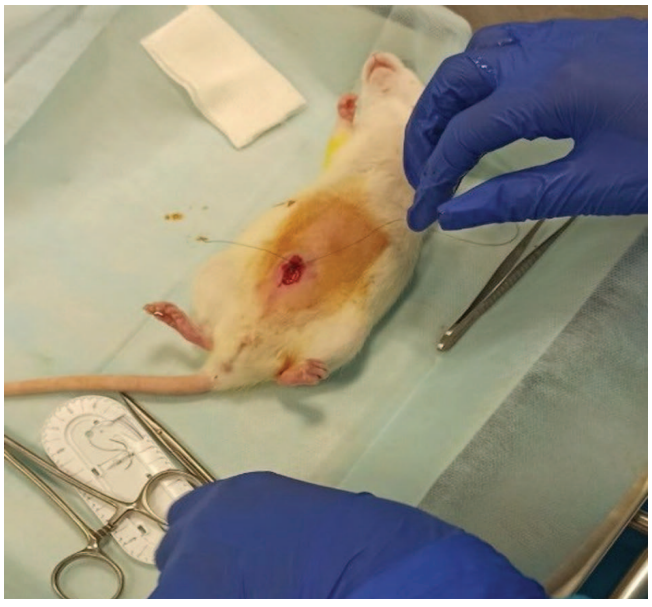

Figure prepared by the authors

Fig. 3. Suturing of the rat peritoneum after implantation of the local hemostatic agent

RESULTS

After intraperitoneal implantation of the studied sample of LHA in rats at a dose of 512 mg/kg bw, no lethal outcomes were registered. The animals did not differ from the control group in appearance and behavior. The death of one male in the control group of falsely operated animals due to postoperative complications was registered. The clinical condition of the experimental animals was satisfactory during the entire period of observation. Signs of intoxication were not observed in any animal. Water and feed consumption in rats from experimental and control groups did not differ.

The effect of the investigated sample on the body weight of sexually mature animals was estimated by a comparative assessment of the body weight of rats after LHA implantation and falsely operated animals at several examination stages: base (one day before implantation of LHA), 1 week, 2 week, 3 week, and 4 week of the experiment.

The data on body weight of rats in the studied groups are presented in Figs. 4 and 5. It is important to note that the initial (base) body weight of both females and males did not differ significantly between the LHA-implanted and control groups.

The comparative assessment of body weight of male rats after implantation of the studied sample and the weight of animals from the control group in the observation period, base (day before implantation), 1 week, 2 week, 3 week, and 4 week, showed no significant differences between the studied groups using the Mann–Whitney test. At the same time, statistically significant differences in the body weight of female rats from the experimental group at 2 weeks of observation ($p = 0.022$), 3 weeks ($p = 0.034$), and 4 weeks ($p = 0.012$) compared to the control were revealed. An analysis of the data presented in Fig. 5, showed that the values of body weight of female rats from the experimental group were statistically significantly lower than those in the control group at 2 weeks by 7.4%, at 3 weeks by 5.2%, at 4 weeks by 9.9%, although the values did not exceed the reference values for this type of animals.

Table 1 shows the data of hematologic parameters in animals of the control and experimental groups. An analysis of the values of hematological parameters in male rats of the experimental group revealed a statistically significant increase in monocyte percentage by 18% and absolute monocyte count of 42% compared to the control group of animals. At the same time, the study and subsequent evaluation of the values of hematologic parameters in females in the experimental group compared to the control revealed a statistically significant decrease in hemoglobin concentration and mean corpuscular volume by 5% and 7%, respectively [25].

Biochemical parameters were studied following 29 days after the onset of the experiment in control animals (males and females), as well as in animals of the experimental group with implanted LHA (Table 2). It was found that the group of male rats after LHA implantation showed a significant increase in the aspartate aminotransferase activity by 18% compared to the control group.

The control group of animals showed no changes in the peritoneal surface. In the experimental group, in one case, a fistula formation of the anterior abdominal wall was noted,

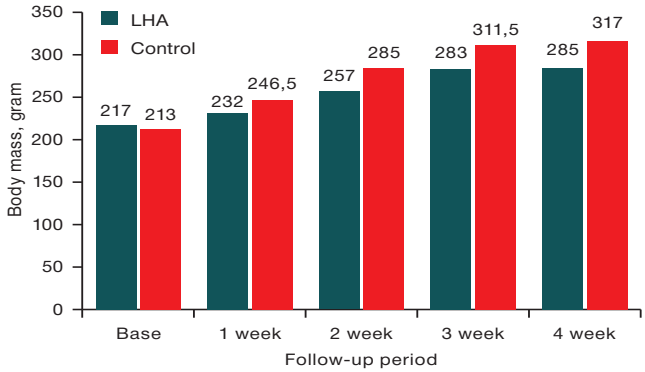

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Fig. 4. Body weight of male rats at different stages of examination
Note: data are presented as median values.

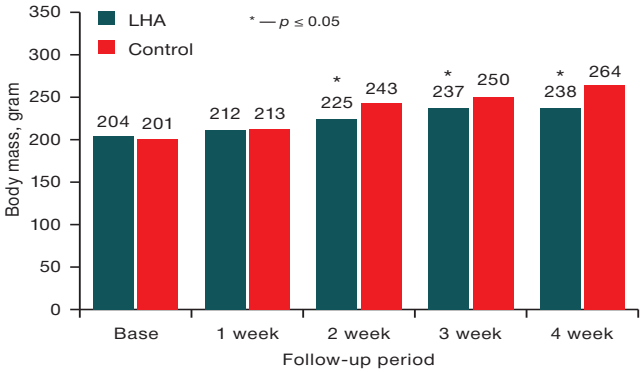

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Fig. 5. Body weight of female rats at different stages of examination
Note: data are presented as median values.

the formation of which was caused by the reaction of foreign body rejection.

When examining the internal organs, thymus had a triangular shape, whitish in color and moderately dense in consistency. The size and shape of the heart were not changed. The surface of the lungs had a pale pink color; the lungs were collapsed at the opening of the chest. The spleen had a dark cherry color, smooth surface, and dense consistency. The stomach had the usual shape and size; the lumen was filled with dense food contents. The mucosa of the small and large intestine was shiny and smooth. The pancreas had a pale pink color and a lobular structure. The size and shape of the liver, kidneys, adrenal glands, testes, and ovaries remained unchanged. The brain substance had a moderate density; no ventricular dilatation was observed.

Table 1. Comparative evaluation of hematological parameters of male and female rats in experimental and control groups 29 days after the onset of the experiment

Indicator	Control (♂)	LHA, 512 mg/kg bw (♂)	Control (♀)	LHA, 512 mg/kg bw (♀)
Number of animals in the group	4	5	5	5
White blood cell count, 10^3/μL	9.68 [8.17; 11.7]	10.84 [10.19; 12.71]	7.51 [7.43; 8.16]	7.99 [7.09; 8.94]
Red blood cell count, 10^6/μL	7.58 [7.52; 8.2]	7.58 [7.55; 7.91]	7.36 [7.31; 7.37]	7.42 [7.31; 7.63]
Hemoglobin, g/dL	14.95 [14.2; 15.95]	14.80 [14.80; 15.00]	14.30 [14.10; 14.50]	13.60* [13.0; 13.8]
Hematocrit, %	42.25 [41.25; 46.0]	42.50 [42.40; 43.20]	41.90 [41.40; 42.20]	40.30 [39.0; 41.2]
Mean corpuscular volume, fL	55.20 [54.5; 56.65]	55.90 [54.6; 56.4]	57.20 [57.0; 57.3]	53.40* [52.60; 55.6]
Platelet count, 10^3/μL	787.00 [686; 928]	826.00 [601; 978]	983.00 [962; 1022]	970.00 [946.0; 1092.0]
Mean platelet volume, fL	6.85 [6.55; 7.75]	6.90 [6.70; 7.10]	7.10 [7.0; 7.20]	7.20 [7.10; 7.30]
Neutrophil %	28.20 [20.75; 38.25]	25.80 [23.20; 25.90]	21.10 [20.50; 32.40]	35.50 [28.80; 47.40]
Lymphocyte %	66.95 [56.0; 72.85]	66.60 [65.30; 68,20]	70.70 [61.40; 74.90]	59.40 [46.40; 65.10]
Monocyte %	3.45 [2.70; 3.65]	4.20* [4.10; 4.50]	2.80 [2.70; 3.00]	2.70 [2.10; 2.70]
Eosinophil %	0.85 [0.75; 1.60]	0.80 [0.80; 1.10]	1.20 [1.20; 1.50]	1.10 [0.90; 2.10]
Basophil %	0.90 [0.75; 1.20]	1.00 [0.80; 1.20]	0.70 [0.60; 1.80]	0.40 [0.40; 0.50]
Unidentified cells, %	0.75 [0.60; 1.00]	1.10 [1.00; 2.00]	0.90 [0.90; 1.10]	0.90 [0.60; 0.90]
Abs. neutrophil count, 10^3/μL	3.22 [2.01; 3.86]	2.64 [2.33; 2.80]	2.06 [1.50; 2.54]	3.25 [2.30; 3.55]
Abs. lymphocyte count, 10^3/μL	6.82 [5.02; 7.80]	7.08 [6.78; 7.28]	5.48 [5.01; 5.74]	4.15 [3.18; 5.35]
Abs. monocyte count, 10^3/μL	0.30 [0.28; 0.33]	0.52* [0.40; 0.55]	0.21 [0.20; 0.24]	0.19 [0.17; 0.25]
Abs. eosinophil count, 10^3/μL	0.09 [0.08; 0.15]	0.09 [0.07; 0.14]	0.09 [0.09; 0.11]	0.08 [0.08; 0.09]
Abs. basophil count, 10^3/μL	0.10 [0.08; 0.11]	0.11 [0.10; 0.11]	0.05 [0.05; 0.18]	0.03 [0.02; 0.05]
Unidentified cells, 10^3/μL	0.07 [0.07; 0.09]	0.14 [0.10; 0.19]	0.08 [0.07; 0.09]	0.06 [0.05; 0.07]

Table prepared by the authors using their own data

Note: data are presented as Me [LQ; UQ].
* — statistical significance of differences between control and experimental groups, $p < 0.05$.

Table 2. Comparative evaluation of biochemical parameters of male and female rats of experimental and control groups 29 days after the onset of the experiment

Indicator	Control (♂)	LHA, 512 mg/kg bw (♂)	Control (♀)	LHA, 512 mg/kg bw (♀)
Number of animals in the group	4	5	5	5
Total protein, g/L	81.4 [75.9; 87.5]	74.5 [71.9; 77.0]	77.8 [77.6; 85.9]	80.4 [79.0; 81.0]
Urea, mmol/L	6.0 [5.3; 7.3]	8.7 [7.6; 9.0]	7.5 [7.0; 8.9]	10.0 [8.1; 10.8]
Creatinine, μmol/L	54.0 [50.5; 62.5]	55.0 [55.0; 55.0]	50.0 [49.0; 52.0]	64.0 [63.0; 70.0]
Glucose, mmol/L	8.8 [8.2; 9.9]	9.2 [9.0; 9.4]	10.5 [9.8; 10.7]	9.8 [9.5; 9.9]
Cholesterol, mmol/L	2.5 [2.2; 3.0]	2.6 [2.1; 2.8]	2.8 [2.4; 3.1]	2.3 [2.2; 2.7]
Total bilirubin, μmol/L	1.7 [1.4; 1.7]	1.5 [1.3; 2.2]	1.2 [0.9; 1.4]	0.6 [0.2; 1.1]
Aspartate aminotransferase (AST), U/L	166.0 [148.6; 171.3]	196.5* [195.9; 200.6]	73.7 [54.4; 74.6]	102.0 [73.3; 114.4]
Alanine aminotransferase (ALT), U/L	76.0 [66.5; 86.0]	81.0 [79.0; 90.0]	70.0 [69.0; 77.0]	69.0 [65.0; 71.0]
Alkaline Phosphatase, U/L	349.0 [265.5; 428.5]	297.0 [275.0; 315.0]	188.0 [182.0;224.0]	236.0 [210.0;283.0]

Table prepared by the authors using their own data

Note: data are presented as Me [LQ; UQ].
* — statistical significance of differences between control and experimental groups, $p < 0.05$.

When evaluating the mass ratios of internal organs of male and female rats after implantation of LHA at a dose of 512 mg/kg bw and control animals using the Mann–Whitney test, no statistically significant differences were found between the studied groups.

A histological examination of the brain, heart, lungs, kidneys, thymus, testes, ovaries, adrenal glands, and spleen preparations of control animals and animals with LHA implantation in the abdominal cavity 29 days after the onset of the study revealed no significant differences between the groups. In the liver of animals of both groups, moderately or sharply expressed fatty dystrophy of hepatocytes was registered: in seven cases in the experimental group with LHA implantation and in six cases in control animals. At the same time, in one animal from the experimental group and in one animal from the control group, minor necroses with moderately expressed lympho-macrophage infiltration were registered. These changes could be caused by the influence of postoperative stress [26].

A histological examination of the peritoneum surface in the experimental group revealed fragments of LHA surrounded by a layer of lymphocytes and leukocytes and young fibrous connective tissue. Leuko-lymphocytic infiltration was also noted (Fig. 7), which are signs of local irritation. In the control group, an insignificant accumulation of lymphocytes and macrophages was registered.

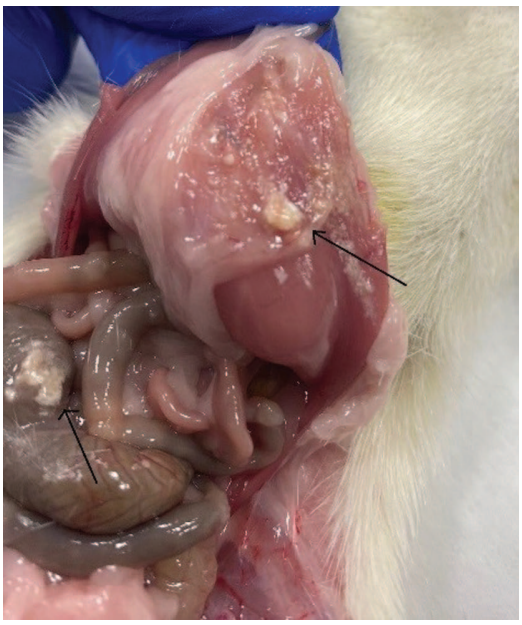

Figure prepared by the authors

Fig. 6. Fragments of the local hemostatic agent in the abdominal cavity of the rat
Note: fragments are indicated by arrows.

DISCUSSION

The conducted study found that intraperitoneal implantation of the tested sample at a dose of 512 mg/kg bw does not lead to the death of animals or to the development of any signs of intoxication.

The animals in the experimental group did not differ from those in the control groups in terms of appearance and behavior. The general condition of the experimental animals was satisfactory throughout the entire experiment;

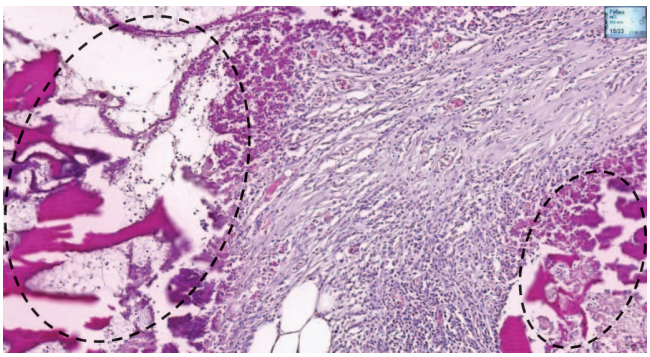

Figure prepared by the authors

Fig. 7. Histological section of rat peritoneum after implantation of local hemostatic agent
Note: on the peritoneal surface, fragments of LHA surrounded by a layer of lymphocytes and leukocytes and young fibrous connective tissue (dashed line). Haematoxylin and eosin staining. Magn. ×100.

the indices of water and feed consumption did not differ from the control. Body weight indices of the male rats did not differ from the control, while those of the female rats were statistically significantly lower compared to the control at several stages of examination. At the same time, the body weight of female animals within their group did not decrease throughout the study. Differences in the body weight of females of experimental and control groups could be related to the effect of postoperative stress.

When analyzing hematological parameters in animals during the experiment, a statistically significant increase in the proportion of monocytes and absolute number of monocytes in male rats of the experimental group compared to the control group was found, probably associated with the inflammatory reaction occurring in the abdominal cavity of rats.

In female rats with implanted LHA, a statistically significant decrease in hemoglobin concentration and mean corpuscular volume was observed compared to the control, apparently caused by the local irritant reaction of the peritoneum. The established increase in the aspartate aminotransferase activity in the group of male rats after LHA implantation compared to the control group is likely to be related to the damage to the animals' liver due to postoperative stress [26].

It should be noted that the fluctuations of hematologic and biochemical parameters in animals did not exceed the reference intervals established for this type of animals [25].

The evaluation of the mass ratios of internal organs of rats after implantation of LHA at a dose of 512 mg/kg bw showed the absence of statistically significant differences between the studied groups. Morphologic macroscopic studies revealed no changes in the structure of internal organs in experimental animals; 29 days after the onset of the experiment, fragments of LHA were observed in the peritoneum and in the mesentery, which formed a dense conglomerate. According to the results of histological evaluation of the local action of LHA implantation, signs of a local irritating effect (compared to the control group of animals) were observed. The formation of a fistula of the anterior abdominal wall in one of the males of the control group suggests that the presented LHA samples should be removed after hemostasis is achieved.

CONCLUSION

The conducted study showed that intraperitoneal implantation of LHA at a dose of 512 mg/kg bw did not cause animal death or signs of intoxication. The body weight of female rats was statistically significantly lower than that of control rats at 2, 3, and 4 weeks of observation. However, the values did not exceed the reference values for this kind of animals.

An intraperitoneal implantation of LHA at a dose of 512 mg/kg bw to male rats, compared to the control, resulted in an increase in the absolute number of monocytes, as well as an increase in the activity of AST, not exceeding the reference values. This could be associated with the local irritating effect of the sample and/or postoperative stress.

The pathomorphological studies revealed no changes in the structure of internal organs of the animals. LHA fragments were found in the peritoneum and mesentery of rats, which formed a dense conglomerate. The histological evaluation of tissues showed signs of a local irritating effect.

The data obtained indicate normal tolerance of animals (rats) to implantation of the studied hemostatic agent in the abdominal cavity at a dose of 512 mg/kg bw, validating its further studies in terms of toxicity and specific activity.

Additional studies are needed to evaluate the biocompatibility and possible biodegradation time of the topical hemostatic agent. The study of biocompatibility and biodegradation of LHA based on chitosan should be carried out on larger laboratory animals (swine) with the purpose of subsequent extrapolation of the obtained results to humans.

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EFFECT OF COMBINATIONS OF ANTIBIOTICS, PHAGES, AND DEPOLYMERASE ON BIOFILMS OF THE DRUG-RESISTANT *KLEBSIELLA PNEUMONIAE* STRAIN

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Introduction. *Klebsiella pneumoniae* poses a serious threat to global healthcare due to the high proportion of multidrug-resistant isolates. Moreover, the formation of biofilms by bacteria significantly complicates the treatment of infections.

Objective. To evaluate the effectiveness of the individual and combined action of antibiotics and bacteriophages or polysaccharide depolymerase on biofilms of a clinically significant strain *K. pneumoniae*.

Materials and methods. The work used the *K. pneumoniae* strain with multidrug resistance (9faiz), 4 antibiotics of various classes (gentamicin, levofloxacin, meropenem and chloramphenicol), 3 bacteriophages of various genera (Dlv622, Seu621 and FRZ284), and 1 polysaccharide depolymerase (Dep622). Experiments were carried out on the formed biofilms by treating 24-hour *K. pneumoniae* films with antimicrobial agents individually or in combinations. The ability of the strain to form biofilms was evaluated by staining with crystalline violet. The comparison between the average optical density values was carried out using a *t*-test and was considered significant at $p \leq 0.05$.

Results. The individual use of antibiotics peak concentrations (C_{max}) or depolymerase concentration of 100 MED (minimum effective dose — MED) did not lead to a significant decrease in biofilm biomass, whereas bacteriophages in a titer of 5×10^9 PFU/mL (plaque-forming unit per mL) statistically significantly reduced its biomass by 27–31% ($p < 0.05$). Most combinations of phages and antibiotics did not lead to a significant increase in the efficiency of biofilm destruction. Only the combination of phage FRZ284 with gentamicin statistically significantly showed an additional decrease in biofilm biomass by 27% ($p < 0.05$). Combinations of depolymerase with all antibiotics except meropenem resulted in a significant increase in biofilm biomass by 27–39% ($p < 0.05$).

Conclusions. The results show the need for individual selection of antimicrobial combinations to combat *K. pneumoniae* biofilms due to the possible effect of synergy and antagonism effects on the outcome of therapy.

Keywords: virulent bacteriophages; *Klebsiella pneumoniae*; synergy; biofilms; depolymerase; antibiotics

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ВЛИЯНИЕ КОМБИНАЦИЙ АНТИБИОТИКОВ, ФАГОВ И ДЕПОЛИМЕРАЗЫ НА БИОПЛЕНКИ ЛЕКАРСТВЕННО-УСТОЙЧИВОГО ШТАММА *KLEBSIELLA PNEUMONIAE*

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Введение. *Klebsiella pneumoniae* представляет серьезную угрозу глобальному здравоохранению из-за высокой доли изолятов с множественной лекарственной устойчивостью. Более того, формирование бактерией биопленок значительно усложняет лечение инфекций.

Цель. Оценка эффективности индивидуального и комбинированного действия антибиотиков и бактериофагов или полисахарид-деполимеразы на биопленки клинически значимого штамма *K. pneumoniae*.

Материалы и методы. В работе использовали штамм *K. pneumoniae* с множественной лекарственной устойчивостью 9faiz, 4 антибиотика различных классов (гентамицин, левофлоксацин, меропенем и хлорамфеникол), 3 бактериофага различных родов (Dlv622, Seu621 и FRZ284) и 1 полисахарид-деполимеразу (Dep622). Эксперименты проводили на сформированных биопленках путем обработки 24-часовых пленок *K. pneumoniae* антимикробными агентами индивидуально или в комбинациях. Способность штамма образовывать биопленки оценивали окрашиванием кристаллическим фиолетовым. Сравнение между средними значениями оптической плотности проводилось с помощью *t*-теста и считалось значимым при $p \leq 0{,}05$.

Результаты. Индивидуальное применение антибиотиков в пиковых концентрациях (C_{max}) или деполимеразы в концентрации 100 МДК (минимальная действующая концентрация) не приводило к значимому снижению биомассы биопленки, тогда как бактериофаги в титре 5×10^9 БОЕ/мл статистически значимо снижали ее биомассу на 27–31% ($p \leq 0{,}05$). Большинство комбинаций фагов и антибиотиков не приводило к значимому повышению эффективности разрушения биопленок; лишь сочетание фага FRZ284 с гентамицином статистически значимо показало дополнительное снижение биомассы биопленки на 27% ($p \leq 0{,}05$). Комбинации деполимеразы со всеми антибиотиками, кроме меропенема, приводили к значимому увеличению биомассы биопленки на 27–39% ($p \leq 0{,}05$).

Выводы. Результаты показывают необходимость индивидуального подбора антимикробных комбинаций для борьбы с биопленками *K. pneumoniae* из-за возможного влияния эффектов синергии и антагонизма на исход терапии.

Ключевые слова: вирулентные бактериофаги; *Klebsiella pneumoniae*; синергия; биопленки; деполимераза; антибиотики

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INTRODUCTION

Klebsiella pneumoniae is an opportunistic gram-negative bacterium which poses a serious threat to global health due to the rapid spread of multidrug-resistant (MDR) strains. Resistant strains cause a significant number of nosocomial infections, such as pneumonia, urinary tract, and bloodstream infections, characterized by a high mortality rate reaching 50% [1].

The therapy of infections caused by *K. pneumoniae* is difficult due to the pathogen's ability to form biofilms. Biofilms are organized communities of microorganisms attached to various biotic and abiotic substrates by means of an exopolymer matrix consisting of proteins, polysaccharides, and extracellular DNA. According to research, about 60–80% of chronic infections are associated with biofilm formation [2]. In addition, biofilms are often formed on invasive medical devices, which increases the risk of acute infections in patients undergoing inpatient treatment [3].

Cells inside biofilms are characterized by a slow metabolism and a reduced growth rate, as well as an increased frequency of horizontal transfer of mobile genetic elements. The latter often contain genes for virulence and antibiotic resistance, allowing biofilm cells to more effectively resist antimicrobial drugs and avoid the immune response of the host body. In combination, such features increase the resistance of biofilms to antibiotics by 100–1000 times compared with planktonic forms of microorganisms [2, 4]. As a result, standard antibiotic therapy regimens demonstrate insufficient effectiveness against biofilm-associated infections, thus necessitating the development of new therapeutic approaches.

One of the promising approaches to controlling biofilms is the use of bacteriophages or their derivatives, such as polysaccharide depolymerases. Due to the action of polysaccharide depolymerases, bacteriophages are capable of degrading the extracellular matrix of biofilms. This violates the integrity of the biofilm structure and increases the sensitivity of bacteria to antimicrobial drugs and environmental factors [5]. This offers opportunities for the use of bacteriophages as independent therapeutic agents, and in combination with clinically used antibiotics.

The combined use of antimicrobial agents can lead to various types of interactions, including synergism, additive effect, or antagonism. Synergy is observed in cases where the combined effect of the use of several antimicrobial agents exceeds the sum of their individual effects. The additive effect implies that the total effect of the agents is equal to the sum of their individual effects, i.e., they do not affect each other's effectiveness. Antagonism is characterized by the fact that the overall effect of a combination of antimicrobial agents is less than the sum of their individual effects and indicates that the action of one of them somehow interferes with the action of the other [6].

It has been shown on planktonic cells that phages or polysaccharide depolymerases in combination with antibiotics predominantly demonstrate synergism. This leads to a more effective elimination of bacteria than when compared with monotherapy with antimicrobial drugs [4, 7–9]. However, a number of publications also report that some combinations of antibacterial drugs demonstrate antagonistic interaction [7, 10]. Nevertheless, the effect of such combinations on biofilms remains insufficiently studied, underlining the need for in-depth research of possible effects arising from the combined action of phages and their enzymes with antibiotics.

The aim of the study was to evaluate the effectiveness of individual and combined action of antibiotics and bacteriophages or polysaccharide depolymerase on biofilms of a clinically significant strain of *K. pneumoniae*.

MATERIALS AND METHODS

Bacterial strains

The study was carried out on the *K. pneumoniae* 9faiz strain from the collection of the Laboratory of Molecular Genetics of Lopukhin Federal Research and Clinical Center of Physical-Chemical Medicine. The strain seeded from biological material in 2019 belonged to the capsule type KL23 and the clinically significant sequence type ST39, previously determined according to standard typing methods [11, 12].

The bacterial culture was grown on Lysogeny broth (LB) in the Lennox modification (Dia-M, Russia). The bacterial culture was incubated for 18 h at a temperature of 37°C.

The minimum inhibitory concentration (MIC) of antibiotics was determined by the method of micro-dilution in accordance with the Russian recommendations "Determination of the sensitivity of microorganisms to antimicrobial drugs" [13]. According to this document, microorganisms are classified as sensitive under the standard dosage regimen (S: MIC ≤ the limit value of sensitivity), sensitive with increased exposure to antimicrobial intermediate (I: the limit value < MIC ≤ boundary value), or stable (R: MIC > boundary value) (Table 1). The inhibitory effect of antibiotics was evaluated using a FlexA-200 flatbed spectrophotometer (Allsheng, China) based on the optical density (OD) of a bacterial culture at a wavelength of 620 nm. Antibacterial drugs of four classes were used for the work: aminoglycosides — gentamicin (GEN); fluoroquinolones of the third generation — levofloxacin (LVX); carbapenems — meropenem (MEM) and amphenicols — chloramphenicol (CMP); and a set of HiMedia reagents (India).

K. pneumoniae Kp9068 and *K. pneumoniae* Kp284 strains were used as host strains for phage lysate growth [14, 15].

Bacteriophages

Three previously described *K. pneumoniae* bacteriophages were used in the work: Dlv622, Seu621, and FRZ284 (GenBank MT939252, MT939253 and MZ602148) belonging to the genera Drulisvirus, Mydovirus, and Jiaodavirus, respectively [14, 15]. Dlv622 and Seu621 phages have capsule specificity, while FRZ284 is characterized by a wide range of hosts and is not associated with the capsule type. Phage lysates in LB sterilized by filtration through a 0.22 μm syringe filter (Merk, Millipor) were used for the experiments. The finished sterile lysates were stored in the refrigerator at a temperature of +4°C.

Table 1. Limits of *K. pneumoniae* sensitivity to antibiotics and the maximum permissible concentrations of these drugs in blood serum

Antibiotics	Sensitivity limits, μg/mL			Peak serum antibiotic level (C_{max}), μg/mL
	≤ S	I	R ≥	
Gentamicin	4	8	16	16 [16]
Levofloxacin	2	4	8	6 [17]
Meropenem	1	2	4	28 [18]
Chloramphenicol	8	16	32	25 [19]

Table prepared by the authors using their own data

The phage titer was determined by spot testing [20]. The ability of phages to infect and lyse the studied strain was analyzed based on the calculation of efficiency of plating (EOP) in accordance with the standard methodology [21]. The EOP value was calculated as the ratio of the bacteriophage titer on the studied strain to the phage titer on the host strain. If the phage did not form single plaques, but caused a halo to appear on the surface of Petri dishes, which disappeared with a decrease in phage concentration, we referred to this phenomenon as lysis from the outside. The lytic activity of phages was measured using Appelman titration according to the classical method with modifications [22]. The suspension of bacteriophages was sequentially diluted tenfold in LB broth. Then 190 μL of each dilution was added to the wells of a flat-bottomed ventilated 96-well plate (Thermo Scientific, Denmark), and 10 μL (5×10^5 CFU/mL) of bacterial culture was inoculated at the logarithmic growth stage (OD600 = 0.3). This was additionally diluted 100 times with fresh LB. After 24-h incubation of the tablet at 37°C, the optical density was measured at a wavelength of 620 nm using a FlexA200 tablet spectrophotometer. The Appelman titer was defined as the highest dilution of phage lysate, in which the optical density did not increase when compared to the negative control, indicating the absence of visible bacterial growth.

Polysaccharide depolymerase

The recombinant polysaccharide depolymerase Dep622 of the tail fibrillation of the bacteriophage Dlv622, obtained as a purified protein, was used in the work, as described earlier in [14]. The ability of Dep622 to degrade capsular polysaccharides of strain 9faiz was evaluated using a technique similar to the titration of bacteriophages. For this purpose, the suspension of recombinant polysaccharide depolymerase was serially diluted in LB medium in steps of 2 (1392.64–0.085 μg/mL). Semi-liquid LB-agar (LB-broth with the addition of 0.7% agarose) containing 100 μL of the night culture of the studied strain was distributed over the surface of a Petri dish. Aliquots (5 μL) of each dilution were applied to the surface of the agar and left until the drops dried. Petri dishes were incubated at 37°C for 24 h. The appearance of translucent spots was identified on the bacterial lawn at the sites of application of the enzyme. They increased in diameter after 48 h of incubation, indicating the enzymatic activity of depolymerase. The minimum effective concentration (MED) was defined as the concentration of the final dilution at which the action of the enzyme was still observed.

A method for evaluating the effectiveness of biofilm formation

Biofilms of the 9faiz strain were grown in a 96-well plate. For this purpose, 190 μL of LB nutrient medium was introduced into each well and 10 μL (5×10^5 CFU/mL) of bacterial culture was inoculated at the logarithmic growth stage (OD600 = 0.3). This was additionally diluted 100 times with fresh LB broth. A pure LB medium was used as a negative control to assess the absence of bacterial culture growth. The tablet was incubated in a thermostat for 24 h at 37°C. Upon completion of incubation, the medium was removed, and the biofilms were washed from the remnants of planktonic cells, and then washed three times with sterile saline solution (0.9%). The biofilms obtained were stained with a solution of crystalline violet in accordance with the standard procedure [23]. The biofilm was incubated with 0.1% aqueous alcohol solution of crystalline violet (Himmed, Russia) for 30 min at room temperature. After incubation, the unbound dye was removed by triple rinsing with distilled water. For subsequent analysis, the bound dye in each well was eluted by adding 200 μL of 96% ethanol. The optical density of the solution was measured on a FlexA200 spectrophotometer at a wavelength of 575 nm. Based on the results of optical density measurement, the ability of the strain to form biofilms was evaluated and classified as follows: non-forming biofilms (OD ≤ ODc); weakly forming biofilms (ODc < OD ≤ 2×ODc); moderately forming biofilms (2×ODc < OD ≤ 4×ODc); or abundantly forming biofilms (OD > 4×ODc). Optical density optical density cut-off (ODc) was defined as the arithmetic mean of the optical density of the negative control plus three standard deviations [23].

Study of individual and combined effects of antibiotics and bacteriophages/polysaccharide depolymerases

Biofilms of the 9faiz strain were grown and washed according to the previously described algorithm, after which the medium was changed to a fresh LB medium containing the studied antibiotics, phages or depolymerase, both individually and in combinations. The concentrations of antibiotics corresponded to the peak concentration (C_{max}) reached in blood serum after administration of the standard therapeutic dose (Table 1). Phage lysate was added at a concentration of 5×10^9 BOE/mL, and recombinant polysaccharide depolymerase at a dosage of 100 MED (68 mg/mL). The amounts of antimicrobial agents were selected based on a well volume of 200 μL.

LB medium without additives was used for positive control of bacterial culture growth in the absence of antimicrobial effects. In the case of individual exposure, one antibacterial agent was added, and for combined exposure, an antibiotic and a bacteriophage or depolymerase were added in pairs (a total of 16 combinations). The biofilms with antimicrobial agents were incubated for 24 h. Then the biofilms were washed and stained with crystalline violet, as described above.

The optical density of the stained biofilms was measured using a FlexA200 spectrophotometer at a wavelength of 575 nm. All experiments were conducted in three biological repeats, each of which included five technical repeats for each combination.

The normality of the distribution was checked by the Shapiro–Wilk test. The statistical significance and reliability of the differences were determined by means of Student's *t*-test. The difference between the averages was considered significant at $p < 0.05$. Data analysis and visualization were performed using the GraphPad Prism 8 software package.

RESULTS

Characteristics of the strain and its resistance to antimicrobial agents

Planktonic cells of the 9faiz strain demonstrated resistance to all the studied antibiotics. The MIC values were as follows: gentamicin — 128 μg/mL; levofloxacin — 128 μg/mL; meropenem — 32 μg/mL; and chloramphenicol — 128 μg/mL. 9faiz has also been characterized as a strain which abundantly forms biofilms.

Bacteriophages Dlv622 and Seu621 showed a low level of seeding efficiency on strain 9faiz (EOP = 0.01). When titrated according to Appelman, Dlv622 and Seu621 at concentrations of 5×10^9 BOE/mL also showed weak activity without completely suppressing the growth of planktonic cells. When determining the effectiveness of seeding, FRZ284 showed lysis from the outside. However, when titrated according to Appelman at a concentration of 5×10^9 BOE/mL, it suppressed bacterial growth by 80%.

Recombinant depolymerase Dep622 deposited on bacterial lawn of strain 9faiz formed translucent zones resembling a halo of phage plaque, indicating enzymatic activity. Based on the results, MED Dep622 was determined as 0.68 μg/mL.

Individual and combined action of antimicrobial agents on biofilms

With the individual use of bacteriophages Dlv622, Seu621 or FRZ284 at a concentration of 5×10^9 BOE/mL, biofilm biomass significantly decreased by 27–30% when compared with the control (Fig. 1). At the same time, the use of antibiotics in C_{max} concentrations or depolymerase Dep622 in 100 MED did not lead to significant destruction of the biofilm.

The combined use of bacteriophage Dlv622 with the studied antibiotics did not lead to a statistically significant decrease in biofilm biomass when compared to the control (Fig. 2A). However, the combination of Dlv622 with levofloxacin or meropenem showed statistically significant differences from the individual action of the phage, thus reducing its effectiveness by 30% and 34%, respectively. This indicated a possible antagonistic interaction of these antimicrobial agents.

The use of bacteriophage Seu621 with the studied antibiotics did not lead to a change in biofilm biomass. The results did not significantly differ either from the individual action of the phage or from the control ($p = 0.05$) (Fig. 2B).

The combined use of FRZ284 with levofloxacin, meropenem, or chloramphenicol significantly reduced biofilm biomass when compared to the control. However, these results did not differ from the individual action of the phage (Fig. 2B). In combination with gentamicin, FRZ284 showed a significant decrease in biofilm biomass: 58% relative to the control; and 39% compared to the individual action of the phage. This indicates a synergistic (presumably) potentiation between the phage and the antibiotic.

Depolymerase Dep622 in combination with gentamicin, levofloxacin and chloramphenicol significantly increased biofilm biomass by 27%, 28%, and 39%, respectively, when compared with the control and individual action of the enzyme. This indicates a potential antagonism between the antimicrobial agents studied herein (Fig. 2G). The combination of Dep622 with meropenem did not significantly affect the biofilm biomass when compared to the control and individual action of the enzyme.

DISCUSSION

The study was conducted on a strain of *K. pneumoniae* 9faiz belonging to the capsule type KL23, often associated with resistance to carbapenems. Among isolates producing carbapenemases, the proportion of KL23 can reach 9–17% [24]. In addition, the strain belonged to the sequence type ST39, characterized by a high level of resistance to carbapenems. It was also associated with several outbreaks of carbapenem-resistant strains of *K. pneumoniae* in Russia and Greece [25]. Antibiotic resistance tests have demonstrated resistance to *K. pneumoniae* 9fa belonging to the

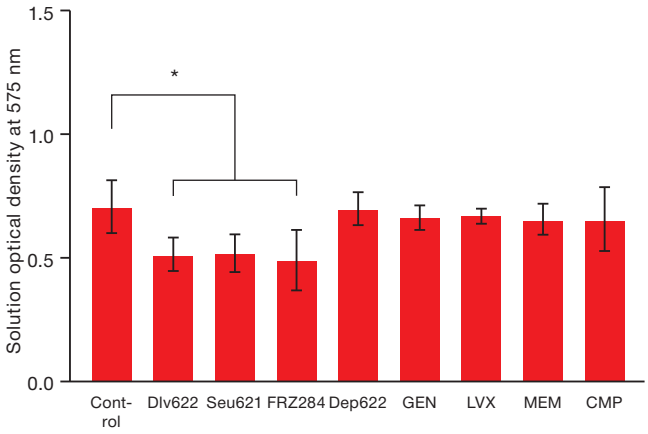

Figure prepared based on the authors' own data

Fig. 1. Changing the biomass of the biofilm of the *K. pneumoniae* strain under the individual action of various antibacterial agents. * — $p \le 0.05$.

