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МЕДИЦИНА ЭКСТРЕМАЛЬНЫХ СИТУАЦИЙ

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Содержание

CALCULATION OF REFERENCE INTERVALS OF BLOOD PARAMETERS IN CHILDREN AND ADOLESCENTS: PROJECTS REVIEW

Grishina ZhV ✉, Klyuchnikov SO, Feshchenko VS, Zholinskiy AV

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The review of the currently existing projects focused on calculating the reference intervals of blood parameters in large samples of children of different gender and age discusses the urgent issues of calculating pediatric reference intervals of biochemical markers, the paper provides comparison of the reference intervals established within the framework of different projects. The limitations, future prospects and harmonization of pediatric reference intervals, including for juvenile athletes, are provided.

Keywords: reference intervals, blood parameters, pediatrics, children, juvenile athletes, sports medicine

Funding: the study was performed as part of the applied research project "Developing the Guidelines on Increasing the Efficiency of the Measures for Medical and Biological Support of Juvenile Athletes of Russian National Teams" (code "Deti-22"), the Federal Research and Clinical Center of Sports Medicine and Rehabilitation of the Federal Medical Biological Agency State Assignment №107.002.22.14 of 11.07.2022.

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РАСЧЕТ РЕФЕРЕНТНЫХ ИНТЕРВАЛОВ ДЛЯ ПОКАЗАТЕЛЕЙ КРОВИ У ДЕТЕЙ И ПОДРОСТКОВ: ОБЗОР ПРОЕКТОВ

Ж. В. Гришина ✉, С. О. Ключников, В. С. Фещенко, А. В. Жолинский

Федеральный научно-клинический центр спортивной медицины и реабилитации Федерального медико-биологического агентства, Москва, Россия

В обзоре существующих на сегодняшний день проектов по расчету референтных интервалов показателей крови на больших выборках детей разного пола и возраста обсуждены актуальные вопросы расчета педиатрических референтных интервалов для биохимических маркеров, проведено сравнение значений референтных интервалов, полученных в разных проектах. Представлены ограничения, будущие перспективы и гармонизация педиатрических референтных интервалов, в том числе для несовершеннолетних спортсменов.

Ключевые слова: референтные интервалы, показатели крови, педиатрия, дети, несовершеннолетние спортсмены, спортивная медицина

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The child's organism is distinguished from the adult's organism not only by physical condition, but also by the organs maturity, features of metabolism, immune and endocrine responsiveness, etc. The dynamics of physiological processes that take place in the child's organism are accompanied by changes in the concentrations of many blood biomarkers, including hormones [1].

In fact, objective assessment and subsequent interpretation of the results of laboratory and instrumental tests considering certain age periods of the child are often complex and not always unambiguous. This negatively affects selection of the tactics for management of children and organization of treatment and preventive care [2, 3]. The reference intervals, being a fundamental medical instrument allowing one to correctly interpret blood test results and distinguish between normal physiological changes and the onset of the disease process in the child's organism, can help to solve this problem [2].

In terms of statistics, reference interval (RI) represents the limits of the range that includes the percentage (usually 95%) of values obtained from the healthy population. The reference limits are determined by calculating the 2.5th and 97.5th percentiles of the test results [2, 4, 5]. Hence, 5% of the results may be interpreted as abnormal.

According to the regulation, statistical methods for calculation of reference intervals are selected based on the distribution of reference values: parametric methods are used for the normally distributed data, while nonparametric methods are used in case there is no null hypothesis of the dataset distribution. Calculation of the 95% confidence interval is possible when the distribution of reference values is normal; conversely, nonparametric methods, specifically the rank test, are used when calculating reference intervals for the samples characterized by non-normal distribution of values obtained from the reference groups of healthy subjects [5].

When using the conventional "direct" approach, RI is usually defined as an interval denoted by two reference limits (2.5th and 97.5th percentiles) obtained from the sample represented by reference population [4, 5].

Currently, indirect methods of RI calculation that involve analysis of the laboratory parameter datasets have been widely implemented. The complex statistical algorithms that

Table. Projects focused on calculating the reference intervals of blood parameters in the populations of healthy children and adolescents

Project	Country	Age groups	Gender	Statistical methods, calculated parameters	Assessed biomarkers	References
AABC	Australia and New Zealand	All	M, F	Direct method, RI	Enzymes and ions	12, 13
Caliper	Canada	Under 18	M, F	Direct method, RI	Common blood analytes, endocrine markers, tumor markers, vitamins, biomarkers of metabolic disorders	8, 10, 14, 15–22
CHILDx	USA	0.5–17 years	M, F	Direct method, RI	Enzymes, hormones, vitamins, bone turnover markers, coagulation indicators	23–28
COPENHAGEN	Denmark	5–20 years	M, F	Direct method, RI	Common blood analytes	29
KiGGS	Germany	Under 18	M, F	Direct method, RI, median	Biochemical markers, immunological markers, thyroid hormones, noncommunicable disease markers	30–34
LOOK	Australia	8, 10, 12 years	M, F	Direct method, RI, median	Cardiac markers, common blood analytes	35, 36
NHANES	USA	all	M, F	Indirect method, 2,5; 25; 50; 75; 97.5 percentiles	Lipid profile, immunological and hematological markers, vitamins, inflammatory markers	37–44
NORIP	Scandinavian countries	Under 18	M, F	Direct method, RI	Tumor markers, common blood analytes	45–48
Referent-20	Russia	Professional athletes aged 14–17	M, F	Indirect method, RI; 5; 10; 25; 50; 75; 90; 95 percentiles	Common blood analytes, metabolic markers	11, 49, 50

Note: AACB — Australasian Association of Clinical Biochemists; CALIPER — Canadian Laboratory Initiative on Paediatric Reference Intervals; CHILDx —Children's Health Improvement through Laboratory Diagnostics; COPENHAGEN — The Copenhagen Puberty Study; KiGGS — German Health Interview and Examination Survey for Children and Adolescents; LOOK — Lifestyle of Our Kids; NHANES — National Health and Nutrition Examination Survey; NORIP —Nordic Reference Interval Project.

are essential for development and implementation of exclusion criteria for unhealthy subjects have become the main problem that restricts the use of indirect methods.

It should be noted that it is rather difficult to calculate and verify RIs. In 2008, specialists of the Clinical and Laboratory Standards Institute (CLSI) and International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) prepared the guidelines (C28-A3) on the RI calculation and verification [6]. The accurately determined RIs are crucial for correct diagnosis and selection of the treatment method, while the general population-based RIs of blood parameters that have not been adapted for children may result in erroneous diagnosis, inadequate treatment, higher health expenditures, etc.

Analysis of the literature data on pediatric RIs has revealed the main directions of contemporary research focused on calculating RIs of blood parameters in children: bone markers [7], markers of cardiovascular disorders and the risk of metabolic syndrome [8], thyroid hormones and growth hormone [9], inborn features of metabolism [10].

In foreign countries, the results of several large-scale projects focused on calculating RIs of blood parameters in multiple samples of healthy children have been published over the recent years (Table). Unfortunately, there are still no such projects in our country. An exception is the project involving calculation of RIs of blood parameters in juvenile athletes that was implemented at the Federal Research and Clinical Center of Sports Medicine and Rehabilitation of FMBA in 2020. It is comparable with the abovementioned foreign projects in terms of the sample size [11].

KiGGS project

The KiGGS project launched at the Robert Koch Institute (RKI) in Germany has become one of the largest projects focused on calculating RIs of blood parameters in children and adolescents in Europe. RIs of multiple laboratory parameters of blood serum and urine were defined withing the framework of the KiGGS project using the sample of healthy children [30–34]. The sample consisted of 17,641 blood and urine samples obtained from children aged 0–17. A total of 43 blood parameters were assessed that were divided into three major categories based on the parameter association with nutrition, the risk of noncommunicable diseases, and immune status. The median values and RIs were calculated for such indicators, as total cholesterol, low density lipoproteins (LDL), high density lipoproteins (HDL), triglycerides, calcidiol. The median values, 25th and 75th percentiles were defined for thyroid hormones in 12,756 subjects over the age of three. KiGGS was also focused on studying the biomarker interplay. For example, a positive correlation between the thyroid-stimulating hormone and the concentrations of the lipid metabolism indicators, except HDL, was revealed.

The important objective of the KiGGS project was to enroll a large number of subjects (to obtain a representative sample) to be able to divide them into age groups for differential assessment with sufficient statistical significance. The fact that the sample included children and adolescents of different ethnic groups living in Germany was a challenge, since such a large population of these individuals could skew the results of the blood parameter RI calculation for the European population. After the sample was formed, the share of children and adolescents without German citizenship in the sample was 8.4%, which was insignificant. The authors plan to resume the project and calculate RIs of blood parameters in children stratified by gender, age, German federal state, migrant status, etc.

NORIP project

The Scandinavian NORIP project launched as early as in 1998 was the other project focused on calculating pediatric

reference standards [45–48]. The common inclusion criteria for the sample used to calculate RIs of blood parameters was healthy individuals under the age of 18. Blood serum, plasma and whole blood samples were obtained from 3036 healthy children in Denmark, Finland, Iceland, Norway, and Sweden. RIs (central 95% range) were calculated for 25 blood serum indicators, including enzymes. The sample was stratified by age and gender.

COPENHAGEN project

In the COPENHAGEN project (2006–2008), the researchers assessed 21 blood parameters using 1421 blood samples obtained from healthy children (596 boys and 825 girls) aged 5–20 [29]. Nonparametric statistical methods were used to calculate the 95% RIs. The RIs were obtained for both genders and six age groups. Furthermore, RIs of the oldest group of children, who took part in the COPENHAGEN project, were compared with the results of the youngest group of the NORIP project. Many RI values calculated in these projects were similar. Some differences in such parameters, as alkaline phosphatase, lactate dehydrogenase, and creatinine levels were observed. These differences are quite natural, since the listed levels increase or decrease with age as the child grows. The feature of the COPENHAGEN project was that the sample of children included healthy school students with no complaints or deviations in health status, while in other projects blood was collected from children who were on outpatient treatment. Moreover, RIs calculated in this project were compared taking into account the measurement method.

CHILDx project

The CHILDx project had been implemented in the USA since 2002, in which pediatric RIs were calculated in the cohort of healthy children 6 months to 17 years of age [23–28]. RIs for a broad range of blood parameters were defined: vitamins, enzymes, hormones, coagulation parameters, and bone tissue markers. In 2005, blood samples of 902 healthy children and adolescents aged 7–17 were used to determine RIs of the coagulation parameters (prothrombin time; partial thromboplastin time; factors VIII, IX and XI; von Willebrand factor) within the framework of the CHILDx project. Eventually, several significant differences between the pediatric and adult RIs of coagulation blood parameters were found, which once again confirmed the need to calculate RIs adapted for children. For example, the median prothrombin time in children was 14.0 s, which was almost 1 s more compared to the median value obtained for adults (13.2 s).

The other CHILDx study conducted in 2011 was focused on calculating RIs of such blood parameters, as enzyme, prealbumin, and uric acid levels using blood serum samples of 1765 healthy children and adolescents [25]. The sample included different age groups. The mean values, median, and significant differences between age and gender groups were calculated (gender differences were defined for about 35% of parmeters). For example, gender differences at the age of 6–8, 12–14, and 15–17 were determined for aldolase levels. Only the levels of amylase enzyme showed no significant gender differences in any of the studied age group. At the same time, there were differences in the levels of ceruloplasmin and uric acid between groups of children aged 12–14 and 15–17. Significant gender differences in the creatine kinase levels were revealed in all age groups, except for the group aged 6–8.

NHANES project

The NHANES project that was also executed in the USA involved studying the impact of age, gender, body mass index, socioeconomic background, and ethnicity on various health parameters, including blood parameters. For that the data of laboratory studies and questionnaire surveys were acquired, and thousands of new participants were endolled every year [37–44]. For example, the study published in 2000 considered the upper 95th percentile of the C-reactive protein (CRP) concentration for the sample of more than 22,000 healthy children and adults taking into account their age, gender, and ethicity [43]. Women usually had higher CRP levels than men. CRP levels were also higher in older people than in children. Furthermore, in 2004 RIs of blood parameters were calculated using the sample of 25,000 healthy people aged 10–75, stratified by age, gender, and ethnicity, as part of this project [41]. Moreover, RIs of the levels of vitamins and lipid metabolism were determined for various age groups.

LOOK project

The large-scale Lifestyle of Our Kids (LOOK) study conducted in Australia was focused on assessing the impact of physical activity on the health of 3528 healthy children and adolescents. In of the most significant studies conducted as part of the project, the central 95% RIs and median values of 37 blood parameters were calculated using the sample of 852 healthy children stratified by gender and age [36]. Blood was collected from the same children at the age of 8, 10, and 12. The following important patterns were revealed:

– the alkaline phosphatase (AP) activity was higher in girls than in boys aged 8 and 10, however, in boys it became higher by the age of 12;

– the creatine kinase activity was higher in boys than in girls in all age groups;

– the cholesterol levels were higher in girls than in boys at the age of 10 and 12, while HDL levels were higher in boys in all age groups. The concentration of triglycerides was higher in girls in all age groups;

– the urate levels were significantly higher in boys at the age of 12;

– the ferritin levels were higher in boys than in girls at the age of 12 (the differences can be explained by the fact that 50 girls out of 256 menstruated at the time of blood collection);

– the glucose concentrations in boys and girls were almost the same, these progressively increased with age in children of both genders. The insulin levels were higher in girls than in boys and progressively increased with age.

In the LOOK study conducted in 2012, the data of 854 children were used to calculate the RIs of the blood NT-proBNP (N-terminal pro-brain natriuretic peptide B-type) levels [36]. The median, confidence intervals, and 95% RI were calculated based on gender for three age groups (8, 10, and 12 years). It was shown that the NT-proBNP concentration decreased between the age of 8–12 in healthy children.

AACB project

In the project on harmonizing the common RIs calculated using blood samples of healthy people at the hospitals of Australia and New Zealand (AACB), 123 laboratories presented RIs used by each laboratory and new test results stratified by gender in 2014 [12, 13]. These data were used to reveal the differences between RIs and analytical techniques applied in

the laboratories. Linear regression was used to compare the results of calculating the upper and lower RI limits. The AACB team defined the position of the values obtained for the new sample relative to the RI of each laboratory and its position relative to the upper and lower RI limits. Then the results of different laboratories were compared with each other. The project provided valuable information about the RIs of blood parameters used in Australia and New Zealand.

CALIPER project

The Canadian Laboratory Initiative on Paediatric Reference Intervals (CALIPER) is one of the large-scale projects focused on calculating pediatric RIs of blood parameters and aimed at building a database of pediatric RIs to be used in pediatric centers of the country and all over the world [8, 10, 14, 15–22]. In this prospective study involving thousands of healthy children and adolescents, RIs stratified by gender and age were calculated for many routinely assessed and specific biochemical markers. During the initial phase, the CALIPER project included 2809 blood plasma and serum samples obtained from apparently healthy children with stable metabolism who attended outpatient clinics. More than 50 blood parameters were assessed by biochemical methods and enzyme-linked immunoassay. Preliminary RIs were developed based on the data obtained in accordance with CLSI and the IFCC C28-A3 guidelines.

Initially, RIs were determined for five age groups stratified by gender. This phase provided the basis for further projects within the framework of CALIPER. However, the CLSI/IFCC C28-A3 guidelines stipulate that the sample for the RI determination must consist of at least 120 healthy people per parameter. The CALIPER initial pilot studies involved apparently healthy children who attended outpatient clinics and had some disease that could affect their blood parameters. Furthermore, the sample size was not enough for each parameter. Later the researchers improved the sites of blood collection: along with clinics, blood was collected in community centers, nursery schools, churches, and schools. The first of these studies was focused on determining RIs by age group for more than 40 most often assessed blood parameters. This phase of the project that represented the first of multiple CALIPER studies made it possible to start filling the gaps in pediatric RIs of blood parameters, including bone tissue markers, cardiovascular risk markers, and metabolic markers [8].

The study results showed that RIs of many blood parameters varied between the age ranges. However, the age ranges are not necessarily correlated to the generally accepted age-related development staged. For example, RI calculation has shown that it is necessary to divide the sample into seven age ranges for AP (0 to 14 days, 15 days to < 1 year, 1 year to < 10 years, 10 to < 13 years, 13 to < 15 years, 15 to < 17 years, and 17 to < 19 years), while three age ranges are enough for the alanine aminotransferase (ALT) (0 to < 1 year, 1 year to < 13 years, 13 to < 19 years) [8].

As for endocrine markers, a sample of healthy children was formed in 2013 within the framework of the CALIPER project in order to calculate RIs of seven reproductive hormones. In this study, RIs of reproductive hormones specific for certain Tanner stage (estradiol, testosterone, progesterone, sex hormone binding globulin, prolactin, follicle stimulating hormone, and luteinizing hormone) were determined. The Tanner stages are used to monitor the child's pubertal development. It is extremely important to have RIs of the hormones specific for Tanner stages, since all children enter puberty at different age. The Tanner stages are a five-item scale, where stage I corresponds to prepubertal stage, and stage V corresponds to postpuberty. The Tanner stage was defined by a subjective method: the study participants viewed the images of the Tanner stages I to V and assessed their own development relative to these images [14].

After determining the RIs of reproductive hormones, pediatric RIs of other biochemical markers, age-specific RIs of steroid hormones, and RIs of vitamins A, E [15], and D [16] were defined. Along with these large studies performed as part of the CALIPER project, some smaller but practically relevant studies were conducted. These were aimed to analyze the effects of freezing conditions on the samples and analyte stability, daily fluctuations of marker concentration in blood, and the effects of fasting on the concentrations of some biomarkers [17–19].

The age-specific and gender-specific RIs of tumor markers, biomarkers of metabolic disorders, testosterone indices, and specific biochemical markers were determined to further expand the CALIPER database. To date, the CALIPER has built a reliable comprehensive database of pediatric RI of more than 100 blood parameters considering age and gender [20–22].

The reviewed projects aimed at calculating RIs of blood parameters in children and adolescents explore a wide variety of blood parameter concentrations depending on age and gender. However, the impact of additional parameters, such as ethnicity, body mass index (BMI), amount of physical activity, etc., on the parameter concentrations is still poorly understood.

Childhood obesity in one more urgent issue related to calculating RIs. It is necessary to consider how the blood concentrations of substances change with BMI [51]. The reference population should consist of subjects that are representative of local population, however, some factors, such as BMI, change constantly with time. This makes it more difficult to obtain a representative sample. Blood parameters that depend on BMI can change with the increase in the average BMI of the sample. Therefore, it's important to know which blood parameters are affected by BMI and whether such changes are physiological or have some clinical significance (for example, as indicators of the metabolic syndrome subclinical progression) [51].

Referent-20 project

Among projects focused on calculating pediatric RIs of blood parameters, the Referent-20 project executed in 2020 at the Federal Research and Clinical Center of Sports Medicine and Rehabilitation of FMBA of Russia should be noted. The sample of thousands of professional juvenile athletes (2986 boys and 2181 girls), formed based on the results of in-depth medical examination of the Russian national team members that was performed in 2015–2019 in the clinic of the Federal Research and Clinical Center of Sports Medicine and Rehabilitation, was studied [11, 49, 50]. RIs of blood markers typical for certain components of metabolism and common blood analytes (a total of 26 parameters) were calculated within the framework of the project.

The athletes were divided into groups based on gender, age (14–15 and 16–17 years), and sports specialization: cyclic sports (the “endurance” group (distance runners) and the “speed + endurance” group (sprinters), speed-sthrength sports (technical types of track and field), complex coordination sports, team sports, and martial arts. When calculating RIs of the assessed blood parameters, the results of althletes not admitted to training based on the results of in-depth medical examination due to functional capabilities of the body and health status were excluded [11, 49].

After the sample was formed considering all exclusion criteria, the distribution of data was tested, outliers were excluded, and RIs of biochemical markers assessed during the in-depth medical examination were calculated. Considering the non-normal distribution of the data on multiple blood parameters, the nonparametric percentile-based statistics was used to determine appropriate RIs [11, 49].