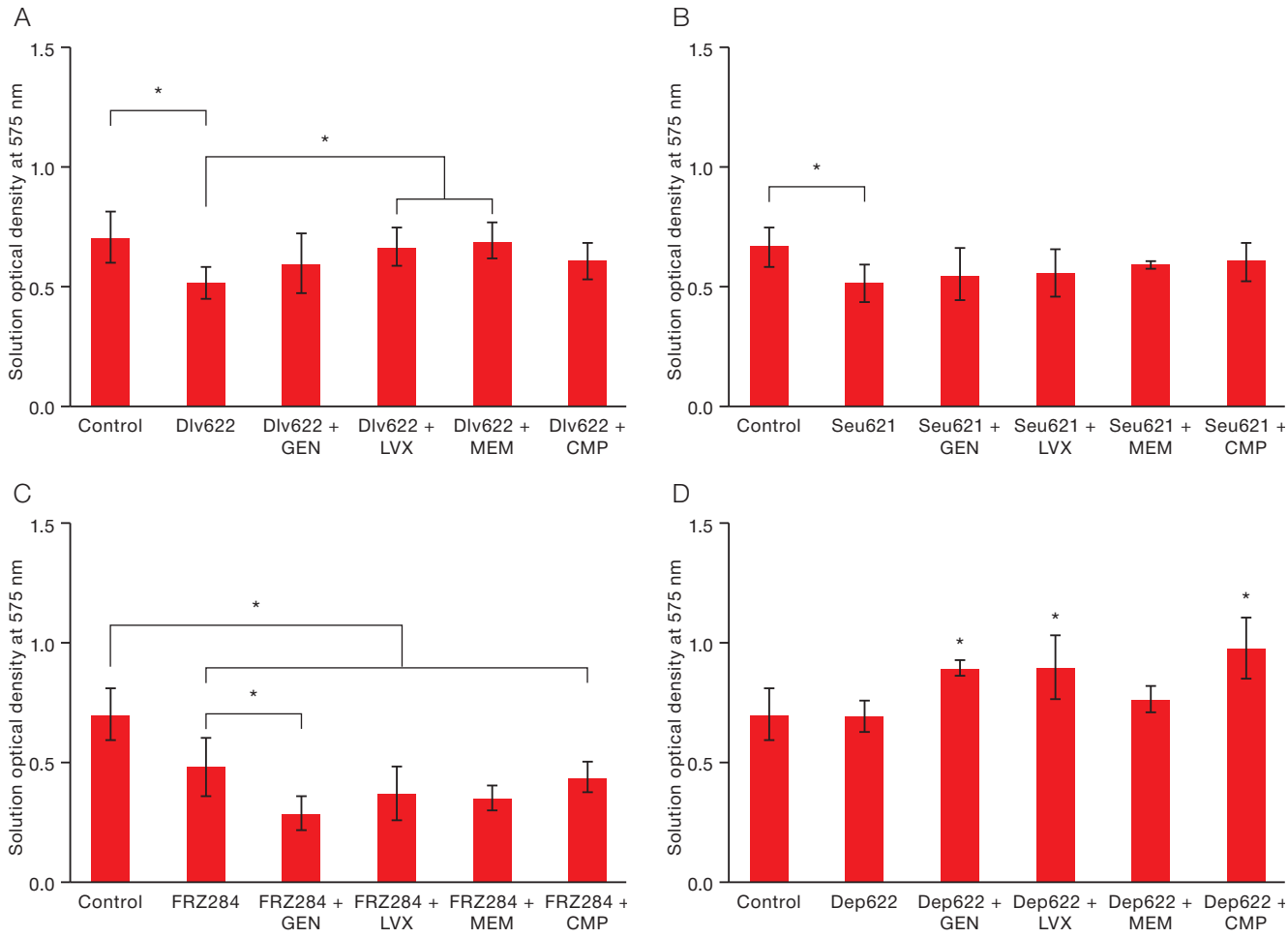

Figure prepared based on the authors' own data

Fig. 2. Changes in the biomass of *K. pneumoniae* 9faiz biofilm under the combined action of various antibacterial agents. * — $p \leq 0.05$

four main classes of antibiotics used to treat klebsiella infection: aminoglycosides, fluoroquinolones, carbapenems, and chloramphenicols. The strain also had a pronounced ability to form biofilms, further emphasizing its pathogenicity and resistance to therapeutic effects with standard doses of antibiotics.

In order to identify a wide range of effects, bacteriophages belonging to various taxonomic groups were used for the study: Dlv622 (Autographiviridae, Slopekvirinae, Drulisvirus); Seu621 (Vequintavirinae, Mydovirus); and FRZ284 (Straboviridae, Tevenvirinae, Jiaodavirus). Bacteriophages Dlv622 and Seu621 have homologous receptor-binding proteins represented by polysaccharide depolymerases specific to *K. pneumoniae* strains with capsule type KL23 [14]. In contrast, the bacteriophage FRZ284 lyzes K strains. pneumoniae is independent of the capsule type and has a receptor-binding protein of unknown specificity [15]. To study the sensitivity of the 9faiz strain, bacteriophages were used at a concentration of 5×10^9 BOE/ml, considered the standard therapeutic dose [26]. The results of the Appelman titration showed that this dose is not sufficient to completely suppress the growth of planktonic cells. In addition, the evaluation of the seeding efficiency revealed that the Dlv622 and Seu621 phages reproduce 100 times less successfully on the 9faiz strain than on the host strain. The FRZ284 phage demonstrates exclusively lysis from the outside.

Due to the resistance of the 9faiz strain to the studied antibiotics and insufficient sensitivity to bacteriophages, monotherapy with antimicrobial agents proved ineffective for the destruction of biofilms, as confirmed during this study. Individual exposure to bacteriophages reduced the biomass of biofilms, although not leading to their complete destruction (Fig. 1). Antibiotics did not statistically significantly change the biomass of biofilms, which can be explained by the use of low concentrations of antimicrobial agents. Antibiotic concentrations significantly lower than MIC were selected for the experiment, albeit corresponding to peak concentrations of the antibiotic in human serum (Table 1). Despite the high resistance of biofilms to antibiotics, exceeding the concentration of C_{max} is unacceptable in the framework of practical therapy. This is due to the potential toxic effect. This highlights the need to take this factor into account when selecting appropriate concentrations of antibiotics for in vitro studies.

The insufficient effectiveness of the individual use of antimicrobial agents emphasizes the need for their combined use. Current studies have established that the combination of antibiotics and bacteriophages against biofilms demonstrates a higher level of efficacy than when compared to

monotherapy. Thus, several studies have shown that the combination of bacteriophages with ciprofloxacin [27], amoxicillin or fosfomycin [9] exhibits a synergistic effect in the destruction of *K. pneumoniae* biofilms. The results of this study also revealed one case of potential synergy. The combined use of bacteriophage FRZ284 with gentamicin significantly reduced the biomass of biofilm of strain 9faiz compared with individual use of bacteriophage (Fig. 2B). For antibiotics in the aminoglycoside class, to which gentamicin belongs, mechanisms for suppressing bacteriophage replication have previously been proposed [10]. This can lead to antagonism. According to our results, the effect of gentamicin probably did not disrupt the replicative cycle of the phage. This may possibly explain the observed synergy and discrepancies with previously described studies. Although there is no data in the literature on the synergy of antibiotics and T4-like bacteriophages of *K. pneumoniae*, including FRZ284, similar cases have been described for combinations of meropenem, ciprofloxacin and colistin with T4-like phages of *Acinetobacter baumannii* [28].

In addition, within the framework of the experiments conducted in this study, two cases of antagonism were identified in which the bacteriophage Dlv622 was combined with levofloxacin or meropenem (Fig. 2A). Despite the availability of data in the literature on the synergistic interaction of antibiotics and bacteriophages, the antagonistic effects identified for the combinations of antimicrobial drugs used in this work against *K. pneumoniae* biofilms have not been previously described. Since levofloxacin inhibits DNA gyrase and topoisomerase IV, the results obtained may indicate a decrease in the rate of DNA replication of the bacteriophage Dlv622. Levofloxacin is also able to stimulate the formation of a thicker biofilm in *K. pneumoniae* [2], which may impede the penetration of the bacteriophage and reduce the effectiveness of its action. At the same time, inhibition of the lytic activity of the bacteriophage by meropenem, which blocks cell wall synthesis, remains difficult to explain due to an insufficient understanding of the mechanism of this effect.

For the remaining combinations of antibiotics and bacteriophages, statistical significance did not allow us to judge the effect. However, focusing on the average values, it can be noted that combinations with bacteriophages Dlv622 and Seu621 led rather to a decrease in the efficiency of destruction of biofilms. On the contrary, combinations of antibiotics with the bacteriophage FRZ284 simply did not lead to an increase in effectiveness. The results obtained indicate the need for a more thorough approach to the selection of antibiotics and bacteriophages for therapy purposes.

The work also evaluated the effectiveness of biofilm destruction through individual exposure to polysaccharide depolymerase and in combination with antibiotics. For this purpose, the recombinant Dep622 protein, a receptor-binding protein of the Dlv622 phage, was used. This effectively destroyed capsule polysaccharides of the *K. pneumoniae* 9faiz strain in in vitro tests. Due to the use of recombinant polysaccharide depolymerases exclusively in laboratory studies, there are currently no standardized therapeutic dosages for enzymes of this group in the literature. Nevertheless, experiments on animal models have not revealed any toxic effects when using depolymerases in various concentrations [4]. This study used the maximum multiple dose (100 MED), which could be achieved in the well of the tablet without significant dilution of the medium.

In the literature, data on the combination of polysaccharide depolymerase with antibiotics showed both synergy effects and the absence of any effects [29–30]. The results obtained demonstrated that the individual action of Dep622 is not sufficient to destroy biofilms of the 9faiz strain. Moreover, the combination of polysaccharide depolymerase with gentamicin, levofloxacin, and chloramphenicol significantly increased biofilm biomass (Fig. 2D), indicating a possible antagonism between antimicrobial agents. Similar effects of antagonism between depolymerases and antibiotics have not been previously described, thus requiring further study.

CONCLUSION

The results obtained demonstrated the inefficiency of monocomponent approaches and established a variety of effects arising from the combined use of antibiotics and bacteriophages or polysaccharide depolymerase against biofilms. In particular, one case of synergy and several cases of potential antagonism between antimicrobial agents have been identified, which is insufficiently covered in the existing literature.

Considering that bacteriophage therapy does not currently require the discontinuation of a course of antibiotics, the potential antagonism between the two antimicrobial agents may become a significant problem in the treatment of biofilm-associated infections, underlining the need for further research in this direction.

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FEATURES OF OCCUPATIONAL MORBIDITY OF URANIUM MINING WORKERS

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Introduction. Preservation of the health and professional longevity of people of working age is a priority task of the Russian healthcare system. In this regard, it is extremely important to study the structure and dynamics of occupational pathology for the scientific justification and development of measures aimed at managing the risks to the health of workers in enterprises with extremely dangerous working conditions and to prevent the development of occupational diseases.

Objective. To study specific features in the structure of occupational morbidity of workers of a uranium mining enterprise over a 50-year period.

Materials and methods. The work was performed on the model of a large uranium mining enterprise within the Rosatom State Corporation in Russia with extremely dangerous working conditions. Cases of occupational diseases detected in workers from the beginning of the operation of the company from 1970 to 2019 were analyzed. The structure of occupational diseases was characterized, and the share contribution of the main nosological forms over 5-year periods was analyzed.

Results. The main nosological forms of occupational diseases in underground miners of a uranium ore mining enterprise were identified. A steady trend towards an increase in the total number of cases of diseases was noted. There was a gradual increase in the proportion of diseases of the musculoskeletal system and peripheral nervous system, a decrease in the proportion of "dust" pathology, an increase and subsequent decrease in the proportion of sensorineural hearing loss, as well as a stable contribution to the structure of occupational pathology of malignant neoplasms.

Conclusion. Indicators of a priori occupational health and safety risk of uranium mining workers reflect the levels of their occupational morbidity. Musculoskeletal system disorders and occupational malignancies dominate in the structure of occupational diseases. This morbidity is many times higher than the national average.

Keywords: occupational pathology structure; occupational risk; diseases of the musculoskeletal system; occupational malignancies

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ОСОБЕННОСТИ ПРОФЕССИОНАЛЬНОЙ ЗАБОЛЕВАЕМОСТИ РАБОТНИКОВ УРАНОДОБЫВАЮЩИХ ПРОИЗВОДСТВ

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Введение. Одной из приоритетных задач здравоохранения Российской Федерации является сохранение здоровья и профессионального долголетия лиц трудоспособного возраста. В связи с этим крайне актуально изучение структуры и динамики профессиональной патологии для научного обоснования и разработки мероприятий по управлению рисками для здоровья работников предприятий с особо опасными условиями труда и профилактике развития профессиональных заболеваний.

Цель. Изучение особенностей структуры профессиональной заболеваемости работников уранодобывающего предприятия за 50-летний период.

Материалы и методы. Работа выполнена на модели крупного уранодобывающего предприятия России с особо опасными условиями труда ГК «Росатом».

Проведен анализ случаев профессиональных заболеваний, выявляемых у работников от начала работы предприятия за период с 1970 по 2019 гг. Охарактеризована структура профессиональных заболеваний, в динамике по 5-летним периодам проанализирован долевой вклад основных нозологических форм.

Результаты. Выявлены приоритетные нозологические формы профессиональных заболеваний у подземных горнорабочих предприятия по добыче урановых руд. Отмечена устойчивая тенденция к увеличению общего числа случаев заболеваний с постепенным увеличением доли заболеваний опорно-двигательного аппарата и периферической нервной системы, снижению доли «пылевой» патологии, росту и последующему снижению доли нейросенсорной тугоухости; стабильный вклад в структуру профессиональной патологии злокачественных новообразований.

Заключение. Показатели априорного профессионального риска работников уранодобывающего предприятия отражают уровни их профессиональной заболеваемости. В структуре профессиональных заболеваний преобладают заболевания костно-мышечной системы и профессиональные злокачественные новообразования, многократно превышающие среднероссийские показатели.

Ключевые слова: структура профессиональной патологии; профессиональный риск; заболевания костно-мышечной системы; профессиональные злокачественные новообразования

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INTRODUCTION

Ensuring the medical component of industrial safety, preserving the health and professional longevity of people of working age comprise priority tasks of the healthcare of the Russian Federation. Strategically important industries need highly qualified personnel, whose training requires significant time and material resources. In this regard, the health of workers in certain industries with particularly dangerous working conditions is of great medical, social, and economic significance [1–2].

The importance of this area was emphasized by M.V. Mishustin, the Chairman of the Government of the Russian Federation, at the opening of the All-Russian Week of Occupational Safety — 2021. He emphasized that the rapidly changing world poses new challenges, which lead to previously unknown risks associated with occupational diseases arise. Therefore, timely identification of signs of the initial development of a possible occupational disease is an urgent task.

According to the definition by the WHO, occupational risk is the prognostic probability of the frequency and severity of adverse reactions to exposure to harmful factors of the working environment and the work process. Production factors (noise, vibration, severity of the labor process) that exceed sanitary and hygienic standards have a negative impact on the body of workers. They can cause a risk of developing occupational diseases, exacerbate the course of a number of common diseases, and determine disability and the onset of disability [3–5].

It is difficult to overestimate the importance of nuclear energy in Russia. Currently, nuclear power plants here generate about 20% of the total electricity amount. Power generation at nuclear power plants, extraction of raw materials, and enrichment of nuclear fuel are carried out at the enterprises of the Rosatom State Corporation, one of the largest producers of natural uranium in the world [6–7].

The majority of studies into the health status of uranium mining workers in Russia were carried out in the late twentieth and early twenty-first centuries. They recorded the presence of such priority factors of working conditions as industrial aerosols, intense noise, local and general transport, technological vibration, physical overload, functional overstrain of the musculoskeletal system, and ionizing radiation [8–12]. The main forms of occupational diseases of workers at that time were pathologies of the respiratory system (silicosis, pneumoconiosis), vibration disease, and occupational hearing loss [13–18].

Therefore, research aimed at studying the features of occupational health disorders of workers exposed to harmful production factors during the extraction of uranium ore and the development of measures to manage the risks of their development are both justified and relevant.

In this paper, we study the structure of occupational morbidity of workers of a uranium mining enterprise over a 50-year period.

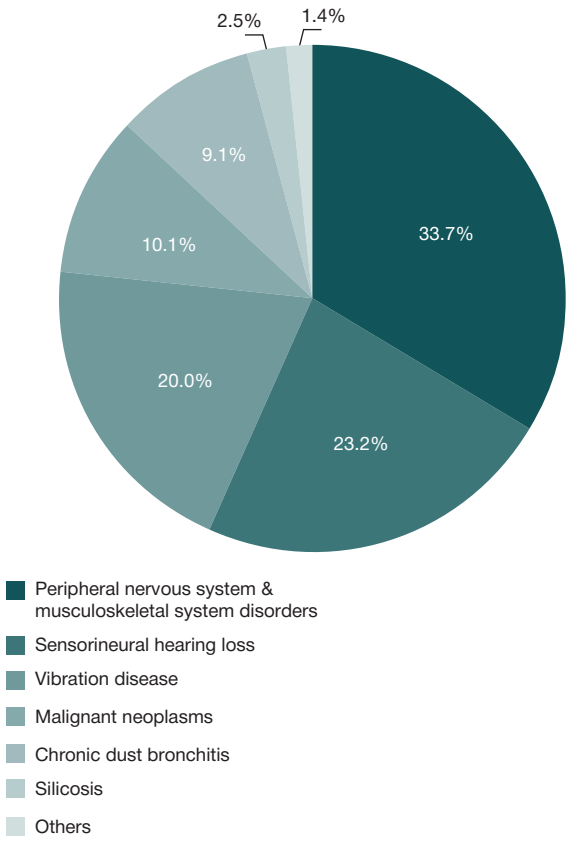
MATERIALS AND METHODS

The work was performed based on a model of a large uranium mining enterprise within Rosatom State Corporation in Russia with extremely dangerous working conditions.

Cases of occupational diseases detected in workers from the beginning of the company's work for the period 1970–2019 were analyzed. The structure of occupational diseases was characterized, and the contribution of the main nosological forms (silicosis, vibration disease, sensorineural hearing loss, chronic dust bronchitis, pathology of the musculoskeletal system and peripheral nervous system, malignant neoplasms) was analyzed over 5-year periods [19–20].

RESULTS AND DISCUSSION

The conducted research established that at the workplaces of the uranium mining enterprise studied here, the current main occupational health risk factors are intense noise, local and general vibration, as well as physical overload



The figure was prepared by the authors based on archival data

Fig. 1. The structure of nosological forms of occupational pathology identified in uranium mining workers for the period 1970–2019 (number of cases, %)

and functional overstrain of the musculoskeletal system due to insufficient mechanization of the labor process and the high prevalence of manual labor among workers of the main professions. Radiation factors (gamma radiation, radon and its short-lived daughter decay products, long-lived alpha-nuclides of a number of natural uranium) have an additional negative impact on the health of miners. The data obtained is comparable with the information available in previously published literature sources [21–23].

Currently, industrial aerosols have an insignificant impact on the occupational risk structure of miners of the uranium mining enterprise, largely due to the use of various dust suppression methods.

The results of a retrospective analysis of cases of occupational diseases based on archived data from the results of preventive medical examinations for the period 1970–2019 are presented in Table 1.

In total, from 1970 to 2019, 1134 cases of occupational diseases were recorded among workers of the uranium mining enterprise. There was a fairly steady increase in the number of occupational diseases, with more than half of all cases (53.9%) detected in the past 10 years, in the 2010–2019 period.

Occupational morbidity is characterized by the following priority groups of pathology: diseases of the peripheral nervous system (PNS) and musculoskeletal system (MSS), formed mainly due to overstrain of organs and systems in the process of performing work functions (33.7%); sensorineural hearing loss (SNHL) caused by noise exceeding the maximum permitted level (23.2%); vibration disease (VD) due to exposure of local and/or general vibration (20.0%); respiratory diseases due to exposure of industrial aerosols (11.6%), among which cases of silicosis (2.5%) and chronic dust bronchitis (CDB) (9.1%) were isolated; malignant neoplasms (MNs) — 10.1% of cases (Fig. 1).

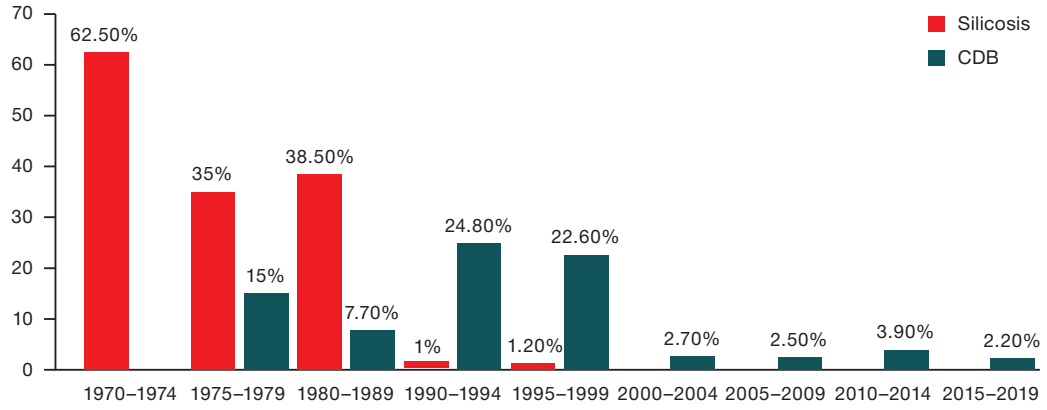
The conducted analysis allowed us to establish specific features in the dynamics of the development of occupational pathology in workers of the enterprise studied herein (Figs. 2–6).

Thus, in the period from 1970 to 1989, more than half of the cases of occupational diseases (up to 62.5%) were due to respiratory pathology, silicosis, and chronic dust

Table 1. Cases of occupational diseases over 5-year periods in 1970–2019 (absolute values)

Cases	VD	MNs	SNHL	PNS & MSS	Silicosis	CDB	Others	Total
1970–1974	2		1		5		0	8
1975–1979	8	2	10		14	6	0	40
1980–1984		2	4		5	1	1	13
1985–1989		7	5		1	27	5	45
1990–1994	4	5	56	8	1	25	2	101
1995–1999	7	25	27	4	1	19	1	84
2000–2004	9	28	23	11		2	0	73
2005–2009	57	9	59	27	1	4	2	159
2010–2014	85	20	51	162		13	2	333
2015–2019	55	17	27	170		6	3	278
Total in 1970–2019	227	115	263	382	28	103	16	1134

The table was prepared by the authors based on archival data



This graph is based on the authors' own data

Fig. 2. Dynamics of the proportion of silicosis and chronic dust bronchitis in the structure of occupational diseases detected in uranium mining workers during the 1970–2019 period

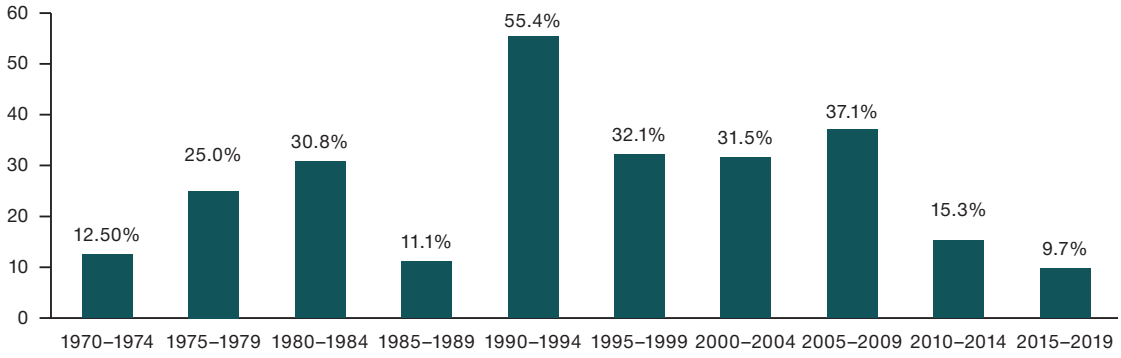


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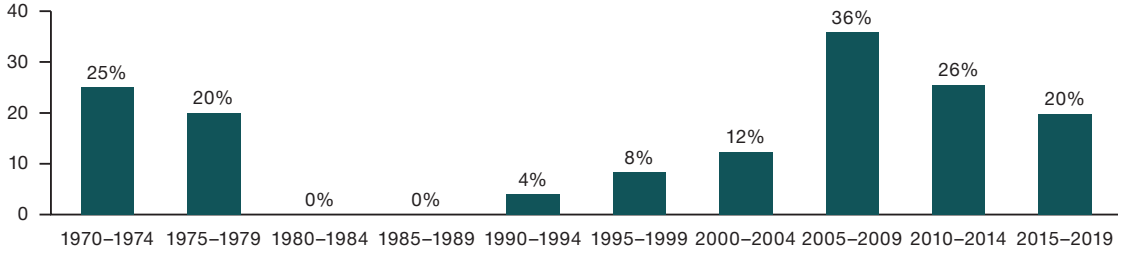
Fig. 3. Dynamics of the proportion of sensorineural hearing loss in the structure of occupational diseases detected in workers of a uranium mining enterprise during the 1970–2019 period



The graph is based on the authors' own data

Fig. 4. Dynamics of the proportion of vibration disease in the structure of occupational diseases detected in workers of a uranium mining enterprise during the 1970–2019 period

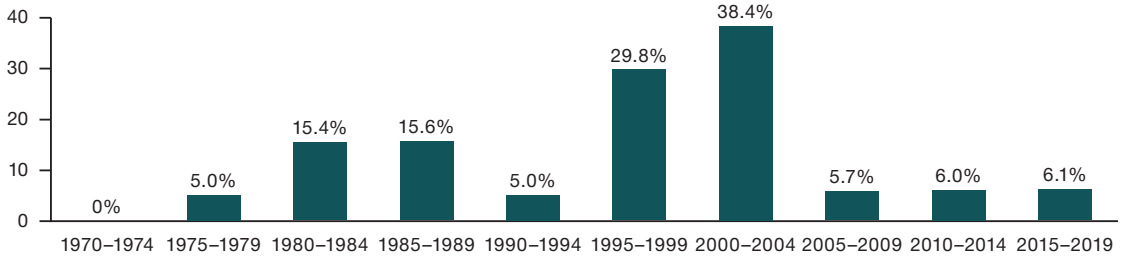


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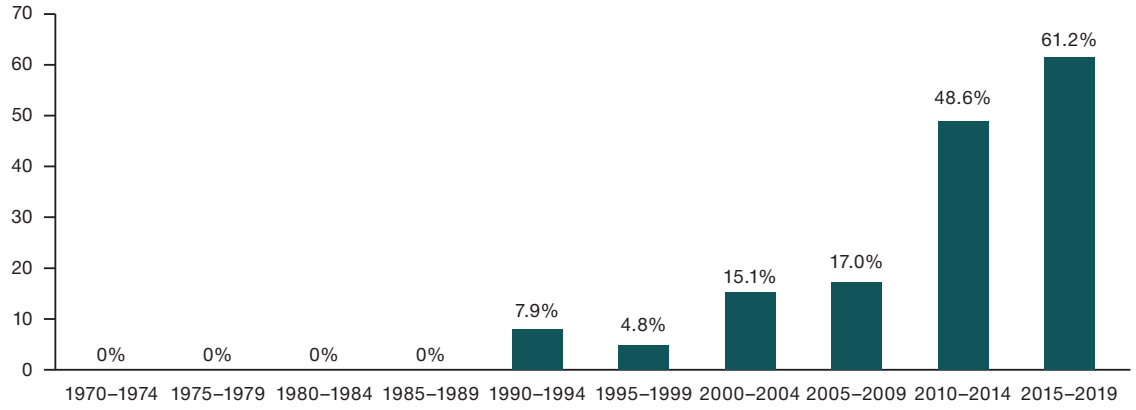
Fig. 5. Dynamics of the proportion of malignant neoplasms in the structure of occupational diseases detected in workers of a uranium mining enterprise during the 1970–2019 period



The graph is based on the author's own data

Fig. 6. Dynamics of the proportion of diseases of the peripheral nervous system and musculoskeletal system in the structure of occupational diseases detected in workers of a uranium mining enterprise during the 1970–2019 period

bronchitis (CDB). Prior to 1984, silicosis prevailed among occupational diseases of the respiratory system; however, since 1985, chronic dust bronchitis has become the priority form of pathology with up to 60% of all cases of occupational diseases (OD). Silicosis was detected only in isolated cases.

Cases of silicosis have not been detected since 2000. CDB accounted for 2.7% to 3.9% of cases of all occupational diseases. It is important to note that cases of silicosis were found in those workers who, prior to starting work in the divisions of a uranium mining enterprise, had already had a history of work experience in «dusty» professions at other mining enterprises.

The proportion of sensorineural hearing loss in the structure of occupational pathology fluctuated over a wide range: the maximum (55.4% of cases) in the period from 1990 to 1994 with a fairly significant decrease in indicators to 9.7–15.3% in the period from 2010 to 2019. The proportion of vibration disease (VB) in the structure of occupational diseases was 20–25% from 1970 to 1979. In the period from 1980 to 1989, cases of VB were not recorded. Since 1990, an increase in the incidence of VB was noted: from 4% in 1990–1994 to 35.8% in 2005–2009. Over the following 10 years, i.e., 2010–2019, the proportion of cases of VB returned to the level of 19.8–25.5%.

It is important to note that in the structure of the occupational pathology of workers of a uranium mining enterprise, cases of malignant neoplasms (MNs) were regularly detected (with the exception of 1970–1974). Respiratory pathology prevailed among MNs (lung cancer — most of all).

The proportion of MNs was no more than 0.6% of cases in the structure of occupational pathology in the Russian Federation over the past 15–20 years. However, in the case of uranium miners, MNs was at least 5–6%. In some periods, this figure reached 29.8–38.4% of cases (1995–2004). Malignant neoplasms are mainly represented by cases of lung cancer (94 cases, 81.7%) and some variants of hemoblastosis (21 cases, 18.3%).

Between 1989 and 2019, in the Russian Federation, slightly over 1000 cases of occupational cancer were detected. The data obtained indicates that cases of occupational cancer detected in uranium enterprise workers account for almost 10% of all cases of occupational cancer in the entire country.

Since 2005, there has been an increase in the incidence of diseases of the musculoskeletal system and peripheral nervous system: the most frequently recorded since 2010. In 2019, this figure reached more than 60%.

Intensive morbidity rates (the number of cases per 10,000 workers) demonstrated a fairly significant increase in the number of cases (56.3% of cases per 10,000 workers in harmful working conditions). A significant spread of occupational morbidity indicators has been established over the years. The highest number emerges in the last 10 years, which can be attributed to increased knowledge of medical professionals and improved diagnosis of various forms of occupational pathology. The occupational disease detection rate is currently the highest (66.6–69.5% of cases per year). A feature of the morbidity rate of uranium mining workers is the almost threefold increase in cases of vibration disease, MSS and PNS disorders, reaching 60%, in the period of 2005–2019.

The data from the retrospective analysis of the structure of occupational morbidity of uranium mining workers demonstrates their features in comparison with the general structure of occupational morbidity of workers in the Russian Federation [24]. More than a third of all cases are occupational diseases of the peripheral nervous system and musculoskeletal system. Almost a quarter are sensorineural hearing loss. One fifth consists of vibration disease which is comparable with previously available information in the literature [25]. However, the frequency of development and detection of respiratory diseases is insignificant when compared to previously available data (more than 10% of cases) [26].

In addition, a feature of the dynamics of the development of certain nosological forms of occupational

pathology among uranium mining workers during the first 10-year period analyzed was the predominance of the number of cases of occupational respiratory diseases (silicosis and chronic dust bronchitis with a predominance of silicosis). Currently, dust bronchitis due to exposure to industrial dust is the predominant form of occupational respiratory disease. The predominance of cases of respiratory pathology among workers in the first decades of the operation of the enterprise can be explained by the fact that in the initial period a significant proportion of workers, including underground miners, had previously worked in underground mining facilities in other regions of the country. As our own qualified personnel were trained, the proportion of workers exposed to fibrogenic dust at other enterprises significantly decreased. This led to a change in the structure of occupational respiratory pathology.

The proportion of malignant neoplasms is rather high (for 50 years it amounted to 10.1% of cases), being significantly higher than the national average [24]. Regular detection of cases of malignant neoplasms (mainly of the respiratory system with a predominance of lung cancer) deserves special attention. These figures significantly exceed the national data and account for almost 10% of all cases of occupational cancer in the whole country. This is an alarming fact and requires special attention from industry management, enterprises, and medical professionals. At the same time, the high detection of oncological pathology can be attributed to the orientation of the occupational pathology service towards the significance of a priori risk in workers engaged in underground mining of uranium ore. This deserves a positive assessment of the work of the medical service [27].

Currently, the share of professional hearing loss among uranium mining workers has significantly decreased. This is probably due to an increase in the effectiveness of noise-canceling measures.

CONCLUSION

The specifics of the working conditions of uranium mining workers at the present stage (features of a priori occupational risk) are reflected in the indicators of occupational morbidity of this contingent. Occupational diseases of the musculoskeletal system prevail, and the levels of occupational diseases are many times higher than the national average.

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POTENTIAL RISKS OF OCCUPATIONAL EXPOSURE TO INNOVATIVE BIOPHARMACEUTICALS: A REVIEW

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Introduction. Gene-targeted therapies (gene-targeted, high-tech, and biopharmaceuticals) are developed based on active pharmaceutical ingredients, which are reactive compounds with pleiotropic activity. Such ingredients are associated with health hazards to workers employed at various stages of their production. Clinically significant pharmacological or toxicological effects of innovative medications on employees exposed to these components are unsafe from the perspective of a risk-based approach in occupational medicine.

Objective. Assessment of potential risks of occupational exposure to innovative biopharmaceuticals in production or laboratory conditions and approaches to their hygienic management.

Materials and methods. The relevant scientific publications were searched and retrieved via electronic bibliographic databases both in the Russian language (eLibrary, CyberLeninka) and in the English language (WoS, Scopus, PubMed). Regulatory documents were analyzed using the *Consultant Plus* legal information system.

Discussion. Specific features of production of new-generation biopharmaceuticals (gene-targeted, high-tech, or biotechnological medications) and the associated risks of occupational exposure to workers in pharmaceutical or laboratory production are considered. It was established that employees of such enterprises are exposed to the combined influence of adverse — biological, physical, and chemical — production environment factors. There is a lack of information on the development of analytical methods for identifying gene-targeted components (high-tech or biotechnological medications) in the workplace air and wastewater, as well as on workplace surfaces. The identified problems of occupational health are related to the lack of legislative instruments and knowledge-based management decisions on the identification of risk factors and control ranges of potential work-related effects of innovative biopharmaceuticals. Such approaches should be based on the principles of hygienic regulation aimed at eliminating or reducing negative industrial effects and ensuring the safety and preservation of employee health.

Conclusions. Major methodological approaches to assessing the work-related impact of gene-targeted, high-tech, or biotechnological therapies on employees of pharmaceutical enterprises are determined. These approaches include: (1) toxicological assessment of compounds with the establishment of possible parameters of toxicometry; (2) evaluation of the pharmacological and toxicokinetic features of gene-targeted therapeutical components; (3) development of methods for their quantitative determination in various environments; (4) establishment of biomarkers of exposure and related effects followed by hygienic rationing and justification of preventive measures.

Keywords: advanced therapy medicinal products; gene therapy medicinal product; biotechnology-derived pharmaceuticals; preclinical studies; monoclonal antibodies; occupational hazard; allowable concentrations

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ВОЗМОЖНЫЕ РИСКИ ПРОФЕССИОНАЛЬНОГО ВОЗДЕЙСТВИЯ ИННОВАЦИОННЫХ БИОЛОГИЧЕСКИХ ЛЕКАРСТВЕННЫХ ПРЕПАРАТОВ: ОБЗОР ЛИТЕРАТУРЫ

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Введение. Активные фармацевтические ингредиенты для производства лекарственных препаратов, воздействующих на генетический аппарат (генотерапевтических, высокотехнологических, биотехнологических), являются реакционноспособными соединениями с плейотропной активностью, что сопряжено с рисками здоровью работников, занятых на различных этапах их производства. Клинически значимый фармако/токсикологический эффект инновационных лекарственных препаратов, воздействующих на работающих, имеющих производственный контакт с данными компонентами, с точки зрения риск-ориентированного подхода в медицине труда является небезопасным.

Цель. Оценка потенциальных рисков профессионального воздействия инновационных биологических лекарственных препаратов на работающих в условиях производства/лаборатории и методических подходов их гигиенической регламентации.

Материалы и методы. Поиск научной литературы выполнен в электронных библиографических базах данных на русском (eLibrary, CyberLeninka) и английском (Web of Science, Scopus, PubMed) языках, нормативных документах в справочной правовой системе КонсультантПлюс.

Обсуждение. Рассмотрены отдельные аспекты особенностей разработки биологических лекарственных препаратов нового поколения (генотерапевтических/высокотехнологических/биотехнологических лекарственных средств) и сопряженных с этим рисков профессионального воздействия на работников в условиях фармацевтического или лабораторного производства. Выявлено, что работники подвергаются сочетанному воздействию неблагоприятных факторов производственной среды различной природы: биологических, физических, химических. Отмечается неполнота информации о разработке аналитических методов идентификации компонентов генотерапевтических/высокотехнологических/биотехнологических лекарственных средств в воздухе рабочей зоны, на рабочих поверхностях, в сточных водах. Обозначены актуальные проблемы гигиены труда, связанные с отсутствием законодательных инструментов, научно обоснованных управленческих решений по идентификации факторов риска здоровью работников, диапазонов контроля потенциального производственного воздействия инновационных биологических

лекарственных препаратов на основе принципов гигиенической регламентации, направленной на устранение или уменьшение негативного производственного воздействия и обеспечение безопасности и сохранения здоровья работников.

Выводы. Проведенная работа позволила определить основные методические подходы по оценке потенциального производственного воздействия генотерапевтических/высокотехнологических/биотехнологических лекарственных средств на работников соответствующих фармацевтических предприятий. К таким подходам относятся: токсикологическая оценка соединений с установлением возможных параметров токсикометрии, исследование фармако/токсикокинетических особенностей компонентов генных препаратов, разработка методик их количественного определения в различных средах, установление биомаркеров экспозиции и эффекта с последующим гигиеническим нормированием и обоснованием основных профилактических мероприятий.

Ключевые слова: высокотехнологичный лекарственный препарат; генотерапевтический лекарственный препарат; биотехнологический лекарственный препарат; доклинические исследования; моноклональные антитела; профессиональный риск; предельно допустимая концентрация

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INTRODUCTION

Modern medicine has reached a high level of development in the fields of innovative technologies and innovative medicinal products (MPs). Unfortunately, for a number of diseases, not only etiotropic but also pathogenetic therapeutical approaches are still lacking [1].

Until recently, the pharmaceutical industry has been largely aimed at the creation and industrial production of low-molecular weight MPs. However, the evolving understanding of the possibility of affecting the genetic apparatus of somatic cells to restore or modify the synthesis of certain proteins associated with a particular disease has led to the appearance of innovative biological MPs [2–4] for treating a wide range of conditions.