Comparison of RIs of some blood parameters, for example, exercise tolerance markers, calculated for the sample of juvenile athletes, with RIs of the same parameters determined within the framework of foreign pediatric projects has made it possible to conclude that there are differences in both reference ranges and their minimum and maximum values [11]. For example, comparison of RIs of blood creatinine calculated for the sample of juvenile athletes (sports RI) with RIs calculated within the framework of the Caliper/Norip prject showed that in the sample of male athletes aged 14–15 the maximum values of RIs of this parameter were 30% higher than RIs in the Caliper/Norip boys, while in boys aged 16–17 these values were almost the same. In the sample of female athletes aged 14–15 the maximum values of RIs of creatinine were 13% higher, and in girls aged 16–17 these were 16% higher than RIs obtained in the Caliper/Norip projects. In sports, blood levels of creatinine are also used as an exercise tolerance marker. The increase in maximum values of RIs of creatinine in juvenile athletes compared to ordinary adolescents may be due to significant physical exertion and increased intake of protein foods [11].

This project has also shown that the minimum and maximum values of RI of cortisol calculated for the sample of juvenile athletes are 58–67% higher than in untrained children. Moreover, the calculated RIs of cortisol in boys and girls of the same age, who are involved in sports, are similar. When comparing RIs of cortisol calculated in the Referent-20 project and the Caliper/Norip projects, the following can be noted: boys aged 14–15, who are involved in sports, have maximum values of RIs of cortisol that are 64.7% higher compared to maximum values of RIs of cortisol in boys involved in the Caliper/Norip projects, while in boys aged 16–17 the maximum values of RIs of cortisol are 67.2% higher. Girls aged 14–15, who are involved in sports, have maximum values of RIs of cortisol that are 64.8% higher compared to maximum values of RIs of cortisol in girls involved in the Caliper/Norip projects, while in girls aged 16–17 the maximum values of RIs of cortisol are 66.7% higher. The finding of higher maximum values of RIs of cortisol in juvenile athletes may be due to stress they experience when engaged in professional sports [11].

CONCLUSION

Thus, the review of world's and domestic literature shows that successful attempts to determine RIs of blood parameters in the population of children and adolescents have been done in recent years. However, the question remains about calculating pediatric RIs of blood parameters in specific cohorts of children, for example, athletes, children of various ethnic groups or children with different BMI. Further research focused on determining RIs of blood markers in juvenile athletes based not only on gender and age, but also on the features of sports load, professional experience, and athletic performance, is required. The data obtained in these studies will help the sports medicine physicians to schedule and perform interventions aimed at optimizing the functional state in a timely manner.

References

1. Dyomin VF, Klyuchnikov SO, Balykovoj LA, Samojlova AS, redaktory. Avtorskie lekcii po pediatrii. Detskaya sportivnaya medicina. 2017; 10: 81–99. Russian.
2. Tahmasebi H, Higgins V, Fung A, Truong D, White-Al Habeeb N, Adeli K. Pediatric reference intervals for biochemical markers: gaps and challenges, recent national initiatives and future perspectives. 2017; 28 (1): 43–63.
3. Dudnikova EhV, redaktor. Fiziologicheskie konstanty u detej: ucheb.-metodicheskoe posobie. Rostov-n/D: Izd-vo RostGMU, 2016; 46 s. Russian.
4. Evgina SA, Savelev LI. Sovremennye teoriya i praktika referentnyx intervalov. Laboratornaya sluzhba. 2019; 8 (2): 36–44. Russian.
5. GOST R 53022.3-2008 «Texnologii laboratornye klinicheskie. Trebovaniya k kachestvu klinicheskix laboratornyx issledovanij. Ch. 3. Pravila ocenki klinicheskoj informativnosti laboratornyx testov». M.: Standartinform, 2009; 22 c.
6. Boyd JC. Clinical and Laboratory Standards Institute (CLSI). Defining, establishing, and verifying reference intervals in the clinical laboratory; Approved guideline, CLSI document C28-A3. 2010; 28 (3).
7. Yang L, Grey V. Pediatric reference intervals for bone markers. Clin Biochem. 2006; 39: 561–8.
8. Colantonio DA, Kyriakopoulou L, Chan MK, Daly CH, Brinc D, Venner AA, et al. Closing the gaps in pediatric laboratory reference intervals: a CALIPER database of 40 biochemical markers in a healthy and multiethnic population of children. Clin Chem. 2012; 58: 854–68.
9. Delvin EE, Laxmi Grey V, Vergee Z, CALIPER Working Group. Gap analysis of pediatric reference intervals related to thyroid hormones and the growth hormone-insulin growth factor axis. Clin Biochem. 2006; 39: 588–94.
10. Konforte D, Shea JL, Kyriakopoulou L, Colantonio D, Cohen AH, Shaw J, et al. Complex biological pattern of fertility hormones in children and adolescents: a study of healthy children from the CALIPER cohort and establishment of pediatric reference intervals. Clin Chem. 2013; 59: 1215–27.
11. Grishina ZhV, Klyuchnikov SO, Yashin TA, Makarova GA, Lomazova EV, Bushueva IE, i dr. Referentnye intervaly bioximicheskix pokazatelej krovi u yunyx sportsmenov. Voprosy prakticheskoj pediatrii. 2022; 17 (1): 71–78. Russian.
12. Tate JR, Sikaris KA, Jones GR, Yen T, Koerbin G, Ryan J, et al. Harmonising adult and paediatric reference intervals in australia and new zealand: an evidence-based approach for establishing a first panel of chemistry analytes. Clin Biochem Rev. 2014; 35: 213–35.
13. Jones GR, Koetsier SD. RCPAQAP First Combined Measurement and Reference Interval Survey. Clin Biochem Rev. 2014; 35: 243–50.
14. Bailey D, Colantonio D, Kyriakopoulou L, Cohen AH, Chan MK, Armbruster D, et al. Marked biological variance in endocrine and biochemical markers in childhood: establishment of pediatric reference intervals using healthy community children from the CALIPER cohort. Clin Chem. 2013; 59: 1393–405.
15. Raizman JE, Cohen AH, Teodoro-Morrison T, Wan B, Khun-Chen M, Wilkenson C, et al. Pediatric reference value distributions for vitamins A and E in the CALIPER cohort and establishment of age-stratified reference intervals. Clin Biochem. 2014; 47: 812–5.
16. Yazdanpanah M, Bailey D, Walsh W, Wan B, Adeli K. Analytical measurement of serum 25-OH-vitamin D(3), 25-OH-vitamin D(2) and their C3-epimers by LC-MS/MS in infant and pediatric specimens. Clin Biochem. 2013; 46: 1264–71.
17. Brinc D, Chan MK, Venner AA, Pasic MD, Colantonio D, Kyriakopolou L, et al. Long-term stability of biochemical markers in pediatric serum specimens stored at –80 degrees C: a CALIPER

Substudy. Clin Biochem. 2012; 45: 816–26.
18. Bailey D, Bevilacqua V, Colantonio DA, Pasic MD, Perumal N, Chan MK, et al. Pediatric within-day biological variation and quality specifications for 38 biochemical markers in the CALIPER cohort. Clin Chem. 2014; 60: 518–29.
19. Pasic MD, Colantonio DA, Chan MK, Venner AA, Brinc D, Adeli K. Influence of fasting and sample collection time on 38 biochemical markers in healthy children: a CALIPER substudy. Clin Biochem. 2012; 45: 1125–30.
20. Bevilacqua V, Chan MK, Chen Y, Armbruster D, Schodin B, Adeli K. Pediatric population reference value distributions for cancer biomarkers and covariate-stratified reference intervals in the CALIPER cohort. Clin Chem. 2014; 60: 1532–42.
21. Teodoro-Morrison T, Kyriakopoulou L, Chen YK, Raizman JE, Bevilacqua V, Chan MK, et al. Dynamic biological changes in metabolic disease biomarkers in childhood and adolescence: A CALIPER study of healthy community children. Clin Biochem. 2015; 48: 828–36.
22. Raizman JE, Quinn F, Armbruster DA, Adeli K. Pediatric reference intervals for calculated free testosterone, bioavailable testosterone and free androgen index in the CALIPER cohort. Clin Chem Lab Med. 2015; 53: e239–e243.
23. Flanders MM, Crist RA, Roberts WL, Rodgers GM. Pediatric reference intervals for seven common coagulation assays. Clin Chem. 2005; 51: 1738–42.
24. Kushnir MM, Rockwood AL, Roberts WL, Pattison EG, Owen WE, Bunker AM, et al. Development and performance evaluation of a tandem mass spectrometry assay for 4 adrenal steroids. Clin Chem. 2006; 52: 1559–67.
25. Clifford SM, Bunker AM, Jacobsen JR, Roberts WL. Age and gender specific pediatric reference intervals for aldolase, amylase, ceruloplasmin, creatine kinase, pancreatic amylase, prealbumin, and uric acid. Clin Chim Acta. 2011; 412: 788–90.
26. Johnson-Davis KL, Moore SJ, Owen WE, Cutler JM, Frank EL. A rapid HPLC method used to establish pediatric reference intervals for vitamins A and E. Clin Chim Acta. 2009; 405: 35–38.
27. Meikle AW, Kushnir MM, Rockwood AL, Pattison EG, Terry AH, Sandrock T, et al. Adrenal steroid concentrations in children seven to seventeen years of age. J Pediatr Endocrinol Metab. 2007; 20: 1281–91.
28. Wyness SP, Roberts WL, Straseski JA. Pediatric reference intervals for four serum bone markers using two automated immunoassays. Clin Chim Acta. 2013; 415: 169–72.
29. Hilsted L, Rustad P, Aksglaede L, Sorensen K, Juul A. Recommended Nordic paediatric reference intervals for 21 common biochemical properties. Scand J Clin Lab Invest. 2013; 73: 1–9.
30. Kohse KP. KiGGS — the German survey on children's health as data base for reference intervals and beyond. Clin Biochem 2014; 47: 742–3.
31. Kamtsiuris P, Lange M, Schaffrath RA. The German Health Interview and Examination Survey for Children and Adolescents (KiGGS): sample design, response and nonresponse analysis. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz. 2007; 50: 547–56.
32. Kohse KP, Thamm M. KiGGS-the German survey on children's health as database for reference intervals. Clin Biochem. 2011; 44: 479.
33. Thierfelder W, Dortschy R, Hintzpeter B, Kahl H, Scheidt-Nave C. Biochemical measures in the German Health Interview and Examination Survey for Children and Adolescents (KiGGS). Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz. 2007; 50: 757–70.
34. Witte T, Ittermann T, Thamm M, Riblet NB, Volzke H. Association between serum thyroid-stimulating hormone levels and serum lipids in children and adolescents: a population-based study of german youth. J Clin Endocrinol Metab. 2015; 100: 2090–97.
35. Southcott EK, Kerrigan JL, Potter JM, Telford RD, Waring P, Reynolds GJ, et al. Establishment of pediatric reference intervals on a large cohort of healthy children. Clin Chim Acta. 2010; 411: 1421–7.
36. Koerbin G, Abhayaratna WP, Potter JM, Apostoloska S, Telford RD, Hickman PE. NTproBNP concentrations in healthy children. Clin Biochem. 2012; 45: 1158–60.
37. Mortensen ME, Caudill SP, Caldwell KL, Ward CD, Jones RL. Total and methyl mercury in whole blood measured for the first time in the U.S. population: NHANES 2011–2012. Environ Res. 2014; 134: 257–64.
38. Kamycheva E, Goto T, Camargo CA, Jr. Celiac disease is associated with reduced bone mineral density and in- creased FRAX scores in the US National Health and Nutrition Examination Survey. Osteoporos Int. 2016.
39. Breslow RA, Wideroff L, Graubard BI, Erwin D, Reichman ME, Ziegler RG, et al. Alcohol and prostate cancer in the NHANES I epidemiologic follow-up study. First National Health and Nutrition Examination Survey of the United States. Ann Epidemiol. 1999; 9: 254–61.
40. Patel MA, Mener DJ, Garcia-Esquinas E, Navas-Acien A, Agrawal Y, Lin SY. Tobacco smoke exposure and eustachian tube disorders in US children and adolescents. PLoS One. 2016; 11: e0163926.
41. Cheng CK, Chan J, Cembrowski GS, van Assendelft OW. Complete blood count reference interval diagrams derived from NHANES III: stratification by age, sex, and race. Lab Hematol. 2004; 10: 42–53.
42. Hollowell JG, van Assendelft OW, Gunter EW, Lewis BG, Najjar M, Pfeiffer C. Centers for Disease Control and Prevention, National Center for Health Statistics. Hematological and iron-related analytes-reference data for persons aged 1 year and over: United States, 1988–94. Vital Health Stat. 2005; (247): 1–156.
43. Wener MH, Daum PR, McQuillan GM. The influence of age, sex, and race on the upper reference limit of serum C-reactive protein concentration. J Rheumatol. 2000; 27: 2351–9.
44. Kant AK, Graubard BI. Race-ethnic, family income, and education differentials in nutritional and lipid biomarkers in US children and adolescents: NHANES 2003–2006. Am J Clin Nutr. 2012; 96: 601–12.
45. Rustad P, Felding P, Lahti A, Hyltoft Petersen P. Descriptive analytical data and consequences for calculation of common reference intervals in the Nordic Reference Interval Project 2000. Scand J Clin Lab Invest. 2004; 64: 343–70.
46. Rustad P, Felding P, Franzson L, Kairisto V, Lahti A, Martensson A, et al. The Nordic Reference Interval Project 2000: recommended reference intervals for 25 common biochemical properties. Scand J Clin Lab Invest. 2004; 64: 271–84.
47. Urdal P, Bolann B, Marstein S, Rustad P, Steensland H, Asberg A. Updated reference intervals for clinical chemical components. Tidsskr Nor Laegeforen. 2004; 124: 1515–7.
48. Rustad P, Felding P, Lahti A. Nordic Reference Interval Project 2000. Proposal for guidelines to establish common biological reference intervals in large geographical areas for biochemical quantities measured frequently in serum and plasma. Clin Chem Lab Med. 2004; 42: 783–91.
49. Makarova GA, Grishina ZhV, Chernuxa SM, Bazanovich SA, Yadgarov MYa, Feshhenko VS. Centil'nye gradacii morfologicheskix i bioximicheskix pokazateleĭ krovi u sportsmenov: osobye podxody k analizu i ocenke. Lechebnaya fizkul'tura i sportivnaya medicina. 2020; 1 (155): 14–21. Russian.
50. Grishina ZhV, Makarova GA, Bazanovich SA, Chernuxa SM, Yadgarov MYa, Feshhenko VS, i dr. Skrytye narusheniya metabolizma u vysokokvalificirovannyx sportsmenov. Sportivnaya medicina: nauka i praktika. 2020; 10 (4): 64–75. Russian.
51. Wilasco MI, Goldani HA, Dornelles CT, Maurer RL, Kieling CO, Porowski M, et al. Ghrelin, leptin and insulin in healthy children: Relationship with anthropometry, gender, and age distribution. Regul Pept. 2012; 173: 21–26.

Литература

1. Дёмин В. Ф., Ключников С. О., Балыковой Л. А., Самойлова А. С., редакторы. Авторские лекции по педиатрии. Детская спортивная медицина. 2017; 10: 81–99.
2. Tahmasebi H, Higgins V, Fung A, Truong D, White-Al Habeeb N,

Adeli K. Pediatric reference intervals for biochemical markers: gaps and challenges, recent national initiatives and future perspectives. 2017; 28 (1): 43–63.
3. Дудникова Э. В., редактор. Физиологические константы у детей: учеб.-методическое пособие. Ростов-н/Д: Изд-во РостГМУ, 2016; 46 с.
4. Евгина С. А., Савельев Л. И. Современные теория и практика референтных интервалов. Лабораторная служба. 2019; 8 (2): 36–44.
5. ГОСТ Р 53022.3-2008 «Технологии лабораторные клинические. Требования к качеству клинических лабораторных исследований. Ч. 3. Правила оценки клинической информативности лабораторных тестов». М.: Стандартинформ, 2009; 22 с.
6. Boyd JC. Clinical and Laboratory Standards Institute (CLSI). Defining, establishing, and verifying reference intervals in the clinical laboratory; Approved guideline, CLSI document C28-A3. 2010; 28 (3).
7. Yang L, Grey V. Pediatric reference intervals for bone markers. Clin Biochem. 2006; 39: 561–8.
8. Colantonio DA, Kyriakopoulou L, Chan MK, Daly CH, Brinc D, Venner AA, et al. Closing the gaps in pediatric laboratory reference intervals: a CALIPER database of 40 biochemical markers in a healthy and multiethnic population of children. Clin Chem. 2012; 58: 854–68.
9. Delvin EE, Laxmi Grey V, Vergee Z, CALIPER Working Group. Gap analysis of pediatric reference intervals related to thyroid hormones and the growth hormone-insulin growth factor axis. Clin Biochem. 2006; 39: 588–94.
10. Konforte D, Shea JL, Kyriakopoulou L, Colantonio D, Cohen AH, Shaw J, et al. Complex biological pattern of fertility hormones in children and adolescents: a study of healthy children from the CALIPER cohort and establishment of pediatric reference intervals. Clin Chem. 2013; 59: 1215–27.
11. Гришина Ж. В., Ключников С. О., Яшин Т. А., Макарова Г. А., Ломазова Е. В., Бушуева И. Е. и др. Референтные интервалы биохимических показателей крови у юных спортсменов. Вопросы практической педиатрии. 2022; 17 (1): 71–78.
12. Tate JR, Sikaris KA, Jones GR, Yen T, Koerbin G, Ryan J, et al. Harmonising adult and paediatric reference intervals in australia and new zealand: an evidence-based approach for establishing a first panel of chemistry analytes. Clin Biochem Rev. 2014; 35: 213–35.
13. Jones GR, Koetsier SD. RCPAQAP First Combined Measurement and Reference Interval Survey. Clin Biochem Rev. 2014; 35: 243–50.
14. Bailey D, Colantonio D, Kyriakopoulou L, Cohen AH, Chan MK, Armbruster D, et al. Marked biological variance in endocrine and biochemical markers in childhood: establishment of pediatric reference intervals using healthy community children from the CALIPER cohort. Clin Chem. 2013; 59: 1393–405.
15. Raizman JE, Cohen AH, Teodoro-Morrison T, Wan B, Khun-Chen M, Wilkenson C, et al. Pediatric reference value distributions for vitamins A and E in the CALIPER cohort and establishment of age-stratified reference intervals. Clin Biochem. 2014; 47: 812–5.
16. Yazdanpanah M, Bailey D, Walsh W, Wan B, Adeli K. Analytical measurement of serum 25-OH-vitamin D(3), 25-OH-vitamin D(2) and their C3-epimers by LC-MS/MS in infant and pediatric specimens. Clin Biochem. 2013; 46: 1264–71.
17. Brinc D, Chan MK, Venner AA, Pasic MD, Colantonio D, Kyriakopolou L, et al. Long-term stability of biochemical markers in pediatric serum specimens stored at –80 degrees C: a CALIPER Substudy. Clin Biochem. 2012; 45: 816–26.
18. Bailey D, Bevilacqua V, Colantonio DA, Pasic MD, Perumal N, Chan MK, et al. Pediatric within-day biological variation and quality specifications for 38 biochemical markers in the CALIPER cohort. Clin Chem. 2014; 60: 518–29.
19. Pasic MD, Colantonio DA, Chan MK, Venner AA, Brinc D, Adeli K. Influence of fasting and sample collection time on 38 biochemical markers in healthy children: a CALIPER substudy. Clin Biochem. 2012; 45: 1125–30.
20. Bevilacqua V, Chan MK, Chen Y, Armbruster D, Schodin B, Adeli K. Pediatric population reference value distributions for cancer biomarkers and covariate-stratified reference intervals in the CALIPER cohort. Clin Chem. 2014; 60: 1532–42.
21. Teodoro-Morrison T, Kyriakopoulou L, Chen YK, Raizman JE, Bevilacqua V, Chan MK, et al. Dynamic biological changes in metabolic disease biomarkers in childhood and adolescence: A CALIPER study of healthy community children. Clin Biochem. 2015; 48: 828–36.
22. Raizman JE, Quinn F, Armbruster DA, Adeli K. Pediatric reference intervals for calculated free testosterone, bioavailable testosterone and free androgen index in the CALIPER cohort. Clin Chem Lab Med. 2015; 53: e239–e243.
23. Flanders MM, Crist RA, Roberts WL, Rodgers GM. Pediatric reference intervals for seven common coagulation assays. Clin Chem. 2005; 51: 1738–42.
24. Kushnir MM, Rockwood AL, Roberts WL, Pattison EG, Owen WE, Bunker AM, et al. Development and performance evaluation of a tandem mass spectrometry assay for 4 adrenal steroids. Clin Chem. 2006; 52: 1559–67.
25. Clifford SM, Bunker AM, Jacobsen JR, Roberts WL. Age and gender specific pediatric reference intervals for aldolase, amylase, ceruloplasmin, creatine kinase, pancreatic amylase, prealbumin, and uric acid. Clin Chim Acta. 2011; 412: 788–90.
26. Johnson-Davis KL, Moore SJ, Owen WE, Cutler JM, Frank EL. A rapid HPLC method used to establish pediatric reference intervals for vitamins A and E. Clin Chim Acta. 2009; 405: 35–38.
27. Meikle AW, Kushnir MM, Rockwood AL, Pattison EG, Terry AH, Sandrock T, et al. Adrenal steroid concentrations in children seven to seventeen years of age. J Pediatr Endocrinol Metab. 2007; 20: 1281–91.
28. Wyness SP, Roberts WL, Straseski JA. Pediatric reference intervals for four serum bone markers using two automated immunoassays. Clin Chim Acta. 2013; 415: 169–72.
29. Hilsted L, Rustad P, Aksglaede L, Sorensen K, Juul A. Recommended Nordic paediatric reference intervals for 21 common biochemical properties. Scand J Clin Lab Invest. 2013; 73: 1–9.
30. Kohse KP. KiGGS — the German survey on children's health as data base for reference intervals and beyond. Clin Biochem 2014; 47: 742–3.
31. Kamtsiuris P, Lange M, Schaffrath RA. The German Health Interview and Examination Survey for Children and Adolescents (KiGGS): sample design, response and nonresponse analysis. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz. 2007; 50: 547–56.
32. Kohse KP, Thamm M. KiGGS-the German survey on children's health as database for reference intervals. Clin Biochem. 2011; 44: 479.
33. Thierfelder W, Dortschy R, Hintzpeter B, Kahl H, Scheidt-Nave C. Biochemical measures in the German Health Interview and Examination Survey for Children and Adolescents (KiGGS). Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz. 2007; 50: 757–70.
34. Witte T, Ittermann T, Thamm M, Riblet NB, Volzke H. Association between serum thyroid-stimulating hormone levels and serum lipids in children and adolescents: a population-based study of german youth. J Clin Endocrinol Metab. 2015; 100: 2090–97.
35. Southcott EK, Kerrigan JL, Potter JM, Telford RD, Waring P, Reynolds GJ, et al. Establishment of pediatric reference intervals on a large cohort of healthy children. Clin Chim Acta. 2010; 411: 1421–7.
36. Koerbin G, Abhayaratna WP, Potter JM, Apostoloska S, Telford RD, Hickman PE. NTproBNP concentrations in healthy children. Clin Biochem. 2012; 45: 1158–60.
37. Mortensen ME, Caudill SP, Caldwell KL, Ward CD, Jones RL. Total and methyl mercury in whole blood measured for the first time in the U.S. population: NHANES 2011–2012. Environ Res. 2014; 134: 257–64.
38. Kamycheva E, Goto T, Camargo CA, Jr. Celiac disease is associated with reduced bone mineral density and in- creased FRAX scores in the US National Health and Nutrition Examination Survey. Osteoporos Int. 2016.
39. Breslow RA, Wideroff L, Graubard BI, Erwin D, Reichman ME, Ziegler RG, et al. Alcohol and prostate cancer in the NHANES I epidemiologic follow-up study. First National Health and Nutrition

Examination Survey of the United States. Ann Epidemiol. 1999; 9: 254–61.
40. Patel MA, Mener DJ, Garcia-Esquinas E, Navas-Acien A, Agrawal Y, Lin SY. Tobacco smoke exposure and eustachian tube disorders in US children and adolescents. PLoS One. 2016; 11: e0163926.
41. Cheng CK, Chan J, Cembrowski GS, van Assendelft OW. Complete blood count reference interval diagrams derived from NHANES III: stratification by age, sex, and race. Lab Hematol. 2004; 10: 42–53.
42. Hollowell JG, van Assendelft OW, Gunter EW, Lewis BG, Najjar M, Pfeiffer C. Centers for Disease Control and Prevention, National Center for Health Statistics. Hematological and iron-related analytes-reference data for persons aged 1 year and over: United States, 1988–94. Vital Health Stat. 2005; (247): 1–156.
43. Wener MH, Daum PR, McQuillan GM. The influence of age, sex, and race on the upper reference limit of serum C-reactive protein concentration. J Rheumatol. 2000; 27: 2351–9.
44. Kant AK, Graubard BI. Race-ethnic, family income, and education differentials in nutritional and lipid biomarkers in US children and adolescents: NHANES 2003–2006. Am J Clin Nutr. 2012; 96: 601–12.
45. Rustad P, Felding P, Lahti A, Hyltoft Petersen P. Descriptive analytical data and consequences for calculation of common reference intervals in the Nordic Reference Interval Project 2000. Scand J Clin Lab Invest. 2004; 64: 343–70.
46. Rustad P, Felding P, Franzson L, Kairisto V, Lahti A, Martensson A, et al. The Nordic Reference Interval Project 2000: recommended reference intervals for 25 common biochemical properties. Scand J Clin Lab Invest. 2004; 64: 271–84.
47. Urdal P, Bolann B, Marstein S, Rustad P, Steensland H, Asberg A. Updated reference intervals for clinical chemical components. Tidsskr Nor Laegeforen. 2004; 124: 1515–7.
48. Rustad P, Felding P, Lahti A. Nordic Reference Interval Project 2000. Proposal for guidelines to establish common biological reference intervals in large geographical areas for biochemical quantities measured frequently in serum and plasma. Clin Chem Lab Med. 2004; 42: 783–91.
49. Макарова Г. А., Гришина Ж. В., Чернуха С. М., Базанович С. А., Ядгаров М. Я., Фещенко В. С. Центильные градации морфологических и биохимических показателей крови у спортсменов: особые подходы к анализу и оценке. Лечебная физкультура и спортивная медицина. 2020; 1 (155): 14–21.
50. Гришина Ж. В., Макарова Г. А., Базанович С. А., Чернуха С. М., Ядгаров М. Я., Фещенко В. С. и др. Скрытые нарушения метаболизма у высококвалифицированных спортсменов. Спортивная медицина: наука и практика. 2020; 10 (4): 64–75.
51. Wilasco MI, Goldani HA, Dornelles CT, Maurer RL, Kieling CO, Porowski M, et al. Ghrelin, leptin and insulin in healthy children: Relationship with anthropometry, gender, and age distribution. Regul Pept. 2012; 173: 21–26.

METHODOLOGICAL ASPECTS OF DRUG DEVELOPMENT AND PRECLINICAL RESEARCH IN THE INTERESTS OF ARTIC MEDICINE

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There is an inextricable link between exploration and development of the Arctic territories and emergence of associated problems of medical and biological nature. It is necessary to design and develop emergency care and prevention drugs and medical devices for use in the Arctic. This review presents an analysis of additional requirements for drugs intended for the Far North and compares methods of modeling extreme conditions in animals. We outline medical and biological problems of the region highlight key areas of Arctic pharmacology: choice of pharmaceutical form, use of cryoprotectants and design of adaptogens. The study mainly revolves around the search for information on modeling extreme environmental factors in animal experiments, as this is a key stage in preclinical studies of drugs for the Arctic medicine. We present the relevant directions of further work promoting the subject: development of the hypoxia and hypothermia assessment criteria, development of modeling methods employing large laboratory animals, improvement of the equipment used.

Keywords: hypoxia, hypothermia, photoperiodism, extreme environmental factors, animal research

Author contribution: Volkova MV — development of the concept, collection and analysis of the published papers, manuscript authoring; Biryukov SA — manuscript editing.

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МЕТОДИЧЕСКИЕ АСПЕКТЫ РАЗРАБОТКИ И ДОКЛИНИЧЕСКИХ ИССЛЕДОВАНИЙ ЛЕКАРСТВЕННЫХ ПРЕПАРАТОВ В ИНТЕРЕСАХ АРКТИЧЕСКОЙ МЕДИЦИНЫ

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Освоение арктических территорий неразрывно связано с возникновением медико-биологических проблем. Необходима разработка медицинских изделий и лекарственных средств для профилактики или оказания неотложной помощи в условиях Арктики. В обзоре представлен анализ дополнительных требований к лекарственным препаратам, предназначенным для использования в условиях Крайнего Севера, и аналитическое сравнение методов моделирования экстремальных состояний у животных. Обозначены медико-биологические проблемы региона и акцентировано внимание на основных направлениях арктической фармакологии: выборе лекарственной формы, использовании криопротекторов и разработке адаптогенов. Основное внимание уделено поиску информации по моделированию экстремальных факторов окружающей среды в экспериментах на животных, так как это является ключевым звеном в доклинических исследованиях препаратов для арктической медицины. Показаны актуальные направления дальнейшей работы для развития данного направления: разработка критериев оценки состояния гипоксии и гипотермии, разработка методов моделирования на крупных лабораторных животных, совершенствование технического оснащения.

Ключевые слова: гипоксия, гипотермия, фотопериодизм, экстремальные факторы внешней среды, исследования на животных

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Almost 18% of the territory of the Russian Federation belongs to the Arctic region (AR). These are the lands from the Franz Josef Land to the Wrangel and Herald Islands, comprising about a third of the entire area of the Arctic shelf. The important transport corridors running through the AR condition the significance of this territory for the Russian Federation. Another reason for development of this region is production of hydrocarbons. With all the other factors considered, the Arctic is significant geopolitically on the global scale [1–4].

People permanently residing in the region back exploration and development of the AR: they power social and economic development, scientific research, infrastructural projects and environmental safety [3–5]. At the same time, development of the territories of the Far North is inextricably linked with problems of medical and physiological character that stem from the impact of severe natural and climatic conditions (cold, increased electromagnetic activity, radiation, specific photoperiodism etc) on the human body [4, 6–8]. Arctic medicine as a field of medical science was delineated with the aim of development of effective prevention and treatment measures; it studies the internal mechanisms of adaptation of the human body, seeks and describes specifics of the course of various diseases and develops methods for their treatment [9].

This review made use of the PubMed and Google Scholar resources. We gave preference to papers published over the past 10 years and indexed in the Scopus and Web of Science databases. The key words cited above were used in the search for publications.

The purpose of this review is to present an analysis of additional requirements for drugs intended for the Far North and compare the methods of modeling extreme conditions in animals.

Medical and biological problems of the Arctic region

Climatic and geographic, psychological and physiological factors peculiar to the AR have a significant impact on life of people. They trigger involvement of all physiological reserves

of the body and a complex restructuring of the homeostatic systems [10–12].

Extremely low ambient temperatures exacerbate the need for heating, the body produces more heat at the expense of efficiency of physical work, which goes down [13]. Metabolism of a human being changes: the need for proteins and fats, fat-soluble vitamins (A, D, E etc) increases [6, 13]. This translates into special nutrition and work/rest requirements [14].

In addition, conditions of the AR support development of tissue hypoxia, which can have various physicochemical or physiological causes (changes in the structure of membranes of erythrocytes that deliver oxygen to tissues etc) [14–15]. The increased need for oxygen on the part of tissues affects the respiratory system and triggers adaptive changes aimed at improvement of gas exchange, i.e., growth of the alveolar surface of the lungs and volume of the pulmonary capillaries [14].

The influence of the Arctic's low temperatures and rarefied atmosphere should be considered in combination, since these two factors can be mutually potentiating [15]. In particular, cold causes vasoconstriction and decreases the intensity of blood flow in general and to the skin in particular, which results in local cooling of hands and feet, face and upper respiratory tract, a process that negatively affects physical performance and changes the functions of external respiration [16–17]. In addition, combined with cold stress, hypoxia contributes to the development of ischemic changes in cardiomyocytes, thus affecting the cardiovascular system [15].

Photoperiodism is another factor that, similarly to exposure to low temperatures, significantly affects physiological state of a person in the AR [18–19]. Almost all cellular functions and physiological systems of the body are under circadian control, a "mechanism" that keeps body's activity at the optimal level, supports energy conservation and maintenance of internal homeostasis [20]. The circadian rhythm (CR) is driven from within, but it is synchronized with external stimuli, including, in the first place, light of a certain portion of the spectrum and intensity [21–22]. There is a pronounced seasonal asymmetry of light availability and insufficient ultraviolet radiation in the polar zone. Combined with the effects of the cold, stress associated with the light alters functioning of the body's hormonal systems [21, 23]. A mismatch between the CR and external signals can lead to the development of metabolic, immune and mental diseases, as well as undermine the effectiveness of wound healing, body detoxification etc [24]. A person may start suffering from desynchronosis, which manifests in deterioration of physical and mental performance, sleep disturbances and unpredictability of human behavior [10, 13, 25].

In the context of studying the AR-associated factors, changes in the body's CR should be considered in conjunction with hypothermia. Body temperature rises during the day and goes down at night, which ensures the optimal course of physiological processes. Various diseases and specifics of their course are also associated with body temperature fluctuations, e.g., high temperature typically indicates a significant systemic inflammatory response [26].

In the AR, the light as direct solar radiation and as rays reflected from the snow is an aggressive factor. The small angle of incidence typical for the region reinforces the effect of light in the visible and ultraviolet spectra. Lack of prevention measures and protection equipment leads to burns of the eye's conjunctiva and cornea, a condition called snow blindness [27].

Another medical and biological problem peculiar to the AR is the wide spread of infectious diseases, including those of emerging from the wilderness in a given location (anthrax, etc.). The two key reasons behind this problem are the large hosts blood-sucking dipterans in the summer and crowded accommodation conditions with people spending most of their time in an artificial environment [6, 28]. Poor water quality also plays a significant role. Meltwater contains heavy metals, organic pollutants and various microorganisms. Consumed for a long period of time, such water disrupts the water-salt exchange in the body, contributes to the leaching of salts and slows down tissue recovery in case of injuries [29].

The diseases that are more common in the AR and less spread in the moderate climate belt deserve a special mention [1]. General hypothermia and the resulting tissue hypoxia and ischemia-reperfusion syndrome hinder healing of various injuries of the skin and soft tissues, including frostbite. Hypothermia aggravates the injuries by disrupting plasma coagulation and platelet function, suppressing the immune response and increasing the risk of sepsis. Damage to local vessels worsens hypoxia in the injured tissues, which prevents collagen synthesis and angiogenesis and slows down tissue regeneration significantly. As a result, surgical intervention as per the standard protocols may be delayed, with such a delay increasing the risk of death of a seriously injured patient [30–31].

Extreme climatic conditions, low population density, remoteness and inaccessibility of the areas comprising the region affect the setup of medical care (MC) systems, including those involved in rescue of people injured in emergency situations [30]. The specifics of provision of MC is also a reason behind the need for development of the new methods of treatment.

Cold climate makes most medical manipulations impossible. Therefore, design of modern equipment, medical devices/items (MDI) and drugs suitable for use in the AR is strategically important, with resistance to extreme low temperatures being one of the key requirements and resistance to humidity and radiation, wind loads and precipitation, as well as stability under multiple cycles of exposure to adverse factors being additional requirements.

The use of drugs and MDI for treatment of diseases in the AR can be divided into three categories. The first group includes drugs necessary for first aid when condition of the patient is life-threatening. The drugs (and MDI) of this group relieve pain, stop massive bleeding and prevent asphyxia; they are used outdoors under the most aggressive conditions. The second group can be considered comprised of drugs intended for use in a limited space, e.g., a first-aid post. In this case, the impact of winds, precipitation and low temperature is minimal, and it mainly manifests during transportation. In addition to first-aid drugs, preventive medicines can be also allocated to this group. The third group includes drugs, MDI and, potentially, cell products used in an in-patient hospital outside the AR. The selection criteria for this group are conditioned by the combined effect of the factors of the Far North on the body, which translates into complication of the pathogenesis of diseases.

Prevention and treatment drugs

Prevention and first aid in the North require a certain set of drugs and MDI. The following groups of essential drugs can be distinguished: cardiovascular drugs, analgesics and antispasmodics, antibiotics and antiviral drugs, drugs for treatment of respiratory diseases and frostbite, adaptagens adaptogens and preventive drugs (e.g., for eye protection), vitamins.

There is a number of limiting factors to be taken into account when selecting drugs for the AR. Many drugs cannot be frozen. Liquid drugs are designed primarily for use at above-

zero ambient temperatures. The stability of a drug can suffer during transportation over hundreds of kilometers, which is often done in vehicles not equipped specifically for the purpose. Low temperatures, high humidity, bright lighting and mechanical shocks, as well as uncontrolled freeze-thaw cycles directly affect the quality of the drug. It is also necessary to factor in the possible reinforcement of adverse effects the drug may have on the body.

From the usability point of view, hard and soft liquid drugs are the most convenient. Low temperatures make drugs more fragile and violate the integrity of the film shell of pills and capsules. Various band aids and dressings, if exposed to low temperatures, can lose their adhesive and functional properties. If the patient needs care at the place of accident, it becomes increasingly hard to apply them because of the many layers of clothing. Also, it may be difficult to use powders and granules to make liquid preparations.

For first aid, injectable drugs (e.g., painkillers) are most effective. They are less resistant to repeated freeze-thaw cycles. Cryoprotectants (alcohols, polymeric compounds etc) can be used to reduce the defrosting time and/or prevent freezing, as well as to protect the active component from degradation [32]. A cryoprotectant cannot be toxic, it should not accumulate in the body and be quickly eliminable in order to prevent the development of side effects [33]. Another important requirement for such protection is lack of any negative influence on the active agent.

Propylene glycol, a polyhydric alcohol completely metabolized by the body, is one of the promising cryoprotectants [33–34]. Today, it is used as an additive to diazepam (200 mg of propylene glycol per 1 ml of the drug, which is 19% of the volume) and other drugs for intramuscular or intravenous administration. Preliminary studies have shown that propylene glycol added to the anesthetic at a concentration below 40% decreases the freezing point to values below minus 25 °C while not affecting efficacy of the active ingredient in any way (unpublished data).

Another direction AR medicine takes in addition to the path of improvement of the already existing drugs is design of adaptogens, biologically active substances of plant or animal origin intended to enable adaptation of the body (its cold resistance and cognitive capabilities in particular) to extreme conditions and reinforce its physical capacity. Design of adaptogens is a difficult task: there is no specific pharmacological target and it is necessary to produce a complex effect on various organs and systems. Another problem with adaptogens is the unpredictable intensity of their effect, which differs depending on who made the drug [35].

Only a small number of drugs and MDI are produced specifically for use in the Arctic. The reason behind this situation is the set of additional requirements for such products that condition their use when there is an impact from a combination of external factors.

Simulation of extreme conditions in animal experiments

Climatic testing rigs and subsequent application of the advanced physicochemical and biochemical methods can enable *in vitro* assessment of stability of the drugs and MDI after exposure to aggressive factors inherent in the Far North.

There are rigid limitations for *in vivo* studies. It is necessary to develop and implement models that allow assessing the functions of cardiovascular, nervous, endocrine and immune systems of the body, as well as external respiration. Such studies could support the methods of regulation of adaptive capabilities of a person in the conditions of the AR [4, 36].

Hypoxia is a factor that significantly handicaps performance. It was established experimentally that a gas mixture with the content of oxygen at 10% alters the pulmonary ventilation pattern from the first minutes it is breathed, with hyperventilation and increased respiratory minute volume being the compensatory mechanisms to manifest earliest [37]. Therefore, investigation of the body's adaptive capabilities should start with experiments involving hypoxic hypoxia associated with a change in the barometric pressure of the inhaled air or a less oxygen-rich breathing mixture [38]. Rodents are the main experimental animals in this context; they are placed in hermetic chambers of various sizes with controlled level of rarefaction of the atmosphere or gas mixture. However, there is no single methodological approach to assessment of severity of the induced hypoxia that would apply to all the various models used by researchers. When used to evaluate the action of a drug on the body, hypoxic hypoxia tests only confirm the antihypoxic effect of the drug and describe its intensity, but report no specific level thereof [38].

The range of variations is greater for simulation of hypothermia in models. A body's temperature can be decreased by cooling the air in the hermetic chamber, by immersion in cold water [39–40], by wrapping the body of an immobilized animal in ice or by placing it on a cold surface [41]. These methods can also be combined. The specific conditions of the experiment define how the decrease of the animal's body temperature and specific features of the model affect experimental results (Table 1).