Active pharmaceutical ingredients currently used for the production of gene-targeted MPs are reactive compounds with pleiotropic activity. Such ingredients can carry health risks for workers involved in various stages of their production [5]. Although the effects of MPs on patients may be desirable or acceptable, any clinically significant pharmacological or toxicological effect of a gene-targeted MP on people working with these components is unsafe from the perspective of a risk-based approach in occupational medicine [6–7]. The priority direction of preventive medicine consists in identification of unsafe production factors and evaluation of safe exposure limits to hazardous factors at the workplace for the purpose of their hygienic control, based on the principles of safety for workers, the environment, and the public.

In this work, we aim to assess potential risks of occupational exposure of workers to innovative biological medicinal products in production or laboratory conditions and to determine approaches for their hygienic management.

MATERIALS AND METHODS

Scientific publications on the topic under study were searched and retrieved via electronic bibliographic databases in the Russian (eLibrary, CyberLeninka) and English (WoS, Scopus, PubMed) languages. Regulatory documents were analyzed using the *Consultant Plus* legal information system. Search queries were carried out using the following keywords: advanced therapy medicinal product (ATMP), gene therapy medicinal product (GTMP), biotechnology-derived pharmaceuticals, preclinical studies, monoclonal antibodies (mAbs), occupational hazard, and allowable concentration. The search depth was 10 years. The inclusion criteria were: availability of structured information on preclinical and clinical safety of ATMPs, GTMPs, biotechnology-derived pharmaceuticals, quantitative methods of their identification in different environments, as well as specific features of production environment control.

RESULTS AND DISCUSSION

Modern therapeutic strategies are aimed at individualization of therapeutic effects, adapting the medication and dose to the needs of a particular patient. Such strategies can be realized through the use of biological or biotechnological products capable of modifying gene sequences or controlling their expression, as well as those changing the biological properties of cells and, accordingly, the production of therapeutically active proteins in the body for their therapeutic or preventive effects [8–9].

According to the report “Gene Therapy Development & Manufacturing 2023”, more than 3150 biological, biotechnological, and gene-targeted new-generation MPs are currently undergoing various R&D stages, with more than two dozen drugs having been approved for clinical use by MP regulatory authorities of different countries [10].

In the Russian Federation, the R&D, expertise, production, and introduction of biological or biotechnological MPs into healthcare practice is an actively growing direction. At the same time, gene therapy is still defined as a set of genetic engineering (biotechnological) and medical methods aimed at introducing changes in the genetic

apparatus of human somatic cells in order to treat diseases [11]. Recently, the regulative documentation in this sphere has undergone revision, evidenced by the adoption of the Federal Scientific and Technical Program for the Development of Genetic Technologies for 2019–2027. This program is aimed at solving the problems of accelerated development of genetic technologies, including genetic editing technologies [12–14].

At the same time, according to the Federal Law No. 61-FZ "On Circulation of Medicinal products" dated 12.04.2010 (ed. 30.01.2024) clause 6.3, art. 4, the following definition is introduced: "advanced therapy medicinal product is a gene therapy medicinal product (GTMP) for medical use or an MP based on somatic cells for medical use or a tissue-engineered MP (tissue engineering product)." Clause 7.2 of art. 4 of this law and the EAEU Decision No. 78 of 03.11.2016 "On the Rules for Registration and Examination of Medicinal Products for Medical Use" defines GTMP as follows: "a biological MP containing an active substance with recombinant nucleic acid or consisting thereof, administered to a person for the purpose of regulating, restoring, replacing, adding, or deleting a genetic sequence. Clause 7.1 of art. 4 of the same law specifies the definition of biotechnological MPs, namely, the production of which is carried out using biotechnological processes and methods. The latter feature DNA recombinant technology, technology for controlled expression of genes encoding biologically active proteins in prokaryotes and eukaryotes, including modified mammalian cells), hybridoma and monoclonal antibody methods.

Along with the abovementioned, according to the RF Federal Law No. 180-FZ of 23.06.2016, the term "biomedical cell product — a complex consisting of a cell line (cell lines) and auxiliary substances, which do not include transplantation objects, as well as advanced therapy medicinal product, including GTMPs" applies to biomedical cell products. Requirements for production conditions, validation of the production process, quality control of target and intermediate products of GTMPs are reflected in the National Standard of the Russian Federation GOST R 52249-2009 "Rules of production and quality control of medicinal products" and the State Pharmacopoeia of the Russian Federation XIV OFS.1.7.1.0011.18 "Biotechnological medicinal products." Methodology and requirements for conducting preclinical studies, stability studies of biotechnological pharmaceuticals, and safety of pharmaceutical substance and finished dosage form are described in the national standard GOST R 57688-2017 "Medicinal products for medical use. Stability studies of biotechnological/biological medicinal products."

It should be noted that the aforementioned legal framework regulates the processes of biological or biotechnological MP circulation, being primarily aimed at ensuring the quality and safety of biological or biotechnological MPs. However, it does not guarantee the health safety of personnel having occupational contact with harmful or hazardous factors at the workplace.

In the Russian Federation, the legislative basis for occupational medicine, i.e., a branch aimed at protecting the health of the working contingent in contact with harmful or hazardous factors of the working environment (physical, chemical, biological, and occupational factors), is formed by a wide range of existing normative and methodological or legal documents, which were significantly amended in the period from 2021 to 2022. These amendments reflect modern requirements for occupational safety and health protection of the working population in accordance with international standards and new achievements in science and technology.

Thus, according to the Sanitary Rules and Regulations 3.3686-21 "Sanitary and Epidemiological Requirements for the Prevention of Infectious Diseases", mandatory requirements for a set of organizational, sanitary, and anti-epidemic, therapeutic and preventive, laboratory-diagnostic, engineering and technological measures are established, along with the order of accounting, storage, transfer and transportation, conditions and algorithm of work at the molecular, cellular levels for the creation of modified or genetically engineered variants of biological agents, diagnostic tests, and diagnostic procedures.

An important point in the recently adopted Decree of the President of the Russian Federation of March 11, 2019 No. 97 "On the Fundamentals of the State Policy of the Russian Federation in the Field of Ensuring Chemical and Biological Safety for the Period up to 2025 and Beyond" was the definition of strategic directions of the state policy in the field of ensuring biological safety. These include maintaining an acceptable level of risk of negative impact of hazardous biological factors on the population and the environment; development of hygienic standards and methods for indicating the content of biological agents in the environment; implementation of modern mechanisms for managing chemical and biological risks; implementation of a set of measures to prevent and minimize biological risks, increase the protection of the population and the environment from the negative impact of hazardous biological factors, as well as assessment of the effectiveness of these measures.

The issues concerning the methodology of development and scientific substantiation of criteria for hygienic assessment of the impact of biological production factors are of particular significance. It should be noted that at the legislative level, there are currently no clear indications of the procedure of industrial control and algorithms for hygienic rationing of harmful factors of the working environment arising at various stages of development and production of GTMP, ATMP, biotechnological MPs, both in the workplace air and in environmental objects.

Industrial biotechnologies, according to GOST R 52249-2009 National Standard "Rules of production and quality control of medicines", represent multistage technological processes, based on the use of different strains and serotypes of living microorganisms, cell cultivation or extraction of material from living organisms, a wide range of raw materials, the formation of a wide range of intermediate and end products of microbial synthesis. This determines the complex nature of harmful effects associated with the process of biological and microbial synthesis and, therefore, the combined nature of harmful effects of biological and other production factors on the body of workers [10, 11].

The general biotechnological scheme of pharmaceutical production includes five stages: strain selection, selection and preparation of nutrient medium, cultivation of strain-producers (fermentation), obtaining inoculum, isolation and purification of the target product. Biotechnological pharmaceuticals require a high degree of purity, which is achieved by successive purification operations, such as separation, destruction of cell membranes (biomass disintegration), separation of cell walls, separation and purification of the product, fine purification and separation of preparations. It should be noted that separation and purification of the product with subsequent separation of preparations and isolation of the target product from the culture fluid or homogenate of destroyed cells is carried out by precipitation (salting), extraction, or adsorption. During this precipitation process, physical (heating, cooling, dilution, concentration) and chemical methods (using inorganic and organic substances — ethanol, methanol, acetone, isopropanol) are applied [11, 12, 13, 14], which creates an additional load in terms of air pollution at the workplace by chemical organic and inorganic compounds.

During production, pharmaceutical ingredients may be released into the workplace air, as a rule, in trace amounts or in high concentrations in emergency situations. Such events may occur in case of non-compliance with sanitary and hygienic requirements, e.g., insufficient sealing of equipment at various stages of the technological process. This may result in contamination of the workplace air, clothing, skin of workers, surfaces of equipment, building structures, industrial sites, and the broader environment. In the air of industrial premises, harmful substances can be found in the form of gases, vapors, aerosols, as well as in the form of mixtures. These substances enter the body mainly through the respiratory tract (inhalation), gastrointestinal tract (orally), skin (transcutaneously), and through the mucous membrane of the eyes in some cases [10, 12, 13, 14].

The risk of inhalation exposure to components of biologic or biotech MPs (detailed in WHO/CDS/CSR/ISR/99.2. Department of Communicable Disease Surveillance and Response) is possible through the following manufacturing manipulations: bacteriological loop calcination, seeding on agar dishes, pipetting, swab preparation, opening cell culture vessels, blood or serum sample collection, centrifugation; the risk of ingestion of a pathogenic agent is likely when handling samples, swabs and cultures; the risk of subcutaneous infection is likely when using needles and syringes when handling blood or removing infected material.

In recent years, the number of therapeutic agents developed on the basis of genetically engineered monoclonal antibodies (mAbs), such as bevacizumab, cetuximab, daratumumab, omalizumab, rituximab, and trastuzumab, has increased significantly. Such agents occupy one of the leading positions in the global pharmaceutical market in terms of production volume [15–17]. As of November 2021, more than 130 antibody-based drugs have been approved or are under consideration by regulatory authorities. In the world's clinical practice, about 35 mAb medications are used for the treatment of oncological, autoimmune, infectious, and allergic diseases characterized by a long progressive course. In Russia, about 23 mAb medications have been registered and are successfully used [14, 18].

As a rule, monoclonal antibodies are large molecules with a molecular mass > 140 kDa, designed for targeting specific proteins [15, 19, 20]. Bispecific monoclonal antibodies (bsAbs) are next-generation antibodies, typically having molecular masses between 50 and 60 kDa, with higher clinical efficacy and safety by targeting two different immunoregulatory pathways. Monoclonal antibodies are produced using hybridoma technology, recombinant DNA, or other technologies [21–23].

The active component of mAb medicines are highly purified immunoglobulins or their fragments, e.g., F(ab') 2-fragments, characterized by specificity to a strictly defined antigen determinant, produced by one clone of antibody-forming cells. The source of mAb production is cloned cells — immortalized ("immortal") B-lymphocytes in the form of a transplanted cell culture or cell line, obtained on the basis of recombinant DNA technology [18, 22]. Immunoglobulins or their fragments can be altered by various modifications: conjugation with a toxin, inclusion of a radioactive tag, chemical binding of two immunoglobulin molecules or their derivatives to obtain an mAbs with dual specificity, creation of Fc-linked fusion proteins — fusion proteins, etc. [16, 19].

Monoclonal antibodies exhibit unusual characteristics. These are large-molecule proteins that are hydrophilic and labile (both chemically and enzymatically), which allows them to be broken down in the gastrointestinal tract. However, these are stable molecules with a long half-life, usually several days or weeks [20, 21]. In addition, according to Brian A. Baldo, conjugation with polyethylene glycol (PEG) or pegylation of mAb further prolongs their half-life and creates additional safety problems associated with the lack of biodegradability of the PEG component [22].

Taft et al. found that for monoclonal antibodies, due to their inherent high specificity of binding and affinity to their target, the main pathway of elimination is target-mediated distribution of drugs, especially at low doses and concentrations [23]. This is the phenomenon of targeted "binding" of a compound, in particular, a monoclonal antibody to a target cell with a receptor type strictly specific thereto. In this case, a small amount of the drug substance is required for the onset of the therapeutic effect [24], which is a favorable criterion for the clinical use of mAb, although significantly increasing the potential risk of occupational exposure of workers to the conditions of its industrial production.

Brian A. Baldo et al. note that adverse reactions from the immune system obtained during clinical observations of mAb use are hypersensitivity reactions, such as anaphylaxis, skin manifestations, generalized cytokine reactions, decreased immune system function, and autoimmune reactions [25].

According to Lars et al., during various technological stages of development and production, protein preparations, including mAb, can be found in the workplace air in the form of gases, vapors, aerosols, and gas-vapor-aerosol mixtures. Such preparations may cause undesirable

side effects in workers of biopharmaceutical companies [26]. Given the vast surface area (more than 100 m^2) of the lining of the pulmonary epithelium, which is in close contact with a wide network of capillaries, the absorption of foreign substances through the lungs by the inhalation route can occur at a high rate [10, 22, 27]. At the same time, the rate of particle settling in the respiratory tract epithelium directly depends on the size of the respirable fraction. Thus, particles larger than 10 μm settle in the nasopharynx and tracheobronchial section (in these sections of the respiratory tract, the epithelium is thicker and covered with a layer of mucus). This limits systemic absorption, not excluding the development of local reactions. In addition, the ciliated epithelium moves mucus-containing particles to the pharynx, where it is swallowed and enters the gastrointestinal tract [28].

Some studies have established that the transport of molecules larger than 0.6 nm through cell layers into the blood via inhalation of protein drugs (mAb, bispecific antibodies, fusion proteins) is provided by alveolar epithelial cells having pores and vesicles by passive diffusion with further manifestation of their systemic effect on the organism [29]. At the same time, recent studies have described that, for larger proteins (>40 kDa), the dominant mechanism of transmembrane protein transport is receptor-mediated transcytosis via the neonatal Fc receptor (FcRn). This mechanism is expressed in the primate upper airways, rat bronchial and alveolar cells with an inherent ability to bind to high affinity proteins, which plays an important role in the transport of IgG to other tissues [30–32]. At the same time, both transcytosis and paracellular mechanisms may be important for smaller proteins [33–35].

Experimental studies by Dumont et al. found that during inhalation exposure of monkeys to protein preparations (including mAb), the level of absorption of IgG1 Fc-domain complex deposited in the lungs was equal to the blood level of protein preparation/mAb during subcutaneous injection in primates and humans [36]. This creates preconditions for accumulation of these preparations in the lung tissue, which can have a negative impact on the organism of workers in the conditions of production at all stages of the technological process.

In a study by Fahy et al. conducted on healthy volunteers, production inhalation exposure to mAb: E25 or omalizumab with a daily exposure via nebulizer 10 min for 56 days was modeled. It was found that more than 15% of the administered medication dose was actually deposited in the alveoli, and the systemic bioavailability of E25 or omalizumab by inhalation ranged within 1.6–4.3% [37–39]. This finding is important for specialists in occupational medicine, since the mAb aggregated in the lungs, even after partial intracellular enzymatic destruction by pulmonary antiproteases, may initiate a cascade of pathological processes in the lung tissue [40]. This should be taken into account when developing regulations for occupational exposure both in the workplace air and in the biological environments of the corresponding pharmaceutical production facilities.

In [41–42], recommendations for establishing occupational exposure limits for monoclonal antibodies and fusion proteins in the workplace air at the level of ≥1 μg/m^3 by inhalation route of entry, taking into account the systemic bioavailability after inhalation of less than 1% for compounds with molecular mass >10 kDa, were proposed.

GTMPs can be used to deliver therapeutic genes into target cells; however, neither DNA nor RNA in free form can be used to achieve this goal due to a rather rapid degradation of nucleic acid in serum under the influence of nucleases. Therefore, vector genetic constructs have been developed for gene delivery into eukaryotic cells since the early 1980s [43]. To date, five major classes of viral vectors have been tested as gene delivery vectors for clinical use, including retroviruses, adenoviruses, adeno-associated viruses, lentiviruses, and herpes simplex viruses [44–45].

According to the data of several clinical and preclinical studies, a number of side effects have been found in GTMPs/ATMPs based on viral vector systems. Thus, some researchers noted that genetic changes mediated by drugs using retroviral vectors with replication deficiency caused manifestations of insertional mutagenesis and malignant transformation of hematopoietic progenitor cells with the development of acute myeloid leukemia and lymphoproliferative diseases [46].

The studies by Ott et al. provided evidence for retroviral vector-induced negative effects on hematopoietic activity, manifested in restoration of oxidative antimicrobial activity in phagocytes after gene transfer, significant gene transfer into neutrophil cells with the formation of a large number of functional phagocytes, and expansion of gene-corrected myelopoiesis with progression toward myelodysplasia [47–48].

Unlike GTMP using retroviral vectors, adenoviral vector-based preparations do not replicate and are not oncogenic. However, they exhibit a pronounced immunogenicity [49] with the activation of immunocompetent cells. These, in turn, begin to secrete cytokines and chemotaxis factors that attract neutrophils, macrophages, and natural killer cells to the focus and trigger an immune response with the production of specific antibodies after several days. Various target cells *in vitro* and several mouse models *in vivo* found that some first-generation adenoviral vectors, which retain a significant part of the genome, are capable of initiating dose-dependent apoptosis, i.e., exhibit direct cytotoxicity [50–51]. Several episodes of inflammatory reaction to adenoviral vectors, including the development of severe hepatotoxicity with lethal outcome, have also been reported in clinical trials [52].

Adeno-associated vectors are among the most common vectors used in gene-targeted therapy, although their use can result in undesirable inadvertent activation or inhibition of endogenous gene expression and infection in primates and humans [53–54].

Lentiviral vectors are derived from HIV-1 and are capable of affecting both dividing and non-dividing cells, making them a potential vector for gene transfer *in vivo*. Most lentiviral vectors retain the ability to integrate into the genome of infected cells; deletion of many HIV proteins reduces the probability of formation of a virus capable of replication in the human body [55]. To obtain pseudotyped lentiviral vectors, envelope glycoproteins of viruses considered to be

potential agents of biological weapons (Ebola, Marburg, Ross River hemorrhagic fever viruses, etc.) are used. Therefore, their use in research is still associated with potential risks, and the long-term safety of these clinical interventions is still being evaluated [56].

Herpesvirus-based vectors provide long-term transgene expression, are neurotrophic and highly effective in studying retrograde and anterograde transport in CNS. However, they are inherently capable of inducing cytopathic (toxic) effects and immune system responses [52].

At present, in the USA and EU countries, recommendations on medical and occupational protection when working with viral vector systems or gene therapy products under the conditions of pharmaceutical enterprises or laboratories and medical institutions are limited to general rules of biosafety when working with biological agents taking the levels of biological risk into account [13]. In the Russian Federation, due to the lack of wide industrial production of gene preparations, there are no coordinated and clear algorithms for assessing exposure to GTMP/ATMP/biotechnological components. In addition, the question of scientific justification for the principles of hygienic aerosol rationing of pharmaceutical components of GTMP/ATMP / biotechnological MPs for controlling the air of the production environment of enterprises or laboratories of the biotechnological industry remains open.

CONCLUSIONS

Our study outlines a number of aspects regarding the development of new-generation biological medicinal products (GTMP/ATMP/biotechnological medication) and associated risks of occupational exposure of workers in the conditions of pharmaceutical or laboratory production. The conducted literature review revealed that workers are exposed to the combined effect of adverse factors of production environment of a biological, physical, and chemical nature. There is a lack of information on the development of analytical methods for the identification of GTMP/ATMP components or biotechnological medications in the workplace air, wastewater, on working surfaces, etc. Only single reports were found in the available literature.

The conducted work allowed us to determine the key methodological approaches to assessing the potential industrial impact of GTMP, ATMP, and biotechnological medications on the employees of pharmaceutical companies. These include toxicological assessment of compounds with the establishment of possible toxicometry parameters; analysis of pharmacological and toxicokinetic features of the components of gene preparations; development of methods for their quantitative determination in different media; establishment of biomarkers of exposure and effect, with subsequent hygiene rationing and justification of key preventive measures.

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THE LONG TIME SUSPENSION TRAUMA: A REVIEW

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Introduction. Suspension trauma, also referred to as a harness-induced pathology or suspension syndrome, occurs when an individual is suspended motionless in a harness. Potentially fatal outcomes of such a condition consist in venous pooling, cerebral hypoperfusion, and rhabdomyolysis.

Objective. To review literature sources on the key mechanisms of suspension injury and potential methods for improving the safety of people at risk.

Results. This condition, recognized since the 1970s, affects individuals involved in activities requiring harness use, such as climbing and industrial work. Recent studies have emphasized the need for immediate horizontal positioning during rescue to restore blood flow and prevent complications. Proper management of hyperkalemia and rhabdomyolysis has become a crucial focus in treatment protocols. Additionally, recognition of the role of the Bezold–Jarisch reflex in cardiovascular collapse highlights the importance of comprehensive rescue strategies. Advances in harness design are also noted as significant preventive measures.

Discussion. The findings indicate that while early management strategies focused on preventing sudden blood return to the heart by maintaining an upright position, more recent insights emphasize the importance of prompt horizontal repositioning. The role of neurocardiogenic factors, such as the Bezold–Jarisch reflex and the influence of rhabdomyolysis-related hyperkalemia, on outcomes has been recognized. This shift reflects an increased awareness of comprehensive rescue protocols that might mitigate risks associated with reflow syndrome and cardiovascular instability.

Conclusions. The progress in understanding suspension injury has significantly improved prevention and treatment protocols. Immediate adjustment of the victim to a horizontal position, proper treatment of complications (for example, hyperkalemia), and improved design of safety systems — all play a key role in minimizing deaths. Further studies should be aimed at investigating the main pathogenetic mechanisms of suspension syndrome and development of advanced rescue methods for improving the safety of people at risk.

Keywords: harness-induced pathology; harness use; industrial safety; prolonged suspension; recreational accidents; suspension shock; suspension syndrome; suspension trauma

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ТРАВМА ПОДВЕШИВАНИЯ В ТЕЧЕНИЕ ПРОДОЛЖИТЕЛЬНОГО ВРЕМЕНИ: ОБЗОР

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Введение. Травма подвешивания, также известная как синдром зависания в обвязке или синдром подвешивания, возникает, когда человек подвешивается неподвижно в страховочных стропах, что приводит к потенциально фатальным последствиям, таким как венозный застой, церебральная гипоперфузия и рабдомиолиз.

Цель. Изучить по данным литературы основные механизмы возникновения травмы подвешивания и потенциальные методы повышения безопасности лиц в группе риска.

Результаты. Это патологическое состояние, впервые упоминаемое еще в 1970-х годах, возникает у людей, занимающихся видами деятельности, требующими использования страховочных систем, такими как альпинизм и промышленные работы. Согласно последним исследованиям в ходе оказания помощи для восстановления кровотока и предотвращения осложнений необходимо немедленно перевести пострадавшего в горизонтальное положение. Надлежащая коррекция гиперкалиемии и рабдомиолиза стала ключевым направлением в протоколах лечения. Кроме того, признание роли рефлекса Бецольда — Яриша в развитии сердечно-сосудистого коллапса подчеркивает важность комплексных стратегий спасения. В качестве важных превентивных мер также отмечаются достижения в разработке более совершенных и безопасных страховочных систем.

Обсуждение. В то время как первоначальные стратегии лечения были направлены на предотвращение внезапного возврата крови к сердцу путем поддержания вертикального положения, последние исследования подчеркивают важность своевременного горизонтального положения. Роль нейрокардиогенных факторов, таких как рефлекс Бецольда — Яриша и влияние гиперкалиемии, связанной с рабдомиолизом, на исход подчеркивают эволюционирующее понимание патофизиологии. Этот сдвиг отражает возросшую осведомленность о комплексных протоколах спасения, которые снижают риски, связанные с синдромом восстановленного кровотока и сердечно-сосудистой нестабильностью.

Выводы. Прогресс в понимании травмы подвешивания значительно улучшил протоколы профилактики и лечения. Немедленное переведение пострадавшего в горизонтальное положение, надлежащее лечение осложнений (например, гиперкалиемии) и усовершенствованная конструкция страховочных систем играют ключевую роль в минимизации летальных исходов. Продолжение изучения основных механизмов патогенеза синдрома подвешивания и разработка новых методов спасения имеют большое значение для дальнейшего повышения безопасности лиц в группе риска.

Ключевые слова: патология страховочной системы; использование страховочных систем; охрана труда; длительное подвешивание; несчастные случаи на отдыхе; шок подвешивания; синдром подвешивания; травма подвешивания

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INTRODUCTION

Suspension trauma, also referred to as harness-induced pathology or suspension syndrome, is a potentially fatal condition that occurs when an individual remains suspended in a harness for a prolonged period of time. Suspension trauma occurs relatively rare in well-regulated industrial settings due to effective prevention strategies and proper equipment.

Over an 11-year span of observation, 5.8 million hours of using a harness by qualified personnel in different fields were recorded. During this period, no episodes of syncope or injuries associated with prolonged suspension were registered [1]. This could be related to efforts from employers to ensure the safety of their staff members. Only a few cases of this complication during the mentioned time span were reported from healthy individuals in sporting accidents and recreational activities in cases of poor training and improper use of harness [2, 3]. The mortality rate associated with suspension trauma varies widely depending on several factors, including the duration of suspension, the condition and comorbidities of the patient, and the promptness of rescue. Although accurate statistical data is lacking, some studies suggest that the risk of severe injury or death increases significantly following approximately 15 minutes of suspension. If the patient is not rescued within half an hour, the mortality rate can increase up to 50%. Timely rescue and pertinent management are crucial for improving patient outcomes [4].

In this review, we carry out an analysis of the evolving understanding of suspension trauma, focusing on key scientific advancements, treatment protocols, and the pathophysiological mechanisms involved. Importantly, no cases of suspension trauma from Poland have been documented so far.

Historical Development

The first accounts of suspension trauma were reported during the Second International Conference of Mountain Rescue Doctors in 1972. This pivotal moment raised awareness about the dangers associated with immobile suspension in a harness, particularly for climbers and cave explorers. One report at that conference informed that out of the 10 individuals who had been trapped in a suspended position, two died before rescue, three died immediately afterward, and five were pronounced dead over the next several days [2, 4]. Early hypotheses attributed these fatal cases to circulatory collapse, encouraging researchers to investigate the underlying causes and outcomes of suspension trauma.

The term “suspension trauma” has gained widespread recognition, particularly in high-risk industries and activities involving harness use. It was initially thought that the harness itself induced a “tourniquet effect,” compressing major veins and arteries, which in turn led to circulatory shock [3]. However, this initial assumption was challenged by later research indicating that venous pooling, rather than mechanical compression from the harness, was a significant factor in the development of suspension trauma [1, 5].

Pathophysiology

The physiological response to prolonged vertical suspension is complex, involving several mechanisms. When a person is suspended motionless in a vertical position, gravity causes blood to pool in the lower extremities. This pooling leads to a decreased venous return to the heart, resulting in a significant reduction in cardiac output and cerebral perfusion. During movement, venous pressure in the foot typically measures approximately 25 mmHg; however, in the state of immobility, it can exceed 90 mmHg [6]. This pooling can result in up to 20% of circulating blood volume

being retained in the lower limbs, leading to a reduction in venous return to the heart, which decreases preload and, consequently, cardiac output. As cardiac output declines, there is a reduction in systemic blood pressure, which severely compromises cerebral perfusion [7]. The body attempts to compensate for this situation by increasing the sympathetic tone, which causes the heart rate to accelerate. However, these compensatory mechanisms are often insufficient to counteract the effects of sustained venous pooling. As cerebral blood flow continues to drop, the brain experiences reduced oxygen delivery, leading to presyncopal symptoms such as dizziness, light-headedness, blurred vision, nausea, and sweating. Given that the individual remains suspended and corrective actions are not taken, these symptoms can quickly progress to syncope (fainting) due to critical hypoperfusion. If unconsciousness ensues, there is a significant risk of death, particularly if the suspension position compromises airway patency or worsens cardiovascular instability [1, 5].

The Bezold–Jarisch reflex, which triggers bradycardia and hypotension in response to reduced blood flow to the heart, is thought to play a key role in the rapid collapse experienced by those affected by suspension trauma [6, 7]. Once the individual loses consciousness, they cannot move into a horizontal position, exacerbating the problem and leading to further reductions in cerebral perfusion and oxygenation [3, 1].

However, the hypothesis that a reduction in cardiac preload, and consequently a decrease in cardiac output, is the primary cause of loss of consciousness and other injuries has not been confirmed so far. Recent reviews highlight the importance of neurocardiogenic mechanisms leading to reduced cerebral perfusion and loss of consciousness. In the suspension trauma syndrome, significant effects on systemic hemodynamic parameters, such as compensatory tachycardia and reduced stroke volume — typically associated with low cardiac preload — are not observed [8].

While the Bezold–Jarisch reflex may be linked to suspension syndrome, experimental studies have provided sufficient evidence. The mechanoreceptors responsible for this reflex, located in the left ventricle, respond to poor ventricular filling [9]

The mechanism of post-rescue death remains to be unclear. The available literature indicates that the sudden return of acidotic blood, which accumulates in the veins of the lower body, to the heart is capable of temporarily depressing cardiac contractility. However, this is not associated with heart rhythm abnormalities. Published review papers highlight rhabdomyolysis as a key concern in the suspension trauma syndrome, primarily resulting from reduced blood flow and muscle damage, leading to the release of substances such as myoglobin and potassium. The higher risk of death due to suspension trauma can relate to the suspension duration of more than 30 minutes, height of more than five feet, and older age.

Clinical Observations and Case Reports

Several case studies have provided information on the clinical manifestations of suspension trauma. For example, a notable case from 2011 involved a climber found unresponsive in his harness after being suspended for several hours. The autopsy results revealed the death to result from mechanical asphyxia, a consequence of immobility and venous pooling [10]. Another series of incidents reported climbers who experienced “rescue death” shortly after being rescued, highlighting the delayed effects of suspension trauma [12].

In addition to venous pooling and circulatory collapse, rhabdomyolysis — the breakdown of muscle tissue due to immobility — has been frequently associated with suspension trauma. This condition releases myoglobin into the bloodstream, which can lead to acute renal failure and complicate treatment [2, 13]. Elevated potassium levels (hyperkalemia) caused by rhabdomyolysis can also lead to fatal cardiac arrhythmias [13].

Management and Treatment

For decades, the standard treatment protocol for suspension trauma has emphasized the importance of keeping victims in an upright position after rescue. This approach was based on the belief that lying the victim down would cause a sudden return of pooled blood to the heart, leading to cardiac overload and rescue death [5, 9]. The protocol was formulated in 1970s on the basis of observational studies and opinions of experts largely from nonmedical fields [2, 3, 9]

The study by Pasquier et al. (2010) demonstrated the significance of airway management and fluid resuscitation, consistent with ALS guidelines, over other interventions in rescuing injured individuals. However, there is a lack of scientific evidence to support the claim that positioning a patient horizontally during assistance increases the risk of death [14].

The guidelines of the International Commission for Mountain Emergency Medicine (ICAR MedCom) have also been updated. In the latest version, one of the key recommendations is to position victims horizontally in a proper manner. This measure restores cardiac output and prevents the effects of venous stasis. Additionally, initiating resuscitation procedures at the earliest sign of cardiac arrest is a top priority, including treatment for hyperkalemia and potential rhabdomyolysis [15].

Prevention and Harness Design

An essential component in preventing suspension trauma is the use of appropriate harnesses with multiple attachment points and adjustable straps, as well as devices in the type of leg loops. Such equipment allows for greater mobility, reducing the risk of venous blood pooling.

Equally important is the proper education and training of both harness users and rescue teams. Users should be informed of early symptoms of suspension trauma, such as hot flashes, sweating, and dizziness, and be reminded to move their legs while suspended [3, 1]. Rescue teams should be trained to quickly reposition the injured individual and minimize the duration of immobility [1, 5].

Research Trends and Future Directions

The current understanding of suspension trauma has improved significantly. However, treatment and prevention measures are constantly under discussion. One of the key areas of debate is whether there are specific variables, such as duration of suspension, type of harness, or pre-existing medical conditions, that increase the risk of severe outcomes, including rescue death [1, 9, 16]

A promising area of research focuses on the role of reflow syndrome, also known as rescue death. This syndrome describes the potential for sudden cardiac failure following the return of pooled, deoxygenated blood to the heart [8, 17, 18]. Earlier studies hypothesized that rescue death could result from placing the victim in a horizontal position too quickly. More recent findings, however, suggest that this phenomenon is more likely related to hyperkalemia and acidosis, both of which can arise from the release of muscle breakdown products during rhabdomyolysis [2, 13, 19]. Continued research into these mechanisms, including studies of human physiology during suspension, is needed to further refine rescue protocols. Understanding of the key mechanisms, such as the role of inflammatory cytokines and oxidative stress in reperfusion injuries, and the effects of prolonged suspension on electrolyte imbalances and muscle degradation are promising research directions for optimizing rescue techniques and improving patient outcomes.

According to current studies, including randomized controlled trials, rapid recovery of blood flow to the brain and heart can be safely achieved by laying the victim flat, countering earlier concerns about rapid reperfusion injury [16]. However, challenges in conducting long-term human studies due to ethical concerns limit the analytical data about severe cases and long-term consequences.

The evolving prevention strategies include improved harness designs that allow suspended individuals to acquire semi-horizontal positions or activate leg muscles to prevent venous pooling. Research into the ergonomic design of harnesses continues, with efforts focused on improving comfort, reducing pressure on the femoral veins, and facilitating faster self-rescue or external rescue. These advances will likely reduce the number of fatal and severe cases of suspension trauma in the future [1, 19].

CONCLUSION

Suspension trauma, a condition once poorly understood, is now recognized as a complex medical phenomenon that requires timely and informed intervention to prevent fatal outcomes. Over the years, the pathophysiological processes occurring in suspension trauma, such as venous pooling, cerebral hypoperfusion, and the Bezold–Jarisch reflex, have been elucidated. Modern harness designs, improved rescue techniques, and updated medical protocols have significantly reduced the risk of death associated with suspension trauma, although further research is required to optimize treatment and prevention strategies.

While earlier practices focused on keeping victims upright after rescue, contemporary guidelines recommend immediate horizontal positioning and followup care to manage conditions such as hyperkalemia and rhabdomyolysis. As research continues and harness technology advances, the risks posed by suspension trauma are expected to diminish. At the same time, awareness and preparedness remain critical in minimizing the dangers faced by workers and adventurers using harness systems.

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LUNG CANCER IMMUNOTHERAPY: STATUS QUO, PROBLEMS, AND PROSPECTS

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Introduction. Lung cancer is the leading cause of cancer mortality in men and women. Due to its high prevalence and significant recurrence rate after standard therapy, the search for new methods of lung cancer treating is an urgent task. A promising treatment strategy is immunotherapy that elicit immune response against tumor cells.

Objective. Evaluation of the clinical efficacy and prospects for the safe use of immunotherapy in malignant neoplasms of the pleural cavity.

Discussion. The introduction of immunotherapeutic approaches, including adoptive cell therapy with tumor-infiltrating lymphocytes (TIL) or CAR-T cells, the development of neoantigen vaccines, oncolytic viruses, in combination with chemotherapy and blockade of immune checkpoints (ICP) have shown optimistic results in preclinical studies and are currently at different stages of clinical trials for safety and efficacy.

Conclusions. Immunotherapy of lung cancer is a promising area of adjuvant therapy. For clinical introduction, immunotherapeutic approaches should be further investigated to increase their effectiveness and minimizing side effects by combining different therapies, improving bioengineered and cellular drugs, and reducing the cost of treatment.

Keywords: lung cancer; adoptive immunotherapy; chimeric T-cell antigen receptor; tumor-infiltrating lymphocytes; immune checkpoint inhibitors; oncolytic viruses

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ИММУНОТЕРАПИЯ РАКА ЛЕГКОГО: STATUS QUO, ПРОБЛЕМЫ И ПЕРСПЕКТИВЫ

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Введение. Рак легкого является основной причиной онкологической смертности и у мужчин, и у женщин. Ввиду высокой распространенности и значительной частоты рецидивов после стандартной терапии поиск новых методов лечения рака легкого является актуальной задачей. Одним из обнадеживающих направлений стала иммунотерапия, целью которой является активация цитотоксического иммунитета против опухолевых клеток.

Цель. Оценка клинической эффективности и перспектив безопасного использования иммунотерапии при злокачественных новообразованиях плевральной полости.

Обсуждение. Внедрение иммунотерапевтических подходов, включающих адоптивную клеточную терапию опухоль-инфильтрирующими лимфоцитами (TIL) или CAR-T- клетками, разработку онковакцин, онколитических вирусов, в комбинации с химиотерапией и блокированием иммунных контрольных точек (ИКТ) показало положительные результаты на стадии доклинических исследований и находится на разных этапах клинических испытаний безопасности и эффективности.

Выводы. Иммунотерапия рака легкого является перспективным направлением адъювантной терапии. Клиническая трансляция иммунотерапевтических подходов нуждается в повышении их эффективности и минимизации побочных эффектов путем комбинации различных методов терапии, совершенствования биоинженерных и клеточных препаратов, а также снижения стоимости лечения.

Ключевые слова: рак легкого; адоптивная иммунотерапия; химерный антигенный рецептор антигена Т-клеток; опухоль-инфильтрирующие лимфоциты; ингибиторы иммунных контрольных точек; онколитические вирусы

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INTRODUCTION

Lung cancer remains the leading cause of cancer deaths worldwide (18.4% of total cancer deaths), resulting in significant socioeconomic losses. According to estimates by GLOBOCAN and the International Agency for Research on Cancer (as of 2018), 2.09 million new cases and 1.76 million deaths from lung cancer were registered, which exceeded the data of 2012 [1]. Due to the long-term asymptomatic course and nonspecific initial symptoms, along with an insufficiently developed strategy of active cancer screening, almost half of patients are diagnosed with this nosology at the metastatic stage of the disease, when radical surgical treatment is almost impossible [2]. According to Kelsey et al., one third of patients diagnosed with the disease and treated in the early stages develop relapse and resistance to chemotherapy [3]. In this regard, the search for new therapeutic methods for lung cancer remains to be an urgent clinical task.

Immunotherapy as a whole represents a broad scientific direction in oncopathology treatment. This direction involves activation of antitumor immunity through the use of antibodies, cytokines, immune cells, chimeric T-cell receptors, inhibitors of immune control points, etc. Immunotherapy has shown its efficacy and safety in the treatment of oncohematological diseases and melanoma [4, 5]. In relation to other solid tumors, clinical studies have shown inconsistent results; however, the prospects of this approach are beyond doubt [6].

The aim of immunotherapy for lung cancer is to enhance the targeted cytotoxicity of immune cells mainly due to specific binding to tumor-associated antigens [7]. This aim is hard to achieve due to the ability of tumor cells to avoid the effects of the immune system by secreting immunosuppressive cytokines, loss of expression of antigens of the main histocompatibility complex, and expression of molecules inhibiting T-cell activation, i.e., cytotoxic T-lymphocyte glycoprotein 4 (CTLA-4), programmed cell death protein 1-PD-1, programmed death ligand 1 (PD-L1) [8]. Due to the immunosuppressive tumor microenvironment, early attempts at immunotherapy for non-small cell lung cancer (NSCLC) proved ineffective [9]; however, the development of molecular biology and immunogenetics over the past ten years contributed to the development of new approaches to overcoming immunosuppression and increasing the focus of the antitumor response, which revived interest in this topic. At present, according to ClinicalTrials.gov, more than 923 studies are being conducted in lung cancer immunotherapy, with this number growing steadily. The types of immunotherapeutic treatment are highly diverse, including, e.g., antitumor vaccines based on sensitized dendritic cells and tumor neoantigens, oncolytic viral therapy, therapy with immune checkpoint inhibitors (ICI), therapy with tumor-infiltrating lymphocytes, CAR-T, CAR-NK therapy, etc. [10].