Physiological features of the rodents should be factored in when aligning body temperatures and states, however, there is no single classification developed for this purpose. Some researchers consider body temperature below 37 °C to be low and 35 °C — critical [46], some take into account

Table 1. Methods of modeling hypothermia in rodents

Animal	Method	t_{ext}, °C	τ	N	t_{rect}, °C	Additional factors	Reference
Mice	contact (metallic plate)	18	8 h	1	33	anesthesia	42
Rats	cold water	12–14	10–20 min	1	34–35	immobilization	41
	hermetic chamber	4–6	160 min	1	36–38	immobilization, humidity 75–80%	
Rats	hermetic chamber	1–2	3.5–4 h	1	14–18	hypoxia (chamber 5 L)	43
Rats	hermetic chamber	2–6	2 days	10	36–37	hypoxia (15% O_2)	44
Rats	hermetic chamber	minus 25	3–9 h	1	30	no	45
Rats	hermetic chamber	4–6	14 days	groups	33	hypoxia (30–50 mg/kg $NaNO_2$), humidity 75–80%, stress factors (light, sound, food restriction)	23

Note: t_{ext} is ambient temperature; τ is the time of keeping under simulated extreme conditions; N is the number of animals kept simultaneously under simulated conditions (in one cage, if a hermetic chambers was used; group — the exact number of animals in a cage is not given); t_{rect} is the rectal temperature of the animals at the end of the keeping period or a limiting value the reaching of which triggered removal of the animal removed from simulated conditions; additional factors list the conditions created while keeping animals in simulated extreme conditions, like immobilization of animals in a plastic case.

the range of moderate hypothermia, 32–35 °C [23]. Thus, modeling hypothermia requires development of the uniform methodological recommendations based on the reference data describing physiological characteristics of the selected animals.

Despite the fact that using cold air to lower the animal's temperature to the desired level is a time-intensive approach, hypothermia is mainly simulated in hermetic chambers (Table 1) because they allow combining this state with other factors, such as hypoxia [15]. High humidity created in the chamber enhances the effect of cold on the animals. One paper reports using light as an additional stress factor.

As mentioned above, same as hypothermia and hypoxia, photoperiodism has a significant effect on living organisms in the AR. Circadian rhythms should be factored in when calculating dosing schedules and evaluating the efficacy of drugs and measuring their blood levels. It has also been confirmed that the acute symptoms of many diseases and conditions manifest at certain times of the day (myocardial infarction, rheumatoid arthritis). Thus, circadian regulation of the molecular processes can affect condition of the patient and results of the therapy [20].

There are three ways of changing the CR in experimental animals: with light, food and temperature. Ambient temperature has a weak synchronizing effect, since animals have internal mechanisms that ensure temperature compensation. It is possible to "reset" the CR with a non-light stimulus. Time-restricted feeding is a popular approach when the goal is to study the CR proper, however, if the feeding period is less than 6 hours, animals cannot eat as much food as they do *ad libitum* [44]. This additional stressor must be taken into account in the experiment.

In our opinion, light is the preferable stress factor for experiments designed to simulate conditions of the AR. As a rule, standard animal keeping conditions imply a 12-hour day and a 12-hour night. Increasing the daylight hours to 22 hours or reducing their duration to 2 hours results in disruption of the CR of animals. Rodents, for example, are night animals, so a longer day translates into a stress for them.

Using the published data, we compared all the available methods and assessed the possibility of combining them (Table 2). Most of the methods are described in single publications only, which highlights one of the main problems faced when compiling guidelines for preclinical studies of drugs intended for the AR: insufficient number of experimental studies. To date, the only document is the antihypoxic drugs activity investigation guidelines [38], which describe modeling of various hypoxic conditions in animals. Unfortunately, we have not found similar guidelines for hypothermia or photoperiodism.

Taking into account the advantages and disadvantages of the methods and the possibility of combining them, the following recommendations for modeling the Arctic factors in preclinical small animal model studies can be formulated. Such research requires keeping the rodents for a long time, therefore, to create hypothermia, it is necessary to use a hermetic chamber. The recommended temperature is 2 through 6 °C. The maximum number of animals in a cage is five, because when the group is larger, the animals huddle together and warm each other. The preferred approach is to keep one animal per cage, but that is not always possible. The humidity in the chamber can be increased in order to amplify the effect of cold. If the study

Table 2. Simulation of extreme conditions of the Arctic

Factor	Method	Advantages	Flaws	Possible combinations with other stressors	Possibility of assessment of physical or behavioral activity*	Experiment duration
Hypoxia	Use of a hermetic chamber	Applicable to a group of animals; use does not require additional skills	Mainly for small laboratory animals; restriction of manipulations with animals is necessary; the parameters are controlled in different points of the chamber	Hypothermia (hermetic chamber, cold water), photoperiodism (light, food)	Tests that could be performed inside the chamber or other short-term (up to 5 minutes) tests outside the chamber	Several days or more
	Inhalation (mask)	Precise composition of the inhaled air	Mainly for large laboratory animals; wear training required	Hypothermia (all), photoperiodism (light)	Depending on the test	Several hours
	Hemic (IV introduction of hemoglobin oxidizer)	Creating a certain level of hypoxia	It is necessary to select concentration and pattern of administration for each animal species due to possible individual intolerance	Hypothermia (all), photoperiodism (light, food)	Carrying out any tests (theoretically)	Several days or more
Hypothermia	Use of a hermetic chamber	Applicable to a group of animals; use does not require additional skills	Mainly for small laboratory animals; the parameters are controlled in different points of the chamber	Hypoxia (all), photoperiodism (light, food)	Tests that could be performed inside the chamber or other short-term (up to 5 minutes) tests outside the chamber	Several days or more
	Immersion in cold water	Rapid achievement of the required body temperature, possibility to combine with physical activity (swimming, swimming with a load)	Higher difference between cold-resistant and cold-sensitive animals	Hypoxia (hermetic chamber, hemic)	Swimming or taking the animal out of the water for a short period of time for other tests	Several hours
	Contact (cold surface, ice)	Convenience of control over physiological parameters when the body temperature drops	Requires anesthesia for each animal individually	Hypoxia (mask, hemic)	Impossible	Several hours

Note: * — indicates the possibility of conducting tests while maintaining the impact of stress factors on the body.

does not include behavioral or long-term experiments (more than 5 minutes) involving removal of the animals from the chamber, it is preferable to induce hypoxia through inhalation, by maintaining a given oxygen level in the hermetic chamber. In case the experimental phase of the study includes periodic tests or repeated daily manipulations, it is advisable to use smaller hermetic chambers containing 2–3 cages with several animals, or individual hermetic chambers, or induce hemic hypoxia. This is necessary to reduce the time spent by animals outside hypoxia and the time needed to restore the required level of oxygen in the air if hermetic chambers are used.

Lack of guidelines and standardized equipment, as well as insufficient amount of practical research, necessitate preliminary experiments with a specific rig before each study. Firstly, this approach allows assessing the effect of hypoxia and hypothermia on the animal's body in each case (with body temperature taken at short intervals, blood sampling etc.) and ensures significant reduction of the number of manipulations during the main experiment while maintaining the value of the resulting information; for example, in a preliminary experiment, temperature of the animals can be measured (rectally) every 6–12 hours for several days, and during the main experiment, the frequency of this operation can be reduced to once a day, with the obtained values compared with the data registered during the preliminary study. Secondly, a preliminary experiment allows identifying animals that are resistant or sensitive to stressful conditions, which is important. Studies [23, 41] have shown that external stress factors affect animals differently: about 10–20% of rats in the experiment proved to be resistant thereto as their body temperature decreased much more slowly than in other rodents. Thus, this approach, depending on the experimental conditions, enables preliminary selection and formation of experimental groups with equal numbers of sensitive and resistant animals. The published data allows a conclusion that identification of resistant animals and determination of the key parameters of the animals' state can last 2–3 days, while the main phase of the experiment can take up to two weeks. It should also be noted that such a two-phase approach implies using the same animals, which necessitates special attention from the bioethical commission to the procedures implemented during the preliminary test. It is extremely important to minimize the negative effect the first phase has on the animals' condition and establish the optimal period between the preliminary and main experiments to ensure their full recovery.

The most popular animal models are rats. It is difficult to plan an experiment and create AR conditions for larger species: their resistance to hypoxia is lower than that of rodents [38]. In this connection, one of the urgent tasks is to scale up existing or develop new methods of modeling extreme environmental conditions that would be suitable for other (larger) laboratory animals.

Criteria of assessment of severity of factors is another area that lacks the required sufficiency. There are physiological and biochemical hypoxia severity assessment criteria described for small animal models [38], but there is nothing of the kind for their larger counterparts. As for hypothermia, it is typically evaluated by rectal temperature alone, and its degree is established based on the literature data that the researcher prefers. There is, however, a consistency peculiar to these data, although not a complete one, which enables comparison of the results obtained. Addition of photoperiodism to the list of factors behind the extreme conditions simulated necessitates development of the main criteria of assessment of light-associated stress in animals, as reflected by, for example, temperature and/or cortisol and melatonin levels [20].

As Table 1 shows, hermetic chambers are a common tool used for modeling the AR conditions. In this connection, a technical problem should be noted: lack of specialized equipment translates into custom rigs designed and made for a given experiment. This problem is important because reproducibility of the results largely depends on standardization of the equipment. The chambers used should maintain values of the parameters (temperature, oxygen level, etc.) uniformly throughout the entire volume of the chamber. A separate task is the selection of the optimal methods of registration of vital signs of the animals since opening the hermetic chamber alters the conditions therein and entails the need for restoration of the required parameter values. Technological aspects should be considered in development of the AR conditions simulation methods designed for other animal species.

There are many factors (age, gender, etc) that shape the body's resistance to extreme influences and diseases. For the most part, simulations of extreme conditions employ healthy animals, but in clinical practice, extreme factors have a greater effect on people with chronic diseases. Therefore, to make extrapolation objective, a group of researchers has suggested [47] working with animals that have a persistent pathological condition, for example, an unpaired variation of the organ that is typically paired. We have not taken into account that study when comparing methods of modeling hypothermia in rodents because it offers insufficient data on the characteristics of the simulated conditions in animals. However, the report confirms that viability of the animals under extreme conditions after nephrectomy decreases, and shows the possibility of implementing this approach in the experiments designed to assess action of a drug.

In connection, modeling of pathological states is an important aspect of preclinical research in the interests of the Arctic medicine. Residents of the AR have their own characteristic diseases, the most common of which is frostbite. It is defined as a complex of pathological changes occurring as a result of local and general cooling of the body. There are several experimental models [48] used in the investigations of the mechanisms of condition development and treatment, as well as assessment of drugs and therapeutic methods. It is possible to induce different degrees of frostbite, but reproducibility for some of them is low. Moreover, in most cases the damaged parts are the limbs, and in the animal experiments it is the trunk that is injured. Therefore, it is necessary to further develop the models in order to open the possibility of combining cryoinjury with other effects and ensure reproducibility of wound characteristics [48–49].

We have found no studies simulating other diseases that may be complicated by the extreme environment factors peculiar to the Arctic. This is another problem in further preclinical studies, since the course of a pathological process without treatment under extreme conditions will require additional modeling and investigation. Assessment of efficacy of a drug will include comparison with its action on animals kept under standard conditions. We have designed models of a lacerated wound [44], frostbite and chemical burn under conditions simulating those of the AR. The latter mice model has shown that inflicting a third-degree burn to animals kept in a climate chamber for a day takes less time compared to animals that were not placed in a hermetic chamber (unpublished data). A possible reason therefor is the significant drop of skin temperature compared to rectal temperature, since temperature of back and limbs of the animals differs from temperature of their rectum by about 0.5 and 6 °C [46]. Similar nuances can also arise in other models of diseases, primarily those related to the skin, musculoskeletal system and respiratory system.

CONCLUSION

Despite the accumulated knowledge and experience, there is no tested and certified set of methods for assessment of impact of cold on the body of an animal [37]. This is also true for *in vivo* investigations of hypoxic conditions. The role of the photoperiod in extreme conditions is often overlooked. In general, the complexity and multitude of factors accounted for in the designs of such experiments translate into lack of a unified approach to them. Yet, hypoxia and hypothermia experiments under controlled conditions are necessary not only for pharmacological but also for physiological studies [37], which, in turn, requires development of a theoretical and regulatory framework, as well as methodological recommendations that, combined, would enable development of reproducible models.

A matter of special importance in design of drugs for the Arctic is their optimal form, improvement of properties of injectable drugs with the help of cryoprotectants. Another topical area that should be highlighted is development of adaptogens.

In our opinion, the most difficult task is modeling the extreme conditions of the AR in animal experiments, with hypothermia, hypoxia and photoperiodism being the key factors. Today, it is possible to compile recommendations for conducting such studies on small laboratory animals, but there are significant gaps in understanding what methods can be used to recreate the impact of the Arctic factors in a large animal model experiment. It is necessary to develop criteria of evaluation of all the listed conditions and standardize the technical base.

A separate area of research is modeling of pathological conditions (diseases) that may be complicated by the effects of aggressive factors peculiar to the AR. Developments in this area can stimulate design of the new, more effective drugs that will be usable not only in the Far North.

There is a positive trend that should be mentioned: every year, there is more and more data published on the capabilities of organisms to adapt to the extreme conditions of the AR. The techniques and hermetic chambers being developed for *in vivo* experiments may also be applicable in other areas, for example, in development of treatment regimens for victims of catastrophes associated with rapid cooling, such as submarine accidents. In terms of the extreme natural factors, these studies have much in common with investigations revolving around the highlands, where the leading role is also played by the oxygen partial pressure drop that stimulates development of tissue hypoxia. Low air temperature with significant daily fluctuations and strong winds increase the risk of respiratory diseases, frostbite and chills [50].

References

1. Azarov II, Butakov SS, Zholus BI, Zetkin AYu, Remmer VN. Opyt soxraneniya zdorov'ya voennosluzhashhix v Arktike v povsednevnoj deyatel'nosti i chrezvychajnyx situaciyax. Morskaya medicina. 2017; 3 (3): 102–111. Russian.
2. Kazakevich EV, Arxipovskij VL, Sereda AP, Abakumov AA. Osobennosti organizacii medicinskoj pomoshhi moryakam v usloviyax Arktiki. Medicina ehkstremal'nyx situacij. 2017; 4 (62): 15–20. Russian.
3. O nacional'nyx celyax i strategicheskix zadachax razvitiya Rossijskoj Federacii na period do 2024 goda: ukaz Prezidenta Rossijskoj Federacii ot 07.05.2018 # 204. Dostupno po ssylke: http://www.kremlin.ru/acts/bank/43027. Russian.
4. Sergejchik OI, Yaroslavskaya EI, Plyusnin AV. Vliyanie faktorov vneshnej sredy na risk serdechno-sosudistyx zabolevanij naseleniya Arktiki. Zhurnal mediko-biologicheskix issledovanij. 2022; 10 (1): 64–72. Russian.
5. Krutikov AV, Smirnova OO, Bocharova LK. Strategy for the Development of the Russian Arctic. Results and Prospects. Artic and North. 2020; (40): 213–26.
6. Solonin YuG, Bojko ER. Mediko-fiziologicheskie problemy v Arktike. Izvestiya Komi nauchnogo centra UrO RAN. 2017; 4 (32): 33–40. Russian.
7. Zhuravel VP. Rossijskaya armiya, MChS i rosgvardiya v Arktike: voprosy bezopasnosti i mezhdunarodnogo sotrudnichestva. Nauchno-analiticheskij vestnik Instituta Evropy RAN. 2018; (5): 158–164. Russian.
8. Watts N, Amann M, Arnell N, Ayeb-Karlsson S, Beagley J, Belesova K, et al. The 2020 report of the Lancet Countdown on health and climate change: responding to converging crises. The Lancet. 2021; 397 (10269): 129–170.
9. Tixonov DG. Arkticheskaya medicina. Yakutsk: Izd-vo YaNC SO RAN, 2010; 320 s. Russian.
10. Pryanichnikov SV. Psixofiziologicheskoe sostoyanie organizma v zavisimosti ot dlitel'nosti prebyvaniya v vysokix shirotax Arktiki. Ehkologiya cheloveka. 2020; 27 (12): 4–10. Russian.
11. Grimaldi S, Partonen T, Haukka J, Aromaa A, Lonnqvist. Seasonal vegetative and affective symptoms in the Finnish general population: testing the dual vulnerability and latitude effect hypotheses. Nordic Journal of Psychiatry. 2009; 63 (5): 397–404.
12. Grishin OV, Ustyuzhaninova NV. Dyxanie na Severe. Funkciya. Struktura. Rezervy. Patologiya. Novosibirsk: Art-Avenue, 2006; 253 s. Russian.
13. Panin LE. Fundamental'nye problemy pripolyarnoj i arkticheskoj mediciny. Sibirskij nauchnyj medicinskij zhurnal. 2013; 33 (6): 5–10. Russian.
14. Nagibovich OA, Uxovskij DM, Zhekalov AN, Tkachuk NA, Arzhavkina LG, Bogdanova EG, i dr. Mexanizmy gipoksii v Arkticheskoj zone Rossijskoj Federacii. Vestnik Rossijskoj voenno-medicinskoj akademii. 2016; (2): 202–5. Russian.
15. Zadnipryanyj IV, Sataeva TP, Tret'yakova OS. Patomorfologicheskie izmeneniya miokarda krys pri vozdejstvii gipobaricheskoj xolodovoj gipoksii. Operativnaya xirurgiya i klinicheskaya anatomiya. 2019; 3 (2): 13–18. Russian.
16. McDonald A, Stubbs R, Lartey P, Kokot S. Environmental Injuries: Hypothermia and Hypothermia. MacEwan University Student Journal. 2020; 4 (1): 1–14.
17. Ovechkina ES, Ovechkin FYu. Patofiziologiya cheloveka v usloviyax severa Rossii. Byulleten' nauki i praktiki. 2021; 7 (8): 185–91. Russian.
18. Kochan TI. Sezonnye izmeneniya pokazatelej uglevodnogo, lipidnogo i belkovogo obmena u zhitelej Evropejskogo Severa. Evrazijskij soyuz uchenyx. 2016; 7–1 (28): 32–35. Russian.
19. Gribanov AV, Anikina NYu, Kotcova ON, Vilova TV. Sezonnaya dinamika cerebral'nogo ehnergoobmena u cheloveka v arkticheskoj zone. Ehkologiya cheloveka. 2021; (5): 13–19. Russian.
20. Gaspar LS, Alvaro AR, Carmo-Silva S, Mendes AF, Relogio A, Cavadas C. The importance of determining circadian parameters in pharmacological studies. British Journal of Pharmacology. 2019; 176 (16): 2827–47.
21. Ulyanovskaya SA, Bazhenov DV, Shestakova VG, Kalinkin MN. Vliyanie klimatogeograficheskix faktorov Severa na adaptivnye reakcii organizma cheloveka. Patologicheskaya fiziologiya i ehksperimental'naya terapiya. 2020; 64 (1): 147–154. Russian.
22. Arendt J, Middleton B. Human seasonal and circadian studies in Antarctica (Halley, 75 S). General and comparative endocrinology. 2018; 258: 250–8.
23. Ganapol'skij VP, Agafonov PV, Matycyn VO. Modelirovanie xolodo-stressovoj dezadaptacii u krys s cel'yu razrabotki metodov ee farmakologicheskoj korrekcii. Rossijskie biomedicinskie issledovaniya. 2022; 7 (1): 3–15. Russian.
24. Finger AM, Dibner C, Kramer A. Coupled network of the circadian