In this article, we evaluate the clinical efficacy and prospects for the safe use of immunotherapy in malignant neoplasms of the pleural cavity.

DISCUSSION

Therapy with tumor-infiltrating lymphocytes (TIL) in lung cancer

Therapy using tumor-infiltrating lymphocytes (TIL) is a type of adoptive cell therapy that involves TIL extraction from the tumor stroma, their subsequent reproduction and activation outside the body (*ex vivo*), and reinfusion back into the patient's body [11]. TILs isolated from the tumor microenvironment can be targeted against various tumor-specific neoantigens, which renders them more effective against heterogeneous lung cancer cells. Due to stimulation by tumor antigens in vivo, TILs possess a significant number of effector memory T-cells expressing chemokine receptors (CCR5 and CXCR3) on their surface, which contributes to more efficient and targeted delivery to the tumor site [12]. Due to the negative selection of the T-cell receptor in the early stages of immune development and the use of autologous cells in patients without gene modifications, TIL therapy exhibits low toxicity [13].

The immune microenvironment in lung cancer is a complex system that includes T-lymphocytes, B-lymphocytes, natural killers, macrophages, dendritic cells, etc. The type, density, and location of immune cells in the tumor microenvironment play a key role in the processes of carcinogenesis, cancer progression, and treatment efficacy [14]. A study of the immune microenvironment in NSCLC revealed that long-term treatment outcomes, such as overall survival, depend on the nature of infiltrating lymphocytes, rather than on their number. Thus, an abundance of $CD8^+$ T-cells expressing cytolytic enzymes, $CD4^+$ T-cells lacking expression of inhibitory receptors, and an increased level of tumor infiltrating B-cells are associated with improved survival rates [15]. Tumor B-cells secrete tumor-specific antibodies that stimulate T-cell responses and support the structure and function of tertiary lymphoid structures. However, B-cells with a variety of effects can become immunosuppressants, producing IL-10 and promoting tumor growth. New immunotherapy strategies should simultaneously activate antitumor B-cells and suppress Breg phenotypes [16]. TILs can also be predictive biomarkers of response to therapy with ICI. A relationship was found between the $CD8^+/CD4^+$ ratio in tumor tissue and the response to treatment with ICI in patients with NSCLC, which can be used for prognostic purposes [17].

Currently, several clinical studies are being conducted to assess the safety and efficacy of administration of both unchanged and genetically engineered TIL to patients with progressive NSCLC (Table 1). The effectiveness of adoptive cell therapy is further enhanced by the use of non-myeloablative lymphodepletion (Cyclophosphamide + Fludara bine) before infusion of TIL, subsequent administration of interleukin-2, as well as due to combination with therapy with ICI. In one of the completed phases I clinical trials (NCT03215810), the safety and efficacy of autologous TIL therapy was proven in 20 patients with progressive NSCLC after ineffective nivolumab monotherapy with an overall response rate of 70% [18]. The results of the remaining studies have yet to be analyzed (Table 1).

It should be noted that therapy with tumor-infiltrating lymphocytes has limitations associated with the complexity and high cost of obtaining a sufficient amount of TIL from tumor tissue for therapy. At the same time, TIL therapy should currently be considered only as adjuvant therapy, i.e., after surgical removal of the tumor, to combat distant metastases. Simplification and cost reduction of TIL production technology is, therefore, extremely important for widespread clinical implementation.

In general, despite the accumulated data on the potential efficacy of TIL therapy in NSCLC, widespread adoption of this technology requires both overcoming technological problems of standardization, simplification and cheapening of TIL production technology. In addition, further clinical trials of TIL in various combinations and at various stages of lung cancer are required to clarify the indications for immunotherapy and identify groups of patients for whom a certain immunotherapy will be most effective.

CAR-T cell therapy for lung cancer

Chimeric T-cell antigen receptor cells (CAR-T) are patient T-cells that, due to genetically modified chimeric antigen receptors, are capable of recognizing antigens on tumor cells and trigger a signaling cascade of activation of effector functions of T-cells. CAR-T cells, divided into five generations according to intracellular signaling structural domains, have an extracellular domain for antigens, a transmembrane domain, and an intracellular domain for signal transmission into the cell [19]. One of the advantages of CAR-T therapy is its specificity, independence from the expression of proteins of the major histocompatibility complex (MHC), which is often suppressed in tumor cells, as well as the ability to provide a stable and long-lasting antitumor response due to the continued proliferation of injected cells in the patient's body [20].

The use of CAR-T-cell therapy in oncohematological diseases has demonstrated impressive results, leading to the approval of the FDA (USA Food and Drug Administration) of this treatment method [21]. The current research is focused on extending the indications for the CAR-T-cell therapy to combat solid tumors.

A meta-analysis that included 22 studies involving 262 patients showed that the overall response rate to CAR-T cell therapy in various solid tumors was 9%. Moreover, various strategies (lymphodepletion before T-cell infusion, transfection method, CAR-T cell persistence, total cell dose, and IL-2 administration) did not significantly affect the effectiveness of treatment [22]. Modest results of CAR-T therapy in relation to solid tumors are often associated with a lack of tumor-specific antigens, a low level of infiltration of CAR-T cells into tumor tissue, and a pronounced

Table 1. Clinical studies of the adaptive cell therapy with autologous tumor-infiltrating lymphocytes in non-small cell lung cancer

№	Diagnosis	Treatment	Phase	*n*	Result	Side effects	Clinical trial ID
1	NSCLC	TIL (LN-145) + IL-2 + non-myeloablative lymphodepletion (Cyclophosphamide + Fludarabine) + Nivolumab	I	20	70% — overall response rate; 10% — complete response; 60% — partial response	Associated with lymphodepletion and with the introduction of IL-2	NCT03215810
2	NSCLC; metastatic melanoma; squamous cell carcinoma of the head and neck	TIL (LN-145) + IL-2 + non-myeloablative lymphodepletion (Cyclophosphamide + Fludarabine) + Pembrolizumab/ Ipilimumab/Nivolumab	II	178	The research is ongoing	No data available	NCT03645928
3	NSCLC	TIL (LN-145) + IL-2 + non-myeloablative lymphodepletion (Cyclophosphamide + Fludarabine)	II	95	The research is ongoing	No data available	NCT04614103
4	stages III and IV NSCLC; metastatic melanoma	Genetically modified TIL (IOV-4001) + IL-2 + non-myeloablative lymphodepletion (Cyclophosphamide + Fludarabine)	I/II	Set	The research is ongoing	No data available	NCT05361174
5	NSCLC; cervical cancer; melanoma	TIL (LM103) + IL-2 + non-myeloablative lymphodepletion (Cyclophosphamide + Fludarabine)	I	15	The research is ongoing	No data available	NCT05366478
6	NSCLC	L-TIL (Liquid Tumor Infiltrating Lymphocytes) + Tislelizumab + Docetaxel	II	33	The research is ongoing	No data available	NCT05878028
7	NSCLC; melanoma; colorectal cancer	Epigenetically reprogrammed TIL (LYL845)	I	108	The research is ongoing	No data available	NCT05573035
8	NSCLC; colorectal cancer; melanoma and others	TIL + IL-2 + non-myeloablative lymphodepletion (Cyclophosphamide + Fludarabine)	I	18	The research is ongoing	No data available	NCT05902520
9	NSCLC	TIL + IL-2 + non-myeloablative lymphodepletion (Cyclophosphamide + Fludarabine) + Aldesleukin	II	85	The research is ongoing	No data available	NCT02133196
10	NSCLC; breast cancer; colorectal cancer; melanoma	TIL (TBio-4101) + IL-2 + non-myeloablative lymphodepletion (Cyclophosphamide + Fludarabine) + Pembrolizumab	I	60	The research is ongoing	No data available	NCT05576077

Table prepared by the authors according to the ClinicalTrials.gov data

immunosuppressive tumor microenvironment [23]. In addition, this method of immunotherapy leads to serious side effects, including cytokine storm and neurotoxicity [24]. In order to resolve the problem of low recruitment of T-cells into the tumor site, CAR-T cells were injected into the tumor, which showed encouraging results in an experimental mouse model [25]. Methods of molecular modifications in T-cells can also be used to enhance targeted delivery [26]. In order to overcome the immunosuppressive microenvironment, attempts have been made to combine CAR-T cell therapy with ICI therapy [27]. Toxicity and optimal therapeutic dosage remain to be determined.

The first step in successful adoptive T-cell therapy is to select the optimal tumor-associated antigen (TAA) for CAR-T cells. Most of the antigens used in CAR-T therapy for lung cancer (EGFR, MSLN, MUC1, PSCA, CEA, D-L1, CD80/CD86, ROR1, and HER2) are also expressed in normal human tissues, which can lead to non-targeted toxic effects [28]. Recently, a new target for lung cancer has been found in the form of LunX (lung-specific protein X), an antigen belonging to the family of clone proteins of the palate, lungs, and nasal epithelium. [29]. Unlike other antigens, LunX is often highly expressed in NSCLC cells, although not being expressed in normal lung tissues [30]. Preclinical studies evaluating the effectiveness of LunX-CAR-T therapy on a xenograft model of lung cancer have shown promising results. It has been experimentally proven that LunX-CAR-T cells inhibit the growth of LunX-positive tumor cells and prolong the survival of mice [31]. In parallel, CAR-T cells targeting c-Met, a transmembrane receptor with tyrosine kinase activity expressed mainly in epithelial cells, are being developed [32]. Preliminary studies have shown that c-Met-directed CAR-T cells demonstrate pronounced antitumor activity both *in vitro* and *in vivo* against NSCLC, offering promising treatment routes [33].

Currently, phase I and II clinical trials of CAR-T therapy for NSCLC are underway, targeting various targets (epidermal growth factor, mesothelin, PD-L1, mucin-1) in combination with or without immunotherapy, with varying efficacy and toxicity [34–36]. In particular, the response to EGFR-CAR-T-cell therapy for EGFR + NSCLC was noted in two patients out of 11 (18%) [34]. In another phase I clinical trial study with intrapleural administration of mesothelin-targeted CAR-T in combination with pembrolizumab therapy for lung cancer and pleural mesothelioma, a good response was observed in only two patients out of 27 (7%) [35]. MUC1-CAR-T-cell therapy in 20 patients with NSCLC led only to stabilization of the disease without visible signs of improvement in 11 patients, while the remaining patients showed disease progression [36]. A number of studies have been discontinued due to the high toxicity of CAR-T-cell drugs.

Note should be made that despite intensive research, immunotherapy with CAR-T cells has not yet shown any significant clinical effect in the fight against lung cancer. In addition, in its current form, such an immunotherapy is burdened with a rather high toxicity. The research into CAR-T cell therapy for solid tumors in general and lung cancer in particular is in its nascent stage, requiring additional efforts in assessing the possibility of its clinical application.

Inhibitors of immune control points in lung cancer

Immunotherapy using checkpoint inhibitors (ICI) of the immune system is one of the most significant breakthroughs in the treatment of oncological diseases. Indeed, a number of multicenter studies have shown its efficacy in increasing the median survival rate in numerous malignancies, including lung cancer. This technology is associated with the inhibition of immunosuppressive proteins CTLA-4 and PD-1/PD-L1, which, in turn, activates cellular antitumor immunity [37]. The interaction of PD-1, located on the surface of thymocytes and other elements of the immune system, with its PD-L1 ligand on tumor cells suppresses the activity of T-cells, reducing their ability to recognize and destroy tumors. Lung cancer often uses this mechanism to avoid the immune response. CTLA-4 is another inhibitory receptor on T-cells, the blockade of which contributes to an increase in the number of activated T-cells and memory T-cells, enhancing the immune system attack on the tumor [38]. The following immune control molecules are being evaluated as potential targets for cancer immunotherapy: molecule 3 containing T-cell immunoglobulin and mucin domain (TIM-3), transmembrane glycoprotein type I (B7-H3), immunoglobulin suppressing activation of T-cells in the V domain (VISTA), lymphocyte activation gene-3 (LAG-3), T-cell immunoglobulin, and ITIM domain (TIGIT) [39].

At the moment, over 300 clinical trials aimed at studying the effectiveness of the ICI in lung cancer therapy have been successfully completed. On their basis, the European Medicines Agency and the USA Food and Drug Administration approved one CTLA-4 inhibitor drug (ipilimumab), five PD-1 inhibitor drugs (nivolumab, pembrolizumab, cemiplimab, sintilimab, camrelizumab), and two PD-L1 inhibitors (durvalumab, atezolizumab) for the treatment of NSCLC. Some other drugs undergo different stages of approval. In the nearest future, new drugs in each of the ICI groups are likely to appear [40].

Randomized clinical trials in patients with PD-L1-positive tumors with an expression of at least 50% showed single-component immunotherapy with ICI to be superior to adjuvant chemotherapy in terms of both toxicity and overall survival [41, 42]. The KEYNOTE-024 trial (phase III, 305 patients) revealed that pembrolizumab, as a first-line therapy in patients with metastatic NSCLC with PD-L1 expression>50%, significantly improved overall survival rates with a lower level of side effects compared to platinum-containing chemotherapy [43]. In addition, KEYNOTE-042 trial (1,274 patients, phase III) [44] and IMpower110 trial (phase III, 572 patients with metastatic NSCLC who had not previously received chemotherapy and whose PD-L1 expression was at least ≥1%) confirmed that ICI therapy provides a significant improvement in the survival of patients with various degrees of PD-L1 expression. However, a particularly pronounced effect was noted in individuals with higher expression levels [45]. This indicates the expediency of selecting immunotherapy as the primary treatment method in patients with locally advanced unresectable or metastatic NSCLC with PD-L1 expression of more than 1%.

When developing advanced treatment methods for lung cancer, special attention is paid to the potential of combining immunotherapy and chemotherapy. According to the results of the 5-year clinical trial KEYNOTE-189 phase III (NCT02578680) in 616 randomized patients with untreated metastatic NSCLC without EGFR/ALK changes on combined immunotherapy and chemotherapy (n = 410 pembrolizumab plus pemetrexed plus platinum), the 5-year overall survival rate was 19.4%. At the same time, the use of only mono-chemotherapy (n = 206 placebo plus pemetrexed plus platinum), the 5-year overall survival rate was 11.3%. Among 57 patients who completed 35 cycles of taking pembrolizumab, the objective response rate was 86.0% [46]. A meta-analysis of 66 studies showed that neoadjuvant immunotherapy for resectable non-small cell lung cancer is safe and effective. In comparison with chemotherapy alone, chemoimmunotherapy improved therapeutic response and survival rates to a greater extent [47]. These data continue to confirm that the combination of ICI therapy with chemotherapy improves the survival of patients with NSCLC, regardless of PD-L1 expression. A phase III CheckMate 9LA large trial demonstrated positive results in overall survival with nivolumab plus ipilimumab compared with chemotherapy in patients with NSCLC, regardless of PD-L1 expression. This prompted the use of a dual immunotherapy approach without chemotherapy [48]. Combination of immunotherapy with other therapeutical approaches to achieve the best effect and reduce side effects deserves further study.

The most common side effects of ICI therapy related to immunity are skin and endocrine disorders, such as rash, itching, and thyroid dysfunction [49]. There is an increasing amount of literature data on cardiovascular toxicity, in particular myocarditis, which requires a more comprehensive assessment of the baseline parameters of the cardiovascular system and optimization of risk factors [50]. Fatal cases are rare, ranging from 0.36% with single-agent immunotherapy to 1.23% with combined immunotherapy [51].

It should be noted that despite significant clinical improvements, most patients ultimately do not respond to ICI therapy due to the development of primary or secondary resistance [52]. A retrospective study of 1201 patients with NSCLC treated with PD-1 inhibitors showed that 78% of 243 cases developed secondary resistance after the initial response [53]. In 74% of patients with NSCLC with an effective initial response to immunotherapy, disease progression was observed within five years. The mechanism of resistance to immunotherapy is rather complex, being most likely associated with changes in the interaction between cells and surrounding cell populations within the tumor microenvironment (TME) [54]. Research into cellular interactions within TME and creation of reliable methods for evaluating immune cells and their effect on the tumor may shed light on the mechanism of overcoming resistance and increasing the effectiveness of ICI therapy. Nevertheless, checkpoint inhibitors have already significantly changed treatment approaches to lung cancer in a positive way. Along with advancement of theories and technologies, more effective treatment options can be expected.

Oncolytic phytotherapy for lung cancer and some other tumors of the pleural cavity

Oncolytic virotherapy (OVT) is another type of immunotherapy for malignant neoplasms that has the potential to overcome the immunosuppressive microenvironment and improve clinical outcomes. Oncolytic viruses (OV) are focused on selective damage and reproduction in tumor cells. This process destroys tumor cells, activating simultaneously the systemic immune response against cancer [55]. Cell death, accompanied by the release of molecules such as DAMPs and PAMPs, as well as cytokines, stimulates the activation and recruitment of antitumor immune cells, including $CD4^+$ and $CD8^+$ T-lymphocytes [56]. The current research focuses on various viruses, including adenoviruses, herpesviruses, measles viruses, Coxsackie viruses, polioviruses, reoviruses, Newcastle disease virus, etc. Malignant tumor cells may be susceptible to infection and replication of the virus as a result of their defective virus perception mechanisms. Some viruses do not require the presence of specific receptors [57]. Individual viruses are purposefully modified to make them oncospecific, e.g., by introducing a defect in the thymidine kinase sequence, in which replication is possible only in tumor cells with a high content of this enzyme [58].

Currently, the only oncolytic virus, which is a genetically modified form of the herpes simplex virus type 1, has been approved by the USA FDA for the treatment of malignant melanoma [59]. A systematic review and meta-analyses evaluating the efficacy and safety of OVT in solid tumors showed that the objective response rate was significantly higher in patients receiving monotherapy with oncolytic adenovirus H101 or combination with chemotherapy, compared to patients receiving chemotherapy alone [60]. According to the ClinicalTrials.gov data more than 20 studies are currently being conducted (Table 2), mainly the first or second phase of clinical trials, with an assessment of the efficacy and safety of oncolytic virotherapy for lung cancer and some other malignant tumors of the pleural cavity, in particular pleural mesothelioma. The effectiveness of intra-tumor administration of ADV/HSV-tk oncolytic virus was shown in 28 patients with metastatic non-small cell lung cancer in combination with stereotactic radiation therapy and further ICI immunotherapy (valciclovir and pembrolizumab) (NCT03004183). Disease stabilization was observed in 10 patients (37.5%), disease progression was observed in 10 patients (37.5%), 6 patients (21.4%) had a partial response, and 2 patients (7.1%) achieved a complete response. The results of another study (NCT02053220) showed that intravenous administration of ColoAd1 adenovirus for resectable NSCLC led to stimulation of the local antitumor immune response in the form of infiltration by $CD8^+$ T-cells [61]. At present, clinical trials of oncolytic virotherapy for lung cancer remain to be launched, requiring data predicting its potential therapeutic efficacy.

The clinical efficacy of OVT as a monotherapy remains limited, attracting research attention to exploring various combined treatment tactics. With respect to the combination of OVT with standard methods of lung cancer

treatment, one meta-analysis involving 1494 patients (the combination therapy group — 820 patients; the traditional treatment group — 674 patients) showed that the OVT in combination significantly improves the objective response in patients compared to standard therapy [62]. OVT is a particularly attractive option as adjuvant therapy to increase overall survival, due to the possibility of targeting residual tumor foci and modulating the suppressed immune system after surgery [63]. There is also evidence that combined radiation therapy and oncolytic virotherapy can enhance their individual antitumor effects, selectively destroying lung tumor cells [64].

Depending on the location and availability of the tumor, the virus can be injected directly into the tumor (single or repeated injections) or systemically (intravenous or intraarterial injection). Intra-tumor administration may be limited by the extracellular matrix, which serves as a barrier preventing the penetration and spread of the virus. Another difficulty in delivering the virus is the activation of antiviral immunity when administered systemically. Introduced viruses are detected by the host's immune system and inactivated by neutralizing antibodies, which reduces their replication and effectiveness. Attempts were made to circumvent this problem by encapsulation of oncolytic adenovirus into extracellular vesicles, which significantly increased *in vitro* infection rates and enhanced the effect of suppressing tumor growth in experimental models of human lung cancer [65]. Such approaches can be integrated into clinical practice to improve the effectiveness of systemic drug delivery, overcoming the immune response.

The modern possibilities of designing recombinant viruses are of great interest. The large viral genome VV allows the introduction of up to 50 kb of foreign genes, as a result of which the effect of OVT can be enhanced by the tumor-selective expression of therapeutic biological drugs, including antibodies, cytokines, chemokines, and ligands. Cytokine genes are among the most commonly used immunomodulatory genes due to the capacity of cytokines to recruit and regulate T-cell homeostasis [66]. Viruses encoding IL-2, IL-12, IL-15, TNF, or other cytokines have been designed to stimulate an increase in the lymphoid cell population after local administration. Studies have demonstrated successful and safe delivery of IL-2 into the tumor microenvironment, reducing tumor load and increasing the number of $CD8^+$ lymphocytes [67]. It was confirmed that IL-15 performs important functions in the activation and survival of T-lymphocytes, natural killer (NK), and NK-T-lymphocytes, with the combination of IL-15 and IL-15Ra enhancing their biological activity [58].

Recombinant virus design techniques may be the key to developing new approaches to treating lung cancer and improving immunotherapy. Due to their good safety profile and a variety of antitumor mechanisms, such approaches are appropriate for combination therapy. Viral infections and tumor lysis processes transforms cold tumors into hot tumors, increasing the infiltration and involving immune cells in the TME. OVT in combination with ICI demonstrate a powerful synergistic effect. The development of strategies for combination therapies requires care, since ICI can affect the ability of OV replication. In order to achieve optimal results, it is necessary to harmonize both treatment methods, avoiding potential risks associated with OV gene activation [68].

In general, oncolytic virus therapy shows broad clinical prospects for future effective treatment strategies of lung cancer. The versatility and relative safety of agents suggest that they are a powerful tool for optimizing combined immunotherapy. Continued clinical research in these directions is required.

CONCLUSION

Lung cancer is characterized by a pronounced immunosuppressive tumor microenvironment. This impedes both the antitumor immune response and the antitumor effectiveness of currently existing methods of adoptive cellular immunotherapy. At the same time, the combined effect of selective ICI, enhanced/targeted TIL, CAR-T and TCR-T, and recombinant oncolytic viruses on the tumor and its microenvironment can overcome the antitumor immune response and become decisive in suppressing tumor growth and improving clinical outcomes.

Each of the discussed methods individually have a number of advantages and disadvantages. This is why a combined and personalized approach to lung cancer immunotherapy seems to be justified. The development of technologies for recombinant oncolytic viruses that cause production of activating cytokines and chemokines by microenvironment cells, along with inhibition of the CTLA-4 and PD-1/PD-L1 signaling axes, as well as the creation of genetically-engineered cytotoxic cells, will undoubtedly raise adoptive immunotherapy to a new level capable of reverting the course of metastatic lung cancer.

Table 2. Clinical studies of oncolytic virotherapy for pleural cavity malignant neoplasms

№	Diagnosis	Treatment	Phase	Selection	Result	Side effects	Clinical trial ID	Event location
1	2	3	4	5	6	7	8	9
1	Solid tumors (lung cancer, head and neck cancer, melanoma, etc.)	Intra-tumor injection of recombinant adenovirus LIF N	1	28	Status unknown	No data available	NCT05180851	Shanghai, China
2	Metastatic NSCLC	Stereotactic radiation therapy in combination with intracellular administration of EBV/HSV-tk oncolytic virus, ICI therapy (valciclovir and pembrolizumab)	2	28	Complete response of 2 patients (7.1%); partial response of 6 patients (21.4%); stabilization of the disease of 10 patients (37.5%); disease progression of 10 patients (37.5%). The overall survival rate is 12.9%.	There are no cases of toxicity to the administration and no serious side effects from the treatment	NCT03004183	Houston, Texas, USA
3	Malignant pleural mesothelioma	Intrapleural administration of a vaccine strain of measles virus encoding a thyroid carrier of sodium iodide	1	15	The results have not been published	No data available	NCT01503177	Rochester, Minnesota, USA
4	NSCLC	Quaratusugene ozeplasmid (Remorse) in combination with pembrolizumab in patients with previously treated NSCLC	1 и 2	180	Recruitment is underway	No data available	NCT05062980	Houston, Tampa, St. Louis, USA
5	Disseminated small cell lung cancer	Intra-tumor injection of an oncolytic virus (RT-01)	1	20	Recruitment is underway	No data available	NCT05205421	Bengbu, China
6	Recurrent progressive solid tumors	Recombinant herpes simplex oncolytic virus type 1 (R130)	1	24	Recruitment is underway	No data available	NCT05886075	Anqing, Anhui, China
7	Progressive solid tumors	Recombinant herpes simplex oncolytic virus type 1 (R130)	1	20	Recruitment is underway	No data available	NCT05860374	Shanghai, Jiangsu, China
8	Progressive solid tumors	Recombinant herpes simplex oncolytic virus type 1 (R130)	1	20	Recruitment is underway	No data available	NCT05961111	Linyi, Shandong, China
9	Resistant to inhibitors of the NCLR immune checkpoint	Oncolytic adenovirus TILT-123 in combination with pembrolizumab	1	22	Recruitment is underway	No data available	NCT06125197	The location is not specified
10	Progressive malignant pleural mesothelioma	Oncolytic adenovirus H101 in combination with an inhibitor PD-1	1	15	Recruitment is underway	No data available	NCT06031636	Tianjin, Tianjin, China
11	Solid tumors	Intra-tumor injection of MEM-288 and nivolumab	1	61	Recruitment is underway	No data available	NCT05076760	Tampa, USA
12	Resectable NSCLC, resectable bladder cancer, resectable colon cancer, etc.	Intra-tumor injection or intravenous infusion of group B oncolytic adenovirus (ColoAd1)	1	17	High local infiltration of $CD8^+$ cells in 80% of the tested tumor samples, indicating a potential immune response.	There are no cases of toxicity to the administration and serious side effects from the treatment	NCT02053220	Madrid, Spain
13	Non-small cell lung cancer	VSV-IFN-β-NIS + Pembrolizumab + ipilimumab + nivolumab	1 и 2	70	Recruitment is underway	No data available	NCT03647163	Rochester, Minnesota, USA

Table 2 (continued)

№	Diagnosis	Treatment	Phase	Selection	Result	Side effects	Clinical trial ID	Event location
1	2	3	4	5	6	7	8	9
14	HER2 positive tumors	Intra-tumor injection of CAdVEC adenovirus	1	45	Recruitment is underway	No data available	NCT03740256	Houston, Texas, USA
15	Progressive NCLR	Intra-tumor injection of adenovirus (CVA21) in combination with pembrolizumab	1	11	Is unknown	No data available	NCT02824965	Heidelberg, Victoria, Australia
16	Progressive solid tumors	Intravenous injection of herpes virus T3011	1 и 2	74	Recruitment is underway	No data available	NCT05598268	Beijing, China
17	Metastatic solid tumors	Intra-tumor or intravenous injection of TBio-6517 (Oncolytic smallpox vaccine virus) in combination with pembrolizumab	1 и 2	27	Stopped	No data available	NCT04301011	USA
18	Metastatic solid tumors	Intra-tumor injection BT-001 (TG6030), alone and in combination with pembrolizumab	1 и 2	48	Recruitment is underway	No data available	NCT04725331	Brussels, Belgium
19	Metastatic solid tumors	Intra-tumor injection of recombinant GM-CSF vaccine; RAC VAC GM-CSF (JX-594)	1	23	Recruitment is underway	No data available	NCT00625456	USA
20	Malignant pleural mesothelioma	Intrapleural HSV1716, an oncolytic virus, is a type I mutant herpes simplex virus (HSV) deleted in the RL1 gene, which encodes the ICP34.5 protein	1 и 2	12	Completed	No data available	NCT01721018	Glasgow, United Kingdom
21	Common solid tumors with neuroendocrine features	Intra-tumor injection of picornavirus Seneca Valley Virus (SVV001)	1	60	Recruitment is underway	No data available	NCT00314925	USA

Table prepared by the authors according to the ClinicalTrials.gov data

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THE ROLE OF INFORMATION SUPPORT IN IMPROVING MEDICAL SUPPORT FOR SEAFARING PERSONNEL

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Introduction. Managing the process of collecting, analyzing, and systematizing the results of medical examinations of seafaring personnel in medical organizations of the Federal Medical and Biological Agency (FMBA) of Russia requires development of a specialized information system.

Objective. To carry out a comprehensive analysis of the activities of FMBA organizations, to develop on its basis and implement into practical use an information system for ensuring the continuity of all types of medical support for seafaring personnel; to create a digital Register of the results of medical examinations of seafarers.

Materials and methods. During the 2014–2023 period, the principles of organizing the work of medical organizations that provide medical care to seafaring personnel were studied. A methodology for recording the results of medical examinations of seafarers with the purpose of forming an information Register was developed. An analysis of medical support provided to people working on ships in 2023 was conducted based on the reports of FMBA structural units, including 35 health centers, 70 ship doctors, and 14 doctors of diving medicine.

Results. In March 2023, the Head Center for Health Protection of Seafarers, FMBA, implemented a pilot project on creation of a Register in the Northwestern Federal District. Within its framework, a methodology for generating a seafarer identification number in the Register was developed and 26,125 records of information about persons who underwent preliminary and periodic medical examinations were analyzed. The implementation of the proposed methodology for the formation of registers in all districts allowed an information system in the amount of 38,993 conclusions to be drawn based on medical examinations conducted in 2022–2023.

Conclusion. A single informational resource in conjunction with the Register for all 35 medical organizations that provide medical support to seafaring personnel, including conducting preliminary and periodic medical examinations, forms the basis for further improvement of scientific approaches to the organization and development of maritime and diving medicine in the Russian Federation.

Keywords: maritime medicine; industrial medicine; information support; Federal Medical and Biological Agency; FMBA; Register of medical examinations of seafaring personnel

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РОЛЬ ИНФОРМАЦИОННОГО СОПРОВОЖДЕНИЯ В СОВЕРШЕНСТВОВАНИИ МЕДИЦИНСКОГО ОБЕСПЕЧЕНИЯ ПЛАВСОСТАВА

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Введение. Управление процессом получения, анализа и систематизации результатов медицинских осмотров плавсостава в медицинских организациях ФМБА России требует создания специальной информационной системы.

Цель. На основании комплексного анализа результатов работы медицинских организаций ФМБА России разработать и внедрить в практическое применение информационную систему для обеспечения бесшовной преемственности в оказании всех видов медицинского обеспечения плавсостава, создать цифровой регистр медицинских освидетельствований плавсостава.

Материалы и методы. За период 2014–2023 гг. изучены организационные принципы работы медицинских учреждений, оказывающих медицинскую помощь плавсоставу. Отработана методика организации персонифицированного учета результатов медосмотров данного контингента для формирования регистров. Анализ работы по медицинскому обеспечению лиц, работающих на судах, выполнен по итогам деятельности 35 здравпунктов, 70 судовых врачей, 14 врачей водолазной медицины в структуре ФМБА России в 2023 году.

Результаты. В Головном центре охраны здоровья моряков ФМБА России в марте 2023 года реализован пилотный проект по созданию регистра в Северо-Западном федеральном округе, разработана методика формирования идентификационного номера моряка в регистре, изучено 26 125 записей сведений о лицах, прошедших предварительные и периодические медицинские осмотры. Использование предложенной методики формирования регистров во всех округах позволило получить в информационной системе данные о медицинских освидетельствованиях за 2022–2023 гг. в количестве 38 993 заключений.

Заключение. Единый информационный ресурс в комплексе с регистром по всем 35 медицинским организациям, осуществляющим медицинское обеспечение плавсостава, в том числе проведение предварительных и периодических медицинских осмотров, составляет основу для дальнейшего совершенствования научных подходов к организации и развитию морской и водолазной медицины в Российской Федерации.

Ключевые слова: морская медицина; промышленная медицина; информационное обеспечение; Федеральное медико-биологическое агентство; регистр медицинских освидетельствований плавсостава

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INTRODUCTION

In recent years, the question of restoring a unified system of medical support for maritime and river fleet workers in Russia has been discussed at various governmental levels [1]. Although existing previously, this system has disintegrated into individual companies and organizations as a result of adverse social and economic changes in the country.

These requirements are outlined in the International Labour Organization (ILO) convention adopted in 2006 and were ratified in the Russian Federation in 2012. Currently, the Russian Federation lacks a unified approach to the organization of information support for medical surveillance of marine specialists [2]. Medical organizations of Federal Medical and Biological Agency (FMBA) of Russia involved in medical examination of seafaring personnel apply the Unified Departmental Medical Information and Analytical System of FMBA of Russia (hereinafter referred to as UDMIAS), as well as medical information systems integrated with UDMIAS (hereinafter referred to as MIS) [3].

In the above systems, accounting of performed periodic and preliminary medical examinations was implemented in accordance with the order of the Ministry of Health of Russia as of 28.01.2021 No. 29n "On Approval of the Procedure for mandatory preliminary and periodic medical examinations of employees, provided for in part four of Article 213 of the Labor Code of the Russian Federation, the list of medical contraindications to work with harmful and (or) hazardous production factors, as well as work in the performance of which mandatory preliminary and periodic medical examinations are carried out" [4].

However, the mentioned guiding document lacks a procedure for preliminary and periodic medical examinations of persons working on ships. It should be noted that other regulatory acts of the Russian Federation did not fully present the procedure for medical examinations of shipboard personnel. The only current document establishing medical contraindications to work on a ship was the Decree of the Russian Federation Government as of 24.06.2017 No. 742 "On approval of the list of diseases that prevent work on marine vessels, inland navigation vessels, as well as on mixed (river-sea) navigation vessels" [5].

In order to meet the requirements of the time, at the end of 2022, the Ministry of Health of Russia issued the order No. 714n dated 01.11.2022 "On Approval of the Procedure for medical examination for medical contraindications to work on a ship, including chemical and toxicological studies of the presence of narcotic drugs, psychotropic substances, and their metabolites in the human body, and the form of a medical report on the absence of medical contraindications to work on a ship." This order defined a procedure for medical examination of seafaring personnel and created prerequisites for optimal organization of accounting of the results of medical examinations of shipboard personnel in information systems, based on reliable statistical data [6]. The timeliness of this activity agrees with the opinion of a number of authors who have carried out a comprehensive legal monitoring of national normative and methodological documents regulating the activities of seafarers' health care centers, assessed the normative regulation of the issue of medical support of crewmembers and stressed the need to regulate the principles of organization of medical care and sanitary and hygienic support of ships during voyages, taking into account the modern achievements of medicine and informational technologies, including distant ones [7, 8].

Thus, the accounting of preliminary and periodic medical examinations of seafaring personnel in the UDMIAS information systems and medical information systems integrated with UDMIAS used by medical organizations of FMBA has become particularly relevant.

In this study, we aim to develop and implement an information system to ensure continuity in the provision of all types of medical support to seafaring personnel with the creation of a digital Register of their medical examinations based on a system analysis of the activities of FMBA medical organizations.

MATERIALS AND METHODS

For the period from 2014 to 2023, specific aspects, shortcomings, and prospects of information support of medical care for seafaring personnel were studied. Data on the morbidity of swimming and diving personnel for the year of 2023 were systematized.

An analysis of the data set reflecting the activity of FMBA medical organizations (MOs) on medical care of seafaring personnel in 2023 showed the presence of 35 health posts (HP), 70 shipboard doctors, and 14 doctors of diving medicine. A questionnaire survey of information systems of all MOs integrated in the study was conducted. In order to ensure effective medical support to seafaring personnel, to coordinate interaction between the profile FMBA departments and subordinate institutions, as well as to create an information resource reflecting the results

of implemented activities, the Head Center for Health Protection of Seafarers (hereinafter referred to as the Head Center) was established in 2021 on the basis of Sokolov Northwestern District Scientific and Clinical Center by the order of the Head of the FMBA of Russia.

One of the main tasks of the Head Center is to provide information and analytical support for the health protection of shipboard personnel, in particular, to create and maintain a register of medical organizations and a digital Register of medical examinations of seafaring personnel, as well as to create, improve, and provide data and technological support for the information system.

In 2022, the Head Center set a task for FMBA medical organizations to organize personalized registration of medical examinations of seafaring personnel and to supply this information for compiling a Register. Due to the varying degrees of technical readiness of MOs in terms of running personalized registration of medical examinations for seafaring personnel using a medical information system (MIS), the Head Center provided them with the necessary information tools, as well as with a prototype module based on the MIS used in Sokolov Northwestern District Scientific and Clinical Center.

RESULTS

The conducted research established that creation of a unified health monitoring circuit for seafaring personnel and divers in the system of FMBA of Russia requires analysis of aggregate information reflecting the results of MO activities in all Federal districts.

The primary data received from FMBA medical organizations revealed the impossibility of separating exclusive information on the seafaring personnel from the total amount of data on preliminary and periodic medical examinations. This was certainly a precondition for obtaining unreliable demographic data and results on the morbidity of seafaring personnel. Interaction with the MOs also showed that personalized records of medical examinations of seafaring personnel were not carried out; sometimes, only paper records were kept.

At the first stage, a conceptual approach to the implementation and exploitation of an information and analytical system of maritime medicine was outlined, including the introduction of standard protocols for data exchange with MO medical information systems, installation and configuration of licensed software, creation of information resources and measures to ensure information security.

Pursuant to the order of the Ministry of Health of Russia from 01.11.2022 No. 714n [6], a pilot project to create a Register in the Northwestern Federal District (NWFD) was launched in 2022 and implemented in March 2023. The structure of the information support system for maintaining the Register of maritime medicine center was developed, see Fig. 1.

A methodology for forming a seafaring personnel identification number in the register consisting of 12 digits was developed. In the format of “12XXXX-XXXXXX-XXXXXXXXX”, the first two numbers denote the subject of the Russian Federation, in which the seafarer underwent a physical examination, “XX34-5XXXXXXXX-XXXXXX” the third-fifth numbers — the MO registration number in the MO register, “XXXXX-X678-9ABX” the sixth to eleventh numbers — the seafarer serial number, “XXXXX-XXXXX-XXXXXXC” the twelfth number — the control sum of the previous numbers (Fig. 2).

At first, the results of compiling a Register in the NWFD were obtained (Fig. 3). As shown in Fig. 3, at the first stage, 26,125 records of information on persons who underwent preliminary and periodic medical examinations were collected by the MOs in the Northwestern Federal District and forwarded to the Head Center.

An analysis of the data presented in Fig. 3 shows that the data contain generalized information on both members of the seafaring personnel and employees of shore-based services working for JSC Atomflot and FSUE Rosmorport. After excluding the shore service employees from the database, 8,259 records were included in the Register based on the attribute of attachment to a watercraft, which amounted to 32% of the previously submitted data. Unique codes of information on seafaring personnel from 7800-0000-0016 to 7800-0001-2277 in accordance with the above-mentioned methodology were inserted in the respective records (Fig. 2). The results of the pilot project were presented at a meeting of the Maritime Medicine Section of the Scientific and Expert Council of the Maritime Board of the Government of the Russian Federation in May 2023 in Vladivostok.

At the next stage, a Register of medical organizations of FMBA of Russia, engaged in medical support of seafaring personnel, including preliminary and periodic medical examinations of this contingent, was created. The Register included 35 FMBA medical organizations located in 8 Federal Districts. The map of their location is shown in Fig. 4.

Based on the results of the pilot project, regulations on the provision of information by medical organizations concerning the results of medical examinations of shipboard personnel to the Head Center for Health Protection of Seafarers, FMBA (hereinafter referred to as the Regulations) were finalized.

The methodological documents developed in the course of the project, as well as a prototype module based on the MIS used by Northwestern District Scientific and Clinical Center named after L.G. Sokolov, were implemented in 2023 in the work of all the FMBA medical organizations included in the register. Since December 2023, these measures have made it possible to provide a complete personalized record of data on periodic and preliminary medical examinations of seafaring personnel, medical reports on the absence of contraindications to work on a watercraft, collection, reconciliation, and analysis of statistical data on the morbidity of seafaring personnel, which fully meets the modern requirements of the health care system [9].

The scope of data storage in the register of medical examinations of seafarers was developed and justified: a seafarer’s passport data (32 fields) and medical data on the medical examination performed in accordance with the order of the Ministry of Health of Russia No. 714n dated 01.11.2022 (60 fields), including data on the medical care provided, therapeutic and preventive measures, results of laboratory and instrumental tests. The included information was rather extensive and formed not only in the MOs

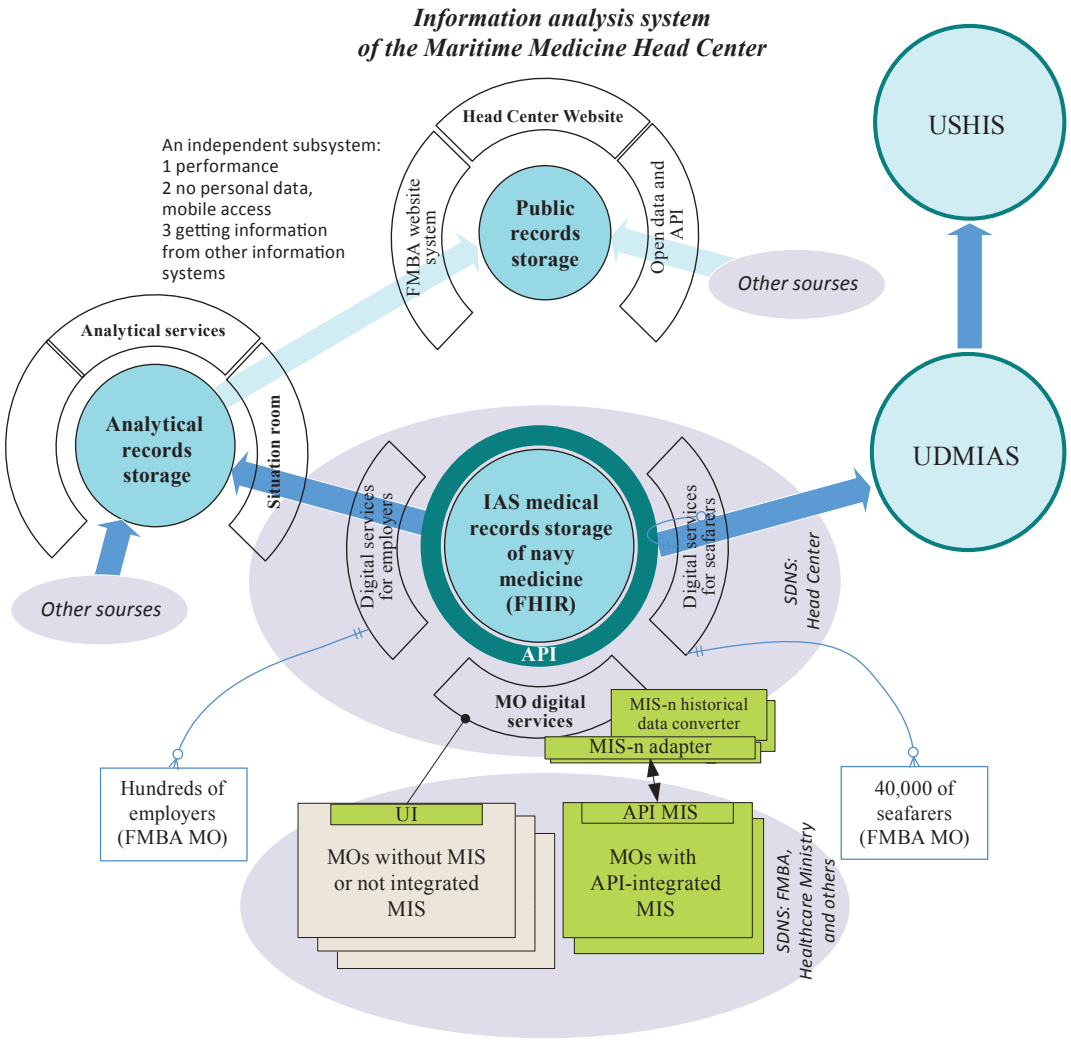


Figure prepared by the authors using their own data

Fig. 1. Schematic diagram of the prototype of the information and analytical system of the Head Center

conducting seafaring personnel medical examinations. Therefore, it was decided to obtain complete medical data upon request in Uniform State Health Information System (USHIS) and UDMIAS, without storing it in the Register. The composition of the Register of medical data is shown in Fig. 5.

The results obtained were used to compile a general Register for all 35 FMBA medical organizations that provide medical support to seafarers, including data on preliminary and periodic medical examinations (Table 1).

In the 2022–2023 period, 38,993 records on medical examinations of seafarers were processed (Table 1). The work is underway: at the end of the first quarter of 2024, 9504 more medical examinations were conducted by all


МЕДИЦИНСКОЕ СВИДЕТЕЛЬСТВО О СОСТОЯНИИ З

Seafarer Information 7800-0000-2801

Информация о моряке (лице, работающем на судне)

Surname: Nikitin First name: Oleg

Фамилия Имя

Figure prepared by the authors using their own data

Fig. 2. Seafarer identification number on the health status medical certificate

RESULTS: uploading data to the Register of medical examinations of seafarers and the Register of Medical Organizations

1. uploaded seafarers' examinations

№	Institution name	dirty data	net data	%
1	Head Center for the Seafarers' Health Protection Sokolov Northwestern District Scientific and Clinical Center	3 166	1 227	39%
2	Semashko Northern Medical Clinical Center	18 758	5 318	28%
3	Murmansk Multidisciplinary Center	4 201	1 714	41%
	TOTAL	26 125	8 259	32%

2. registered medical organizations

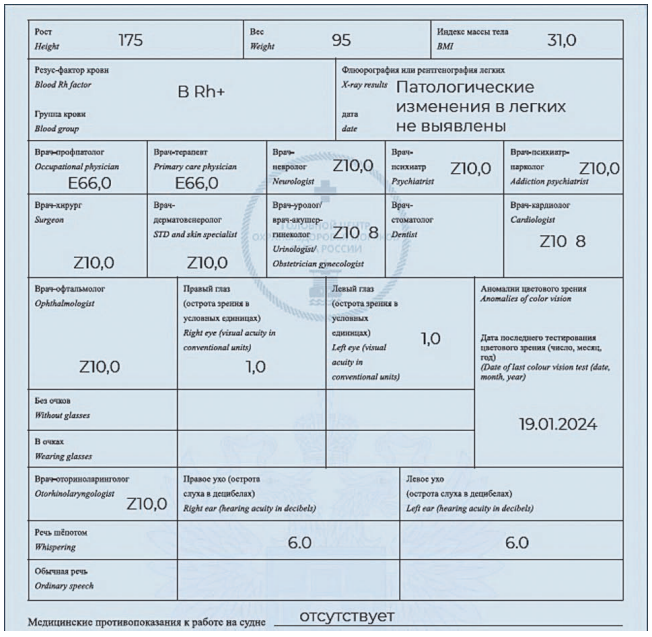
№	Institution name	code	seafarers' codes
1	Head Center for the Seafarers' Health Protection Sokolov Northwestern District Scientific and Clinical Center	78000	c 7800-0000-0016 no 7800-0001-2277
2	Semashko Northern Medical Clinical Center	29000	c 2900-0000-0012 no 2900-0005-3188
3	Central Medical Sanitary Department No. 58	29001	-
4	Murmansk Multidisciplinary Center	51000	code not allocated

Figure prepared by the authors using their own data

Fig. 3. Results of compiling a Register of seafaring personnel medical examinations in the MOs of FMBA of Russia in the Northwestern Federal District

Figure prepared by the authors using their own data

Fig. 4. Schematic location of 35 FMBA medical organizations engaged in medical support of seafaring personnel.



Рост / Height: 175	Вес / Weight: 95	Индекс массы тела / BMI: 31,0		
Резус-фактор крови / Blood Rh factor: B Rh+; Группа крови / Blood group		Флюорография или рентгенография легких / X-ray results: Патологические изменения в легких не выявлены; дата / date		
Врач-профпатолог / Occupational physician: E66,0	Врач-терапевт / Primary care physician: E66,0	Врач-невролог / Neurologist: Z10,0	Врач-психиатр / Psychiatrist: Z10,0	Врач-психиатр-нарколог / Addiction psychiatrist: Z10,0
Врач-хирург / Surgeon: Z10,0	Врач-дерматовенеролог / STD and skin specialist: Z10,0	Врач-уролог/ врач-акушер-гинеколог / Urinologist/ Obstetrician gynecologist: Z10 8	Врач-стоматолог / Dentist	Врач-кардиолог / Cardiologist: Z10 8
Врач-офтальмолог / Ophthalmologist: Z10,0	Правый глаз (острота зрения в условных единицах) / Right eye (visual acuity in conventional units): 1,0	Левый глаз (острота зрения в условных единицах) / Left eye (visual acuity in conventional units): 1,0	Аномалии цветового зрения / Anomalies of color vision; Дата последнего тестирования цветового зрения (число, месяц, год) / (Date of last colour vision test (date, month, year): 19.01.2024	
Без очков / Without glasses				
В очках / Wearing glasses				
Врач-оториноларинголог / Otorhinolaryngologist: Z10,0	Правое ухо (острота слуха в децибелах) / Right ear (hearing acuity in decibels)		Левое ухо (острота слуха в децибелах) / Left ear (hearing acuity in decibels)	
Речь шёпотом / Whispering	6.0		6.0	
Обычная речь / Ordinary speech				
Медицинские противопоказания к работе на судне: отсутствует				

Figure prepared by the authors using their own data

Fig. 5. Composition of medical data in the Register of medical examination of seafaring personnel

MOs, with the total number of the seafaring personnel in the Register having reached 39,333 persons. It should be noted that the data on the number of seafaring personnel is preliminary, changing dynamically and requiring additional verification with information from medical organizations due to technical errors in the dates of birth, insurance numbers, personal account numbers, and a number of other documents.

CONCLUSION

The need to restore a unified system of medical services for seafaring personnel is determined by the fundamental document in the field of national maritime policy, i.e., the Maritime Doctrine of the Russian Federation [10]. In the context of changing geopolitical landscapes, the development of maritime medicine becomes the state's priority, with the main role entrusted to the FMBA of Russia.

Systematization of the personal data of seafaring personnel for all 35 medical organizations, providing medical support to this contingent, the use of standard effective tools within a single information contour of the FMBA of Russia, the development of a single information resource will form the basis for further improvement of scientific approaches to the organization and development of maritime and diving medicine in the Russian Federation.

Therefore, the introduction of a unified information system in the structure of FMBA medical institutions is an important step towards the implementation of the Maritime Doctrine of the Russian Federation. The issues of medical care for seafaring personnel fully comply with the requirements of the Convention of the International Labor Organization.

Table 1. Indicators of medical data included into the Register of medical examination of seafaring personnel

№	Indicator	Quantity, units
1	Number of medical organizations in the Register of Medical Organizations and on the Maritime medicine website	35
2	Number of data fields per seafaring personnel in the Register — personal and medical	32 + 60
3	Number of seafaring personnel medical examinations in the Register:	
3.1	For 2022 (information from 20% of medical organizations for half a year)	9949
3.2	For 2023 (information from 100% of medical organizations by the end of the year)	29,044
3.3	Total for 2022–2023	38,993

Table prepared by the authors using own data

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PROSPECTS FOR DIAGNOSIS AND TREATMENT OF MINIMAL TRAUMA AND INJURY OF LARGE JOINTS IN UNDERAGE ATHLETES: A REVIEW

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Introduction. The vast majority of modern sports exert a significant load on the musculoskeletal system (MSS). The ever-growing popularity of sports among underage children, their active participation in various competitions and trainings impose an increased risk of sports injuries, particularly minimal trauma and injury of large joints. Although numerous works have addressed the development of clinical diagnostic and therapeutic methods used for MSS injuries, there is a lack of publications on sports injuries in underage athletes.

Objective. Evaluation of current methods for diagnosis and therapy of minimal trauma and injury of large joints in underage athletes with the purpose of selecting the most promising and effective methods.

Findings. The main causes and mechanisms of injuries are considered. Such injuries are generalized depending on the sports type. A review of available methods for clinical and instrumental research and innovative therapeutical methods is carried out. Platelet-rich plasma therapy (PRP) was found to be the most promising minimally-invasive biotherapy for MSS injuries, particularly with respect to children and adolescent athletes. This method restores the anatomical integrity of damaged elements and relieves pain at rest, during physical exertion, and in a stress test with the possibility of preserving the function of the injured joint and rehabilitation in the shortest possible time. PRP therapy is an alternative to conventional treatment methods, offering new prospects in regenerative and sports medicine.

Conclusions. A comprehensive personalized approach combining clinical examination and instrumental studies is key to ensuring the accuracy and objectivity of the health status of young athletes. Such an approach allows diseases to be identified at an early stage, differential diagnosis to be conducted, and treatment efficacy to be evaluated, taking the specifics of pediatric practice into account.

Keywords: sports medicine; pediatric sports injuries; therapy of minimal trauma and injury of large joints; PRP therapy; underage athletes

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ПЕРСПЕКТИВЫ ДИАГНОСТИКИ И ЛЕЧЕНИЯ МИНИМАЛЬНЫХ ТРАВМ И ПОВРЕЖДЕНИЙ КРУПНЫХ СУСТАВОВ У НЕСОВЕРШЕННОЛЕТНИХ СПОРТСМЕНОВ: СОВРЕМЕННЫЕ ПРЕДСТАВЛЕНИЯ

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Введение. Подавляющее большинство современных видов спорта оказывают значительную нагрузку на опорно-двигательный аппарат (ОДА). Постоянно растущая популярность спорта среди несовершеннолетних детей, их активное участие в различных соревнованиях и тренировках создают повышенный риск получения спортивных травм, особенно минимальных повреждений и травм крупных суставов. Множество работ посвящено клинико-диагностическим и терапевтическим методам, применяющимся при травмах ОДА, однако лишь незначительная их часть касается именно детского спортивного травматизма.

Цель. Оценка существующих методов диагностики и терапии минимальных травм и повреждений крупных суставов у несовершеннолетних спортсменов для выбора наиболее перспективных и эффективных из них.

Обсуждение. Рассмотрены основные причины и механизмы травм, проведена систематизация типов травм в зависимости от вида спорта, выполнен анализ имеющихся современных методов клинико-инструментального исследования и инновационных методов терапии. Выяснено, что наиболее перспективным малоинвазивным методом биотерапии травм и повреждений ОДА, особенно в аспекте применения в детской и подростковой группе спортсменов, является PRP-терапия (терапия богатой тромбоцитами плазмой). Данный метод позволяет существенно восстанавливать анатомическую целостность поврежденных элементов, купировать болевой синдром в покое, при физической нагрузке и в стресс-тесте с возможностью сохранения функции травмированного сустава и реабилитации в кратчайшие сроки. PRP-терапия представляет собой альтернативу традиционным методам лечения, открывая новые горизонты в регенеративной и спортивной медицине.

Выводы. Комплексный персонализированный подход, объединяющий клинический осмотр и инструментальные исследования, является ключевым в обеспечении точности и объективности оценки состояния здоровья юных спортсменов; он позволяет выявить заболевания на ранней стадии, провести дифференциальную диагностику и оценить эффективность лечения с учетом особенностей педиатрической практики.

Ключевые слова: спортивная медицина; детские спортивные травмы; терапия минимальных травм и повреждений крупных суставов; PRP-терапия; несовершеннолетние спортсмены

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INTRODUCTION

The vast majority of modern sports exert a significant load on the musculoskeletal system (MSS). At the same time, the frequency of various MSS diseases among athletes is much higher than among the general population. Thus, the prevalence of injuries as a result of sports among children aged 5–17 years reaches about 35.8%.

The ever-growing popularity of sports among minors, their active participation in various competitions and training create an increased risk of sports injuries, especially minimal damage and injuries to large joints [3]. Such injuries, despite their apparent lightness, may have serious consequences for the sports career of a young athlete if timely and adequate diagnosis and therapy of existing changes are not carried out, especially in the early stages.

Accurate diagnosis and rational tactics of managing the patient with injuries of large joints are the key factors in achieving the maximum efficacy of therapeutic measures [4]. Numerous publications have investigated the questions of clinical diagnostic and therapeutic methods for MSS injuries, with only a few of them addressing specifically children's sports injuries. In order to gain a comprehensive understanding of how protocols should be modified when applied to underage athletes in case of injury, it seems relevant to carry out a review of standard and innovative methods of clinical and instrumental examination and treatment of such injuries.

In this work, we review modern methods of diagnosis and therapy of minimal injuries and damage to large joints in underage athletes in order to select the most promising and effective methods.

FINDINGS

General ideas about sports injury

Sports injuries as a result of intense training and competitive stress may involve serious consequences for athletes. Such events not only disrupt the training and competitive processes, but also lead to long-term rehabilitation, frequently accompanied by temporary or permanent restriction of physical activity. In severe cases, sports injuries can cause premature termination of sporting careers and even lead to disability, significantly impairing the quality of life of athletes [5].

Sports-associated injuries are distinguished by a variety of driving mechanisms, the anatomical and physiological characteristics of the child, and the type of sports. The International Olympic Committee Consensus Group on the Epidemiology of Injuries and Diseases defines sports injury as "a tissue damage or other derangement of normal physical function due to participation in sports, resulting from rapid or repetitive transfer of kinetic energy" [6].

Successful treatment of sports injuries depends on accurate diagnosis, which takes into account the following key factors: the onset of occurrence (acute injury or injury caused by excessive exertion), the type of damaged tissue (tendon, muscle, cartilage, bone), and the severity of the injury (fracture without displacement or with displacement) [7].

In acute trauma, the patient clearly remembers the moment, place, cause, and circumstances of its occurrence, unlike injuries associated with excessive overstrain. The most common acute injuries include sprains and ligament damage (rupture), joint dislocations and fractures. Overstrain injuries, conversely, develop gradually as a result of repetitive microtraumas that occur with excessive and repetitive stress. One feature of such injuries is the gradual manifestation of symptoms, when repeated microtrauma overloads the ability of tissues to auto-healing. This is particularly important in children's practice, given the functional immaturity of tissues, organs, and systems of the child's body and less developed resistance to stress [8].

An increase in training loads, a high level of competition in sports, and the requirements imposed on a child — all these circumstances explain why sports injuries occupy a leading place in the morbidity structure of young athletes. Brenner et al. showed that overexertion injuries and emotional burnout of a child are the two main reasons for ending a sporting career. It is important to understand the mechanisms and underlying processes of injury for selecting an optimal examination tactics

and timely determination of treatment criteria (conservative or operative) [10].

Understanding all aspects of sports injury, taking into account gender and age characteristics and the type of sports, as well as the frequency of injuries and the likelihood of acute injury or injury as a result of overexertion, plays an important role in shaping a personalized approach to the management of athletes, including the development of a set of preventive measures. For a systematic assessment of sports injuries, differentiation is necessary by the localization of the anatomical segment and by the type of damaged injury tissue (ligament, muscle, or bone). For example, injuries to the hip or knee area may involve muscle contusion, muscle compartment syndrome, tendinopathy, and tendon rupture. Most childhood injuries associated with excessive exertion affect the lower extremities, especially the knees, ankles, and feet, and also include damage to muscles and tendons [11]. Injuries from excessive loads (in comparison with acute effects) are about twice more common in the knee joint, while acute injuries are about three times more likely to occur in the ankle joint [12].

According to Sheffield, the knee joint is most often injured in sports such as football and rugby [13]. At the same time, the author pays special attention to the assessment, treatment, and rehabilitation of knee instability and the difficulties faced by the attending physician in the management of young and active patients. Other authors noted that the prevalence of patellar tendinopathy (jumper's knee symptoms) in athletes reaches approximately 14%, while the recurrence rate reaches 45% in volleyball players and 32% in basketball players. The study by Bahr M.A. and Bahr R. found that about 29–44% of elite volleyball players who perform more than 500 jumps per week report jumper's knee symptoms [15]. In the structure of sports injuries, ankle injuries reach 10–12% of all injuries to the musculoskeletal system and 20–25% of all sports injuries to the lower extremities.

According to Sobhani et al., the most common injury in football associated with excessive strain is Achilles tendinopathy. This can be explained by the large amount of running and jumping in this kind of sports [16]. The most common MSS injuries as a result of overexertion are presented in Table 1.

Modern methods of diagnosis of minimal injuries and damage to large joints in children

A key factor in the successful recovery of an athlete after injury is a comprehensive and dynamic clinical and instrumental examination [3, 16, 18], which allows not only the degree of damage to be assessed, but also the progress of rehabilitation to be traced in dynamics. The introduction of instrumental research methods into clinical practice has significantly extended the possibilities of early diagnosis [3, 16]. Timely and accurate diagnosis contributes to the speedy return of the athlete to previous physical activity and professional loads [19].

The most common injuries of the MSS in qualified athletes, including minors, are the so-called minor injuries [20], such as bruises, sprains, chronic microtrauma, degenerative-dystrophic processes, etc. Most of these injuries are manifestations of overtraining or minor sports injuries that do not require specialized diagnosis and treatment. However, minor injuries might disguise the initial manifestations of more significant injuries and pathological conditions (spondylolisthesis, protrusion and herniation of discs, dorsalgia, vertebral ring apophyseal fracture, undiagnosed spinal injuries, etc.), which, in the absence of timely treatment, can lead to more serious health problems for an athlete.

The difficulty in diagnosing minor MSS injuries is associated with their meager and non-specific symptoms. Athletes complain of discomfort in the area of injury or mild pain syndrome without a clear localization. As usual, such "complaints" are not given due importance, with their discomfort attributed to a reaction to training loads, overtraining, etc. Such a situation becomes even more complicated in case of underage athletes due to the specifics of collecting and interpreting complaints in pediatric practice, which requires a more thorough approach to interviewing a young patient by the attending physician. Another diagnostic problem is the low sensitivity and specificity of standard traumatic orthopedic tests to a number of minor MSS

Table 1. Injuries of the musculoskeletal system as a result of overstrain

Tissue	Injury type	Examples of manifestation in sports
Muscles/fascia	Chronic compartment syndrome; Delayed Muscle Soreness (DOMS); Fasciitis	Iliotibial syndrome when running
Tendon	Tendinopathy (includes parathenonitis, tenosynovitis, tendinosis and tendinitis)	Tendinopathy of the Achilles tendon in football players; Patellar tendinosis in volleyball (jumper's knee)
Joint	Synovitis; Injuries to the upper lip; Chondropathy; Injuries to the internal structures of the joint	Superior labrum anterior to posterior lesions of the shoulder (SLAP) in athletes engaged in throwing (baseball, cricket); Damage to the internal structures of the knee joint (running, jumping)
Ligamentous apparatus	Chronic degeneration/microfractures	Collateral ulnar ligament injury in baseball
Bone	Stress reaction, stress fracture; Osteitis, periostitis; Apophysitis	Stress fracture of the metatarsal bone during running and ballet; Medial tibial stress syndrome in running and dancing; Osgood-Schlatter disease; Stress fracture

Table prepared by the authors based on [21]

injuries. Such tests are mostly focused on assessing the passive range of motion and the active functioning of the athlete's musculoskeletal system, without association with a particular sports, which limits their clinical significance and application [21]. As a rule, minor injuries occur when performing specific active movements characteristic of certain sports, which are often impossible to reproduce in a standard clinical examination.

The frequency of minor traumas and injuries of the MSS, including in underage athletes, requires a systematic approach to their clinical and diagnostic examination based on a thorough collection of complaints and anamnesis, permitting timely diagnosis of the existing pathology and developing a personalized treatment approach.

Dietvorst et al. described a diagnostic procedure for anterior cruciate ligament injuries in children and adolescents, confirming the diagnostic value of anamnesis collection, physical examination, and arthroscopy [22]. Endele et al. showed sufficient efficacy of standard clinical, radiological, and ultrasound diagnostics for primary detection of fractures and ligament injuries of the ankle joint in children [23].

A thorough history collection is an indispensable tool for diagnosing MSS injuries, which allows not only the mechanism of injury to be identified, but also a conclusion about the intended type of injury to be made, thus suggesting the most appropriate directions for further examination and treatment.

The pain syndrome may be diffuse or local in nature. When determining the intensity of pain, the Visual Analogue Scale (VAS) is most often used, including in traumatology practice [24]. Physical examination includes examination of the joint for swelling and palpation for soreness. Among the assessed factors are local temperature, soreness, fluctuation, sensitivity disorders (hyperesthesia, hyposthesia, anesthesia), tissue turgor, skin and muscle condition, tissue swelling, crepitation of fragments, patellar balloting, and tendon mobility. Palpation is diagnosed with crepitating and stenosing parathenonitis, clicking joint, and snapping scapula syndrome. In addition, the length and circumference of the limb are measured, and the amplitude of its movements is determined.

In modern clinical practice, preference is given to highly informative research methods. X-ray examinations significantly extends diagnostic capabilities, providing data that cannot be detected by conventional clinical methods. Ultrasound examination (ultrasound), magnetic resonance imaging (MRI), and multispiral computed tomography (MSCT) are currently used as the main methods of choice for visualization of bone and cartilage structures [3, 16]. The publications addressing the issues of visualization highlight the importance of radiography, ultrasound, and additional methods (if necessary) for the diagnosis of MSS injuries.

Ultrasound diagnostics of the joints demonstrates a sufficiently high accuracy in the diagnosis of intra-articular injuries. The most frequently detected injuries the knee joint were found to be damage to the inner and outer menisci. A study into latent intraarticular knee joint injuries in children showed the need to use MRI diagnostics to establish a diagnosis in the absence or minimum amount of radiological data [26]. The importance of magnetic resonance imaging in the diagnosis of damage to the ligamentous apparatus of the knee joint in children was noted in [27].

MRI implies a high soft-tissue contrast, allowing examination in any plane and taking into account the anatomical features of the patient (including three-dimensional images). Moreover, MRI is the only noninvasive diagnostic method with high sensitivity and specificity in detecting edema and infiltration of bone tissue. Thus, even minimal damage to the menisci can be detected, including in pediatric practice. MRI diagnostics allows the physician to establish an accurate diagnosis and prescribe appropriate treatment.

Thus, modern diagnostics of sports injuries is based on an integrated approach, including medical history, which determines the nature of the injury, the circumstances of the injury, the duration of injury (acute or chronic), clinical examination (with provocative tests), as well as additional examination methods (CT, MRI, ultrasound, radiography of damaged limb segments, and standard laboratory tests, such as clinical and biochemical blood tests). In case of MSS injuries in children, along with applying standard approaches to patient management, special attention should be paid to collecting complaints and anamnesis. Indeed, the lack of information or its incorrect interpretation can cause an incorrect diagnosis and, as a result, incorrect examination and treatment tactics.

Therapy of minimal injuries and damage to large joints in children

The method and duration of treatment (conservative or operative) are determined by the specifics of injury (which tissue is damaged, the degree of damage), the age of the young athlete, and the kind of sports he or she is engaged in. The most common sports injuries that do not require specialized medical care include superficial injuries, namely: soft tissue bruises, sprains, ruptures of the ligamentous apparatus, joint damage.

The first stage of treatment of sports injuries in children is based on the P.R.I.C.E and/or R.I.C.E protocols: Protection, Rest, Ice, Compression, Elevation (PRICE). Within this framework, Protection is understood as limiting or excluding the load with the help of crutches, a cane, partial immobilization of the injured area with a bandage, splint or bandage. Rest provides for restriction of movements or "relative" rest, when actions that load the injured area to such an extent that pain occurs, or which can slow or prevent healing, are excluded. Ice cryotherapy is applied in acute injuries to reduce swelling and pain. Compression includes the use of a compression bandage, i.e., an elastic bandage for easy support of damaged tissue. Elevation is understood as placing the damaged area above the level of the heart in order to reduce the accumulation of fluid in the damaged limb or joint and, as a result, reduce the level of pain [28].

RICE is the basis for the treatment of acute soft tissue injuries, promoting a conservative approach during the first 24–48 hours after injury. The purpose of this protocol is to minimize bleeding, reduce swelling, and alleviate discomfort at the site of injury, which measures potentially

speed up the recovery process [28]. Scientific discoveries and advances in clinical practice have suggested that RICE cannot be a versatile approach for all injury treatment scenarios [29, 30].

New data confirm the use of more active recovery strategies based on the following principles: Movement, Exercise, Analgesia, Treatment (MEAT); Protection, Optimal Loading, Ice, Compression, Elevation (POLICE), and Protection, Elevation, Avoid anti-inflammatories, Compression, Education and Load, Optimism, Vascularization, and Exercise (PEACE and LOVE) [31, 32]. Thus, the above principles emphasize the importance of early motor activity, individual exercises, and comprehensive care to improve healing and functional recovery. At the same time, the fundamental elements of RICE still retain their value, especially when providing emergency care after injuries [33].

Various treatment methods are aimed at restoring the anatomical integrity and functionality of joints, minimizing pain syndrome, preventing the development of complications and chronic joint diseases, and (most importantly) ensuring a safe and speedy return to sports.

In modern clinical practice, the following methods of treatment of minimal injuries and damage to large joints are distinguished:

1. Conservative treatment

- Immobilization of the joint: fixation of the joint with plaster, orthoses or bandages, which is necessary to stabilize the joint and prevent further damage;
- Pharmacotherapy: the use of painkillers, anti-inflammatory, and other drugs to relieve pain and reduce the inflammatory process. Drug therapy may be effective for mild injuries; however, it may not always completely eliminate the symptoms. In addition, some drugs may have side effects, such as allergic reactions and gastrointestinal disorders [35, 36];
- Comprehensive medical rehabilitation, including rehabilitation programs: physical therapy (a set of exercises aimed at restoring the amplitude of movement in the joints, muscle strength, coordination of movements and balance), massage, physiotherapy (to reduce inflammation, relieve pain, and accelerate the rehabilitation process).

Conservative treatment of minimal injuries and damage to large joints in underage athletes has a number of advantages. Such a treatment approach is non-invasive, requiring no surgical intervention. This reduces the risk of postoperative complications, infections, and scarring of tissues, being associated with minimal risk (compared to surgery, conservative treatment is less risky for young athletes, especially during periods of active growth) and cost-effectiveness. However, conservative treatment has a number of limitations, e.g., it may not be effective enough for severe injuries. In addition, conservative treatment requires a long recovery period and increases the risk of developing chronic instability, which can lead to repeated injuries. Thus, conservative treatment is a promising method of treating minimal injuries and damage to large joints in underage athletes; however, in some cases, surgical intervention may be required.

2. Surgical treatment

Surgical intervention is used for severe injuries requiring restoration of the integrity of ligaments, cartilage, or bones, as well as for stabilizing joints. Indications for surgical intervention include a complete or partial rupture of ligaments, not amenable to conservative treatment, broken bones of the joint that needs fixing fragments, and inflammatory processes that are not amenable to conservative treatment, cartilage defects, causing pain, limited mobility and threatening the destruction of the articular surface, permanent dislocations or subluxations, which are not amenable to conservative therapy [39]. Surgical intervention allows the anatomy of the joint to be restored and its stability to be ensured. It may be necessary for restoring function, preventing repeated injuries, reducing pain, improving joint mobility, and improving the quality of life of an athlete. The disadvantages of this treatment include the risk of complications and a long period of rehabilitation.

Thus, surgery for joint injuries in athletes, including minors, is a serious intervention that requires careful planning and an individual approach.

3. Minimally invasive methods (intra-articular injections)

PRP therapy (Platelet Rich Plasma) is an innovative treatment method that is actively used in various fields of medicine, especially in orthopedics and sports medicine [40, 41]. This method is based on the use of autologous (own) blood plasma of the patient, enriched with platelets [40]. Platelets contain growth factors that stimulate tissue regeneration, accelerate healing, and promote recovery after injury or surgery [41]. One of the main advantages of PRP therapy is rapid recovery after the procedure [40, 41]. Patients can return to sports activities within a few days after this procedure. In addition, PRP can help improve blood circulation and metabolism in the tissues of the joint, which also contributes to their recovery. This method is safe and associated with a minimal risk of allergic diseases. It is important that the composition of PRP can be adapted to the individual needs of the patient. This makes this method versatile for various types of injuries and diseases. In addition, it has become possible to use PRP in professional sports, despite the content of growth factors in the composition. Growth factors are independently considered as doping and, in accordance with the decision of the Anti-Doping Agency, were the reason for deterring the use of PRP until 2011 in sports medicine for muscle damage [42].

Thus, there are several options for the treatment of MSS injuries, while the choice of a specific method depends on the type of damaged tissue, the nature and severity of the damage, the age of the athlete, and the type of sports, as well as the type of injury (acute or related to overstrain/overstrain). In order to alleviate pain, shorten the rehabilitation period, and return to high-performance sports as soon as possible in the absence of indications for surgical treatment, minimally invasive methods,

including PRP therapy, both as monotherapy and in combination with conservative treatment, are undoubtedly a priority in application [43].

PRP therapy as an innovative method of treating minimal injuries and damage to large joints in pediatric practice and sports medicine

Modern knowledge about the anatomy and physiology of child development, taking into account age-related features and growth processes, combined with advanced examination methods (MRI, CT, ultrasound) have brought the diagnosis of injuries and injuries to a qualitatively new level, allowing even minimal damage to be detected and appropriate pathogenetically justified treatment to be prescribed [3, 18].

Conservative treatment, including rest, immobilization, and physiotherapy, has undoubtedly proved its effectiveness over time. However, modern conditions are increasingly requiring faster healing of the damaged part and timely return to athletic fitness, including in high-performance sports [34–36]. This has become an incentive to search for new treatment methods.

In the field of orthopedic medicine, the search for innovative therapies aimed at relieving pain, accelerating recovery, and promoting tissue regeneration has led to the emergence of regenerative therapy as a promising direction. Regenerative therapy is based on the use of innovative cellular technologies and products to repair damaged tissues and organs. As part of the development of regenerative therapy, the use of orthobiologics products, i.e., biological substances that contribute to faster recovery of damaged tissues, is of great importance. These include hyaluronic acid, platelet-rich plasma (PRP), mesenchymal stem cells, bone marrow aspirate concentrate (BMAC), and cultured mesenchymal stem cells [44]. Orthobiological products are naturally found in the body; however, they can help accelerate the healing process in higher concentrations.

PRP therapy is among the most recent methods of biotherapy of MSS injuries. This approach shows positive results in relieving pain syndrome, improving functional state, and shortening the rehabilitation period in patients with injuries of the musculoskeletal system [46–48]. PRP is an orthobiological treatment method based on the use of biologically active platelet molecules. At baseline levels, platelets function as a natural reservoir of growth factors, including platelet-derived growth factor (PDGF), epidermal growth factor (EGF), transforming growth factor beta-1 (TGF-β1), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (FGF), hepatocyte growth factor (HGF), and insulin-like growth factor (IGF-I). PRP is commonly used in orthopedic practice to accelerate tissue healing as a result of injuries, including those related to sports.

According to Russian and foreign literature, PRP is a generalizing term for a group of human autologous blood products. PRP includes products derived from autologous blood, such as platelet-rich plasma and autologous conditioned plasma. Platelet-derived products are classified into pure PRP (Pure Platelet-Rich Plasma/P-PRP), plasma enriched with growth factors (Plasma Rich Growth Factors/PRGF), leukocyte and platelet plasma, pure platelet-rich fibrin (P-PRF), and leukocyte (Leukocyte-Platelet-Rich Fibrin/L-PRF), and platelet fibrin (Advanced/A-PRF). The composition of these products may vary depending on the content of cells and fibrin, as well as on the density of the fibrin network [52].

In 2009, the first classification of platelet concentrates was proposed [53]. This classification is simple and based on the content of certain blood components and their quantity. This classification divides products according to two main parameters: cellular composition (mainly leukocytes) and fibrin architecture. This separation made it possible to identify the four main families for product rearrangement given below.

1. Pure platelet-rich plasma (P-PRP) or leukocyte-poor platelet-rich plasma products are preparations without leukocytes and with a low-density fibrin network after activation. By definition, all products of this family can be used in the form of liquid solutions or in the form of an activated gel. Therefore, it can be injected (in the form of a solution) or applied as a gel to the surface of a wound or suture (similar to the use of fibrin adhesives). This family includes platelet-rich Plasma (PRP) and autologous conditioned plasma (ACP).

2. Plasma products enriched with leukocytes and platelets (L-PRP), which are preparations with leukocytes and a low-density fibrin mesh after activation. By definition, like P-PRP, all products of this family can be used in the form of liquid solutions or in the form of an activated gel [54].

3. Pure platelet-rich fibrin (P-PRF) or leukocyte-poor and platelet-rich fibrin are preparations without leukocytes, but with a high-density fibrin network. By definition, these products exist only in the form of a highly activated gel and cannot be injected or used as traditional fibrin adhesives.

4. Leukocyte- and platelet-rich fibrin (L-PRF) products are preparations with leukocytes and with a high-density fibrin network [55].

The classification described above covers all forms of platelet concentrates. In traumatology and orthopedics, another classification has been proposed, which is based on the use of platelet-enriched plasma (PRP only).

Mishra et al. proposed to classify PRP products taking into account the concentration of platelets and leukocytes specifically for use in therapeutic practice in athletes [49]. This classification divides PRP into four types depending on the presence or absence of white blood cells, as well as whether PRP is activated or not. According to this classification, type 1 PRP is an L-PRP solution, type 2 PRP is an L-PRP gel, type 3 PRP is a PPRP solution, and type 4 PRP is a P-PRP gel. This design classification is similar to the general one published in 2009; however, the division of PRP products is limited by cellular composition and activation, which makes it more understandable for clinical use [49]

The only new parameter in the above classification is the assessment of platelet concentration, and type A PRP

is five times (or more) higher than the concentration of platelets in the blood, and type B PRP is only five times higher than the concentration of platelets in the blood. The latter parameter is controversial, since the concept of accounting for platelet concentrations in an PRP product has been largely rejected in previous years for a logical reason, i.e., platelet concentration depends only on the volume of liquid serum used to maintain platelets in suspension. The amount of serum varies greatly depending on the protocol and the expected use, having no influence on the intended effect. The concept of the absolute platelet count would be more logical, although the effect of this parameter on clinical results has not received confirmation in publications. From this point of view, the fivefold threshold does not have a generally accepted meaning and justification [56].