clocks: a driving force of rhythmic physiology. FEBS letters. 2020; 594 (17): 2734–69.
25. Xasnulin VI, Xasnulin PV. Sovremennye predstavleniya o mexanizmax formirovaniya severnogo stressa u cheloveka v vysokix shirotax. Ehkologiya cheloveka. 2012; (1): 3–11. Russian.
26. Coiffard B, Diallo AB, Mezouar S, Leone M, Mege J-L. A tangled threesome: circadian rhytnm, body temperature variations, and the Immune System. Biology. 2021; 10 (1): 65.
27. Kelexsashvili LV, Volchenkova AV, Sokolova AS. Gigienicheskoe obosnovanie metodov korrekcii sindroma «snezhnaya slepota» v usloviyax Arktiki. V sbornike: Materialy III Dal'nevostochnogo medicinskogo molodezhnogo foruma; 2–4 oktyabrya 2019; Xabarovsk. Aktual'nye voprosy sovremennoj mediciny. 2019: 317–320. Russian.
28. Soldatov EA, Golota AS, Kornilova AA, Krassij AB, Levando KK, Chuvashev ML. i dr. Medicinskoe obespechenie v Arktike: 2015 g. Voenno-medicinskij zhurnal. 2016; 337 (5): 44–51. Russian.
29. Miroshnichenko YuV, Kononov VN, Rodionov EO, Mustaev OZ, Soldatov EA, Chuvashev ML, i dr. Vliyanie osobyx mediko-geograficheskix uslovij Arktiki na obespechenie soedinenij i voinskix chastej medicinskim imushhestvom. Farmaciya i farmakologiya. 2017; 5 (4): 368–79. Russian.
30. Kotenko PK, Shevcov VI. Analiz mediko-social'nyx faktorov, opredelyayushhix perspektivnyj oblik sistemy okazaniya medicinskoj pomoshhi postradavshim v chrezvychajnyx situaciyax v Arkticheskoj zone Rossijskoj Federacii. Morskaya medicina. 2019; 4 (4): 44–54. Russian.
31. Moffatt SE. Hypothermia in trauma. Emergency Medicine Journal. 2013; 30 (12): 989–996.
32. Raja R, Bryant SJ, Wilkinson BL, Bryant G. The need for novel cryoprotectants and cryopreservation protocols: Insights into the importance of biophysical investigation and cell permeability. Biochimica et Biophysica Acta (BBA)-General Subjects. 2021; 1865 (1): 129749.
33. Bozkurt Y, editor. Cryopreservation biotechnology in biomedical and biological sciences. BoD-Books on Demand; 2018.
34. Warner RM, Brown KS, Benson JD, Eroglu A, Higgins AZ. Multiple cryoprotectant toxicity model for virification solution optimization. Cryobiology. 2022; 108: 1–9.
35. Kurkin VA, Petruxina IK, Akushskaya AS. Issledovanie nomenklatury adaptogennyx lekarstvennyx preparatov, predstavlennyx na farmacevticheskom rynke Rossijskoj Federacii. Fundamental'nye issledovaniya. 2014; 8 (4): 898–902. Russian.
36. Pomatto LCD, Davies KJA. Adaptive homestasis and the free radical theory of ageing. Free Radical Biology and Medicine. 2018; 124: 420–30.
37. Golubev VN, Korolev YuN, Belokopytova EV. Individual'naya strategiya adaptacii dyxatel'noj sistemy cheloveka kak reakciya na gipoksicheskuyu gipoksiyu. Izvestiya Rossijskoj Voenno-medicinskoj akademii. 2019; 38 (3): 172–7. Russian.
38. Karkishhenko NN, Karkishhenko VN, Shustov EB, Kanapadze GD, Revyakin AO, Semenov XX. Biomedicinskoe (doklinicheskoe) izuchenie antigipoksicheskoj aktivnosti lekarstvennyx sredstv. M.: Nauchnyj centr biomedicinskix texnologij FMBA, 2017; 97 s. Russian.
39. Bobrov IP, Lepilov AV, Guldaeva ZN, Dolgatov AYu, Alymova EE, Kryuchkova NG, i dr. Tuchnokletochnaya infil'traciya legkix krys posle gipotermii. Sovremennye problemy nauki i obrazovaniya. 2019; (1): 1–8. Russian.
40. Klichxanov NK, Ismailova ZhG, Astaeva MD. Intensivnost' svobodnoradikal'nyx processov v krovi krys pri glubokoj gipotermii i v xode samosogrevaniya. Acta Biomedica Scientifica. 2016; 1 (5): 104–9. Russian.
41. Shustov EB, Kapanadze GD, Fokin YuV, Matveenko EL. Metodicheskie osobennosti biomedicinskix issledovanij vliyaniya farmakologicheskix sredstv na ustojchivost' organizma k ostroj obshhej gipotermii. Biomedicina. 2017; (3): 4–15. Russian.
42. Akimov AG, Egorova MA. Vliyanie gipotermii na impul'snuyu aktivnost' nejronov pervichnoj sluxovoj kory domovoj myshi (Mus musculus). Integrativnaya fiziologiya. 2022; 3 (1): 100–9. Russian.
43. Aksyonova GE, Logvinovich OS, Fialkovskaya LA, Afanasev VN, Ignatev DA, Kolomijceva IK, i dr. Vliyanie gipotermii na aktivnost' ornitindekarboksilazy v tkanyax krys. Doklady akademii nauk. 2009; 428 (4): 547–9. Russian.
44. Volkova MV, Boyarincev VV, Trofimenko AV, Rybalkin SP, Kovaleva EV, Biryukov SA, i dr. Ehffektivnost' primeneniya mezenximal'nyx stromal'nyx kletok dlya lecheniya rvano-ushiblennyx ran v usloviyax gipotermii i gipoksii. Izvestiya Rossijskoj voenno-medicinskoj akademii. 2022; 41 (3): 261–8. Russian.
45. Lycheva NA, Shaxmatov II, Kiselev VI, Vdovin VM. Izuchenie otstavlennogo vliyaniya gipotermii na parametry sistemy gemostaza u krys. Sibirskij nauchnyj medicinskij zhurnal. 2014; 34 (4): 25–29. Russian.
46. Abrashova TV, Gushhin YaA, Kovaleva MA, Rybakova AV, Selezneva AI, Sokolova AP, i dr. Fiziologicheskie, bioximicheskie i biometricheskie pokazateli normy ehksperimental'nyx zhivotnyx. SPb: LEMA, 2013; 116 s. Russian.
47. Shormanov IS, Kosenko MV, Petrovskij AK, Petrovskaya AYu, Andreeva LA, Fedorov VN, i dr. Farmakologicheskaya podderzhka adaptivnyx vozmozhnostej organizma v usloviyax ehksperimental'nogo operacionnogo stressa. Medicinskij vestnik Bashkortostana. 2016; 11 (2): 47–52. Russian.
48. Bajkalov GI, Ershov KI, Baxareva KI. Ehksperimental'nye metody modelirovaniya otmorozhenij. Sibirskij medicinskij vestnik. 2020; (3): 44–49. Russian.
49. Handford C, Thomas O, Imray CHE. Frostbite. Emergency Medicine Clinics. 2017; 35 (2): 281–99.
50. Soldatov EA, Anisimov AS, Blinov VA, Belkin NV. Sovershenstvovanie medicinskogo obespecheniya meropriyatij po podgotovke voennosluzhashhix dlya vypolneniya zadach v vysokogornoj mestnosti. Izvestiya Rossijskoj voenno-medicinskoj akademii. 2017; 36 (2): 3–8. Russian.

Литература

1. Азаров И. И., Бутаков С. С., Жолус Б. И., Зеткин А. Ю., Реммер В. Н. Опыт сохранения здоровья военнослужащих в Арктике в повседневной деятельности и чрезвычайных ситуациях. Морская медицина. 2017; 3 (3): 102–111.
2. Казакевич Е. В., Архиповский В. Л., Середа А. П., Абакумов А. А. Особенности организации медицинской помощи морякам в условиях Арктики. Медицина экстремальных ситуаций. 2017; 4 (62): 15–20.
3. О национальных целях и стратегических задачах развития Российской Федерации на период до 2024 года: указ Президента Российской Федерации от 07.05.2018 № 204. Доступно по ссылке: http://www.kremlin.ru/acts/bank/43027.
4. Сергейчик О. И., Ярославская Е. И., Плюснин А. В. Влияние факторов внешней среды на риск сердечно-сосудистых заболеваний населения Арктики. Журнал медико-биологических исследований. 2022; 10 (1): 64–72.
5. Krutikov AV, Smirnova OO, Bocharova LK. Strategy for the Development of the Russian Arctic. Results and Prospects. Artic and North. 2020; (40): 213–26.
6. Солонин Ю. Г., Бойко Е. Р. Медико-физиологические проблемы в Арктике. Известия Коми научного центра УрО РАН. 2017; 4 (32): 33–40.
7. Журавель В. П. Российская армия, МЧС и росгвардия в Арктике: вопросы безопасности и международного сотрудничества. Научно-аналитический вестник Института Европы РАН. 2018; (5): 158–164.
8. Watts N, Amann M, Arnell N, Ayeb-Karlsson S, Beagley J, Belesova K, et al. The 2020 report of the Lancet Countdown on health and climate change: responding to converging crises. The Lancet. 2021; 397 (10269): 129–170.
9. Тихонов Д. Г. Арктическая медицина. Якутск: Изд-во ЯНЦ СО РАН, 2010; 320 с.
10. Пряничников С. В. Психофизиологическое состояние организма в зависимости от длительности пребывания в

высоких широтах Арктики. Экология человека. 2020; 27 (12): 4–10.
11. Grimaldi S, Partonen T, Haukka J, Aromaa A, Lonnqvist. Seasonal vegetative and affective symptoms in the Finnish general population: testing the dual vulnerability and latitude effect hypotheses. Nordic Journal of Psychiatry. 2009; 63 (5): 397–404.
12. Гришин О. В., Устюжанинова Н. В. Дыхание на Севере. Функция. Структура. Резервы. Патология. Новосибирск: Art-Avenue, 2006; 253 с.
13. Панин Л. Е. Фундаментальные проблемы приполярной и арктической медицины. Сибирский научный медицинский журнал. 2013; 33 (6): 5–10.
14. Нагибович О. А., Уховский Д. М., Жекалов А. Н., Ткачук Н. А., Аржавкина Л. Г., Богданова Е. Г. и др. Механизмы гипоксии в Арктической зоне Российской Федерации. Вестник Российской военно-медицинской академии. 2016; (2): 202–5.
15. Заднипряный И. В., Сатаева Т. П., Третьякова О. С. Патоморфологические изменения миокарда крыс при воздействии гипобарической холодовой гипоксии. Оперативная хирургия и клиническая анатомия. 2019; 3 (2): 13–18.
16. McDonald A, Stubbs R, Lartey P, Kokot S. Environmental Injuries: Hypothermia and Hypothermia. MacEwan University Student Journal. 2020; 4 (1): 1–14.
17. Овечкина Е. С., Овечкин Ф. Ю. Патофизиология человека в условиях севера России. Бюллетень науки и практики. 2021; 7 (8): 185–91.
18. Кочан Т. И. Сезонные изменения показателей углеводного, липидного и белкового обмена у жителей Европейского Севера. Евразийский союз ученых. 2016; 7–1 (28): 32–35.
19. Грибанов А. В., Аникина Н. Ю., Котцова О. Н., Вилова Т. В. Сезонная динамика церебрального энергообмена у человека в арктической зоне. Экология человека. 2021; (5): 13–19.
20. Gaspar LS, Alvaro AR, Carmo-Silva S, Mendes AF, Relogio A, Cavadas C. The importance of determining circadian parameters in pharmacological studies. British Journal of Pharmacology. 2019; 176 (16): 2827–47.
21. Ульяновская С. А., Баженов Д. В., Шестакова В. Г., Калинкин М. Н. Влияние климатогеографических факторов Севера на адаптивные реакции организма человека. Патологическая физиология и экспериментальная терапия. 2020; 64 (1): 147–154.
22. Arendt J, Middleton B. Human seasonal and circadian studies in Antarctica (Halley, 75 S). General and comparative endocrinology. 2018; 258: 250–8.
23. Ганапольский В. П., Агафонов П. В., Матыцын В. О. Моделирование холодо-стрессовой дезадаптации у крыс с целью разработки методов ее фармакологической коррекции. Российские биомедицинские исследования. 2022; 7 (1): 3–15.
24. Finger AM, Dibner C, Kramer A. Coupled network of the circadian clocks: a driving force of rhythmic physiology. FEBS letters. 2020; 594 (17): 2734–69.
25. Хаснулин В. И., Хаснулин П. В. Современные представления о механизмах формирования северного стресса у человека в высоких широтах. Экология человека. 2012; (1): 3–11.
26. Coiffard B, Diallo AB, Mezouar S, Leone M, Mege J-L. A tangled threesome: circadian rhytnm, body temperature variations, and the Immune System. Biology. 2021; 10 (1): 65.
27. Келехсашвили Л. В., Волченкова А. В., Соколова А. С. Гигиеническое обоснование методов коррекции синдрома «снежная слепота» в условиях Арктики. В сборнике: Материалы III Дальневосточного медицинского молодежного форума; 2–4 октября 2019; Хабаровск. Актуальные вопросы современной медицины. 2019: 317–320.
28. Солдатов Е. А., Голота А. С., Корнилова А. А., Крассий А. Б., Левандо К. К., Чувашев М. Л. и др. Медицинское обеспечение в Арктике: 2015 г. Военно-медицинский журнал. 2016; 337 (5): 44–51.
29. Мирошниченко Ю. В., Кононов В. Н., Родионов Е. О., Мустаев О. З., Солдатов Е. А., Чувашев М. Л. и др. Влияние особых медико-географических условий Арктики на обеспечение соединений и воинских частей медицинским имуществом. Фармация и фармакология. 2017; 5 (4): 368–79.
30. Котенко П. К., Шевцов В. И. Анализ медико-социальных факторов, определяющих перспективный облик системы оказания медицинской помощи пострадавшим в чрезвычайных ситуациях в Арктической зоне Российской Федерации. Морская медицина. 2019; 4 (4): 44–54.
31. Moffatt SE. Hypothermia in trauma. Emergency Medicine Journal. 2013; 30 (12): 989–996.
32. Raja R, Bryant SJ, Wilkinson BL, Bryant G. The need for novel cryoprotectants and cryopreservation protocols: Insights into the importance of biophysical investigation and cell permeability. Biochimica et Biophysica Acta (BBA)-General Subjects. 2021; 1865 (1): 129749.
33. Bozkurt Y, editor. Cryopreservation biotechnology in biomedical and biological sciences. BoD-Books on Demand; 2018.
34. Warner RM, Brown KS, Benson JD, Eroglu A, Higgins AZ. Multiple cryoprotectant toxicity model for virification solution optimization. Cryobiology. 2022; 108: 1–9.
35. Куркин В. А., Петрухина И. К., Акушская А. С. Исследование номенклатуры адаптогенных лекарственных препаратов, представленных на фармацевтическом рынке Российской Федерации. Фундаментальные исследования. 2014; 8 (4): 898–902.
36. Pomatto LCD, Davies KJA. Adaptive homestasis and the free radical theory of ageing. Free Radical Biology and Medicine. 2018; 124: 420–30.
37. Голубев В. Н., Королев Ю. Н., Белокопытова Е. В. Индивидуальная стратегия адаптации дыхательной системы человека как реакция на гипоксическую гипоксию. Известия Российской Военно-медицинской академии. 2019; 38 (3): 172–7.
38. Каркищенко Н. Н., Каркищенко В. Н., Шустов Е. Б., Канападзе Г. Д., Ревякин А. О., Семенов Х. Х. Биомедицинское (доклиническое) изучение антигипоксической активности лекарственных средств. М.: Научный центр биомедицинских технологий ФМБА, 2017; 97 с.
39. Бобров И. П., Лепилов А. В., Гулдаева З. Н., Долгатов А. Ю., Алымова Е. Е., Крючкова Н. Г. и др. Тучноклеточная инфильтрация легких крыс после гипотермии. Современные проблемы науки и образования. 2019; (1): 1–8.
40. Кличханов Н. К., Исмаилова Ж. Г., Астаева М. Д. Интенсивность свободнорадикальных процессов в крови крыс при глубокой гипотермии и в ходе самосогревания. Acta Biomedica Scientifica. 2016; 1 (5): 104–9.
41. Шустов Е. Б., Капанадзе Г. Д., Фокин Ю. В., Матвеенко Е. Л. Методические особенности биомедицинских исследований влияния фармакологических средств на устойчивость организма к острой общей гипотермии. Биомедицина. 2017; (3): 4–15.
42. Акимов А. Г., Егорова М. А. Влияние гипотермии на импульсную активность нейронов первичной слуховой коры домовой мыши (Mus musculus). Интегративная физиология. 2022; 3 (1): 100–9.
43. Аксёнова Г. Е., Логвинович О. С., Фиалковская Л. А., Афанасьев В. Н., Игнатьев Д. А., Коломийцева И. К. и др. Влияние гипотермии на активность орнитиндекарбоксилазы в тканях крыс. Доклады академии наук. 2009; 428 (4): 547–9.
44. Волкова М. В., Бояринцев В. В., Трофименко А. В., Рыбалкин С. П., Ковалева Е. В., Бирюков С. А. и др. Эффективность применения мезенхимальных стромальных клеток для лечения рвано-ушибленных ран в условиях гипотермии и гипоксии. Известия Российской военно-медицинской академии. 2022; 41 (3): 261–8.
45. Лычева Н. А., Шахматов И. И., Киселев В. И., Вдовин В. М. Изучение отставленного влияния гипотермии на параметры системы гемостаза у крыс. Сибирский научный медицинский журнал. 2014; 34 (4): 25–29.
46. Абрашова Т. В., Гущин Я. А., Ковалева М. А., Рыбакова А. В., Селезнева А. И., Соколова А. П. и др. Физиологические, биохимические и биометрические показатели нормы экспериментальных животных.СПб: ЛЕМА, 2013; 116 с.
47. Шорманов И. С., Косенко М. В., Петровский А. К., Петровская А. Ю., Андреева Л. А., Федоров В. Н. и др. Фармакологическая поддержка адаптивных возможностей организма в условиях

экспериментального операционного стресса. Медицинский вестник Башкортостана. 2016; 11 (2): 47–52.
48. Байкалов Г. И., Ершов К. И., Бахарева К. И. Экспериментальные методы моделирования отморожений. Сибирский медицинский вестник. 2020; (3): 44–49.
49. Handford C, Thomas O, Imray CHE. Frostbite. Emergency Medicine Clinics. 2017; 35 (2): 281–99.
50. Солдатов Е. А., Анисимов А. С., Блинов В. А., Белкин Н. В. Совершенствование медицинского обеспечения мероприятий по подготовке военнослужащих для выполнения задач в высокогорной местности. Известия Российской военно-медицинской академии. 2017; 36 (2): 3–8.

IDENTIFICATION OF PHOSPHONYLATED PEPTIDES USING A MALDI TARGET FUNCTIONALIZED WITH LANTHANUM STEARATE

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As markers of intoxication, adducts of blood proteins with organophosphorus compounds (OPs) allow establishing the fact of poisoning and, furthermore, enable identification of the OPs by the attached residue. This study aimed to develop a method of specific and selective extraction of blood protein adducts carrying OPs on the surface of a matrix-assisted laser desorption/ionization (MALDI) target functionalized with multimolecular structures based on lanthanum stearate using metal affinity chromatography. We have shown the ability of adsorbent to retain both full-size and dealkylated adducts of blood proteins with OPs. The developed method allowed extraction and identification of peptides of human serum albumin and human butyrylcholinesterase modified with various OPs (after incubation of human blood plasma with OPs in concentrations from 1 to 100 ng/mL), which makes this approach applicable for the purposes of OPs identification in the context of investigation of real cases of intoxication.

Keywords: Lanthanum stearate, Langmuir–Blodgett films, serum albumin adducts, butyrylcholinesterase adducts, organophosphorus compounds

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ИДЕНТИФИКАЦИЯ ФОСФОНИЛИРОВАННЫХ ПЕПТИДОВ С ПРИМЕНЕНИЕМ МАЛДИ-МИШЕНИ, ФУНКЦИОНАЛИЗИРОВАННОЙ СТЕАРАТОМ ЛАНТАНА

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Аддукты белков крови с фосфорорганическими соединениями (ФОС) как маркеры интоксикации позволяют не только установить факт отравления, но и идентифицировать ФОС по присоединившемуся остатку. Целью работы было разработать методику специфичной и селективной экстракции аддуктов белков крови с ФОС на поверхности матрично-активированной лазерной десорбцией/ионизацией (МАЛДИ) мишени, функционализированной мультимолекулярными структурами на основе стеарата лантана, с помощью металл-аффинной хроматографии. Показано, что сорбент способен удерживать как полноразмерные, так и деалкилированные аддукты белков крови с ФОС. С помощью предложенной методики были экстрагированы и идентифицированы пептиды сывороточного альбумина и бутирилхолинэстеразы человека, модифицированные различными ФОС, после инкубации плазмы крови человека с ФОС в диапазоне концентраций 1 × 100 нг/мл, что позволяет использовать этот подход для идентификации ФОС при расследовании реальных случаев интоксикаций.