PRP exhibits a pronounced anti-inflammatory, analgesic, pro-regenerative, and anti-apoptotic effect, stimulates the growth and migration of fibroblasts and osteoblasts. Therefore, it is increasingly used to treat the consequences of MSS injuries. Numerous studies have confirmed the effectiveness of PRP in patellar tendinopathy, lateral epicondylitis, rotator cuff injury, tendon and muscle injuries of various localization [50, 51].

Thus, PRP therapy is a promising method for treating damage to large joints, which can become an alternative to conventional methods of treatment, such as conservative therapy and surgery.

CONCLUSION

Modern medicine pays particular attention to the diagnosis of trauma in young athletes, focusing, among other things, on rehabilitation measures after minimal injuries and damage to large joints. Understanding the circumstances of such injuries (causes, onset, mechanisms), as well as the precise definition of damaged tissue in a specific segment of an athlete's limb, are key for determining an optimal examination tactics, developing an individual treatment plan, and predicting both the recovery process and the possibility of returning to full-fledged athletic activity. Special attention should be paid to young athletes whose musculoskeletal system is only in the formation stage, thus being more vulnerable to damage.

Modern instrumental methods extend the range of tools for preventing the development of complications and selecting an optimal treatment approach. Clinical examination and history analysis offer the primary overview of the patient's condition, allowing the physician to identify the main complaints and symptoms. Instrumental research methods, such as radiography, CT, MRI, and ultrasound, provide a more detailed information about the structure and function of organs and body systems, which complements the overall clinical picture, allowing the physician to gain a systemic view of the pathological process and make an accurate diagnosis.

An integrated approach based on clinical examination and instrumental studies is a key factor in ensuring an accurate and objective assessment of the patient's condition. This, in turn, makes it possible to identify diseases at an early stage, conduct differential diagnosis, and evaluate the treatment efficacy.

Along with standard conservative and surgical approaches to the treatment of minimal trauma and MSS injuries in athletes, including children and adolescents, an increasing attention is currently paid to minimally invasive treatment based on orthobiological products. The latter are capable of accelerating the healing process of damaged cells, tissues and organs, reducing the rehabilitation period, which is especially important for young athletes in their early sporting carriers.

Platelet-rich plasma (PRP) therapy is the most promising minimally-invasive method of biotherapy for MSS injuries, especially as applied to adolescent athletes. This method proves effective in restoring the anatomical integrity of damaged elements and relieving pain at rest, during physical exertion, and in a stress test. PRP provides the possibility of preserving the function of the injured joint and rehabilitation in the shortest possible time. PRP therapy is an alternative to conventional treatment methods, offering new opportunities in the fields of regenerative and sports medicine.

Thus, an integrated approach to diagnosis combining clinical examination and instrumental studies, the use of minimally-invasive innovative cellular technologies in underage professional athletes with MSS injuries, including large joints, is becoming an indispensable tool in modern sports medicine.

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PROSPECTIVE DIRECTIONS IN HUMAN HEALTH MONITORING DURING LONG-TERM SPACEFLIGHTS

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Introduction. The increasing duration of spaceflights and the associated prolonged exposure of space crewmembers to unfavorable microgravity conditions necessitate the development of improved approaches to diagnosing the health status directly during the flight. This study is aimed at searching and selecting promising biological markers suitable for studying directly during spaceflights.

Objective. To review the current status of the abovementioned problem and to identify biochemical and molecular markers most promising for biomedical research in spaceflight conditions.

Methods. A literature review of methods currently used for monitoring the level of biological markers characterizing variations in the immune, excretory, reproductive, musculoskeletal, and blood coagulation systems caused by spaceflight conditions was carried out.

Findings. Data concerning biological markers used for monitoring the health status of space crewmembers were analyzed. The authors argue that protein markers reflecting bone tissue remodeling hold particular promise. The decrease in bone tissue density developed as a result of microgravity carries potential risks of traumatism, thus making screening diagnostics of the state of the musculoskeletal system a key focus of laboratory diagnostics. The conducted literature review suggests that P1NP and osteocalcin may serve as the most informative markers of new bone tissue formation, while collagen C-telopeptide, pyridine cross-links, and tartrate-resistant acid phosphatase may serve as markers of bone tissue lysis.

Keywords: aerospace medicine; bone remodeling; molecular markers; bone mineralization; micro-RNA; spaceflight; microgravity; thrombosis

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ПЕРСПЕКТИВНЫЕ НАПРАВЛЕНИЯ МОНИТОРИНГА СОСТОЯНИЯ ЗДОРОВЬЯ ЧЕЛОВЕКА В УСЛОВИЯХ ДЛИТЕЛЬНОГО КОСМИЧЕСКОГО ПОЛЕТА

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Введение. В связи с увеличением длительности космических полетов растет и продолжительность пребывания членов экипажа в неблагоприятных условиях микрогравитации, что требует разработки подходов, направленных на диагностику состояния здоровья непосредственно в процессе полета. Данное исследование направлено на поиск и выбор перспективных биологических маркеров, целесообразных для изучения в условиях космического полета.

Цель. Изучить современное состояние проблемы и определить биохимические и молекулярные маркеры, наиболее перспективные для направления медико-биологических исследований, выполняемых в условиях космического полета.

Результаты. Проведен анализ данных литературы, посвященных изучению методов контроля уровня биологических маркеров, характеризующих вызываемые условиями космического полета изменения иммунной, выделительной, репродуктивной систем, опорно-двигательного аппарата и системы свертывания крови.

Выводы. В настоящем обзоре рассмотрены данные, касающиеся биологических маркеров, позволяющих контролировать состояние здоровья космонавтов. По мнению коллектива авторов, наиболее перспективными являются белковые маркеры, отражающие перестройку костной ткани. Развивающееся в результате микрогравитации снижение плотности костной ткани потенциально несет риски травматизма, поэтому скрининговая диагностика состояния опорно-двигательной системы является актуальной проблемой лабораторной диагностики. Исходя из данных литературы, наиболее информативными маркерами образования новой костной ткани могут служить P1NP и остеокальцин, а ее лизиса — С-телопептид коллагена, пиридиновые сшивки и тартрат-резистентная кислая фосфатаза.

Ключевые слова: космическая медицина; ремоделирование кости; биологические маркеры; минерализация кости; космос; невесомость; тромбоз

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INTRODUCTION

The growing diversity and amount of works performed by crewmembers during spaceflights, including long-term technical and biological experiments, increase the duration of their stay on the Earth's orbit. Astronauts encounter long-term unfavorable effects associated with gravity, such as hypodynamia, prolonged stay in a closed environment, increased noise, radiation, mental workloads, as well as restricted diets [1]. The continued quest of the mankind for longer-term manned missions will require even longer stays under the conditions hostile to the human body. Measures aimed at maintaining the physical and mental health of astronauts should be based on the knowledge of the behavior of human organs and systems under conditions of long-term spaceflights. The current level of technical development makes it possible to create laboratory instruments adapted for work in space conditions, permitting timely assessment of numerous parameters of body functions and life processes followed by correction, if necessary, the diet, physical load, and living conditions.

Various approaches can be applied to assess the functional state of the body. These include measurements, both invasive and noninvasive, undertaken during the flight and zero-G conditions, as well as collecting and storing samples for analysis after returning to the Earth. Conducting measurements directly in zero-G conditions makes it possible to avoid additional manipulations associated with preservation, storage, and delivery of biological samples, thus excluding the influence of storage and transportation conditions on target components in these biospheres.

Much attention has been paid to the development and application of noninvasive methods for monitoring astronauts' health status, such as Doppler blood cell composition detection [2], assessment of body fluid distribution [3], bicycle ergometry, etc. Unfortunately, such methods prove ineffective for evaluating minor changes in parameters and analyzing specific markers of the clinical status of the human body. The widespread approach involving collection of biological samples during the flight with their subsequent transportation to the Earth is inapplicable for many clinical and laboratory parameters due to the impossibility of their analysis after freezing and/or long-term storage of biospecimens. In addition, this approach allows analysis of changes in laboratory parameters only after a prolonged period of time, thus excluding the possibility of introducing the necessary adjustments to the experiment protocol along with monitoring and adjusting the health status of astronauts in real time [4].

Therefore, the search for biological markers that could reflect changes in human health during spaceflights represent a highly relevant research task. This will contribute to the development and improvement of valid diagnostic test systems for assessing the functional state of human health during spaceflights.

In this work, we aim to review prospective directions of modern biomedical research conducted in spaceflight conditions.

STUDY RESULTS

Current state of the problem

At present, a large number of studies focus on investigating astronauts' health status. However, most of them involve the stage of clinical and laboratory analysis after the astronauts return to the Earth. The results of such studies, although replenishing the knowledge base in the field of space medicine, are unsuitable for assessing the state of human health during the flight. The diagnostic value of such samples is minimal due to their inability to reflect the dynamics of changes in the state of astronauts' organs and systems at the moment of their stay in space. It should be noted that in zero-G conditions, the long-term storage and delivery of biological samples containing whole cells to the Earth conditions is hard to organize. In addition, a number of biological substrates, protein-containing ones in particular, should be analyzed directly during the flight due to the inadmissibility of their freezing.

The low gravity environment of space expeditions makes biological fluids change their behavior, thus changing the requirements for biosampling. Invasive biosubstrate collection using conventional techniques (syringe or open technique) becomes difficult. In space conditions, blood collection procedure is associated with venipuncture, which carries risks of low-dispersed aerosol formation in the air or development of infectious complications. Hence, the use of biological media available through minimally invasive or self-administered collection methods becomes relevant for analysis. Such biosubstrates include saliva, sweat prints, and urine samples. If blood components are to be tested, aliquot volumes should be minimized and the limitations of vacuum sampling systems should be considered.

In addition, preferences for a certain technique for introducing samples into the working area of the test system change. Thus, incubation of freely poured liquid when performing conventional enzyme-linked immunosorbent assay (ELISA) becomes unrealistic. Adhesive/capillary interactions of liquid with the solid phase keep under microgravity conditions, which currently allows the use of diagnostic systems in the form of test strips on the International Space Station (ISS). However, such test systems have a number of limitations, one of which is the semi-quantitative estimation of concentrations. It should be noted that the rapid development of microfluidic technologies and their successful application in terrestrial conditions makes it possible to assume that the adaptation of these technologies to microgravity conditions will facilitate the transition to the analysis of biological markers, including ELISA methods.

Currently, a number of physiological parameters, such as hemoglobin levels, blood glucose concentration, etc., are measured in space environments using immunochemiluminescence on test strips (Reflotron, F. Hoffmann-La Roche Ltd. / Roche Diagnostics GmbH, Germany) [5]. Other analytes and samples require transportation to terrestrial conditions followed by their analysis upon completion of the flight. Such samples include blood

serum, washes from the internal surfaces of the station and the surfaces of samplers, samples for microbiological studies, e.g., those collected as part of a Chromatomass-spectrum M experiment. Such studies are important for studying the continuously changing microbiome of the station [6], associated with the constant exchange of microflora in astronauts [7], but the urgency of obtaining such results is significantly lower than in the case of monitoring the health of the crew.

Recently, the results of a number of projects on studying astronauts' health using individual indicative markers of various states of the body systems in microgravity conditions have been published. Thus, as part of the Splanch experiment on the International Space Station (ISS), and earlier on the MIR space station, the levels of markers of cardiovascular damage, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured using a modified Reflotron-4 device. Hematological studies in spaceflight conditions were carried out on board the ISS, and earlier on the MIR station for 15 months during three MIR expeditions (15th, 16th, and 17th). These studies included an assessment of the blood cell composition, hematocrit, hemoglobin, reticulocytes, and the leukogram [8]. The analyses were carried out on smears of capillary blood using a Mikrovzor device, which combines a microscope with a television transmitter. In the Russian segment of the ISS, hematocrit was measured using a Hematocrit device. Kunz H, Quiriarte H et al. recorded that the hematocrit level was significantly elevated in the early stages of the flight, remaining unchanged throughout the entire stay in space, which is associated with a decrease in the volume of circulating blood and blood plasma under microgravity conditions. In the early post-flight period, a decrease in hematocrit below the pre-flight level was noted, indicating a loss of the cell pool [9]. Some authors (Mikhailov P.A. et al.) noted the development of functional erythropenia and an increase in the number of abnormal erythrocytes during orthostatic suspension in animal experiments [10].

DISCUSSION OF RESULTS

Influence of spaceflight conditions on the immune system

The need to elucidate the specifics of functioning of crewmembers' immune system is determined by harsh conditions, including confined spaces, hidden cavities, and reduced gravity, which contributes to the aerosol formation in the air. All this forms a favorable environment for the growth and transmission of pathogenic microorganisms, including herpes virus [11]. Paul AM, Mhatre SD et al. studied the leucogram and cytokine profile as an assessment of the cellular component [12–13]. They found a decrease in the number of eosinophils and a slight increase in the number of neutrophils when T-lymphocytes were passivated in vitro, which is probably due to a decrease in the expression of CD3 and IL-2 receptors on the T-lymphocytes surface [14]. Other studies revealed a decrease in the leukocytes number, in particular lymphocytes, monocytes [9], and leukocyte differentiation [15].

Microgravity promotes increased production of tumor necrosis factor and the development of immune cells apoptosis [16], which was confirmed by studies on cell lines. C.A. Savary et al. found that dendritic cells obtained by differentiation of donor $CD34^+$ progenitor cells in a rotating culture cell, modeling microgravity conditions, showed a decreased ability to phagocytose *Candida albicans* fungi and antigen presentation [17]. A number of studies have recorded a decrease in the cytotoxic function of natural killer cells against leukemia cells of the K562 line *in vitro* occurring in the early stages of spaceflights [18].

V.K. Ilyin et al. found that the levels of sIgA, IgM, and IgA immunoglobulins in saliva and gingival fluid decrease during a spaceflight. The noted shifts are highly likely to trigger a decrease in the protective function of saliva, thus contributing to the risk of infectious and inflammatory processes when the main paradontopathogenic strains of pathogens in the oral cavity are detected in the subjects [19].

The studies conducted by C.M. Ott showed that microgravity conditions and prolonged spaceflights stimulate the reactivation of latent herpesviruses, as evidenced by the increased frequency of human herpes virus type 1 detection in the saliva of astronauts and increased incidence of shingles. In addition, researchers diagnosed cytomegalovirus in the urine of 47% of Space Shuttle crewmembers [20].

A number of scientific studies have confirmed the formation of a general imbalance of the immune system in the conditions of spaceflights: about 46% of ISS crew members experienced these immune disorders [21]. A decrease in local immunity and functional activity of natural killer cells was registered, which led to reactivation of latent viruses, in particular, herpes virus. A number of publications (about 17% of reports) noted allergic reactions due to both a shift in the cytokine profile and other spaceflight factors (stress, space radiation) affecting the immune system status [21].

The National Aeronautics and Space Administration (NASA) has also identified changes in the immune response of astronauts during the Apollo (1975) and Skylab (Skylab-3 mission, 1973) missions. Serum samples collected during spaceflights were analyzed for miR-21 microRNAs, the expression of which increases approximately twofold during early T-cell activation. By quantitative polymerase chain reaction (PCR) tests of four biological samples, suppression of miR-21 expression under spaceflight conditions and suppression of expression of 85 genes was detected. In particular, the expression of early growth response protein 3 (EGR3), Fas-ligand of TNF superfamily (FASLG), protein family (BTG2), Spruti homolog 2 (SPRY2), and T-cell GTPase activator protein (TAGAP), whose regulation is carried out specifically by miR-21, was reduced. According to Hughes-Fulford M et al, the change in TAGAP expression can be functionally associated with the development of rheumatoid arthritis and multiple sclerosis, and a decrease in BTG2 gene expression reduces the cellular immune response [22].

Influence of spaceflight conditions on the excretory system

According to the literature, the filtration function of kidneys in microgravity conditions is conventionally estimated by creatinine and urea levels, residual products of protein metabolism. Since creatinine is contained in muscle cells, given the relatively stable muscle mass, its level is not subject to significant fluctuations. Creatinine is excreted by the kidneys, which, in the absence of evidence of muscle injury, allows effective assessment of glomerular filtration rate [23]. Clinical urine analysis is a standard practice of clinical and laboratory diagnostics used to assess the excretory and filtering function of the kidneys and to evaluate the homeostasis of the body. In the practice of space medicine, this analysis is implemented using a Urolux urine analyzer included in the ISS onboard equipment, which uses 10-zone test strips. Measurements are carried out by the method of reflectance photometry. The parameters evaluated include urine specific gravity, acidity (pH), presence of leukocytes, nitrite, protein, glucose, ketone bodies, urobilinogen, bilirubin, and blood elements (erythrocytes, leukocytes) [24]. Currently, urine biochemical analysis is another routine comprehensive method for assessing the excretory system status, including under spaceflight conditions.

Keith Siew et al. reported a reversible renal tubular remodeling caused by adaptation to changes in the blood electrolyte composition and redistribution of body fluid due to cranial displacement. An increase in the secretion of calcium, phosphorus, and magnesium ions in the excreted urine was noted, which is presumably associated with bone resorption [25].

Influence of spaceflight conditions on the blood coagulation system

Thrombosis in microgravity conditions is a relevant problem in modern astronautics. An ultrasound examination study conducted in 2019 found that six out of 11 ISS crewmembers had asymptomatic blood flow disorders in the head and neck vessels, with one of the astronauts having an occlusive thrombosis of the left internal jugular vein [26]. Thrombosis can be caused both a decrease in the velocity of blood flow through the vessels associated with hypodynamia and biochemical changes in the blood and endothelium. Changes in the blood protein composition affect the thickness and functional state of the vascular glycocalyx [27] and alter the rheological blood properties, increasing its viscosity, leading to an increased risk of thrombosis. In the early post-flight period, an increase in the parameters indicative of increased thrombosis potential, such as soluble fibrin monomer complexes (SFMCs) [28], factor XI, fibrinogen, fibrinopeptide A, plasminogen activator inhibitor serpin-3, etc., was observed [29].

Thrombosis formation may also be influenced by the planned use of oral contraceptives during flight to achieve medical amenorrhea [30], practiced for hygienic and water-saving reasons in female astronauts. According to published data, regular administration of a drug containing drospirenone leads to decrease in the plasma albumin level. In the absence of pharmacotherapy, the plasma protein composition and associated risks of thrombosis showed no sex differences [31]. However, the studied sample of astronauts is currently small, and its further increase during the development of space programs in the future may reveal prerequisites for the occurrence of multidirectional abnormalities in the hemostasis system.

To date, the increased risk of thrombosis caused by prolonged stay in microgravity has been associated with a number of factors that are difficult to correct, such as changes in blood circulation, blood cell composition, and activity of signaling molecules. In order to detect a pathological link in the coagulation system, determination of a large number of biomarkers is required. This task is associated with simultaneous processing of different types of biomaterials and a prolonged stage of sample preparation. Collection of different types of biomaterials under zero-G conditions is undesirable due to technical limitations and additional risks for astronauts' health. These limitations make it necessary to create test systems capable of analyzing a minimum number of integral indicators in the blood coagulation system, sufficient for monitoring the health of astronauts, with the prospect of being capable of determining the mechanisms of a particular pathology. In case of detection of thrombosis of large vessels, it is impossible to provide surgical assistance due to logistic constrains. The use of drug therapy is also problematic due to a limited set of pharmaceuticals and the development of possible complications that can aggravate the astronaut's condition.

Thus, the search for early biomarkers of the hemostasis system state and scientific substantiation of methods for diagnostics/correction and prevention of coagulopathies in space crewmembers is a promising direction of space medicine. However, the current list of biological markers required for complete characterization of coagulation processes is rather extensive, which makes their measurement in flight conditions highly difficult.

MicroRNAs, small non-coding RNA molecules (16–25 nucleotides) that perform regulatory functions in relation to a number of genes, may be potential informative markers of the blood coagulation system. In studies on rodents, as well as in the NASA Twins Study, microRNAs associated with the hemostasis system and more intensively expressed under spaceflight conditions were identified: miR-125, miR-16, and let-7a/7c [32–33]. These microRNAs are associated with the mechanisms of radiation damage in the vascular wall and, therefore, may be predictors of thrombosis. Changes in the level of miR-16, which has anti-inflammatory and antithrombotic effects, were observed in [32]. To date, there has been no precise information regarding the prognostic value of microRNA estimates in the human blood due to the lack of accumulated scientific data. This does not allow microRNA to be used as a biomarker of crew health control. Micro-RNA studies are associated with PCR, which is not yet feasible in zero gravity.

Influence of spaceflight conditions on the reproductive system

The IMMUNO experiment conducted from 2012 to 2017 was aimed at monitoring the reproductive system of astronauts during long-term missions to the ISS. The experimental group included exclusively male individuals, which fact should be taken into account when interpreting the data. The levels of luteinizing hormone (LH) and follicle-stimulating hormones (FSH) affecting testosterone synthesis were measured by interstitial Leydig cells [34]. The levels of activin A, which is responsible for the regulation of FSH synthesis, regulating the immune response and the process of wound healing [35], as well as the antagonistic protein produced by Sertoli cells, inhibin B, which inhibits the synthesis of follicle-stimulating hormone [36], were determined.

The study was supplemented by estimating levels of antisperm immunoglobulins A, G, M (AS-IgA, AS-IgG, AS-IgM) and the sum of antisperm antibodies, as well as the total and free cortisol in saliva [37], testosterone, estradiol, and aldosterone [38]. It is worth noting that all studies were performed after the completion of the spaceflight by the ELISA method. It was found that long-term spaceflights lead to an increase in the estrogen level, which was associated with a decrease in the content of specific and nonspecific transport proteins in the body. At the same time, an increase in the concentration of stress hormone cortisol was noted. The study by I.A. Nichiporuk et al. [37], although failing to definitely establish the totality of factors leading to hormonal imbalance, noted that the changes in the reproductive system are reversible.

Influence of spaceflight conditions on the musculoskeletal system

During a long-term spaceflight, all astronauts are subject to degradation of the musculoskeletal system, which is manifested in a decrease in muscle mass, bone mineralization, and reorganization of collagen in bones, tendons, and ligaments. Most of the large bones, experiencing constant load under the conditions of Earth gravity, are subjected to partial resorption in zero gravity. This leads to both reorganization of the micro- and macrostructure of bone tissue and to demineralization of most bones. The greatest danger is associated with damage to the skeleton of the lower limbs, lumbar vertebral bodies, pelvic bones, highly loaded bones of the skull base, and cervical vertebrae. Less critical is partial demineralization of thin spongy bones that carry less load. The only exception is the bones of the upper part of the skull, whose density increases in zero gravity due to the natural compensatory response to the changing load.

These conditions strongly affect the physical health of astronauts and their ability to perform their main tasks. Under spaceflight conditions, to assess the physical state of an individual, muscle volume measurements [39], dynamometry, myography [40], strain gauging, and bioimpedanceometry were performed. The latter are indirect methods that give no clear picture of the processes occurring in the body. In the pre-flight and early post-flight periods, examinations were performed using a noninvasive method of osteodensiometry [41]. It was found that the influence of microgravity is not limited to the development of osteopenia, being accompanied instead by redistribution of bone mineral density. The state of the muscular system and the effectiveness of training before spacewalking are evaluated by bicycle ergometry; the results are influenced by age, cardiovascular system, and fatigue. Moreover, data on previous measurements of the subject are required.

An optimal approach consists in the estimation of clinical molecular markers, conducted directly during the flight and allowing assessment of both the general state and its changes under the influence of loads and other external factors. Some markers include low molecular weight compounds and those excreted by the kidneys, which makes their estimation in urine accessible. Other markers are unable to pass the renal barrier and their estimation is possible only in blood.

Damage to the muscle system can be estimated by the level of creatine kinase, which was performed in the research [42]. However, this parameter is nonspecific with respect to the type of muscle tissue and can indicate damage to both skeletal muscle and myocardium.

Bone resorption is a relatively slow process carried out by osteoclasts. An activated osteoclast is fixed by specific integrin proteins to the bone matrix, triggering the synthesis of Cathepsin-K. The latter is an acidic protease capable of degrading type I structural collagen, which makes up more than 80% of the organic matter of bone. As a result of this process, large fragments of collagen containing large amounts of pyridine cross-links are released into the bloodstream. Osteoclasts synthesize matrix metalloproteases, whose work in the resorption focus result in the release of large fragments of collagen, consisting of two C-telopeptides of type I collagen, quickly excreted with urine, in the bloodstream. The third important component of bone resorption is the transmembrane transport of matrix degradation products into the cell by tartrate-resistant acid phosphatase.

In the process of resorption, calcium ions are released and their concentration in the blood increases, which may contribute to the development of uro- and nephrolithiasis. The study [43] showed an increased calcium content in morning urine and suggested using its determination to monitor the state of the bone system. However, the concentration of ionized calcium depends not only on the processes occurring in bone tissue, which significantly reduces the diagnostic value of its determination.

Thus, the control of collagen C-telopeptide, pyridine cross-links (pyridinoline and deoxypyridinoline) and tartrate-resistant acid phosphatase are convenient markers of bone resorption.

In parallel to bone resorption, bone tissue is also formed by osteoblasts. The resorption Howship's lacuna is filled by fibroblasts and osteoblasts synthesizing collagen of the first type, forming osteoid. In the process of collagen maturation, N-propeptide (P1NP, amino-terminal propeptide of procollagen type 1) determined in the blood becomes detached, which can be considered as a

marker of bone matrix formation. At the same time, a high mechanical strength of bone is provided by the mineral component, whose formation is affected by osteocalcin, a non-collagen protein, which promotes bone mineralization due to the stacking of oriented hydroxyapatite crystals [44]. The enzyme alkaline phosphatase, the exact function of which remains unknown, is involved in bone matrix formation. Considering that insufficient blood calcium levels may prevent the formation of new bone or provoke a decrease in mineralization, the level of ionized calcium in the blood should be taken into account when interpreting the results obtained [45].

Earlier studies showed a decrease in P1NP and bone alkaline phosphatase already by the eighth day of zero gravity. Simultaneously, such markers of bone resorption as pyridine cross-links and C-telopeptides of collagen type I in blood and urine increased [46].

The influence on bone remodeling processes extends beyond hypodynamia and orthostatic hypotension. The direction of the processes is regulated by the humoral system, including steroid hormones. Spaceflights are associated with a serious physical and psycho-emotional stress, which leads to the production of cortisol. Cortisol interferes with the formation of new bone tissue, shifting the equilibrium of bone remodeling toward impaired bone trophic and resorption [47].

In the context of bone tissue loss of its typical structure under spaceflight conditions, the participation of microRNAs in the regulation of de novo bone tissue formation is of interest. To test the hypothesis about changes in microRNA secretion by human osteoblasts under microgravity conditions, the expression of micro-RNAs in rat femur bone was studied. Thus, 14 microRNAs were identified that significantly decreased their expression under conditions of simulated zero gravity and 5 microRNAs whose expression significantly increased under these conditions. The main targets regulated by these microRNAs were genes of Wnt/β-catenin signaling pathway and estrogen-mediated cell cycle regulation [48]. It is assumed that the results obtained could indirectly indicate the state of bone tissue; however, such results are not available in the literature at the moment.

In 2014–2015, NASA carried out studies of urine compounds reflecting the state of the musculoskeletal system: urea, phosphorus and calcium, and creatinine. The results were presented as a ratio to creatinine as an indicator reflecting the glomerular filtration rate. A temporary decrease in urea/creatinine and phosphorus/creatinine ratios was noted, while an increase in calcium/creatinine ratio was observed [49].

A study of the urine proteome after a prolonged spaceflight showed the disappearance of the following peptides: tyrosine kinase receptor (IPI00296992); cytoskeletal keratin-1 (IPI00009865); G-protein coupled receptor from the C family (IPI00789902); inter-α (globulin) H4 inhibitor (IPI00944960); and SERPING1 gene protein (IPI00879931) [41, 50].

CONCLUSION

The conducted review of published literature has allowed us to identify the most promising markers for assessing the status of human bone, blood coagulation, and immune systems under the conditions of zero gravity (microgravity).

The decreased density of bone tissue under the action of microgravity potentially carries risks of traumatism, e.g., during astronauts' return to the Earth. Therefore, monitoring the state of human bone tissue is a relevant problem of laboratory diagnostics. P1NP, bone alkaline phosphatase, and osteocalcin may serve as the most informative markers of bone tissue formation, along with its lysis — collagen C-telopeptide, pyridine cross-links (pyridinoline and deoxypyridinoline), and tartrate-resistant acid phosphatase. Micro-RNAs are also promising markers of the state of the musculoskeletal apparatus. However, their participation in the regulation of a number of body systems restricts identification of the processes occurring in bone tissue on the basis of individual micro-RNAs. In this regard, a multivariate analysis of a set of several microRNAs appears promising. The difficulty of PCR staging in zero gravity also requires its own technological solution.

The pathologies of the cardiovascular system, the risk of which increases in microgravity conditions, include a decrease in microcirculation and the development of thrombosis. According to the available literature data, the most informative markers of risk and dynamics of thrombotic complications may include soluble fibrin monomer complexes (SFMCs), factor XI, fibrinogen, fibrinopeptide A, and plasminogen activator inhibitor serpin-3. MicroRNA families, such as miR-125, miR-16, and let-7a/7c, are also promising molecular markers of cardiovascular complications.

This review has not considered markers of the state of central nervous system (CNS). Currently, there is a lack of definite molecular and biochemical markers to diagnose a specific neurologic disorder and to identify differences between healthy individuals and patients with CNS disorders. Additionally, CNS dysfunctions in humans are detected already at the stage of selecting candidates for flights.

Longer space exploration projects, including those associated with the prospect of manned interplanetary flights, impose stricter requirements on the control of astronauts' health condition. Although monitoring of the overall health status remains relevant, the limitations associated with zero gravity and space station conditions shift the focus to a gradual introduction of individual diagnostic markers. According to the authors, these include, e.g., the biological markers characterizing the process of bone tissue remodeling, characterized by bone resorption in the lower parts of the body, hyper-mineralization of skull bones and cervical spine. At the same time, analytical systems based on microfluidic technologies seem to be the most promising tool for monitoring these markers during spaceflights.

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CORRELATION OF BLOOD PROTEOME PARAMETERS TO THE NUMBER OF CERTAIN INTESTINAL MICROFLORA BACTERIA IN HEALTHY WOMEN

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Introduction. Human intestinal microflora fulfils a wide range of important functions for the body. It provides non-specific anti-inflammatory defense through the production of bacteriocins, organic acids and substances with bacteriostatic properties. It also stimulates eukaryotic cells to synthesize mucin and substances with antimicrobial activity, thus suppressing the development of inflammatory reactions in intestinal epithelial cells. These bacteria obviously act synergistically with immunocompetent intestinal cells undergoing changes in zero gravity conditions modeled using dry immersion. Regulatory and metabolic changes which occur during model experiments are reflected, inter alia, in the protein composition of the blood.

Objective. Identification of the relationship between the blood protein level and the amount of *E. coli*, *Lactobacillus* spp., *Enterococcus* spp. and *Bifidobacterium* spp. in the intestine using an experimental model of 3-day dry immersion for potential use as clinical recommendations for the correction of intestinal microflora, based on data from the proteomic profile of the blood.

Materials and methods. The study was conducted among six women aged 25–40 years. During 3-day dry immersion, the subjects were completely immersed in an immersion bath containing water at room temperature. Direct contact between the subjects' skin and the water was excluded. During the study, fecal samples and capillary blood samples were taken from each of the participants. In order to assess the protein levels, chromatography-mass spectrometric analysis of samples of dried blood spots was performed using nano-HPLC Dionex Ultimate3000 combined with a timsTOF Pro mass spectrometer. The study of the number of intestinal bacteria was carried out using culture seeding of pre-diluted fecal samples on selective media according to a standard technique, followed by consideration of colonies.

Results. The regression model showed a relationship between the levels of individual proteins and representatives of the intestinal microflora. A statistically significant correlation was found between blood proteins ENO1 (r = 0.71), MYH9 and SPTA1 (r = –0.99) with the amount of *E. coli*; blood proteins EPB41, VCP, C8B, CCT2 (r = 0.74), FAH, YWHAE (r = –0.46) with the amount of *Bifidobacterium* spp. There was also a significant strong positive correlation between *Lactobacillus* spp. and proteins ENO1, CA2 (r =0.74) and S100A6 and HSPA4 (r =–0.87). The CALM2 protein (r = –0.76) correlated with the amount of *Enterococcus* spp.

Conclusions. Protein complexes were identified, the number of which correlated with the number of certain types of intestinal microflora: proteins associated with the immune system; proteins which directly or indirectly affect digestion and mineral metabolism; and proteins which affect cell tolerance to hypoxia.

Keywords: intestinal microflora; blood proteins; dry immersion; chromatography-mass spectrometry

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КОРРЕЛЯЦИЯ ПАРАМЕТРОВ ПРОТЕОМА КРОВИ С КОЛИЧЕСТВОМ НЕКОТОРЫХ БАКТЕРИЙ КИШЕЧНОЙ МИКРОФЛОРЫ У ЗДОРОВЫХ ЖЕНЩИН

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Введение. Микрофлора кишечника человека обладает целым спектром важных для организма функций: осуществляет неспецифическую противовоспалительную защиту посредством продукции бактериоцинов, органических кислот и веществ с бактериостатическими свойствами, стимулирует эукариотические клетки к синтезу муцина и веществ с антимикробной активностью, подавляет развитие воспалительных реакций в клетках эпителия кишечника. Очевидно, эти бактерии действуют синергично с иммунокомпетентными клетками кишечника, претерпевающими изменения в условиях невесомости, моделируемых с помощью «сухой» иммерсии. Регуляторные и метаболические изменения, происходящие во время модельных экспериментов, отражаются в том числе на белковом составе крови.

Цель. Выявление взаимосвязи между уровнем белков в крови человека и количеством *E. coli, Lactobacillus* spp., *Enterococcus* spp. *и Bifidobacterium* spp. в кишечнике с применением экспериментальной модели 3-суточной «сухой» иммерсии для потенциального использования в качестве клинических рекомендаций по коррекции микрофлоры кишечника, основываясь на данных протеомного профиля крови.

Материалы и методы. Исследование проведено с участием 6 женщин возрастом 25–40 лет. Во время 3-суточной «сухой» иммерсии испытуемые находились в иммерсионной ванне полностью погруженными в воду комнатной температуры, исключая прямой контакт кожи испытуемых и воды. В ходе исследования отбирались фекальные пробы и образцы капиллярной крови у каждой из участниц. Для оценки количества белков проводили хромато-масс-спектрометрический анализ образцов высушенных пятен крови с использованием нано-ВЭЖХ Dionex Ultimate3000, совмещенным с масс-спектрометром TimsTOF Pro. Исследование количества кишечных бактерий проводили с помощью культурального посева предварительно разведенных образцов фекалий на селективные среды по стандартной методике с последующим учетом колоний.

Результаты. Регрессионная модель показала связь между уровнями отдельных белков и представителями кишечной микрофлоры. Была выявлена статистически значимая корреляционная взаимосвязь белков крови ENO1 (r = 0,71), MYH9 и SPTA1 (r = -0,99) с количеством *E. coli;* белков крови EPB41, VCP, C8B и CCT2 (r = 0,74) и белков FAH, YWHAE (r = -0,46) с количеством *Bifidobacterium* spp., а также достоверная сильная положительная корреляционная взаимосвязь между *Lactobacillus* spp. и белками ENO1, CA2 (r = 0,74), S100A6 и HSPA4 (r = -0,87). С количеством *Enterococcus* spp. коррелировал белок CALM2 (r = -0,76).

Выводы. Выявлены комплексы белков, количество которых коррелировало с количеством некоторых видов микрофлоры кишечника: белки, связанные с иммунной системой; белки, прямо или косвенно влияющие на процессы пищеварения и минеральный обмен; белки, влияющие на толерантность клеток к гипоксии.

Ключевые слова: микрофлора кишечника; белки крови; «сухая» иммерсия; хромато-масс-спектрометрия

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INTRODUCTION

Normal human intestinal microflora is represented by a wide range of microorganisms, most of which are obligate or facultative anaerobes. Opportunistic infections can occur directly due to obligate pathogens or indirectly due to excessive growth of opportunistic microorganisms. Depletion of the intestinal commensal population may also play a determining role [1]. Factors of space flight, for example, a changed diet, hygienic procedures, psycho-emotional stress, constant microbial metabolism, which inevitably occurs in a hermetically sealed space of a spacecraft, negatively affect the composition of the intestinal microbiota. This process is associated with active reproduction of the conditionally pathogenic component of the microflora, decreasing the number of protective types of microorganisms [2]. This requires the development of means to prevent and reduce the risks of developing dysbiotic conditions, as well as research into better understanding the interrelationships of the intestinal microbiota and other physiological and biochemical indicators of human health. This understanding will subsequently enable the composition of the intestinal microbiota to be influenced through targeted effects on individual processes in the body, for example, on the metabolism of single proteins.

The vast majority of bacteria live in the large intestine. The proximal sections of the small intestine normally contain up to 10^4 CFU/mL of microorganisms, associated with the milieu pH (7.2–7.6) and the bactericidal effect of bile. The commensal microflora, in addition to participating in the digestive processes, fulfils a whole range of important functions for the host body. It provides non-specific anti-inflammatory defense through the production of bacteriocins, organic acids and substances with bacteriostatic properties. It also stimulates eukaryotic cells to synthesize mucin and substances with antimicrobial activity. In addition, the community of commensal microflora specifically suppresses the development of inflammatory reactions in intestinal epithelial cells. These representatives of the intestinal microflora obviously act synergistically with the local immune system [3, 4].

Consequently, the human intestine is not only an important part of the digesting system for digesting food, absorbing water and nutrients, it also plays an essential role in the organization of immune defense [5]. The epithelial cells of the intestinal mucosa are involved in immune regulation. In the intestinal mucosa's own plate, there are T and B cells which protect the body from pathogens. In addition, the cells of the intestinal mucosa produce various cytokines, such as gamma interferon (IFN-γ), tumor necrosis factor α (TNF-α), interleukin 2 (IL-2) and interleukin 6 (IL-6), which are important regulators of both physiological adaptive reactions and congenital immune reactions [6]. In particular, they can participate in inflammatory reactions and mediate differentiation, proliferation and activation of various immune cells [7]. Moreover, the intestinal microflora is necessary for the organization and implementation of various immune reactions [8]. Thus, the microflora of intestinal commensals activates both innate and adaptive immunity in the cells of the intestinal mucosa [9].

In this study, we have attempted to analyze the possible relationship of the blood protein complex with the number of intestinal bacteria in women participating in an experiment with 3-day dry immersion [10].

Previous studies of the human intestinal microflora in a dry immersion experiment established significant deterioration in the state of the microflora, an increase in the proportion of opportunistic microorganisms, and a decrease in the amount of intestinal commensal bacteria [11].

According to some researchers, the regulatory and metabolic changes which occur during dry immersion experiments are reflected in blood protein composition. Mass spectrometry-based studies were performed by means of proteomics methods. Changes were established in the levels of plasminogen, fibronectin, other coagulation and fibrinolysis factors, as well as an increase in the content of fibrinolysis products, and activation of the complement system [12]. Proteomic methods clearly allow identification of proteins which respond to a complex set of dry immersion factors and clarify the molecular mechanisms of changes in various physiological systems.

The aim of the study was to identify the relationship between human blood proteins level and the amount of *E. coli*, *Lactobacillus* spp., *Enterococcus* spp. and *Bifidobacterium* spp. in the intestine. The study was conducted by means of experimental 3-day dry immersion. Its potential use is in clinical recommendations for the correction of intestinal microflora, based on data from the proteomic profile of the blood.

MATERIALS AND METHODS

Experimental design

Six women aged from 25 to 40 years participated in the 3-day dry immersion experiment. During the experiment, the subjects did not take antibacterial drugs or other drugs which can affect fluctuations in microflora levels. At the beginning of the experiment, all participants were assigned to the same phase of the menstrual cycle (follicular phase) in order to avoid differences in estradiol levels and its effects on microflora and plasma proteins. During the period of dry immersion, the subjects were not subjected to any additional influences, in the aim of preventing adaptive changes in physiological systems [10].

The dry immersion experiment is a method of simulating such factors affecting the body in space flight as hypogravity, support unloading, and redistribution of body fluids in the cranial direction. During dry immersion, the female subjects were in the immersion bath completely immersed in water at room temperature. Waterproof film prevented the skin of the subjects from coming into contact with water, permitting the time spent in the bath to be increased (Fig. 1).

The study was conducted on the Dry Immersion bench base of the Institute of Biomedical Problems. Throughout the experiment, the participants remained in a horizontal position without physical exertion and with limited voluntary movements.

Collection of stool samples, cultivation and identification of representatives of the intestinal microflora

Stool samples were taken 1–2 days before the start of the experiment and 1–3 days after the end of the dry immersion, in order to assess the amount of *E. coli*, *Bifidobacterium* spp., *Lactobacillus* spp., *Enterococcus faecium*. A number of tenfold dilutions were prepared from fecal samples in sterile saline solution from 10^{-1} to 10^{-9}. Then 100 µL of the inoculate was sown in Petri dishes with selective nutrient media: De Man–Rogosa–Sharpe agar (MRS for cultivation of bacteria of the genus *Lactobacillus* spp.); Endo medium (for the cultivation of *E. coli*); agar for enterococci; and bifidoagar (manufacturer of all media — Himedia, India). The cultures were grown in a thermostat at 37°C for 48–72 h. Depending on the culture under study, bifidobacteria and lactobacilli were grown under anaerobic conditions. The colonies thus grown were counted using the Stegler SCM-2 colony counter and visually identified [13].

Collecting samples of dry blood stains

Capillary blood samples with a volume of 20 µL for chromatography-mass spectrometric analysis were taken using an automatic scarifier by piercing the terminal phalanx of the ring finger. This was followed by application to special filters

The photo is published with the written consent of the participants of the study

Fig. 1. Volunteer in an immersion bath

(Perkin Elmer) for drying (the “dry spot” method). Blood samples were collected from volunteers 2 days before the start of the experiment and in dynamics for 1st, 2nd, 3rd days during the dry immersion, as well as 2 days after completion. After collection, the capillary blood samples were dried at room temperature for 2 h. Then the dried blood stain samples were stored at minus 20°C.

The dried blood stains were prepared for chromatography-mass spectrometric analysis, in order to determine proteins in human blood as follows: the proteins were extracted in a buffer containing 25 mmol ammonium bicarbonate, 1% sodium deoxycholate and 5 mmol TCEP (tris-(2-carboxyethyl) phosphine hydrochloride) (Thermo Scientific) at a temperature of 60°C, at a shaking rate of 1000 revolutions per minute (thermomixer, Eppendorf) for 1 h. They were then reduced, alkylated, precipitated and cleaved with trypsin, as described in the procedure [14].

Chromatography-mass spectrometric analysis of extracts of dry blood stains

Mixtures of tryptic peptides were separated using liquid chromatography based on nano-HPLC Dionex Ultimate3000 (Thermo Fisher Scientific, USA). They were then analyzed on a timsTOF Pro mass spectrometer (Bruker Daltonics, USA) using the method of parallel accumulation with sequential fragmentation (PASEF) [15].

Statistical analysis

The statistical analysis of the data obtained was carried out using a number of nonparametric techniques. Changes in the intestinal microflora were assessed using the nonparametric Kruskal-Wallis test for related samples. The eubiotic index was calculated by summation of positive and negative quantitative changes in protective and conditionally pathogenic groups of microorganisms. The index reflects positive changes in the composition of the microflora. Statistical processing of the eubiotic index was carried out using a paired two-sample t-test for averages.

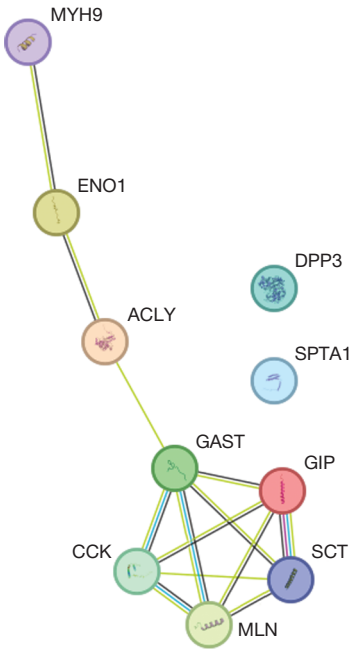
The change in the amount of blood proteins was assessed using discriminant analysis for small samples. The relationship between the level of human blood proteins and the number of intestinal bacteria was suitably described using a regression model. In this model the number of bacteria was the dependent variable, while the number of proteins was the independent variable [16]. The results were processed using the Statistica 12.0 software package. $P \leq 0.05$ was taken as the critical significance level. The STRING database was used to visualize protein relationships.

RESULTS

Approximately 1256 proteins were identified in the samples of dry blood spots of the female volunteers. Their relative levels were determined using the label-free quantification method. The regression model showed a relationship between the number of proteins in the blood described below and the number of bacteria *E. coli*, *Bifidobacterium* spp., *Lactobacillus* spp., *Enterococcus faecium*.

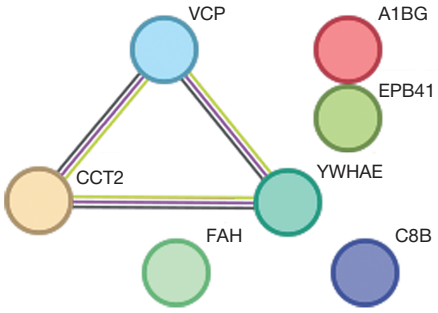
As a result of the regression analysis, the relationship of a number of proteins to the amount of *E. coli* was established (Fig. 2). The amount of ENO1 protein (alpha-enolase) in the blood positively correlated with the amount of *E. coli* ($r = 0.71$), while for the proteins MYH9 (non-muscular myosin with heavy chain IIa), ACLY (ATP citrate lyase), DPP3 (dipeptidyl peptidase 3), SPTA1 (spectrin alpha chain) a strong negative correlation $r = -0.99$ ($p \leq 0.05$) was detected.

The proteins EPB41 (Erythrocyte Membrane Protein Band 4.1), A1BG (Alpha-1-B Glycoprotein), VCP (Valosin Containing Protein), C8B (complement component C8 beta chain), and CCT2 (T-complex protein 1 subunit beta) were statistically significantly correlated with the number of bifidobacteria (*Bifidobacterium* spp.) in the intestine: $r = 0.74$ ($p \leq 0.05$). Furthermore, a weak negative correlation $r = -0.46$ ($p \leq 0.05$) was observed for proteins FAH (enzyme Fumarylacetoacetate hydrolase) and YWHAE (Tyrosine 3-Monooxygenase/Tryptophan 5-Monooxygenase Activation Protein Epsilon). The relevant data is shown in Figure 3.



This figure was prepared by the authors using own data

Fig. 2. Relationship between blood proteins and number of *E. coli* in the intestinal microflora of the volunteers



This figure was prepared by the authors using own data

Fig. 3. Interconnection of proteins which correlate to the number of *Bifidobacterium* spp. in the intestinal microflora of the volunteers

During the study, the data analysis established a significant strong positive correlation between the number of lactobacilli and blood proteins ENO1, CA2 (r = 0.74), and a negative correlation between the number of *Lactobacillus* spp. and proteins S100A6 and HSPA4 (r = –0.87). A negative correlation was also found between the CALM 2 protein (calmodulin) and the number of enterococci in the intestinal flora (r = –0.76) (p = 0.05). Table 1 summarized the data regarding the established correlation of proteins with some representatives of the intestinal microflora.

DISCUSSION

As a result of regression analysis, the relationship of a number of proteins with the amount of *E. coli* was established. The amount of ENO1 protein (alpha-enolase, glycolytic enzyme that catalyzes the conversion of 2-phosphoglycerate to phosphoenolpyruvate) positively correlates to the amount of *E. coli*. The main functions of this protein are participation in glycolysis, cell growth processes, and allergic reactions. In addition, this protein serves as a receptor on the surface of leukocytes, thus stimulating the production of immunoglobulins [17].

It must be noted that *E. coli* plays an important role in the human body. It is capable of producing a number of vitamins (B_1, B_2, B_6, K, etc.) and fatty acids. It participates in the metabolism of cholesterol, bilirubin, choline, bile acids and is involved in the absorption of iron and calcium [3, 5].

E. coli has been shown to produce a substance with immunological similarity to somatostatin [16]. Somatostatin is also produced by D-cells of the small intestine, while somatostatin-28 is involved in the inhibition of insulin, secretin, glucagon, gastrin, and other hormones in the gastrointestinal tract. The main function of somatostatin synthesized in the intestine is to prevent the secretion of hydrochloric acid, slow intestinal motility, and change the level of bile acids [18].

The secretion of the large intestine contains a significant number of rejected epithelial cells, lymphocytes and mucus, although containing a small amount of enzymes. The Lieberkühn gland (crypt), along with intestinal villi, is one of the two most important structural units of the intestinal mucosa. For each villus in humans, there are from 4 to 7 Lieberkühn glands. The maximum number is located in the duodenum. The Lieberkühn glands of the large intestine are lined with a single-layered cylindrical polar epithelium, the height of which is higher at the mouth than at the base. The epithelium of the Lieberkühn glands contains various endocrine cells: I-cells producing cholecystokinin (CCK), S-cells secretin (SCT), K-cells glucose-dependent insulinotropic polypeptide (GIP), M–cells motilin (MLN), and G-cells gastrin (GAST) [19]. The above proteins are biochemically related to one another and to the ACLY protein (ATP-citrate lyase), to the level of which *E. coli* correlates. In addition, ACLY and a number of differentially expressed genes are involved in ErbB (erythoblastic oncogene B) signaling and cholicytokinin/gastrin signaling. ACLY is an important enzyme which binds carbohydrates to lipid metabolism by producing acetyl-CoA from citrate for the biosynthesis of fatty acids and cholesterol [20]. Changes in ACLY levels are probably related to gastrin signaling.

Another protein which affects the amount of *E. coli* is MYH9 (non-muscular myosin with heavy chain IIa, a member of the family of motor proteins). MYH9 is involved in the processes of secretion, cytokinesis and ensures cell mobility. The amount of *E. coli* negatively correlates with the amount of this blood protein, which may also be related to gastrin signaling.

After analyzing the data obtained, it can be concluded that an increase in the level of ENO1 and a decrease in MYH9 and ACLY contribute to an increase in the number of *E. coli*. The ENO1, MYH9 and ACLY genes are co-expressed and involved in gastrin signaling. Gastrin, in turn, is associated with cholecystokinin, secretin, glucose-dependent insulinotropic polypeptide and motilin, produced by the Lieberkühn glands of the colon. At the same time, glucose-dependent insulinotropic polypeptin (incretin) inhibits the absorption of fats, probably causing an increase in their amount in undigested food. As a result, this can lead to an increase in the amount of *E. coli*, for which fatty acids are one of the energy sources [21]. Incretin also inhibits lipoprotein lipase. According to *Scholl RA et al*., a high amount of *E. coli* correlates with a decrease in this enzyme [22].

Positively correlated to the amount of *E. coli*, the ENO1 protein stimulates the production of immunoglobulins, possibly indicating an increase in immune activity locally in the large intestine. One explanation for this relationship is the assumption that the autologous commensal intestinal microflora probably possesses tolerance to locally secreted immunoglobulins. A similar relationship was noted for another obligate representative of the intestinal flora: *Lactobacillus* spp. Summarizing the above, ENO1, MYH9 and ACLY are closely related to the processes of excretion of biologically active substances by cells of the Lieberkühn glands of the colon, in particular incretin (GIP). This, in turn, taking into account its functions, contributes to an increase in the number of *E. coli*.

The protein DPP3 (Dipeptidyl peptidase 3) is a zinc-dependent peptidase and an intracellular serine peptidase.

Table 1. Correlations of proteins with some representatives of the intestinal microflora revealed in the study, ($p \leq 0.05$)

№	A microorganism of the intestinal microbiome	Proteins negatively correlating with this microorganism	*r*	Positively correlating proteins with this microorganism, correlation coefficient	*r*
1	*E. coli*	MYH9, ACLY, DPP3, SPTA1	0.99	ENO1	0,71
2	*Lactobacillus* spp.	S100A6, HSPA4	0.87	ENO1, CA2	0,97
3	*Enterococcus* spp.	CALM2	0.76	-	
4	*Bifidobacterium* spp.	YWHAE, FAH	0.46	VCP, C8B, CCT2, EPB41, A1BG	0,74

This table was prepared by the authors based on their own data

This protein has a site of unique catalytic sequence which ensures the degradation of oligopeptides with residues from 4 to 10 amino acids. In our study, this site had a fairly strong negative correlation to the amount of *E. coli*. The DPP3 protein is known to have a wide range of biological functions. Thus, it participates in the intracellular cleavage of proteins. In addition, in some studies [23] have shown activity of DPP3 in cells of the innate immune system, for example, in polymorphonuclear granulocytes and neutrophils. This activity partly confirms its active participation in the regulation of the immune function of the body. It is interesting to note that our study established a strong negative correlation between the amount of this protein and both the amount of *E. coli* and the number of hemolytic staphylococci.

The study also found that the level of SPTA1 protein had a fairly strong negative correlation to the amount of *E. coli*. However, given the functions of this protein, the possible causes of the interaction of the levels of this protein and the number of *E. coli* representatives remain unclear.

Bifidobacteria are one of the most important components of the intestinal microflora. They are involved in the synthesis of lactate and acetate which regulate the pH of intestinal contents. They also provide increased colonization resistance of the intestinal microflora.

In our study, the strongest associations with the number of bifidobacteria in the intestine were found for the proteins EPB41, A1BG, VCP, C8B, and CCT2. A weak correlation was also established with the number of proteins FAH and YWHAE.

The protein encoded by the EPB41 gene (Erythrocyte Membrane Protein band 4.1) is a multifunctional protein which mediates interactions between the cytoskeleton of erythrocytes and the plasma membrane. The protein encoded by the EPB41 gene binds and stabilizes dopamine receptors D2 and D3 on the plasma membrane of neurons. It also participates in the regulation of calcium ion transport and regulation of intestinal absorption [24]. In the studies conducted, a positive correlation was established between the amount of EPB41 protein and the number of bifidobacteria. It was also noted that with a decrease in the amount of EPB41 protein, there is a violation of calcium absorption in the cells of the small intestine [23]. Thus, with an increase in the amount of EPER41 protein, calcium absorption in the epithelium of the small intestine increases. A similar process is controlled by bifidobacteria which also cause increased absorption of calcium ions. The largest amount of *Bifidobacterium* spp. is found in the large intestine. Bifidobacteria and lactobacilli make up about 20–30% of the microflora of the small intestine, localized mainly in the jejunum [25].

One of the putative functions of the A1BG protein (Alpha-1-B Glycoprotein) expressed in the liver is to participate in cell recognition and regulation of cellular behavior [26]. For this protein, a positive correlation was noted with the number of bifidobacteria. This correlation can be explained by tolerance of the immune system towards intestinal commensal population and an increased immune response against the background of a stress factor (dry immersion). It is important to note that the C8B protein (complement component C8 beta chain, lectin activation pathway), which also plays a key role in the implementation of the mechanisms of innate and adaptive immune response, negatively correlates with the number of bifidobacteria in the intestine.

CCT2 protein (T-complex protein 1 subunit beta, molecular chaperone) promotes protein folding during ATP hydrolysis. As part of the TRiC (chaperonin) complex, it plays a role in the folding of actin and tubulin. According to literature sources, the spectrin cytoskeleton is a target for intestinal bacterial pathogens (for example, pathogenic strains of *E. coli*, *S. Typhimurium*, *L. Monocytogene*) due to increased cell adhesion. It also plays a crucial role in the progression of dysbiotic conditions [27]. A similar mechanism may also enhance adhesion of *Bifidobacterium* spp. on the intestinal epithelium and, thus, promote their growth. In our study, a positive correlation was noted between the amount of this protein and the number of bifidobacteria.

At the same time, YWHAE (Tyrosine 3-Monooxygenase/Tryptophan 5-Monooxygenase Activation Protein Epsilon), involved in the regulation of a wide range of both general and specialized signaling pathways, is also involved in the implementation of various biochemical processes related to signal transmission, such as cell division and regulation of insulin sensitivity. Its level is closely related to the amount of HSF1 protein (Heat shock factor 1). Its production by the cell, in turn, is induced not only by temperature stress, but also by many other provoking factors, namely hypoxic conditions, exposure to xenobiotics, proteotoxic stress [28]. The relationship between the levels of YWHAE blood protein and the amount of *Bifidobacterium* spp. in the intestinal flora is negative. Thus, with increased exposure to stress factors and an increase, respectively, in the YWHAE blood protein, the growth of bifidobacteria is inhibited. This inhibited growth confirms the relationship between stress levels of various etiologies and intestinal flora.

The FAH protein (enzyme Fumarylacetoacetate hydrolase which degrades 4-fumarylacetoacetate to acetoacetate and fumarate, providing the final stage of tyrosine amino acid catabolism), pursuant to its main function, is involved in tyrosine metabolism and is the last of the five enzymes which degrade this amino acid. The effect of this hydrolase and the conversion of 4-fumarylacetoacetate into fumarate and acetoacetate is to increase the blood level of ketone bodies. It has been shown that an excess of ketone bodies, for example, in keto diet, reduces the number of bifidobacteria. Thus, according to Ang QY, there is a negative correlation between them [29]. Our experiment showed a direct positive correlation between the amount of FAH protein and the amount of *Bifidobacterium* spp., although its severity was relatively weak.

At the same time, the VCP protein (valosin-containing protein) ATPase of the transitional endoplasmic reticulum is a component of the protein degradation process associated with the endoplasmic network. It is also responsible for maintaining cell proteostasis, including intestinal endothelium. Some studies have suggested that the VCP protein may contribute to the course of infection caused by toxoplasma [30]. This protein positively correlates to the number of bifidobacteria. In addition, it is co-expressed with CCT2 and YWHAE proteins.

Lactobacilli play an important role in maintaining the colonization resistance of the gastrointestinal tract. Although most bacteria in the gastrointestinal tract live in the large intestine, lactobacilli and enterococci are the dominant flora in the duodenum and jejunum. They are small in number compared to the colon (about 10^3–10^4 CFU/mL). However, they play an important role in immunomodulation and exhibit antagonistic activity against pathogenic microorganisms [31].

Most *Lactobacillus* spp. strains inhabiting the human intestine are homofermentative and form mainly lactic acid as a result of fermentation. They use glycolysis to form lactate from glucose. In relation to oxygen, most lactobacilli are aerotolerant anaerobes, that is, they grow most actively with oxygen deficiency.

In our study, the data analysis established a relationship between the number of proteins ENO1, CA2, S100A6, and HSPA4 and the number of lactobacilli. At the same time, ENO1 and CA2 had a positive correlation, and S100A6 and HSPA4 had a negative correlation to the amount of *Lactobacillus* spp.

Some studies have found that ENO1 is a protein which provides cell tolerance to hypoxia, while catalyzing the conversion of 2-phosphoglycerate to phosphoenolpyruvate during glycolysis. It is also present both in human cells and tissues, and in some species of *Lactobacillus* spp. The relationship between the amount of this protein and the number of lactobacilli is most likely primarily due to an increase in the tolerance of intestinal cells to hypoxia, which, in turn, contributes to an increase in the number of lactobacilli.

The protein CA2, belonging to the family of carbonic anhydrases, plays a crucial role in the functioning of hemoglobin. This is due to the catalysis of the process of hydration of carbon dioxide with the formation of carbonic acid and its subsequent dissociation in water. This leads to a decrease in the pH of the blood, which, in turn, reduces the affinity of hemoglobin to oxygen. Thus, an increase in the amount of this protein is also associated with hypoxia and, as a result, the creation of the most favorable conditions for the reproduction of *Lactobacillus* spp.

A6 S100A6 (S100) calcium-binding protein plays an important role in calcium binding. The level of this blood protein negatively correlates with the number of lactobacillus (*Lactobacillus* spp.), which use calcium ions in the process of citrate metabolism [32]. Consequently, with an increase in the amount of S100A6 protein, the amount of calcium necessary for the metabolism of lactobacilli decreases and the level of *Lactobacillus* spp. decreases.

It has been established that HSPA4 protein (a member of the Hsp110 family of heat shock proteins) possesses the important functions of a molecular chaperone inside the cell: response to an unfolded protein, protein import into the outer membrane of mitochondria, and assembly of protein complexes. Under the influence of many stress factors which cause the disruption of proteostasis processes, a decrease in intestinal commensals also occurs [33, 34]. Our studies established that the correlation of the amount of this protein to the amount of *Lactobacillus* spp. was negative: the greater the level of this blood protein, the higher the stress level and the lower the number of lactobacilli.

Enterococci are one of the important components of the intestinal microflora. A number of drugs currently used as probiotics contain Enterococcus faecium. The number of enterococci in the intestine should normally be 10^6 CFU/mL. Enterococci (along with lactobacilli), in addition to the large intestine, colonize the small intestine, albeit in a noticeably lesser number. *Enterococcus* spp. is classified as lactic acid microorganisms, since they perform fermentation-type metabolism and ferment carbohydrates to form lactic acid, which, in turn, reduces the milieu pH. In addition, enterococci are well-known producers of antimicrobial peptides (enterocins).

The analysis of the data obtained established a negative correlation between the protein CALM2 (calmodulin) in the blood and the number of enterococci in the intestinal flora. Calmodulin is expressed by epithelial cells in almost all parts of the small intestine and probably regulates the concentration of free calcium in microvilli cells. There is evidence that an increased content of calcium and magnesium ions in the medium inhibits enterocin production and enterococcal metabolism [35]. This is probably one of the possible reasons for the negative correlation of the CALM2 blood protein and the amount of *Enterococcus* spp. in the intestinal flora.

CONCLUSIONS

1. The studies identified protein complexes the level of which in the host's blood correlated to the number of protective microorganisms.

2. All proteins correlating with different protective microorganisms can be conditionally divided into four groups depending on the functions and nature of the interaction of these proteins with various microorganisms: proteins associated with the immune system; proteins which directly or indirectly affect the processes of digestion and mineral metabolism; proteins which affect the tolerance of cells to hypoxia; proteins with a high regression coefficient in correlation with some microorganisms, but no obvious functional relationship.

3. The data obtained contributes not only to a fundamental understanding of the relationship between the various processes in the human body, but can also serve as an important starting point for the formation of clinical recommendations for the correction of intestinal microflora based on data from the proteomic profile of blood, or, conversely, correction of blood protein parameters using probiotic and autoprobiotic agents.

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MICROPARTICLES AS QUALITY CRITERIA FOR PLATELET CONCENTRATE

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Introduction. Due to the increased requirement for platelet concentrate use in the treatment and prevention of thrombocytopathy, there is a pressing need for the development, improvement and implementation of new approaches to monitoring its quality parameters and safety assessment.

Objective. To conduct a systematic review and analysis of literature data, in order to identify promising approaches to evaluating an adequate analysis of the quality of platelet concentrate to improve the effectiveness and safety of transfusions.

Discussion. The possibilities and advantages of a rational approach to platelet concentrate transfusion are established, while considering the degree of platelet activation required to optimize the preparation of the component. Special attention was paid to methods for evaluating platelet activation. The detection of microparticles based on dynamic light scattering will make it possible to distinguish activated platelets (with a high content of microparticles) from inactive (with a low content of microparticles) platelets during both therapeutic and preventive transfusions and optimize the use of this scarce blood component.

Conclusions. The ability to differentiate platelet concentrates based on the screening of the content of microparticles formed due to activation will contribute to improving the effectiveness and safety of transfusion therapy.

Keywords: transfusion; platelet concentrate; microparticles; light scattering; refractoriness

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МИКРОЧАСТИЦЫ КАК КРИТЕРИИ КАЧЕСТВА КОНЦЕНТРАТА ТРОМБОЦИТОВ

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Введение. В связи с возросшей потребностью применения концентрата тромбоцитов для лечения и профилактики тромбоцитопатии актуальной задачей является разработка, совершенствование и внедрение новых подходов мониторинга параметров его качества и оценки безопасности.

Цель. Проведение систематического обзора и анализа литературных данных для выявления перспективных подходов к оценке адекватного анализа качества тромбоцитного концентрата для повышения эффективности и безопасности трансфузий.

Обсуждение. Выявлены возможности и преимущества рационального подхода к переливанию концентрата тромбоцитов с учетом степени активации тромбоцитов для оптимизации заготовки компонента. Особое внимание уделено методам оценки активации тромбоцитов. Обнаружение микрочастиц на основе динамического рассеяния света позволит отличать активированные (с высоким содержанием микрочастиц) от неактивированных (с низким содержанием микрочастиц) тромбоцитов при проведении как лечебных, так и профилактических трансфузий и оптимизировать использование этого дефицитного компонента крови.

Выводы. Возможность дифференцировки концентратов тромбоцитов на основе скрининга содержания микрочастиц, образующихся в результате активации, будет способствовать повышению эффективности и безопасности трансфузионной терапии.

Ключевые слова: трансфузия; концентрат тромбоцитов; микрочастицы; светорассеяние; рефрактерность

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INTRODUCTION

In the last two decades in the Russian Federation, due to the improvement and intensification of treatment programs with the use of PC in many fields of medicine, there has been a steady increase in the need to use platelet concentrates (PC).

Platelets are specialized nuclear-free blood cells which play a key role in stopping bleeding and dangerously blocking healthy blood vessels in thrombosis. Inactivated

platelets are flattened spheroids (disks) with a semi-axis ratio 2–8 and a characteristic size of 2–4 μm in diameter. In the activated state, the shape of platelets changes: a part of activated platelets acquires a shape close to spherical. During the transition to the activated state, microparticles with sizes ranging from 50–100 nm are separated from platelets that fall into the intercellular space of blood [1]. An indicator of the proportion of active platelets is the content of microparticles in a given concentrate.

Platelet concentrates are one of the deficit blood components used in hemotransfusions. In order to assess the quality of these concentrates and their proper use, information is needed regarding the proportion of active platelets contained in them [1–4]. This indicator depends on the individual characteristics of the donor, the method of obtaining platelet concentrate from donor blood, as well as the duration and storage conditions of the concentrate. Too large a proportion of microparticles in the concentrate makes it unsuitable for use in transfusion. The possibility of using concentrates with a moderate proportion of active platelets and, consequently, microparticles depends on the characteristics of the patient for whom the concentrate is intended.

In this work, we conduct a systematic review and analysis of published literature to identify promising approaches to assessing adequate platelet concentrate quality assurance to improve the efficiency and safety of transfusions.

DISCUSSION

Properties and functions of platelet microparticles

The formation of microparticles (MPs) or microvesicles is an integral manifestation of cell viability, occurring both in vivo and in vitro. The formation of microparticles is stimulated by pathogens, stress, various damages, and other unfavorable factors [1]. The most active production of microparticles is characteristic of blood formers, as well as endothelial and smooth muscle cells of blood vessels [2]. Among all microparticles in the blood, platelet microparticles (PMs) are the most abundant [3] and account for about 70–90% of the total number of cells. There is a growing scientific and clinical interest in the physiologic role played by platelet microparticles [4]. Compared to platelets, which have an average lifespan of about 10 days, the lifespan of platelet microparticles is measured in minutes [5], while the aspect of removing microparticles from circulation in the bloodstream remains unclear.

Platelet microparticles are 0.1–1 μm fragments released from platelet plasma membranes which undergo activation, stress or apoptosis, with a wide range of biological activities. They have a phospholipid-based (PLP) structure and express functional receptors from platelet membranes. As the most abundant microparticles in the blood, PMs express the procoagulant phosphatidylserine (PS) and likely complement, if not enhance, platelet functions in hemostasis, thrombosis, cancer and inflammation, while also acting as stimulators of tissue regeneration. Their size and structure make PMs indispensable in the system of intercellular interactions with tissue cells as a delivery tool for platelet-carried bioactive molecules, including growth factors, other signaling molecules, and microRNAs.

Considering the reactivity of PMs, they may have different pathophysiologic effects on the cellular environment when interacting with components of the circulatory system. There is also growing evidence that PM production is triggered during donation, component separation, and storage of blood. This may lead to thrombotic and inflammatory side effects in recipients. Evaluation of PMs requires rigorous pre-analytical and analytical procedures, aimed at avoiding the generation of artifacts. At the same it ensures the accurate estimation of the quantity, size redistribution and functional properties of these microparticles [6, 7].

In vivo, PMs can be released from platelets under normal physiologic conditions or as a result of activation, stress, or apoptosis. Surface marker studies have shown that PMs are probably the most abundant MPs in healthy individuals, constituting 70–90% of circulating cells in the blood [6], with a range of approximately 100–1000/μL [7]. Megakaryocytes can also release MPs [8], but determining their proportion requires further studies. The remaining MPs are released by endothelial cells, leukocytes, and erythrocytes. Evaluation of PMs still requires the development of rigorous preanalytical and analytical procedures, in order to ensure the most accurate estimation of abundance, size, and functional properties. The number of PMs increases as a result of activation of the coagulation cascade or complement system, as well as by apoptotic signals or shear forces. The number of PMs increases in some prothrombotic and inflammatory diseases, as well as in some cancers [9].

The quantitative content of microparticles in blood is probably related to the state of balance between their formation and utilization [10]. In various pathological conditions, this balance is disturbed, and an increase in the content of platelet microparticles caused by “chronic activation” of platelets is registered in the blood [11]. Platelet microparticles are heterogeneous and characterized by the presence or absence of mitochondria [12], as well as variation in size. Since PMs can express functional receptors from platelet membranes and are PLP-based nanoparticles, they are increasingly considered as tools in the interaction between platelets and tissue cells [13]. Platelet microparticles contain biologically active molecules, including growth factors and other signaling molecules which can transmit messages to neighboring or target cells [14]. Thus, PMs have the potential to influence the cellular environment interacting with the blood network. This is because they expose the procoagulant surface of PLP and can act as a transporter of previously mentioned bioactive molecules, as well as genetic materials including microRNAs [15]. PM generation is also triggered in vitro during blood cell processing and storage [16], potentially causing side effects during transfusion therapy.

Accurate characterization of PMs requires careful preparation of study samples, in order to avoid experimental artifacts. In addition, when evaluating MPs [17], a reasonable choice of analytical methods combining techniques which characterize the cellular origin, number, size, and functional activity of microparticles should be made.

Microparticles as a versatile quality indicator of platelet concentrates

PCs are widely used in clinical practice. In cancer patients, platelets are used to prevent hemorrhagic syndrome. In trauma and surgical patients, PC transfusions are performed to stop bleeding. In addition, the introduction of platelet-rich plasma injections is based on the immunologic functions of platelets.

The quality requirements for platelets intended to prevent and stop bleeding or accelerate wound healing vary widely. The question is whether a single measurable characteristic can describe platelet quality for all applications. A positive answer is provided by Maurer-Spurej, who presents data regarding the use of microparticle measurement in platelet samples as a versatile quality characterization for production, storage, viability assessment, function, and compatibility of PCs [18].

It has been suggested that viable platelets in the platelet concentrate are lost due to storage lesion changes. However, donor variability is not thought to be a major contributing factor [19]. This implies that patients with indications for transfusion of PC containing long-lasting viable platelets in the bloodstream should be transfused as soon as possible after procurement. Traditionally, in vitro platelet quality assessment has been based on these assumptions [20]. In the event of changes in these parameters both during platelet activation and storage, the measurement of CD62 surface receptor expression, ADP-stimulated aggregation level is performed. It has been shown that the release of microparticles by platelets follows activation, and increases during storage of platelet components [21]. Thus, platelet quality is assessed from the perspective of the manufacturer and regulated, in order to ensure consistency and stability of the manufacturing process [22].

Since PC quality assessment is focused on detecting degradation from the beginning to the end of the regulated 5-day shelf-life [23], normative indices have high resolution for small changes in “resting/viable” platelets, while low resolution for “activated/functional” platelets. The characteristics of donor platelet quality are relevant when assessing the immediate response to transfusion. However, maintaining the duration of response depends predominantly on patient characteristics [24, 25].

The studies of Maurer-Spurej et al. noted that in 17% of cases (regardless of age) transfusion of donor platelet concentrates did not lead to the expected increase in the number of platelets after transfusion in oncohematologic patients [26]. One possible reason for this unexpectedly high rate of adverse clinical outcomes is that platelet viability is highly dependent on donor characteristics. Indeed, it was found that approximately 33% of donors were found to have pre-activated platelets, as evidenced by high levels of microparticles in the harvested platelet concentrate [27]. Patients with oncopathology usually require platelet transfusion not for reasons of active bleeding, but due to a predisposition to develop bleeding due to low platelet counts secondary to underlying disease and/or therapy. In these patients, donor platelets must remain in the circulatory stream for possible activation. Due to reduced viability, pre-activated platelets are not recommended for storage or use in cancer patients [28]. On the contrary, pre-activated platelets with increased hemostatic activity are believed to be effective in stopping acute bleeding, which is especially important for patients with surgery or trauma [29].

Platelets are used in clinical practice for a wide variety of purposes. For this reason, the quality of the platelet concentrate should match the specific transfusion objective. Consequently, the ideal quality parameter for predicting platelet function after PC transfusion should be to distinguish between resting/viable and pre-activated/highly functional platelets with the same resolution across the entire viability/functionality spectrum [28, 29].

The indicator of microparticle content as fragmentation and heterogeneity of platelets can be considered as a quality criterion for platelet production, storage, viability, function, and compatibility [30, 31]. The heterogeneity of platelet concentrates, considering the degree of activation by microparticles, enables the process of procurement to be corrected, and PC to be differentiated for prophylactic and therapeutic transfusions.

If platelets are more viable (i.e., capable of surviving certain storage conditions, transportation, irradiation, and other influences), then they are, by definition, less functional. This fact has been known since the 1970s, when researchers tried to determine the optimal storage temperature for platelet concentrates. Platelets stored at room temperature were found to be more viable, while refrigerated platelets showed better functional activity. This is based on an assessment of the criterion of changes in hemostatic parameters in volunteers receiving aspirin or in patients with thrombocytopenia [32]. Maintenance of platelet viability is an important role of anticoagulants. Concentrates rich in viable platelets are homogeneous in composition, containing predominantly disc-shaped platelets and few (or no) microparticles or microaggregates [27]. In contrast, platelets which have undergone long-term storage and refrigeration, or otherwise activated, are expected to be heterogeneous. They are expected to contain platelets with a high level of polydispersity due to polymorphism, high surface expression of activation markers, large numbers of microparticles, and the presence of microaggregates [33].

The heterogeneity of platelet concentrates increases during storage [34] as well as during pathogenreduction [23] and varies greatly in healthy donors [35]. The greatest contribution to platelet heterogeneity is their microparticle content.

At the present time, two important questions require consideration: firstly, whether microparticles in blood components have a potentially pathogenic effect; and secondly, how the process of preparation, additional processing and storage of blood components affect the release of microparticles. Recently, the importance of microparticles as indicators of PC quality has been heightened due to their potential physiological and pathophysiological roles. The importance of microparticles in transfusion medicine is recognized since microparticles are present in both plasma

and cellular blood products [7]. The pathophysiological effects of PMs related to the regulation of immune responses have raised obvious concerns about the potential deleterious effects associated with blood transfusion in immunocompromised patients [36, 37]. There is strong evidence that both blood cell-derived microparticles and platelet microparticles are generated during the production and storage of all blood components, plasma for transfusion, and red blood cell and platelet concentrates prepared from whole blood by apheresis [38, 39]. PM release is induced by stimuli such as shear stress resulting from activation or apoptosis of cells during storage, as well as their contact with the walls of the storage container [38]. Cellular expression by-products are formed [40], when platelet concentrates are stored at from +20 to –24°C or erythrocyte units at from +1 to –6°C.

Platelet microparticles appear to be present in higher amounts in PC prepared by apheresis, rather than in the platelet concentrate obtained from the leukotrombotic layer. The specifics of the apheresis procedure protocol or the type of cell separator used have been shown to potentially influence the extent of PM release during processing [41]. Platelet microparticles do not appear to be removed by leukoreduction [42]. On the other hand, leukofiltration of whole blood has been found to reduce the risk of subsequent PM formation [43]. Fresh frozen plasma prepared after overnight exposure of whole blood at +4°C contained more PMs than that obtained 8 h after blood collection [44]. At the same time, according to George JN et al., Cryoprecipitate containing highly concentrated PMs had a potentially more pronounced hemostatic effect when compared to the initial plasma [45].

In order to ensure transfusion therapy safety, a better understanding of the PM release mechanism during processing and storage of blood components through careful sample preparation and a combination of analytical techniques [46] is important. Suspected complications of transfusion include: increased risk of infectious complications; as well as renal, respiratory and multi-organ failure [37]. Some authors (Khorana AA, Francis CW et al.) have found a correlation between the increased incidence of venous thrombosis and embolism after administration of platelet concentrates with the presence of PMs [47], known to exhibit higher procoagulant activity (50–100-fold), when compared to equivalent activated platelets [48]. Although PM half-life is thought to be very short due to phagocytosis by macrophages, recent experiments have shown that it can be approximately 5.8 h when platelet concentrates are administered to patients with severe thrombocytopenia [49].

Phosphatidylserine-expressing PMs can activate innate immune cells. This can lead to an inflammatory response mediating transfusion-related acute lung injury (TRALI) [50]. Platelet microparticles may also contribute to immunosuppressive effects occurring during transfusion, indirectly explaining the occurrence of post-transfusion infections or cancer recurrences [40]. Another possible MP side effect is the potential high risk of alloimmunization against blood cell antigens, since they may have a high immunological potential [51].

Platelet refractoriness is a situation in which a patient fails to have the expected clinically significant response to a PC transfusion [52]: a complication seen in 27% of platelet recipients [53]. Refractoriness to platelet transfusions is defined as two consecutive platelet transfusions resulting in an insufficient increase in corrected count increments (CCI). The threshold below which the CCI is considered insufficient depends on the time of measurement. A CCI of less than 5000–7500 platelets/μL measured in a recipient blood sample taken 1 h after transfusion characterizes poor recovery, while a CCI of less than 5000 platelets/μL in a sample taken 24 hours after transfusion characterizes poor survival. Patients with immune refractoriness have a low post-transfusion CCI both 1 and 24 hours after transfusion, which can be eliminated (or not) by selection of appropriate HLA/HPA platelet concentrates [54]. However, an increase in platelet count at 1 h, followed by a significant decrease in platelet count 24 h after transfusion is detectable even in the absence of documented alloimmunization. It has been suggested that inadequate platelet quality is one of the reasons why cancer patients may have particularly poor platelet survival in the period of 24 h after transfusion [12]. In addition to complicating the treatment process itself, the cost of bed days more than doubles when treating patients with platelet resistance, when compared with patients without refractoriness with hospital stays 21 days longer [55].