Ключевые слова: стеарат лантана, пленки Ленгмюра–Блоджетт, фосфорорганические соединения (ФОС), аддукты сывороточного альбумина, аддукты бутирилхолинэстеразы

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Organophosphorus compounds (OPs) form a large class of compounds used in industrial fabrication and agriculture that can inhibit serine esterases and proteases [1]. Highly toxic varieties of OPs are included in Schedule 1 of the Chemical Weapons Convention [2].

OPs adducts to human serum albumin (SA) and human butyrylcholinesterase (BChE) are the key long-lived biomarkers of OPs intoxication. Their half-life is 20–25 days and 11–14 days (SA and BChE, respectively) [3, 4], makes them usable as means of determining the agent in blood samples several weeks after poisoning. The OPs adducts to BChE are the most reliable biomarkers enabling establishing of poisoning with the degree of enzyme inhibition at less than 1% [5]. There have been developed many approaches to determination of these adducts in complex samples that are based on the targeted analysis performed by means of high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) [6–8]. However, MALDI mass spectrometry (MS), although not used as frequently, has certain advantages for screening of blood protein adducts to OPs when the aim is to identify unknown BChE inhibitors. Determination of the mass difference (ΔM) between modified and native peptides allows compiling the gross formula of OPs.

MALDI-MS has grown popular due to its simplicity, reliability, high sensitivity and capability to analyze a wide range of masses

[9–11]. However, its applicability is limited significantly because of the signal suppression effect that emerges when there is a large number of components; it is major hindrance to successful detection of trace amounts of substances. Therefore, this method often requires preliminary sample preparation aimed at either reduction of the number of components in the mixture or analyte enrichment.

Recently, there have been suggested approaches that make the MALDI target not only a sample carrier but also a factor in the analysis protocol, giving it an additional functionality [12]. For this purpose, the surface of the steel substrate is functionalized and thus given the necessary properties [13, 14], which allows conducting various analytical procedures on the target surface before the MS analysis ("lab-on-a-plate"), with the end result being a more effective analysis overall. The "lab-on-a-plate" format reduces the amount of sample needed for the analysis, cuts losses on the adsorbent during extraction, unlocks the possibility of working with very small volume samples and minimizes sample preparation to a few simple steps. A target functionalized with an adsorbent can be used for direct *in situ* enrichment of a sample followed by MALDI-MS.

Collapsed Langmuir monolayers are thin-film adsorbents with unique surface build of metal atoms [15]. They are both hydrophilic and hydrophobic [16] and show sufficient resistance to external influences [17]. Earlier, it was suggested that collapsed monolayers of lanthanum stearate (FLa) may be used in spin columns for extraction of a number of organic and bioorganic compounds [18], including human SA adducts with 2-(fluoromethylphosphoryl)oxy-3.3-dimethylbutane. We have proposed an approach that enables formation of both a collapsed FLa monolayer and its multimolecular structures directly on the MALDI target [19, 20]. The resulting material has a well-developed surface with good adhesion properties. It adheres well to a polished MALDI target and retains its qualities as a metal-affinity adsorbent. The purpose of this study was to investigate the possibility of specific extraction of blood protein adducts with OPs on the surface of a MALDI target functionalized with FLa multimolecular structures in the "lab-on-a-plate" format.

METHODS

Preparation of lanthanum stearate monolayers on a MALDI target

A polished MALDI target (MTP 384 target plate polished steel BC; Bruker Daltonics, Germany) was used as a substrate. We added 0.6 µl (a drop) of lanthanum (III) salt aqueous solution with a concentration of 1 mM onto the surface of the MALDI target. On top of the drop we added 0.6 µl of the saturated solution of stearic acid in n-hexane. The procedure was repeated twice, with three monolayers being the result thereof. After that, we removed the remains of the lanthanum salt solution drop. A drop of 0.6 µl of the lanthanum salt aqueous solution was then again applied to the same target well, followed by three applications of the stearic acid solution. As a result, the MALDI target spot was modified with six FLa monolayers. Seeking to completely remove the lanthanum salt, we washed the applied adsorbent off three times with deionized water by adding 8 µl of water to the spot, leaving it there for 1 minute and then removing with a dispenser.

Preparation of samples of proteins modified with OPs

To prepare the modified serum albumin samples, within two hours after collection we supplemented samples of donated K2 EDTA human blood plasma with a series of isopropyl alcohol dilutions of 2-(fluoromethylphosphoryl)oxy-3,3-dimethylbutane, CAS № 96-64-0 (hereinafter PFMP) to final concentrations of 1 ng/mL — 1 µg/mL, as suggested in a previously published article [5]. The first step of the process of producing samples of modified BChE was to isolate the enzyme from the donated human blood plasma by affinity chromatography on procainamide sepharose. Then we added 1 mg/ml of PFMP to the purified enzyme to the final concentration of 100 µg/ml, which triggered inhibition of over 90% of the enzyme. The incubated and control blood plasma was hydrolyzed with the pepsin, and the incubated and control BChE enzyme was hydrolyzed with trypsin. To assess the specificity and selectivity of adsorption of modified peptides on FLa, we mixed the hydrolyzed incubated and control blood plasma and BChE enzyme in various proportions. With the aim to establish the limits of detection of modified BChE in blood plasma, the enzyme was isolated from 250 µL of plasma using immunoprecipitation [21]. All the experiments were performed in four replicates.

MALDI mass spectrometry

The mass spectra were acquired by means of an UltrafleXtreme tandem time-of-flight mass spectrometer (Bruker Daltonics; Germany). The mass spectra were registered in the positive reflectron mode in the m/z range of 1000–3200. The number of shots accumulated per one spectrum was 20000, the shot frequency — 2000 Hz. We used the Flex Control 3.4 and Flex Analysis 3.4 software to register and interpret the spectral data. The tandem mass spectra were obtained in the LIFT mode, with precursor ions fragmented under the collision-induced dissociation (CID) conditions.

Sample preparation and MALDI-MS of samples of the Seventh Official OPCW Biomedical Proficiency Test

Samples from the OPCW (pooled human blood plasma) were received in the context of participation of the Research Institute of Hygiene, Occupational Pathology and Human Ecology in the Seventh Official OPCW Biomedical Proficiency Test. VX agent (O-ethyl-S-2-diisopropylaminoethyl methylphosphonothiolate, CAS № 50782-69-9) were added to the samples in the OPCW laboratory to a final concentration of 10 ng/ml, and GE agent (O-isopropyl ethyl phosphonofluoride, CAS № 1189-87-3) — to a final concentration of 20 ng/ml.

BChE was isolated from the samples by immunoprecipitation, with the help of magnetic microspheres of the Dynabeads Antibody Coupling Kit (143.11D; Invitrogen, USA) that were covalently coupled with BChE antibodies (Pierce BChE monoclonal mouse antibody HAH 0020102; Thermo, USA). The microspheres were prepared according to the Dynabeads Antibody Coupling Kit manufacturer's protocol. An earlier described technique enabled isolation of BChE from plasma [21].

Trypsin (Sigma-Aldrich; USA) was used for enzymatic hydrolysis of BChE. We washed 100 µl of BChE-containing eluate with water three times in the Amicon Ultra-0.5, 30K centrifuge filters (UFC5030BK; Merck Millipore, USA). Next, the volume of the sample was adjusted to 100 µl with 25 mM of aqueous ammonium bicarbonate solution, then supplemented with 5 µl of trypsin (0.1 mg/ml) and incubated at 37 °C for 4 hours, then we added another 5 µl of trypsin solution (0.1 mg /ml) and incubated the sample at 37°C for 16 hours.

We performed metal affinity chromatography (IMAC) on the FLa-containing adsorbent directly on the MALDI target with deposited monolayers.

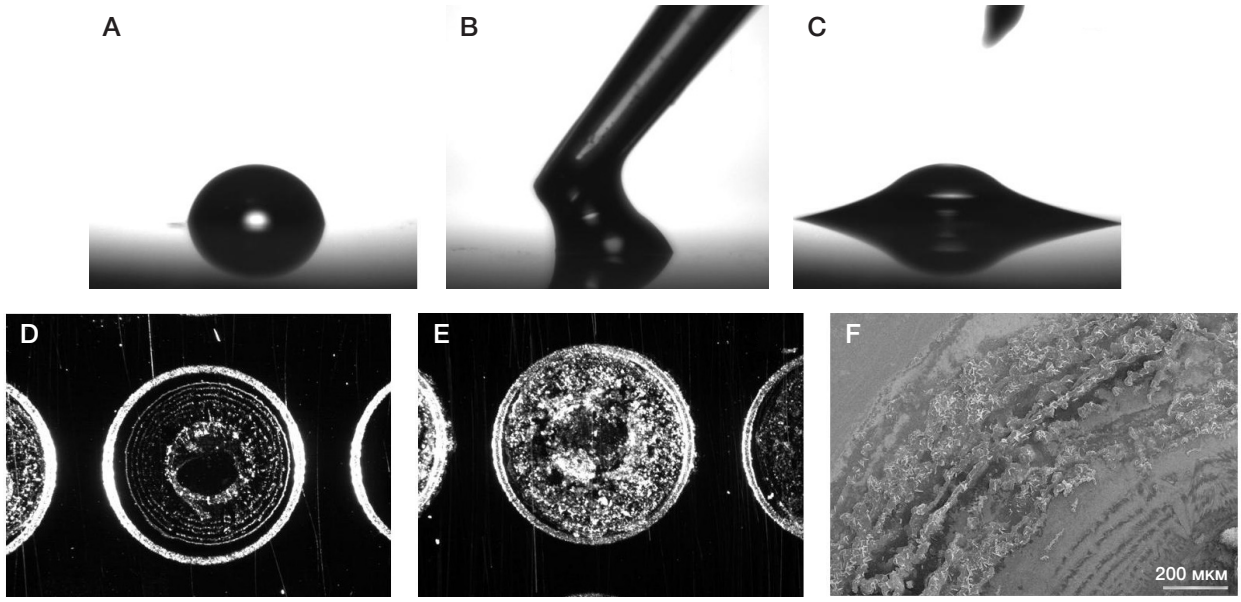

Fig. 1. A. Drop of an aqueous subphase. **B**. Application of the n-hexane stearic acid solution. **C**. Distribution of the organic phase over the substrate. **D**, **E**. Image of MALDI target spots after deposition of one and six FLa layers (SMZ 1500 stereomicroscope with a DS-2MBWc digital camera (Nikon; Japan)). **F**. SEM image of six FLa layers formed on the MALDI target (scanning electron microscope S-3400N; Hitachi, Japan)

Samples of BChE tryptic hydrolyzate modified with OPs were adsorbed in 2.5% aqueous ammonia solution. The duration of this step was 20 minutes. To remove the unbound fraction, we washed the adsorbent twice with 5 µl of 2.5% aqueous ammonia solution and 8 µl of water. To improve desorption of phosphonylated peptides, we added 3 µl of 30% acetonitrile with of 0.1% trifluoroacetic acid (TFA), and after that 2 µl of CHCA matrix (5 mg/ml in 70% aqueous acetonitrile) was added to the target. After IMAC, the FLa monolayers retained adsorbed phosphonylated peptides of blood proteins.

To perform the MALDI-MS analysis of samples of the Seventh Official OPCW Biomedical Proficiency Test, we used SolariX XR 7T (Bruker Daltonics; Germany), a Fourier transform ion-cyclotron resonance mass spectrometer equipped with SmartBeam-II laser (355 nm). The mass spectra were acquired in the positive ion mode in the m/z range of 1000–3200. We used the Data Analysis 5.0 software (Bruker Daltonics; Germany) to process the mass spectral data.

RESULTS

Functionalization of the MALDI target surface

For this work, we selected a polished MALDI target with hydrophobic surface that can carry a drop of water without altering its geometry (Fig. 1A); it was functionalized using the technique published earlier [20]. The volume of solutions allowed keeping n-hexane inside the spot when the solution of stearic acid in n-hexane was applied to the aqueous drop (Fig. 1B), which means the layers formed strictly inside it, too (Fig. 1C-F). We discovered that after successive formation of six monolayers the material no longer stays in the well, and seven or more monolayers adversely affect adhesion of lanthanum stearate films to the MALDI target. The MALDI-MS analysis of the films formed on the target showed that the main structural unit of the adsorbent is the lanthanum distearate ion, as evidenced by the signal with m/z 705.46. The physicochemical parameters of lanthanum stearate films were described earlier [20]. The MALDI target surface was functionalized through formation of adsorbent on the spots based on the multimolecular FLa structures consisting of six collapsed monolayers.

Evaluation of adsorption properties of FLa

Developing a "lab-on-a-plate" approach to metal-affinity extraction, we have shown that drops of widely used IMAC solutions reliably stay on a spot of adsorbent consisting of six collapsed FLa monolayers in the following volumes: 2.5% aqueous ammonia — 7–8 µl; water — 10–12 µl; 30% aqueous acetonitrile — 3–5 µl. A peptic blood plasma hydrolyzate, which has the serum albumin (SA) PFMP-modified by 90% by tyrosine-411, was chosen as a model object. Afterwards, all experiments that included IMAC followed the pattern described below. To equilibrate the phase, we added a drop of 2.5% aqueous ammonia to the adsorbent, kept it there for 5 minutes, then discarded and applied again. Next, we added 1 µl of the sample to the drop and allowed the adsorption process to progress for 20 minutes. The unbound fraction was transferred to an adjacent spot for further MALDI-MS. FLa was washed successively with 2.5% aqueous ammonia, water and 0.1% aqueous TFA. Then we dried the adsorbent and applied 3 µl of 30% aqueous acetonitrile and 2 µL of 5 mg/ml CHCA matrix solution for desorption, which was followed by MALDI mass spectrometry.

To investigate the adsorption capacity of FLa, we diluted the human blood plasma peptic hydrolyzate containing 90% modified serum albumin (initial concentration of SA — 1 mg/ml) with distilled water to the following concentrations: 0.9, 0.8, 0.7, 0, 6, 0.5, 0.4, 0.3, 0.2 and 0.1 mg/mL. IMAC-MALDI-MS showed that after IMAC the signal with m/z 1567.87, corresponding to the VRY(PFMP)TKKVPQVST adduct, was not detected in the mass spectrum of the unbound fraction after topping the adsorbent with 1 µl of a 0.5 mg/ml protein solution. The highly intense m/z 1567.87 signal was registered in the mass spectra recorded for all adsorbent spots; fragment analysis confirmed that this signal belongs to the VRY(PFMP)TKKVPQVST adduct. Based on the degree of protein modification, which is 90%, and with hydrolysis complete (as evidenced by the absence of the LVRY(PFMP)TKKVPQVST signal, m/z 1680.89, that indicates incomplete hydrolysis, in all mass spectra), we can conclude that six layers of FLa are capable of retaining 0.012 µg of the VRY(PFMP)TKKVPQVST adduct.

To assess the specificity and selectivity of the approach, peptic hydrolyzates of modified and unmodified SA were mixed

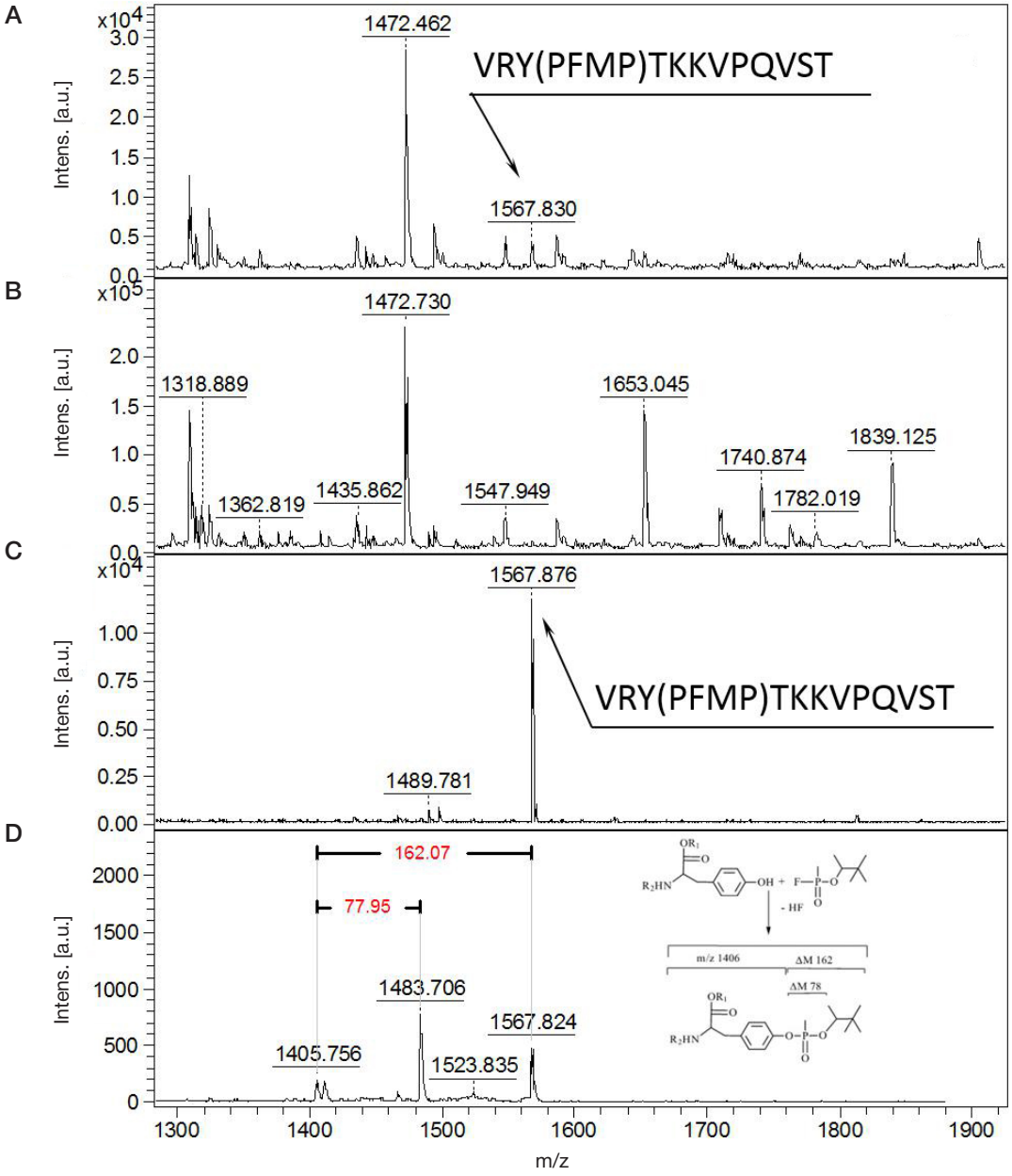

Fig. 2. Mass spectrum of human SA peptic hydrolyzate modified with PFMP at the modified-to-unmodified ratio of 1 : 10. **A**. Mass spectrum of the original sample. **B**. Mass spectrum of the unbound fraction. **C**. Mass spectrum of the adsorbent spot after IMAC. **D**. PFMP modification identification by neutral loss

in such a way that the ratios of VRY(PFMP)TKKVPQVST/ VRYTKKVPQVST were 1 : 10, 1 : 100, 1 : 1000, and 1 : 10,000. We applied a sample with initial SA concentration of 1 mg/ml to the formed and prepared FLa spots in accordance with the data presented in Table 1. The results of the IMAC-MALDI-MS analysis (Table 1; Fig. 2) indicate the high efficiency of the suggested approach to metal-affinity extraction of SA adducts with PFMP in the "lab-on-a-plate" format. We have not detected the adduct signal only in case of application of 1 μl of the mixture at the VRY(PFMP) TKKVPQVST/VRYTKKVPQVST ratio of 1 : 10,000. However, application of even 5 μl of this solution yields detection of the m/z 1567.87 signal with the signal-to-noise ratio (S/N) of 2–3 (various replicates). In case of 1 μl of a 1 : 1000 mixture, the S/N ratio was 5–6. At higher concentrations, the adduct is reproducibly and reliably detected.