Microparticles are markers of prothrombotic inflammation. Patients who become refractory to platelet transfusion often have concomitant fever or systemic inflammation, detectable as increased microparticles [56]. Consequently, homogeneous platelets may be a better choice for cancer patients at risk of developing refractoriness, whereas heterogeneous platelets may lead to the development of complications. Thus, transfusion of platelets from microparticle-rich donors may be the most optimal option. In all likelihood, transfusion of heterogeneous platelets to patients whose immune systems are hyperactivated may push them to a tipping point where they become immune to platelet transfusion. In independent studies by Cortés-Puch et al, and Flegel et al, found that not transfusing heterogeneous platelets prophylactically may prevent refractoriness. A similar concept was analyzed in an experimental study on animals in which the combination of bacterial infection and transfusion of “older” erythrocytes containing high concentrations of microparticles led to an increased risk of mortality [57, 58].

Heterogeneous platelet concentrates contain pre-activated platelets capable of reacting quickly when they enter the bloodstream. Thus, heterogeneous platelets have a more pronounced functional activity and have been shown to stop bleeding faster than homogeneous viable platelets [33].

The introduction of microparticle measurements for the quality and safety control of platelet concentrates could eliminate the influence of pathogen inactivation. It could also contribute to supplement solutions and 7-day storage conditions for PC. Inventory management based on this indicator could lead to optimization of the treatment process and significantly reduce costs.

Evaluation of platelet microparticle content

A number of experimental methods have been used to detect and analyze microparticles of platelet origin. These include: flow cytometry; nanoparticle trajectory analysis; electron microscopy; atomic force microscopy, and dynamic light scattering [59–61].

The most informative of these methods is flow cytometry. This method measures elastic light scattering intensity from a single cell or particle, sequentially passing through the laser beam focusing zone. It subsequently processes this data using special mathematical algorithms. In this case, the characteristics of frontal and lateral light scattering provide an idea of the size and structure of the cell. It also considers the level of fluorescence of chemical compounds included in the cell. This method requires the use of rather complex and expensive equipment. It also requires a large amount of time to be spent on preparation and analysis, so it is of little use for rapid screening of platelet concentrates.

Dynamic light scattering (DLS) was proposed as an express method for the rapid control of microparticle content in platelet concentrates. It is a rather effective method for measuring the size and size distribution of particles in a liquid. In DLS analyzers, the time dependence of the intensity of laser radiation scattered by particles suspended in the liquid is directly measured. Based on this dependence, the distribution of particles in the coordinates “particle diameter — relative intensity of radiation” scattered by particles of a given diameter (intensity distribution) is reconstructed. Two areas can be distinguished in such distributions: in the interval from 0.05 to 0.5 μm, corresponding to microparticles; and in the area from 1 to several μm, corresponding to platelets. This principle is the basis for the evaluation of platelet concentrates using a specialized DLS analyzer ThromboLUX produced by the Canadian company LightIntegra Technology [59].

When using the ThromboLUX technique with the tested platelet concentrate, three measurements are performed: at 37°C; cooling to 20°C; and reheating to 37°C. The study by Maurer-Spurej E. et al. demonstrated the result of measurements of particle distributions by mean of the DLS method [27]. It was found that upon cooling from 37°C to 20°C, the proportion of platelets decreased from 72 to 65%, while microparticles increased from 26 to 31%. Reheating to 37°C restored the former ratio of microparticles and platelets.

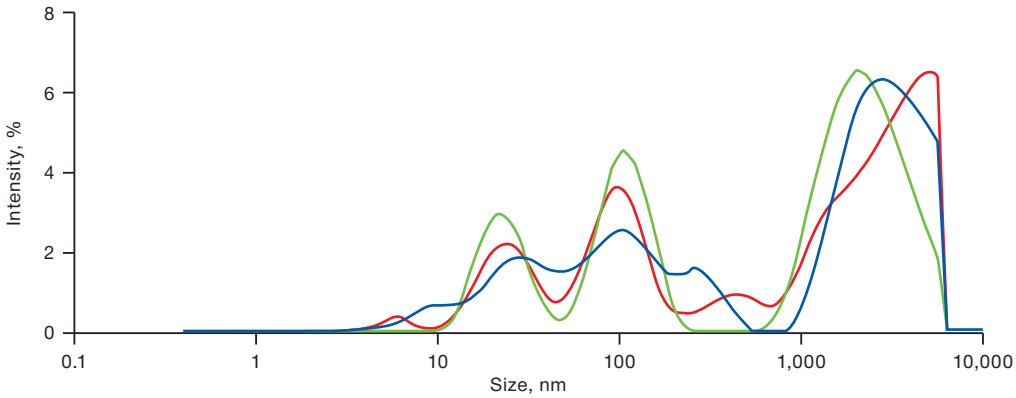
The ThromboLux method has been shown to correlate well with flow cytometry and electron microscopy data [59]. The content of microparticles can be a fairly versatile indicator used to assess the quality of platelets in the concentrate. In an experimental study [18], a scheme for selecting the optimal platelet concentrate for different categories of patients was shown based on the analysis of a large number of data. In particular, the ThromboLux technique has been used in the USA and Canada for the comparative analysis of the quality of PCs obtained from different donors or by different methods from the blood of one donor [60].

Microparticles in platelet concentrates have recently been studied using a versatile DLS analyzer, the Malvern Zetasizer [61]. This instrument collects scattered radiation at an angle of 173° (backscattering technology), enabling measurements to be performed for low-transparent samples. Particle distributions measured for undiluted platelet concentrates are the most informative. Figure 1 shows the scattered intensity distributions of microparticles and platelets measured under the above conditions. Three measurements were made for each of the samples studied. The first measurement was made at a temperature of 37°C, characterizing the initial state of the concentrate. The second measurement characterizes the resistance of platelets to temperature stress, i.e. activation when the temperature is lowered to 20°C. The third measurement was performed after increasing the temperature from 20 to 37°C, its results allowed the ability of platelets to recover from stress to be assessed.

The data presented in Figure 1 shows a large proportion of microparticles in PC at 20°C (green line), and its decrease when heated to 37°C (blue line). Labrie A. et al. analyzed platelet concentrates obtained by different methods: by apheresis; from platelet-enriched plasma; and from leukocyte-phosphorus film. The measured size distributions of microparticles clearly show peaks corresponding to exosomes (smaller particles with a diameter not exceeding 100 μm) and microparticles of platelet origin [59]. However, depending on the method of PC production, the positions of these peaks on the scale of particle diameters differ markedly [60].

In recent years, technologies for the preparation, processing, storage, and use of PCs have developed intensively. They now require new methods for assessing the quality and safety of blood components which meet modern standards [59–63]. The method of microparticle detection in PCs as one of the possibilities to preserve their quality characteristics and safety during subsequent transfusions is shown in Fig. 2.

Figure 2 shows the steps required to routinely manage platelet supply in a blood bank: a sample is obtained from the platelet concentrate; the sample is loaded into a capillary for DLS measurement; a DLS test is performed to identify microparticles; and the reported microparticle content then used to identify activated platelets. It takes the average user 3 min 23 s to prepare the DLS system for the test, obtain and test the sample according to the protocol, and then label the platelet bag. The focus of this protocol is to determine the composition of particles present in platelet transfusions and to use microparticles as biomarkers of platelet activation. Platelet transfusions are labeled as inactivated or activated based on a threshold of 15% microparticle percentage. Towards the end of the shelf life, an increase in the number of circulating platelet-derived MPs is noted in the PC. This indicates excessive platelet activation and/or apoptosis and loss of platelet functional activity, ultimately leading to a decrease in the expected therapeutic effect of the PC. Determination of the number of platelet-derived MPs by DLS method may be a promising method for assessing the quality of PC.



The figure is based on the authors' use of data from references [59]

Fig. 1. Scattered intensity distribution of microparticles and platelets in platelet concentrate
Note: red color — at a temperature of 37°C (initial state); green color — at a temperature of 20°C; blue color — after increasing the temperature from 20 to 37°C.

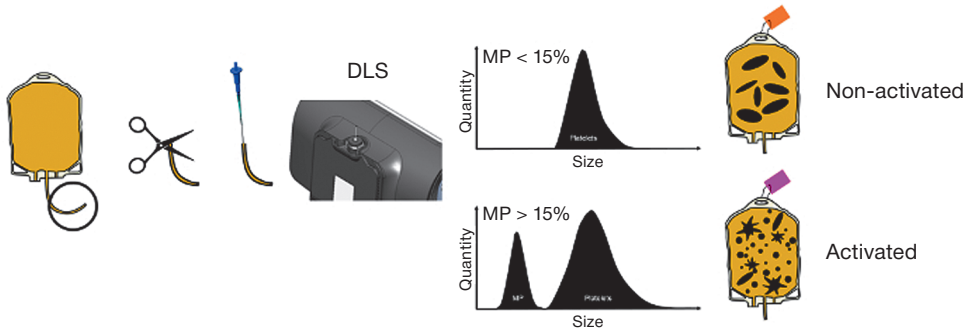


Figure prepared based on data from [60]

Fig. 2. A method for detecting the content of microparticles which permits differentiation between activated and non-activated platelet concentrates [60]

A promising development is the use of a domestic technique involving dynamic light scattering. This technique analyzes the content of microparticles in platelet concentrates as one of the criteria for quality control of blood components. Modern analysis will perform routine screening of microparticles in platelet-rich plasma or platelet concentrates, with validation of the results, leading to the formation of a conclusion on the possibility of using PC for subsequent transfusion.

CONCLUSION

With the development of modern technologies, the search for new methods to determine the quality and safety of blood components, including platelet concentrates, is becoming increasingly important. For routine screening of PC, a rapid and non-invasive test can be performed. Its purpose is to evaluate the characteristics of those platelets which are important for the recipient. The parameter to be investigated could be the determination of microparticle content. By measuring the composition of the platelet concentrate, the component's storage characteristics and resistance to additional stress can be determined, while its optimal use can be justified.

The method presented herein to evaluate the quality and safety of platelet concentrates is based on dynamic light scattering, thus offering several advantages over previous tests. The introduction of the method into clinical practice will enable the effectiveness of PC use before transfusion to be assessed. This is important because multiple transfusions of PC in patients may cause adverse events. Patients who experience blood loss due to trauma or surgery should be transfused with more active platelets containing high levels of microparticles. When transfusing PC to cancer patients, activated platelets are undesirable. In this case, it is recommended that a platelet concentrate with a minimum content of microparticles be used. However, the use of this method in clinical practice requires further study.

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USE OF LACTULOSE IN THE COMPOSITION OF BLOOD CELL CRYOPRESERVATIVES

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Introduction. Cryopreservation allows for long-term conservation of biomaterials. The insufficient efficacy of available cryopreservatives and the toxicity of a number of cryocomponents renders the search for low-toxic biocompatible cryoagents highly relevant.

Objective. Assessment of morphological and functional features of blood cells in a lactulose-based cryopreservative for storing whole blood at moderately low temperatures (minus 20 °C) using leukocyte, platelet, and erythrocytes parameters.

Materials and methods. The study was conducted using peripheral venous blood of 30 female donor volunteers aged 18–23 years. Samples of peripheral venous blood were stabilized by 3-substituted potassium salt of ethylenediaminetetraacetic acid. The cryopreservative was prepared using a 0.9 % sodium chloride solution to maintain the isotonic concentration. Glycerin and dimethyl sulfoxide were used as cell-penetrating cryoprotectors; lactulose disaccharide was used as a non-penetrating cryoprotector. The composition of the obtained cryopreservative was optimized by varying the mass fractions of the components. Clinical blood tests were performed using a Gemalite 1270 automatic hematology analyzer. A computer cytomorphometric study was performed in the MEKOS-C2 hardware and software environment.

Results. The conservation of blood samples using the developed cryopreservative for 24 h at a temperature of minus 20 °C increased the percentage of preserved leukocytes, erythrocytes, and platelets to 88.6±0.41 %, 92.1±0.31 %, and 91.4±0.52 %, respectively. The blood cells retained their physiological activity after thawing compared to blood samples stored at room temperature.

Conclusions. The morphological and functional safety of blood cells in samples stored with the developed cryopreservative was revealed after 24 h of storage at minus 20°C. The advantages of this cryopreservative include the possibility of its long-term storage without loss of cryoprotective properties, stabilizing blood cells to the effects of sub-moderate low temperatures of minus 20 °C, the use of non-toxic lactulose disaccharide that does not penetrate into the cell. The developed cryopreservative proves effective in freezing conditions at minus 20 °C, being affordable in terms of cost (all components are manufactured in the Russian Federation). Further research in this direction will contribute to the development of safer blood donation approaches and reducing complications during transfusion of blood components.

Keywords: cryopreservation; cryoprotectors; erythrocytes; leukocytes; platelets; lactulose; morphofunctional properties

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ИСПОЛЬЗОВАНИЕ ЛАКТУЛОЗЫ В СОСТАВЕ КРИОКОНСЕРВАНТА ДЛЯ СОХРАНЕНИЯ КЛЕТОК КРОВИ

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Введение. Криоконсервация позволяет длительно сохранять биоматериал, однако существует ряд проблем, связанных с недостаточной эффективностью криоконсервантов и токсичностью ряда криокомпонентов, в связи с чем актуален поиск низкотоксичных биосовместимых криоагентов.

Цель. Оценка морфофункциональных особенностей форменных элементов крови в криоконсерванте с лактулозой на основании показателей лейкоцитарных, тромбоцитарных и эритроцитарных параметров для хранения цельной крови при умеренно низкой температуре (–20 °С).

Материалы и методы. Исследование проведено на 30 добровольцах-донорах женского пола в возрасте 18–23 лет. Объект исследования — периферическая венозная кровь, стабилизированная 3-замещенной калиевой солью этилендиаминтетрауксусной кислоты. При приготовлении модельного криоконсерванта был использован 0,9 % раствор хлорида натрия для поддержания изотонической концентрации. В качестве криопротекторов, проникающих в клетку, использовали глицерин и диметилсульфоксид, в качестве не проникающего — дисахарид лактулозу. Оптимизация состава криоконсерванта проводилась за счет варьирования массовых долей компонентов. Общий анализ крови выполняли на автоматическом гематологическом анализаторе «Гемалайт 1270». Компьютерное цитоморфометрическое исследование проводили на аппаратно-программном комплексе «МЕКОС-Ц2».

Результаты. В ходе исследования в условиях сохранения образцов крови с применением разработанного криоконсерванта после 24 ч хранения при температуре –20 °С увеличивался процент сохранности лейкоцитов, эритроцитов, тромбоцитов (88,6 ± 0,41, 92,1 ± 0,31, 91,4 ± 0,52% соответственно) с сохранением форменных элементов крови в физиологически активном состоянии после оттаивания по сравнению с образцами крови, сохранявшимися при комнатной температуре.

Выводы. Выявлена морфологическая и функциональная сохранность форменных элементов крови в образцах с применением разработанного криоконсерванта после 24 ч хранения при температуре –20 °С. Преимущества данного криоконсерванта: возможность его длительного хранения без потери криопротекторных свойств, обеспечение криопротектором стабилизации форменных элементов крови к воздействию субумеренно

низкой температуры –20 °С, применение нетоксичного дисахарида лактулозы, не проникающего внутрь клетки. Разработанный криоконсервант является эффективным в условиях замораживания при –20 °С и доступным (все компоненты производятся на территории Российской Федерации). Исследования в данном направлении позволят более эффективно использовать аутодонорство во избежание ряда осложнений при трансфузии компонентов крови.

Ключевые слова: криоконсервация; криопротекторы; эритроциты; лейкоциты; тромбоциты; лактулоза; морфофункциональные свойства

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INTRODUCTION

Preservation of blood cells outside their native environment is a relevant task in practical healthcare. Cryopreservation is indented for long-term storage of biosamples without changes in their cellular structure and functional activity. The use of liquid nitrogen requires bulky and expensive equipment, as well as regular replenishment of expendable materials, which affects the cost of material storage and transportation of samples [1]. Therefore, effective cryopreservatives for the temperature range from –20°C to –80° are in high demand. A distinctive disadvantage of cryopreservation consists in the possible destruction of cell membranes due to insufficient efficiency of existing cryopreservatives [2]. At the same time, cryoagents should not only possess low toxicity [3, 4], but also prevent the loss of phenotypic and functional properties of the biomaterial. Therefore, the search for low-toxic biocompatible cryoagents represents a highly relevant research task [5].

The highest results are achieved using combined cryopreservatives, including endo- and exocellular cryoprotectants [6]. The toxic effect of cryosystems can be reduced by introducing lipids, proteins, carbohydrates [7–10], amino acids [11], and polyatomic spirits [12] as cryoagents of natural origin. Low-molecular carbohydrates are a promising class of cryoprotectors [13]. Disaccharide trehalose has a pronounced protective effect on cell membranes [14]. Mixtures of sugars and polyols are considered as a natural eutectic system [15]. At present, the literature lacks experimental data on effects of a number of carbohydrates, disaccharide lactulose in particular, on cells when used as cryoprotectants. Lactulose is a safe compound for all age groups, including children under one year of age [16]. In a study of lactulose, no data on toxic, teratogenic, and mutagenic effects were obtained in clinical studies involving humans [17]. At the same time, protective properties of lactulose on microorganisms during their freezing were noted [18, 19].

The need for whole blood and its components is constantly increasing [20]. For analytical purposes, fresh whole blood samples are preferred; however, this implies the necessity of rapid analysis after collection and the limited number of repeat tests that can be performed without additional blood collection [21]. Although the experience of cryopreservation in the field of capillary blood with subsequent analysis by cytometry was described in [22], no similar data on cryopreservation of venous blood can be found. Thus, it appears relevant to analyze the morphological and functional parameters of cryopreserved whole venous blood with the purpose of determining the possibility of increasing the storage duration of stabilized blood samples without changes in analytical techniques and blood parameters.

In this study, we evaluate the morphological and functional features of blood cells preserved with a lactulose-based cryopreservative using leukocyte, platelet, and erythrocytes parameters for whole blood storage at moderately low temperatures (–20°C).

MATERIALS AND METHODS

The study was conducted using blood samples from 30 healthy female donors aged 18–23 years. All participants were distributed according to the phase of the menstrual cycle (follicular phase). Inclusion criteria for the study were the absence of chronic diseases in the period of exacerbation, bad habits, and visible signs of allergic or infectious diseases. All female participants signed informed consent to participate in the study. The research object was peripheral venous blood stabilized with 3-substituted potassium salt of ethylenediaminetetraacetic acid (K3 EDTA) in vitro. Venous blood samples in the amount of 15 mL were collected once in the morning from the elbow vein into specialized vacuum tubes for hematological studies with K3 EDTA.

Experimental samples were divided into three groups. The control group included 10 blood samples, in which the characteristics and parameters of blood elements were investigated at a temperature of +20 ± 1.0°C. Experimental group I included 10 blood samples, in which a model cryopreservative was introduced beforehand. These samples were examined after 4 h, at a temperature of +20 ± 1.0°C. Experimental group II included 10 blood samples, into

which the model cryopreservative was introduced, followed by introducing the samples into a state of cold anabiosis at –20 ± 1.0°C for 24 h to evaluate the preservation of samples after a single freeze/thaw cycle. The samples were then unfrozen for a subsequent examination of the characteristics and parameters of blood elements at +20 ± 1.0°C.

When preparing a model cryopreservative, a 0.9% sodium chloride solution was used to maintain isotonic concentration. Glycerol and dimethyl sulfoxide (DMSO) were used as cell-penetrating cryoprotectants, while disaccharide lactulose was used as a non-penetrating cryoprotectant. The cryopreservative composition was optimized by varying the mass fractions of the components. The final composition of the model cryopreservative had the following ratios of components: 20% of glycerol (analytic grade), 10% of DMSO (chemically pure), 2.5% of lactulose (*Lactusan* trade mark according to TU 9229-004-53757476-04), and isotonic (0.9%) sodium chloride solution up to 100%. The prepared solution was autoclaved (without DMSO) at 1.2 atm for 30 min and stored in a refrigerator at 2–4°C. DMSO is not subject to autoclaving due to its oxidizing in air, which leads to its decomposition into compounds that increases the toxicity of the composition. Sterilization of DMSO was carried out by the sterilizing filtration method with the subsequent storage of DMSO in glass sterile tubes at –10°C. Thawing of DMSO was performed in a UT-4334 water bath (ULAB, Russia) at 37 ± 1°C. DMSO was added to the prepared sterile model cryopreservative immediately before freezing the experimental samples.

The model cryopreservative in the amount of 500 μL was added to the samples of experimental groups I and II using volume changer pipettes with the 2:1 ratio of venous blood/cryopreservative by volume. The tubes were sealed with stoppers, and the contents were stirred for 10 min using an orbital shaker (PSU-10i, Latvia). Following 4 h, group I samples were examined at +20 ± 1.0°C. Group II samples were placed in the freezing chamber of an electric freezer with a temperature of –20 ± 1.0°C and kept for 24 h. Subsequently, the samples were thawed in a water bath UT-4334 (ULAB, Russia) in manual mode at a temperature of +37 ± 1°C for 1 min. Further, the characteristics and parameters of blood elements were investigated at +20 ± 1.0°C. A computer cytomorphometric study of blood cells was performed in the MECOS-C2 hardware and software environment (Medical Computer Systems, Russia). *In vitro* diagnostic tests of the total blood count (TBC) were performed using a Hemalight 1270 automatic hematological analyzer (Dixion, Russia).

The obtained results were processed using the IBM SPSS Statistic 23.0 software statistical package (IBM Corp., Armonk, NY, USA). The distribution of the studied parameters was evaluated using the Shapiro–Wilk W-criterion. The level of statistical significance of intergroup differences when the distribution of indicator values conformed to the law of normal distribution was evaluated using Student's t-criterion for unrelated samples. For indicators with non-normal distribution, the nonparametric Mann–Whitney U-criterion was applied. For indicators with normal distribution, the mean (*M*), error of mean (*m*), and standard deviation (δ) were calculated. Intergroup differences were considered statistically significant at $p \leq 0.05$.

RESULTS

We analyzed the leukocyte dynamics in experimental and control groups (at +20 ± 1.0°C in experimental group I with the cryopreservative and at –20 ± 1.0°C in experimental group II with the cryopreservative). The corresponding data are presented in Table 1.

When analyzing leukocytic indices in both experimental groups, compared to the control, a statistically significant decrease in the number of leukocytes was noted. The most pronounced decrease was observed experimental group II, $(3.94 \pm 0.87) \times 10^9$/L ($p < 0.01$), against $(4.55 \pm 0.83) \times 10^9$/L in experimental group I and $(5.60 \pm 0.92) \times 10^9$/L in the control, respectively. Meanwhile, the value of mean cells was significantly increased in both group I and group II compared to the control, indicating a decrease in leukocyte cell recognition by the blood analyzer. Despite significant intergroup differences, this index ranged within physiologic reference values. The percentage of leukocyte preservation in blood samples with the cryopreservative under study (temperature +20 ± 1.0°C) was 81 ± 0.89%, and 88.6 ± 0.41% when exposed to negative temperatures (–20 ± 1.0°C).

Computer morphometry allows mathematical characteristics of the cell population to be obtained, at the same time as providing an opportunity to estimate the activity of intracellular processes [23]. To assess the functional state of cells, we analyzed computerized cytomorphometry of leukocytes in the control group and experimental groups (at +20 ± 1.0°C in experimental group I with the cryopreservative and at –20 ± 1.0°C in experimental group

Table 1. Leukocyte indices of total blood count (TBC) in the study groups

Total blood counts	Control group $n = 10$	Experimental group I $n = 10$	Experimental group II $n = 10$
Leukocytes, $\times 10^9$/L	5.60 ± 0.92	4.55 ± 0.83*	3.94 ± 0.87*
Lymphocytes, %	38.31 ± 4.21	25.70 ± 3.14	25.70 ± 2.17
Granulocytes, %	56.20 ± 5.23	51.10 ± 4.24	51.10 ± 3.21
Percentage of medium-sized cells, %	6.20 ± 1.43	10.20 ± 1.47*◆	9.20 ± 1.23*◆

Table prepared by the authors based on their own data

Note: data are presented as mean value and standard error of the mean (*M* ± *m*);
* — statistically significant difference between control and experimental groups ($p < 0.01$)
◆ — statistically significant difference between experimental groups I and II ($p < 0.01$)

II with the cryopreservative). The corresponding data are presented in Table 2.

An analysis of the computer cytomorphometry data of leukocytes showed that in group II (samples with the cryopreservative frozen at –20 ± 1.0°C), the optical density of cytoplasm significantly increased up to 1.03 ± 0.01 c.u. both in comparison with the control, 0.66 ± 0.01 c.u., and experimental group I, 0.50 ± 0.01 c.u. This might indicate the increased permeability of endocellular cryoagents into the cell.

Erythrocyte parameters were also analyzed in the control group and experimental groups (at +20 ± 1.0°C in experimental group I with the cryopreservative and at –20 ± 1.0°C in experimental group II with the cryopreservative). The corresponding data are presented in Table 3.

When performing the total blood analysis, values indicating changes in quantitative and calculated indices were obtained. Thus, a significant decrease in the level of total blood hemoglobin in both experimental groups (119.25 ± 4.27 g/L and 111.64 ± 4.42 g/L) compared to the control group was observed. In addition, a decrease in the average hemoglobin content in erythrocytes in both experimental groups, compared to the control, was noted. These indicators showed a decreasing trend. This indirectly points to pre-dilution of the studied sample; however, it can be said that the cryopreservative does not lead to critically significant changes in blood components at room temperature. The recorded decrease in both total hemoglobin and its average content in cells indicates the processes of increased transmembrane permeability, while these indicators did not exceed the reference range of physiologically acceptable values in the experimental groups.

The percentage of erythrocyte preservation in blood samples with the cryopreservative applied (temperature +20 ± 1.0°C) was 89 ± 0.2%, and 92.1 ± 0.31% when exposed to negative temperatures (–20 ± 1.0°C). The conducted computer cytomorphometry of erythrocytes in both experimental groups, compared to the control, revealed a significant increase in the cell area in group I (15 ± 1.22 μm^2) and group II (22 ± 1.45 μm^2), compared to 14 ± 2.1 μm^2 in the control. The value of mean cell diameter tends to increase, comprising 6.11 ± 0.91 μm in group I and 9.04 ± 1.34 μm

Table 2. Difference indices of computerized cytomorphometry of leukocytes in the study groups

Parameter	Control group *n* = 10	Experimental group I *n* = 10	Experimental group II *n* = 10
Cell area, μm^2	70.00 ± 4.16	59.00 ± 5.23	60 ± 5.92
Cell form factor, %	14.11 ± 5.12	18.01 ± 3.84	16.7 ± 4.33
Cell polarization index, %	0.16 ± 0.01	0.31 ± 0.01	0.14 ± 0.01
Optical density of cytoplasm, conventional units	0.66 ± 0.01	0.50 ± 0.01*♦	1.03 ± 0.01*♦
Nucleus area, μm^2	52.00 ± 5.32	40.00 ± 4.88*♦	38 ± 4.41*♦
Core form factor, %	14.30 ± 3.41	13.10 ± 2.88	11.4 ± 3.23
Core polarization, %	0.06 ± 0.01	0.02 ± 0.01	0.25 ± 0.01
Nuclear cell ratio, %	0.74 ± 0.01	0.68 ± 0.01	0.63 ± 0.01
Share of core complementation, %	0.04 ± 0.01	0.02 ± 0.01	0.05 ± 0.01

Table prepared by the authors using their ow data

Note: data are presented as mean value and standard error of the mean (*M* ± *m*);
* — statistically significant difference between control and experimental subjects ($p < 0.01$)
♦ — statistically significant difference between the 1st and 2nd experimental groups ($p < 0.01$)

Table 3. Erythrocytic parameters of TBC in the experimental groups

Total blood counts	Control group *n* = 10	Experimental group I *n* = 10	Experimental group II *n* = 10
Erythrocytes, ×10^{12}/L	4.62 ± 0.24	4.08 ± 0.22	3.75 ± 0.41
Hemoglobin, g/L	131 ± 5.34	119.25 ± 4.27*	111.64 ± 4.42*♦
Average erythrocyte volume, fl	86.3 ± 4.36	84.3 ± 4.11	81.7 ± 3.47
Hematocrit, %	39.3 ± 1.55	35.4 ± 1.45	32.2 ± 1.27
Average hemoglobin content in erythrocytes, pg	28.5 ± 0.3	26.2 ± 1.47*	23.7 ± 1.25*♦
Erythrocyte hemoglobin saturation, g/L	336 ± 29.74	311 ± 23.37	305 ± 19.89
Degree of erythrocyte size deviation, %	12.2 ± 1.65	11.7 ± 1.44	10.5 ± 1.47

Table prepared by the authors using their own data

Note: data are presented as mean value and standard error of the mean (*M* ± *m*);
* — statistically significant difference between control and experimental groups ($p < 0.01$)
♦ — statistically significant difference between the 1st and 2nd experimental groups ($p < 0.01$)

Table 4. Difference indices of computerized cytomorphometry of erythrocytes in the study groups

Parameter	Control group *n* = 10	Experimental group I *n* = 10	Experimental group II *n* = 10
Cell area, μm²	14 ± 2.1	15 ± 1.22*	22 ± 1.45*◆
Average cell diameter, μm	6.11 ± 0.91	7.01 ± 1.12	9.04 ± 1.34
Form factor, %	0.16 ± 0.01	0.31 ± 0.01	0.33 ± 0.01
Polarization, %	0.66 ± 0.01	0.50 ± 0.01	0.47 ± 0.01
Integral optical density (red), μm²	52 ± 2.18	40 ± 2.25	38 ± 1.43
Integral optical density (green), μm²	14.3 ± 2.15	13.1 ± 1.49	12.1 ± 1.43
Integral optical density (blue), μm²	0.06 ± 0.01	0.02 ± 0.01	0.02 ± 0.01

Table prepared by the authors using their own data

Note: data are presented as mean value and standard error of the mean (*M* ± *m*);
* — statistically significant difference between control and experimental groups ($p < 0.01$)
◆ — statistically significant difference between the 1st and 2nd experimental groups ($p < 0.01$)

Table 5. Platelet TBC indices in the study groups

Total blood counts	Control group *n* = 10	Experimental group I *n* = 10	Experimental group II *n* = 10
Thrombocytes, ×10⁹/L	230.08 ± 6.31	198.60 ± 5.36*	181.5 ± 5.71*◆
Average platelet volume, fl	8.24 ± 1.31	7.56 ± 1.47	6.13 ± 1.54
Thrombocrit, %	2.23 ± 0.65	2.10 ± 0.74	2.03 ± 0.14
Large thrombocyte ratio, %	17.38 ± 3.31	21.24 ± 2.36	20.12 ± 3.51

Table prepared by the authors using their own data

Note: data are presented as mean value and standard error of the mean (*M* ± *m*);
* — statistically significant difference between control and experimental groups ($p < 0.01$)
◆ — statistically significant difference between the 1st and 2nd experimental groups ($p < 0.01$)

in group II, compared to 7.01 ± 1.12 μm in the control. The increase in both the area and diameter of erythrocytes indicates a possible increase in the permeability of the applied cryoagent inside the cell. The corresponding data are presented in Table 4.

The conducted comparative analysis of platelet indices found the number of platelets to decrease significantly. Thus, in groups I and II, this value was (198.60 ± 5.36)×10⁹/L and (181.5 ± 5.71)×10⁹/L, respectively, compared to (230.08 ± 6.31)×10⁹/L in the control. The decrease in the number of cells was most likely caused by the increase in the volume of the studied sample against the background of cryopreservative application. At the same time, the coefficient of large platelets in both experimental groups in comparison with the control group showed an increasing trend (see Table 5).

The percentage of platelet preservation in blood samples with the cryopreservative applied (temperature +20 ± 1.0°C) was 86.2 ± 0.31% and 91.4 ± 0.52% when exposed to negative temperatures (–20 ± 1.0°C). Presumably, the cryopreservative activates platelets to some extent, which is accompanied by their increase. However, all the studied parameters were within the range of acceptable physiological values. Therefore, we observed a reaction of platelet adaptation to the impact of a foreign component, i.e., cryopreservative.

The computer cytomorphometry of platelets in the experimental groups found a statistically significant increase in the cell area compared to the control. This parameter was more pronounced in the samples of group I (with the cryopreservative applied) at the level of 11.85 ± 1.15 μm², compared to 9.12 ± 1.12 μm² in group II (with the cryopreservative applied and freezing). It should be noted that despite significant intergroup differences, this index reflects the platelet area activity with subsequent adaptation after freezing. The marked changes were within the range of physiologic reference values. The corresponding data are presented in Table 6.

DISCUSSION

The conducted comparative analysis of TBC indices in the experimental groups with the cryopreservative (regardless of temperature conditions of blood sample preservation) established a reliable decrease in the total number of leukocytes. This finding might be related to changes in the ratio of liquid part of blood and blood elements under the conditions of cryopreservative application, as well as to the decrease in leukocyte cell recognition by the blood analyzer. At the same time, a reliable increase in the percentage of medium cells in samples with the preservation temperature of –20 ± 1.0°C was observed. It might be connected with changes in the leukocyte morphology, indicated by changes in the optical density of leukocyte cytoplasm revealed by computer cytomorphometry. These changes may also be associated with the action of the cryopreservative;

Table 6. Computerized cytomorphometry of platelets in the study groups

Parameter	Control group $n = 10$	Experimental group I $n = 10$	Experimental group II $n = 10$
Cell area, μm^2	7.70 ± 1.23	11.85 ± 1.15*	9.12 ± 1.12*♦
Min. diameter, μm	2.65 ± 0.15	2.83 ± 0.54	2.72 ± 0.71
Max. diameter, μm	4.03 ± 0.43	4.94 ± 0.33	4.81 ± 0.25
Average diameter, μm	3.44 ± 0.27	3.92 ± 0.78	3.88 ± 0.31
Form factor, %	12.90 ± 2.10	14.87 ± 2.88	13.10 ± 1.97

Table prepared by the authors using their own data

Note: data are presented as mean value and standard error of the mean ($M \pm m$);
* — statistically significant difference between control and experimental groups ($p < 0.01$)
♦ — statistically significant difference between the 1st and 2nd experimental groups ($p < 0.01$)

however, the samples subjected to freezing showed the stability of the indicators.

The comparison of erythrocyte indices in the groups with cryopreservative application, both under positive temperatures and during freezing of samples, revealed a significant decrease in the total blood hemoglobin and average hemoglobin content in erythrocytes. However, statistically significant changes in erythrocyte concentration were not revealed, which indicates the effectiveness of the applied cryoprotectant. At the same time, according to the computer cytomorphometry data, the increase in the erythrocyte cell area and the increasing trend of the average cell diameter and erythrocyte shape factor in the samples with cryopreservation and subsequent freezing, confirms the transmembrane penetration of cryoagents inside the cells.

The dynamics of platelet indices showed the platelet concentration to statistically significantly decrease in cryopreserved blood (+20 ± 1.0°C), which is associated with changes in the ratio of cells to the liquid part of the blood. A more pronounced decrease in this index was noted in the samples preserved under negative temperatures, compared to group I (with the cryopreservative at a temperature of +20 ± 1.0°C), while its changes did not exceed the limits of physiological norm. Thus, this blood preservation method can be used in the formation of a cryopreserved bank of platelet hemoconcentrate. The conducted computer cytomorphometry of platelet cells demonstrated a statistically significant increase in cell area. This increase might be explained by the fact that platelets trigger activation processes in response to contact with a foreign agent in the form of cryopreservative. At the same time, in blood samples of group II, also with the cryopreservative but during freezing, we registered a less pronounced increase in the platelet area. This can be explained by the onset of adaptation processes to the cryopreservative when exposed to negative temperatures; however, this observation requires further elucidation.

During preservation of blood samples with the developed cryopreservative for 24 h of storage at –20°C, the percentage of preserved blood cells, i.e., leukocytes, erythrocytes, and platelets comprised 88.6 ± 0.41%, 92.1 ± 0.31%, and 91.4 ± 0.52%, respectively. Importantly, the blood cells maintained their physiological activity after thawing, compared to the blood samples preserved at room temperature. Similar experiments conducted by Kiryanova et al. using a Cryosin solution as a cryopreservative showed the percentage of preserved erythrocytes in the amount of 83.8 ± 4.09% [24]. Works by Isaeva et al. aimed at assessing the viability of nucleus-containing cells in leukoconcentrates at the stages of their obtaining and freezing achieved the level of 86.7% cells in a viable state [25]. The research team of Vetoshkin et al. conducted studies by freezing of donor blood platelets using a combined cryopreservative and obtained the preservation of their functional activity at a level of 63.5–88.8% [26]. Whole blood cells after daily storage at –40°C were also studied, with the morphological and functional preservation of cells amounting to 85.3 ± 0.30% for erythrocytes, 75 ± 0.71% for platelets, and 90.1 ± 0.91% for leukocytes compared to the values registered before freezing [27].

Shirokikh et al. studied the effect of polysaccharide fractions on cryopreserved human venous blood and determined a decrease in the osmolarity of human blood from 281 to 149 mOsm/L. This result was attributed to the interaction of functional groups of polysaccharides with osmotically active substances of blood plasma, leading to a decrease in the osmolarity of the medium and acceleration of water crystallization [28]. Lactulose (as well as other disaccharides) belongs to non-penetrating cryoprotectants. Such compositions create osmotic pressure, which causes dehydration of cells and reduces the degree of ice formation inside cells. The cryo-effect of lactulose is apparently similar to that of trehalose disaccharide, which acts as an inhibitor of ice crystal growth during freezing and recrystallization of ice during thawing, forming a highly viscous glass-like state [29]. Nevertheless, the cryoprotective effect of lactulose has not yet been fully determined, requiring further investigation.

The main results of our study are protected by the patent for invention [30]. The advantages of using the developed cryoprotectant include the possibility of long-term storage without loss of cryoprotective properties and stabilization of blood cell elements under sub-zero temperatures of –20°C. The preparation of cryoprotectant does not require bulky and expensive equipment, as well as toxic components. Disaccharide lactulose is non-toxic compound, not penetrating inside the cell.

CONCLUSION

Our studies have confirmed the possibility of successful morphological and functional preservation of blood cells with the proposed cryopreservative after 24 h of storage

at –20°C. The developed cryopreservative is affordable (all components are produced in the Russian Federation) and effective for freezing blood samples at –20°C. This extends the range of current cryopreservatives applied for analysis of morphological and functional parameters of frozen whole blood samples in large-scale studies and field medicine, for storage of biomaterial in long-term expeditions and remote areas. The works carried out in this direction contribute to the development of effective blood donation approaches, allowing complications in transfusion of blood components to be mitigated.

In our opinion, lactulose deserves further study as a cryocomponent for ensuring the safety of blood cells during long-term storage periods.

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