Table 1. Results of investigation of selectivity of the developed technique with dilution of PFMP-modified SA with human blood plasma

Molar ratio VRY(PFMP)TKKVPQVST/ VRYTKKVPQVST	Volume of sample applied to the adsorbent, μl	Signal-to-noise ratio
1 : 10	1	51
1 : 100	1	35
1 : 1000	5	13
	1	5
1 : 10,000	5	3
	1	–

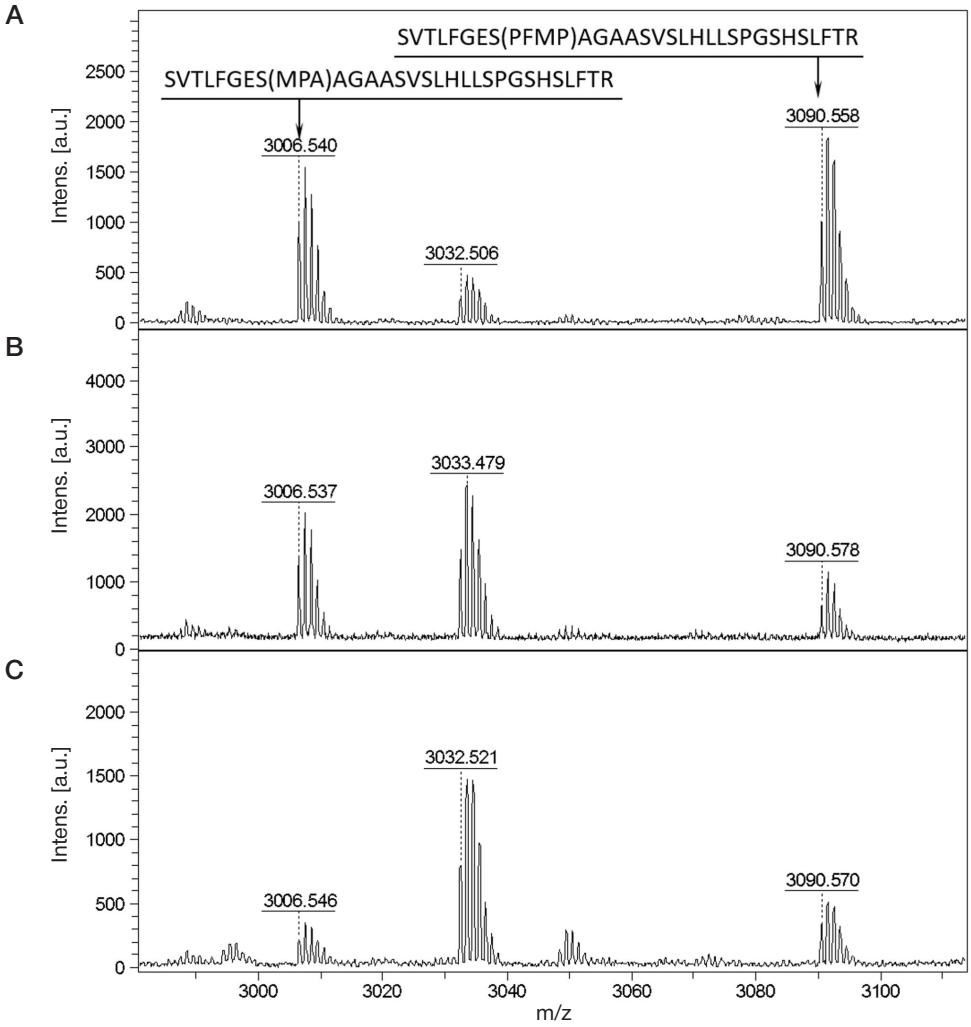

Fig. 3. Results of IMAC(FLa)-MALDI-MS analysis of the tryptic hydrolyzate of BChE (1 mg/ml) modified with PFMP, modified-to-unmodified ratios of 100 : 0 (**A**); 1 : 10 (**B**); 1 : 100 (**C**)

At the next stage of the work, we studied the possibility of extraction of BChE adducts with PFMP by IMAC on FLa, followed by MALDI-MS. The BChE tryptic peptide modified with OPs is a more convenient analyte for MALDI-MS than BChE active site peptic nonapeptide, which is widely used for HPLC-MS/MS analysis. The tryptic peptide containing active site serine-198 consists of 29 amino acid residues: SVTLFGES*AGAASVSLHLLSPGSHSLFTR (m/z 2928.521). However, unlike SA adducts with OPs, BChE adducts are subject to dealkylation (aging) [22, 23], and MS most often detects BChE adduct with a methylphosphonic acid (MPA) residue. We experimented with BChE isolated from human blood plasma that was incubated with PFMP until complete inhibition of the enzyme. One µl of 1 mg/ml tryptic hydrolyzate of the modified BChE protein was applied to FLa, the application followed by IMAC-MALDI-MS, which allows drawing a conclusion (Fig. 3) that FLa formed on the target exhibit specificity towards BChE adducts with both PFMP and MPA. The mass spectrum contains signals at m/z 3006.51, which corresponds to SVTLFGES(MPA)AGAASVSLHLLSPGSHSLFTR adduct, and at m/z 3090.61 which represents the SVTLFGES(PFMP)AGAASVSLHLLSPGSHSLFTR adduct. When the hydrolyzate of modified BChE is diluted with hydrolyzate of unmodified BChE, specificity and selectivity of IMAC-MALDI-MS is noticeably lower compared to the analysis of adducts of human serum albumin with PFMP. However, at the 1 : 100 ratio of the modified form to the unmodified form, signals of BChE adducts with PFMP and MPA are reliably detected at S/N ≥ 6.

Detection of adducts in human blood plasma samples incubated with PFMP

We used human blood plasma incubated with PFMP at concentrations of 1 and 10 ng/ml as samples. The degree of BChE inhibition was < 5 and 40%, respectively. The adduct of SA with PFMP was searched for in the peptic hydrolyzate of the total blood plasma protein; after protein precipitation and reconstitution in distilled water, the concentration of SA was 1 mg/ml. In case of BChE, it was important to isolate its purest form possible, therefore, we resorted to a highly specific method of immunoprecipitation and used 250 µl of blood plasma for the purpose. For IMAC-MALDI-MS, a solution with a BChE concentration of 1 mg/ml was used; Figure 4 and Table 2 show the results thereof. Applying the developed approach to analyze the peptic hydrolyzate of the total blood plasma protein, we encountered the VRY(PFMP)TKKVPQVST adduct signal in the mass spectra of both samples. However, with only 1 µl of the first sample, which was incubated with PFMP at a concentration of 10 ng/ml, applied to the adsorbent, we received S/N of 5 for the signal of VRY(PFMP)TKKVPQVST, whereas the second sample, with the final concentration of PFMP at 1 ng/ml, required enrichment, and we had to apply

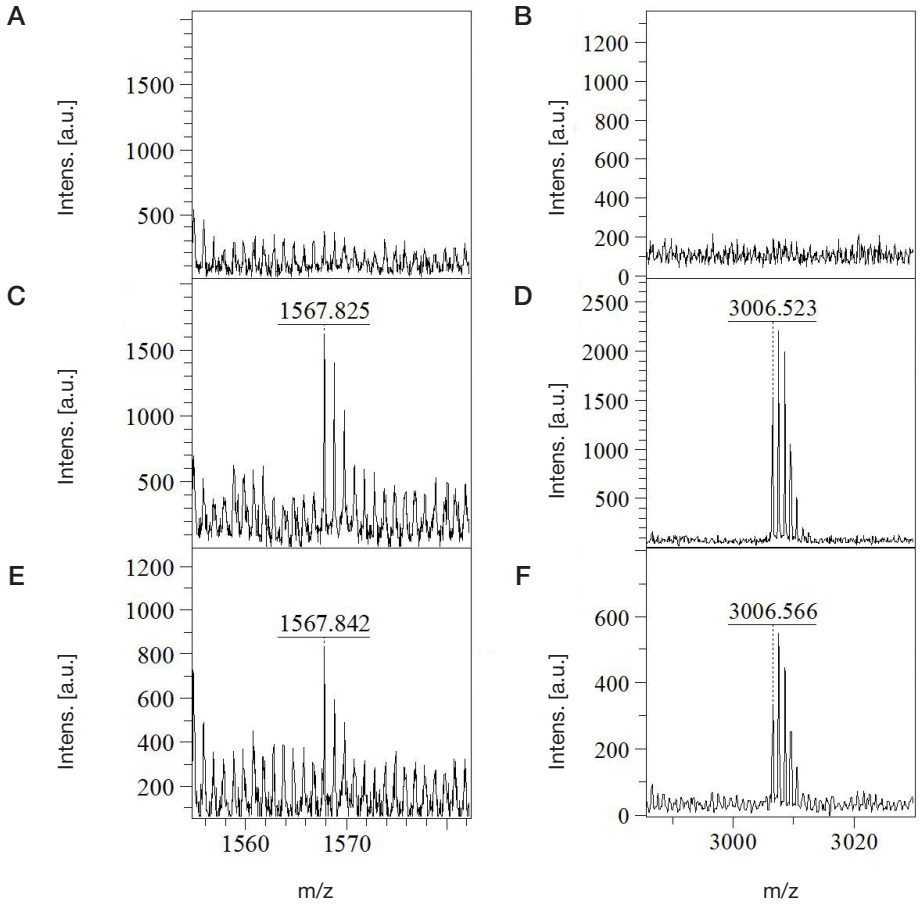

Fig. 4. Results of IMAC(FLa)-MALDI-MS analysis of blood plasma samples incubated with PFMP. The figure shows mass spectra of control samples (**A**, **B**); adducts VRY(PFMP)TKKVPQVST and SVTLFGES(MPA)AGAASVSLHLLSPGSHSLFTR, PFMP concentration 10 ng/ml (**C**, **D**); VRY(PFMP)TKKVPQVST and SVTLFGES(MPA)AGAASVSLHLLSPGSHSLFTR adducts, PFMP concentration 1 ng/ml (**E**, **F**)

5 µl thereof to the adsorbent, after which we registered the signal with the S/N ratio of 4. Moreover, in both blood plasma samples we identified SVTLFGES(MPA)AGAASVSLHLLSPGSHSLFTR, the BChE adduct isolated through immunoprecipitation with dealkylated residue of PFMP. It exhibited good S/N value and required no additional method optimization.

Detection of adducts of OPs with BChE in human blood plasma samples

We applied the suggested approach to detect OPs adducts with BChE when doing the Seventh Official OPCW Biomedical Proficiency Test. Preliminarily, BChE was isolated from 250 µl of blood plasma supplemented with OPs. After immunoprecipitation, we eluted BChE from the adsorbent with 0.6% formic acid, transferred the eluate into a bicarbonate buffer and hydrolyzed it with trypsin. Subsequent IMAC-MALDI-MS revealed a native peptide and modified peptides at m/z 3034.5397 (VX) and m/z 3062.5650 (GE) in the m/z range from 2880 to 3100 (Fig. 5). Other OPs, like VR and CVX, have the molecular weight similar to that of the GE residue. In such a case, a feasible approach to the task of agent identification involves its reactivation from protein adducts and gas chromatography — mass spectrometry as means of detection.

Table 2. Sensitivity of IMAC-MALDI-MS for peptic SA peptides and BChE tryptic peptides modified with PFMP

№	Peptide (m/z)	Ratio PFMP / plasma, ng/ml	Volume of sample applied to the adsorbent, µl	Signal-to-noise ratio
CA				
1	VRY(PFMP)TKKVPQVST (1567,89)	10 : 1	1	5
2	VRY(PFMP)TKKVPQVST (1567,89)	1 : 1	5	4
БХЭ				
3	SVTLFGES(MPA)AGAASVSLHLLSPGSHSLFTR (3006,54)	10 : 1	1	34
4	SVTLFGES(PFMP)AGAASVSLHLLSPGSHSLFTR (3090,56)	10 : 1	1	–
5	SVTLFGES(MPA)AGAASVSLHLLSPGSHSLFTR (3006,54)	1 : 1	1	14
6	SVTLFGES(PFMP)AGAASVSLHLLSPGSHSLFTR (3090,56)	1 : 1	1	–

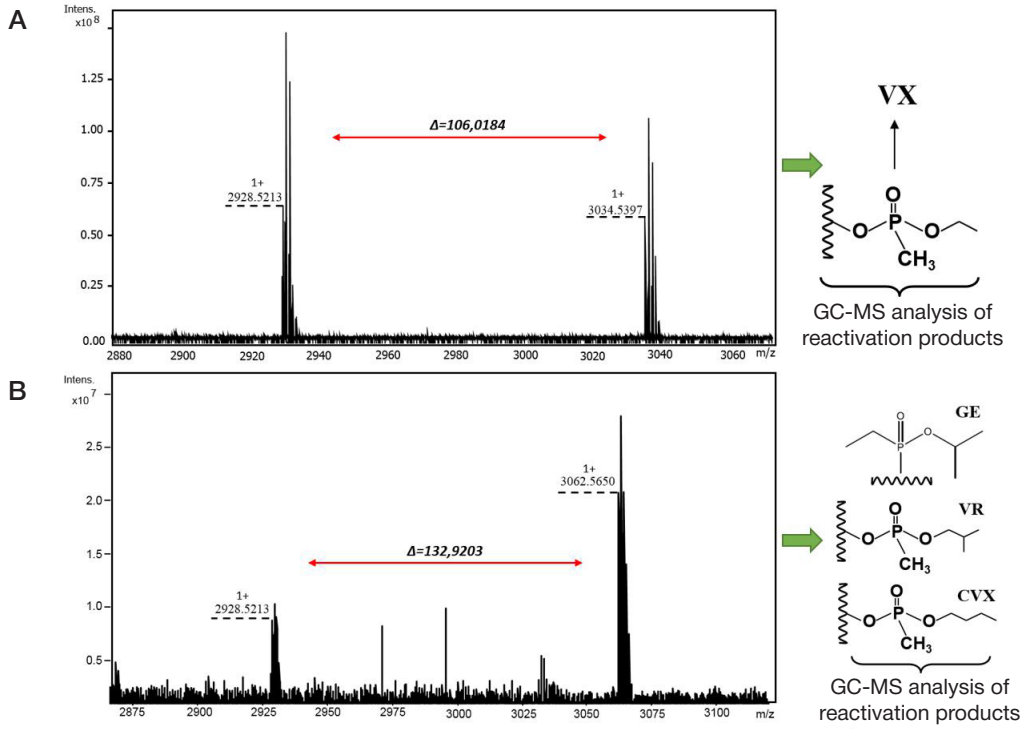

Fig. 5. Results of IMAC(FLa)-MALDI-MS analysis of samples from the Seventh Official OPCW Biomedical Proficiency Test. **A**. MALDI mass spectrum of tryptic hydrolyzate of BChE modified with VX, signal at m/z 3034.5397, SVTLFGES(Vx)AGAASVSLHLLSPGSHSLFTR. **B**. MALDI mass spectrum of tryptic hydrolyzate of BChE modified with GE, signal at m/z 3062.5650 - SVTLFGES(GE)AGAASVSLHLLSPGSHSLFTR

Fourier transform ion-cyclotron resonance mass spectrometry allowed determining the exact mass of the attached residue, which translated into a significantly narrower list of candidate OPs.

DISCUSSION

The suggested approach enables screening for OPs-modified SA and BChE peptides in an array of real blood plasma samples when poisoning by this class of compounds is suspected. In such cases, the concentration of these highly toxic OPs in blood plasma can reach 100 ng/ml. Identification of the mass of attached residue with the help of high resolution MS allows reasonable assumptions about the parent compound.

A number of studies [24–27] take SA adducts with OPs as markers of intoxication that allow not only establishing the fact of poisoning but also enable identification of the OPs by the attached residue. However, the majority of researchers consider the adduct of BChE, which attaches the OPs residue by serine-198, to be the main protein marker [28]. The reason behind this fact is the great difference in concentrations of BChE and SA in human blood plasma: 5 and 40000 μg/ml, respectively. Even when the BChE is inhibited completely, the degree of SA modification will be extremely low. At the same time, in contrast to BChE, SA adducts with OPs do not undergo dealkylation, which in most cases allows either identifying the OPs by ΔM between modified and native peptides or selecting an empirical formula, which greatly facilitates further identification by other methods. Accordingly, the stage of sample enrichment with SA adducts with OPs becomes a compulsory one. Serum albumin is the main component of blood plasma, therefore, enzymatic hydrolyzates of blood plasma can be used to identify SA adducts without protein isolation. Indeed, IMAC on the surface of a MALDI target functionalized with FLa allows specific and selective extraction of adducts of SA with PFMP even from a peptic hydrolyzate of the total blood plasma protein, with SA concentration at 40% and the remaining protein components enhancing suppression of the target signals. It should be noted that for incubation of plasma with OPs we selected PFMP concentrations that do not imply a lethal outcome if OPs in such a dose entered human body. With PFMP concentration of 1 ng/ml, BChE inhibition was below 5%, which often does not even cause clinical manifestations. The suggested "lab-on-a-plate" format revealed presence of a dealkylated adduct, which signals of BChE inhibition, and, furthermore, provided information enabling identification of PFMP by ΔM = 162. Compared to extraction in spin columns, the approach translates into significant reduction of the reagent costs and time expenditures while noticeably increasing sensitivity of the analysis, which is essential when working with extremely limited amounts of biosamples.

Sequential isolation of the BChE enzyme from blood plasma and subsequent IMAC on FLa of the enzyme's tryptic hydrolyzate allow detecting potential covalent adducts of OPs with BChE active site serine by MALDI-MS in the m/z range of 3000–3100. Information about the mass of the attached inhibitor significantly shortens the list of potential substances that caused the poisoning. The probable structural isomers that have similar molecular weight can be identified through targeted chemical analysis by gas chromatography in combination with MS after reactivation from protein adducts, or by analysis of tyrosine adducts of blood plasma proteins that do not dealkylate spontaneously.

The Research Institute of Hygiene, Occupational Pathology and Human Ecology is an accredited laboratory of the OPCW; the developed approach allows rapid screening of potential organophosphorus toxicants in blood plasma when investigating poisoning with this class of compounds.

CONCLUSIONS

We developed a technique of specific and selective extraction of blood protein adducts with OPs on the surface of a MALDI target functionalized with multimolecular structures based on lanthanum stearate using MAC in the "lab-on-a-plate" format. The study has shown that the adsorbent is capable of retaining

both full-length and dealkylated adducts of blood proteins with 2-(fluoromethylphosphoryl)oxy-3.3-dimethylbutane (PFMP). The technique allowed extraction and identification of peptides of SA and BChE modified with PFMP and MPA (after incubation of human blood plasma with OPs in concentrations from 1 to 100 ng/mL), which makes this approach applicable for the purposes of OPs identification in the context of investigation of real cases of intoxication. We applied the suggested approach in the context of doing the Seventh Official OPCW Biomedical Proficiency Test.

References

1. Mangas J, Estevez E, Vilanova TC, França C. New insights on molecular interactions of organophosphorus pesticides with esterases. Toxicology. 2017; 376: 30–43.
2. Konvenciya o ximicheskom oruzhii. Dostupno po ssylke: https://www.opcw.org/sites/default/files/documents/CWC/CWC_ru.pdf.
3. Schaller J, Gerber S, Kämpfer U, Lejon S, Trachsel Ch. Human Blood Plasma Proteins: Structure and Function. John Wiley & Sons, Ltd., 2008; 538 p.
4. Van der Schans MJ, Fidder A, van Oeveren D, Hulst AG, Noort D. Verification of exposure to cholinesterase inhibitors: generic detection of OPCW Schedule 1 nerve agent adducts to human butyrylcholinesterase. J Anal Toxicol. 2008; 32: 125–9. DOI: 10.1093/jat/32.1.125.
5. Dubrovskii Ya, Murashko E, Chuprina O, Beltyukov P, Radilov A, Solovyev N, et al. Mass spectrometry based proteomic approach for the screening of butyrylcholinesterase adduct formation with organophosphates. Talanta. 2019; 197: 374–82. DOI: 10.1016/j.talanta.2019.01.059.
6. Read RW, Riches JR, Stevens JA, Stubbs SJ, Black RM. Biomarkers of organophosphorus nerve agent exposure: comparison of phosphylated butyrylcholinesterase and phosphylated albumin after oxime therapy. Arch Toxicol. 2010; 84: 25–36. DOI: 10.1007/s00204-009-0473-4.
7. Liu C, Huang G, Xia Ha, Liu S, Liu J, Yu H, et al. Yuan. Simultaneous quantification of soman and VX adducts to butyrylcholinesterase, their aged methylphosphonic acid adducts and butyrylcholinesterase in plasma using an off-column procainamide-gel separation method combined with UHPLC–MS/MS. J Chromatogr. 2016; 1036–7: 57–65. DOI: 10.1016/j.jchromb.2016.09.044.
8. Fu F, Guo Y, Lu X, Zhao P, Zou S, Wang H, et al. Forensic analysis of soman exposure using characteristic fragment ions from protein adducts. Human & Experimental Toxicology, 2021; 9603271211001111. DOI: 10.1177/09603271211001111.
9. Awad H, Khamis MM, El-Aneed A. Mass spectrometry, review of the basics: ionization, Applied spectroscopy reviews. 2015; 50: 158–75. DOI: 10.1080/05704928.2014.954046.
10. Greco V, Piras C, Pieroni L, Ronci M, Putignani L, Roncada P, et al. Applications of MALDI-TOF mass spectrometry in clinical proteomics. Expert Review of Proteomics. 2018; 15: 683–96. DOI: 10.1080/14789450.2018.1505510.
11. Israr MZ, Bernieh D, Salzano A, Cassambai Sh, Yazaki Y, Suzuki T. Matrix-assisted laser desorption ionisation (MALDI) mass spectrometry (MS): basics and clinical applications. Clin Chem Lab Med. 2020; 58: 883–96. DOI: 10.1515/cclm-2019-0868.
12. Urban PL, Amantonico A, Zenobi R. Lab-on-a-plate: extending the functionality of MALDI-MS and LDI-MS targets. Mass Spectrometry Reviews. 2011; 30: 435–78. DOI: 10.1002/mas.20288.
13. Ch-J Chen, Ch-Ch Lai, M-Ch Tseng, Yu-Ch Liu, Sh-Yi Line, Fuu-J Tsai. Simple fabrication of hydrophobic surface target for increased sensitivity and homogeneity in matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analysis of peptides, phosphopeptides, carbohydrates and proteins. Analytica Chimica Acta. 2013; 783: 31–38. DOI: 10.1016/j.aca.2013.04.029.
14. Huang H, Sun L, He H, Xia T. A new silicon dioxide-coated MALDI-TOF sample plate for peptide analysis. BioMed Research International. 2020; 8597217: 1–6. DOI: 10.1155/2020/8597217.
15. Gladilovich V, Greifenhagen U, Sukhodolov N, Selyutin A, Singer D, Thieme D, et al. Immobilized metal affinity chromatography on collapsed Langmuir-Blodgett iron (III) stearate films and iron(III) oxide nanoparticles for bottom-up phosphoproteomics. J Chromatogr A. 2016; 1443: 181–90. DOI: 10.1016/j.chroma.2016.03.044.
16. Rozhkova EA, Krasnov IA, Sukhodolov NG, Ivanov NS, Yanklovich AI, Podolskaya EP, et al. Surface behavior of nanostructures (Langmuir-Blodgett Films), Containing Fe(III) ions and their composition determination mass-spectrometry methods. Nauchnoe Priborostr. 2008; 18: 54–60.
17. Miyauchi S, Arisawa S, Yamada T, Yamamoto H, Yamamoto R. Adhesion of fatty acid in Langmuir-Blodgett films. Thin Solid Films. 1989; 178: 347–50. DOI: 10.1016/0040-6090(89)90320-9.
18. Shreyner E, Alexandrova M, Sukhodolov N, Selyutin A, Podolskaya E. Extraction of the insecticide dieldrin from water and biological samples by metal affinity chromatography, Mendeleev Commun. 2017; 27: 304–6. DOI: 10.1016/j.mencom.2017.05.030.
19. Silyavka ES, Selyutin AA, Sukhodolov NG, Shilovskikh VV, Oleneva PA, Mitrofanov AA, et al. Collapsed monomolecular thin films as surface nanomodification techniques for bioorganic MALDI analysis. AIP Conf Proc. 2019; 2064: 030015-1–030015-5. DOI: 10.1063/1.5087677.
20. Gladchuk AS, Silyavka ES, Shilovskikh VV, Bocharov VN, Zorin IM, Tomilin NV, et al. Self-organization of stearic acid salts on the hemispherical surface of the aqueous subphase allows functionalization of matrix-assisted laser desorption/ionization mass spectrometry target plates for on-plate immobilized metal affinity chromatography enrichment. Thin Solid Films. 2022; 756: 139374. DOI: 10.1016/j.tsf.2022.139374.
21. Koryagina NL, Saveleva EI, Karakashev GV, Babakov VN, Dubrovskii YaA, et al. Determination of protein adducts of organophosphorus nerve agents in blood plasma. J Anal Chem. 2016; 71: 849–59. DOI: 10.1134/S1061934816080086.
22. Masson P, Nachon F, Lockridge O. Structural approach to the aging of phosphylated cholinesterases. Chemico-Biological Interactions. 2010; 187: 157–62. DOI: 10.1016/j.cbi.2010.03.027.
23. Jiang W, Murashko EA, Dubrovskii YA, Podolskaya EP, Babakov VN, Mikler J, et al. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of titanium oxide-enriched peptides for detection of aged organophosphorus adducts on human butyrylcholinesterase. Anal Biochem. 2013; 439: 132–41. DOI: 10.1016/j.ab.2013.04.018.
24. Marsillach J, Costa LG, Furlong CE. Protein adducts as biomarkers of exposure to organophosphorus compounds. Toxicology. 2013; 307: 46–54. DOI: 10.1016/j.tox.2012.12.007.
25. Peeples ES, Schopfer LM, Duysen EG, Spaulding R, Voelker T, Thompson CM, et al. Albumin, a new biomarker of organophosphorus toxicant exposure, identified by mass spectrometry. Toxicological Sciences. 2005; 83: 303–12. DOI: 10.1093/toxsci/kfi023.
26. Bao Y, Liu Q, Chen J, Lin Y, Wu B, Xie J. Quantification of nerve agent adducts with albumin in rat plasma using liquid chromatography–isotope dilution tandem mass spectrometry. J of Chromatography A. 2012; 1229: 164–71. DOI: 10.1016/j.chroma.2012.01.032.
27. John H, van der Schans MJ, Koller M, H. Spruit ET, Worek F, Thiermann H, et al. Fatal sarin poisoning in Syria 2013: forensic verification within an international laboratory network. Forensic toxicology. 2017; 36: 61–71. DOI: 10.1007/s11419-017-0376-7.
28. Schopfer LM, Lockridge O. Analytical approaches for monitoring exposure to organophosphorus and carbamate agents through analysis of protein adducts. Drug testing and analysis. 2012; 4: 246–61. DOI: 10.1002/dta.1325.

Литература

1. Mangas J, Estevez E, Vilanova TC, França C. New insights on molecular interactions of organophosphorus pesticides with esterases. Toxicology. 2017; 376: 30–43.
2. Конвенция о химическом оружии. Доступно по ссылке: https://www.opcw.org/sites/default/files/documents/CWC/CWC_ru.pdf.
3. Schaller J, Gerber S, Kämpfer U, Lejon S, Trachsel Ch. Human Blood Plasma Proteins: Structure and Function. John Wiley & Sons, Ltd., 2008; 538 p.
4. Van der Schans MJ, Fidder A, van Oeveren D, Hulst AG, Noort D. Verification of exposure to cholinesterase inhibitors: generic detection of OPCW Schedule 1 nerve agent adducts to human butyrylcholinesterase. J Anal Toxicol. 2008; 32: 125–9. DOI: 10.1093/jat/32.1.125.
5. Dubrovskii Ya, Murashko E, Chuprina O, Beltyukov P, Radilov A, Solovyev N, et al. Mass spectrometry based proteomic approach for the screening of butyrylcholinesterase adduct formation with organophosphates. Talanta. 2019; 197: 374–82. DOI: 10.1016/j.talanta.2019.01.059.
6. Read RW, Riches JR, Stevens JA, Stubbs SJ, Black RM. Biomarkers of organophosphorus nerve agent exposure: comparison of phosphylated butyrylcholinesterase and phosphylated albumin after oxime therapy. Arch Toxicol. 2010; 84: 25–36. DOI: 10.1007/s00204-009-0473-4.
7. Liu C, Huang G, Xia Ha, Liu S, Liu J, Yu H, et al. Yuan. Simultaneous quantification of soman and VX adducts to butyrylcholinesterase, their aged methylphosphonic acid adducts and butyrylcholinesterase in plasma using an off-column procainamide-gel separation method combined with UHPLC–MS/MS. J Chromatogr. 2016; 1036–7: 57–65. DOI: 10.1016/j.jchromb.2016.09.044.
8. Fu F, Guo Y, Lu X, Zhao P, Zou S, Wang H, et al. Forensic analysis of soman exposure using characteristic fragment ions from protein adducts. Human & Experimental Toxicology, 2021; 9603271211001111. DOI: 10.1177/09603271211001111.
9. Awad H, Khamis MM, El-Aneed A. Mass spectrometry, review of the basics: ionization, Applied spectroscopy reviews. 2015; 50: 158–75. DOI: 10.1080/05704928.2014.954046.
10. Greco V, Piras C, Pieroni L, Ronci M, Putignani L, Roncada P, et al. Applications of MALDI-TOF mass spectrometry in clinical proteomics. Expert Review of Proteomics. 2018; 15: 683–96. DOI: 10.1080/14789450.2018.1505510.
11. Israr MZ, Bernieh D, Salzano A, Cassambai Sh, Yazaki Y, Suzuki T. Matrix-assisted laser desorption ionisation (MALDI) mass spectrometry (MS): basics and clinical applications. Clin Chem Lab Med. 2020; 58: 883–96. DOI: 10.1515/cclm-2019-0868.
12. Urban PL, Amantonico A, Zenobi R. Lab-on-a-plate: extending the functionality of MALDI-MS and LDI-MS targets. Mass Spectrometry Reviews. 2011; 30: 435–78. DOI: 10.1002/mas.20288.
13. Ch-J Chen, Ch-Ch Lai, M-Ch Tseng, Yu-Ch Liu, Sh-Yi Line, Fuu-J Tsai. Simple fabrication of hydrophobic surface target for increased sensitivity and homogeneity in matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analysis of peptides, phosphopeptides, carbohydrates and proteins. Analytica Chimica Acta. 2013; 783: 31–38. DOI: 10.1016/j.aca.2013.04.029.
14. Huang H, Sun L, He H, Xia T. A new silicon dioxide-coated MALDI-TOF sample plate for peptide analysis. BioMed Research International. 2020; 8597217: 1–6. DOI: 10.1155/2020/8597217.
15. Gladilovich V, Greifenhagen U, Sukhodolov N, Selyutin A, Singer D, Thieme D, et al. Immobilized metal affinity chromatography on collapsed Langmuir-Blodgett iron (III) stearate films and iron(III) oxide nanoparticles for bottom-up phosphoproteomics. J Chromatogr A. 2016; 1443: 181–90. DOI: 10.1016/j.chroma.2016.03.044.
16. Рожкова ЕА, Краснов ИА, Суходолов НГ, Иванов НС, Янклович АИ, Подольская ЕП, и др. Исследование поверхностных свойств наноструктур (пленок Лэнгмюра-Блоджетт), содержащих ионы железа, и определение их состава с привлечением методов масс-спектрометрии. Научное приборостроение. 2008; 18: 54–60.
17. Miyauchi S, Arisawa S, Yamada T, Yamamoto H, Yamamoto R. Adhesion of fatty acid in Langmuir-Blodgett films. Thin Solid Films. 1989; 178: 347–50. DOI: 10.1016/0040-6090(89)90320-9.
18. Shreyner E, Alexandrova M, Sukhodolov N, Selyutin A, Podolskaya E. Extraction of the insecticide dieldrin from water and biological samples by metal affinity chromatography, Mendeleev Commun. 2017; 27: 304–6. DOI: 10.1016/j.mencom.2017.05.030.
19. Silyavka ES, Selyutin AA, Sukhodolov NG, Shilovskikh VV, Oleneva PA, Mitrofanov AA, et al. Collapsed monomolecular thin films as surface nanomodification techniques for bioorganic MALDI analysis. AIP Conf Proc. 2019; 2064: 030015-1–030015-5. DOI: 10.1063/1.5087677.
20. Gladchuk AS, Silyavka ES, Shilovskikh VV, Bocharov VN, Zorin IM, Tomilin NV, et al. Self-organization of stearic acid salts on the hemispherical surface of the aqueous subphase allows functionalization of matrix-assisted laser desorption/ionization mass spectrometry target plates for on-plate immobilized metal affinity chromatography enrichment. Thin Solid Films. 2022; 756: 139374. DOI: 10.1016/j.tsf.2022.139374.
21. Корягина Н. Л., Савельева Е. И., Каракашев Г. В., Бабаков В. Н., Дубровский Я. А., и др. Определение конъюгированных с белками метаболитов фосфорорганических отравляющих веществ в плазме крови. Журнал аналитической химии. 2016; 71 (8): 883–93.
22. Masson P, Nachon F, Lockridge O. Structural approach to the aging of phosphylated cholinesterases. Chemico-Biological Interactions. 2010; 187: 157–62. DOI: 10.1016/j.cbi.2010.03.027.
23. Jiang W, Murashko EA, Dubrovskii YA, Podolskaya EP, Babakov VN, Mikler J, et al. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry of titanium oxide-enriched peptides for detection of aged organophosphorus adducts on human butyrylcholinesterase. Anal Biochem. 2013; 439: 132–41. DOI: 10.1016/j.ab.2013.04.018.
24. Marsillach J, Costa LG, Furlong CE. Protein adducts as biomarkers of exposure to organophosphorus compounds. Toxicology. 2013; 307: 46–54. DOI: 10.1016/j.tox.2012.12.007.
25. Peeples ES, Schopfer LM, Duysen EG, Spaulding R, Voelker T, Thompson CM, et al. Albumin, a new biomarker of organophosphorus toxicant exposure, identified by mass spectrometry. Toxicological Sciences. 2005; 83: 303–12. DOI: 10.1093/toxsci/kfi023.
26. Bao Y, Liu Q, Chen J, Lin Y, Wu B, Xie J. Quantification of nerve agent adducts with albumin in rat plasma using liquid chromatography–isotope dilution tandem mass spectrometry. J of Chromatography A. 2012; 1229: 164–71. DOI: 10.1016/j.chroma.2012.01.032.
27. John H, van der Schans MJ, Koller M, H. Spruit ET, Worek F, Thiermann H, et al. Fatal sarin poisoning in Syria 2013: forensic verification within an international laboratory network. Forensic toxicology. 2017; 36: 61–71. DOI: 10.1007/s11419-017-0376-7.
28. Schopfer LM, Lockridge O. Analytical approaches for monitoring exposure to organophosphorus and carbamate agents through analysis of protein adducts. Drug testing and analysis. 2012; 4: 246–61. DOI: 10.1002/dta.1325.

EXPERIMENTAL ANIMAL MODEL FOR TREATMENT OF ABSOLUTE UTERINE FACTOR INFERTILITY

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Reproductive organ transplantation was considered as a potential method for treatment of the ovarian factor, tubal factor, and uterine factor infertility before the advent of advanced assisted reproductive technologies. Uterus transplantation can be considered as the method for treatment of absolute uterine factor infertility similar to transplantation of non-vital organs. However, the clinical use of uterus transplantation in humans causes a lot of problems. The study was aimed to develop a program for assessment of various surgical tissue revascularization techniques for restoration of reproductive function in experimental animals with uterine factor infertility. Chinchilla rabbits (n = 20) were selected for experiments because of the fact that all mammals have similar structure of the organs. The innovative technique involving the use of ovarian arteries instead of uterine arteries (as in the standard protocol) was used in laboratory animals to develop the surgical protocol for transplantation of reproductive tissues. The animal study results show that hemodynamic characteristics of blood supply to the transplanted uterus remain unchanged. This makes it possible to use the surgical method in the experiments on the uterus transplant from a deceased donor. The proposed uterus transplantation protocol ensures high transplant survival rate and normal blood supply to the transplant, along with the reduced risk of injury to the donor and reduced complexity of the surgical procedure.

Keywords: uterus transplantation, treatment of uterine factor infertility, organ transplantation, organ donation, comparative analysis

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Compliance with the ethical standards: the study was conducted in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986) and the Rules for Handling Experimental Animals (order № 755 of 12.08.1977 of the Ministry of Health of the USSR).

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МОДЕЛЬ ЛЕЧЕНИЯ АБСОЛЮТНОГО МАТОЧНОГО БЕСПЛОДИЯ НА ЭКСПЕРИМЕНТАЛЬНЫХ ЖИВОТНЫХ

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До появления современных вспомогательных репродуктивных технологий трансплантацию репродуктивных органов рассматривали как потенциальное лечение яичникового, трубного и маточного бесплодия. Трансплантацию матки можно рассматривать как метод лечения абсолютного маточного бесплодия, подобный пересадке нежизненно важных органов. Однако клиническое применение трансплантации матки у человека вызывает много проблем. Целью исследования было разработать программу по оценке различных хирургических методов реваскуляризации тканей для восстановления репродуктивной функции при маточной форме бесплодия на экспериментальных животных. Выбор кроликов породы шиншилла (n = 20) для экспериментальной работы обусловлен идентичностью строения органокомплекса всех млекопитающих. Для разработки хирургического протокола пересадки тканей репродуктивной системы на лабораторных животных применили инновационную методику по использованию яичниковых артерий вместо маточных, как в стандартном протоколе. Согласно результатам, полученным на лабораторных животных, гемодинамические характеристики кровоснабжения пересаженной матки не меняются, что позволяет имплементировать данную хирургическую методику для использования в эксперименте на трупном материале для пересадки матки. Предлагаемый протокол проведения операции по трансплантации матки обеспечивает высокий уровень приживаемости трансплантанта и его нормальное кровоснабжение с минимизацией рисков травматизации донора и снижением сложности операции.

Ключевые слова: пересадка матки, лечение маточного бесплодия, трансплантация органов, органное донорство, сравнительный анализ

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Absolute uterine factor infertility (AUFI) have historically been regarded as an incurable form of infertility, however, the first case of a child born after allogeneic uterus transplantation (UT) was reported in 2014 [1]. The success of this surgical procedure was the substantial progress in treatment of AUFI. AUFI may be caused by past surgical interventions preventing embryo implantation or positive pregnancy outcome [2]. About 20 women per 100,000 female population of reproductive age have AUFI [3, 4]. Uterus transplantation is associated with a number of ethical challenges, which, in turn, can produce medical, psychological, and legal risks for both genetic and biological mothers of the unborn child [4].

Absolute uterine factor infertility (AUFI) can have various causes: uterine agenesis, anomalies of uterine development, congenital uterine anomalies or the history of organ retrieval surgery preventing embryo implantation or positive pregnancy outcome [2, 4].

The absent uterus is the most obvious cause of AUFI. The uterus is most often absent due to the history of hysterectomy used for treatment of such disorders as uterine fibroid, cervical or endometrial cancer, severe adenomyosis [1, 3].

The Mayer–Rokitansky–Küster–Hauser (MRKH) syndrome associated with abnormal formation of Müllerian ducts is the cause of congenital aplasia of the uterus [4]. There is a number of AUFI causes found in women with the preserved uterus, such as congenital uterine anomalies with partial developmental defects and fusion defects of the Müllerian ducts [5].

AUFI may result from complications of intrauterine manipulation or severe endometritis [6]. UT can be the only method for restoring fertility in this group of patients. The estimated prevalence of AUFI is 20,000 cases in women of reproductive age per 100 million population [4].

The cold or warm ischemia is one of the most important issues to be dealt with when treating infertility using UT. Cells of the transplant are damaged since the moment of artery clamping in the donor's body to the moment of reperfusion after revascularization in the recipient's body. The mouse study has shown that spontaneous pregnancy is still possible after 24 h of cold ischemia; the loss of function occurs after 48 ч h of cold ischemia [7]. The sheep uterus that was as large as the human uterus remained viable after 24 h of cold ischemia, viability was assessed within 8 days after the organ auto-transplantation [8]. Sensitivity to warm ischemia was studied in rats [9], Macaca fascicularis macaques [10], and sheep [11]. The organ remained viable after 5, 4, and 3 h of ischemia, respectively. The innate regenerative potential that includes, among other things, organ-specific stem cells, ensures the ability of the uterus to compensate possible ischemia injury during transplantation [7–9, 12].

Immunosupression is one more important issue. The rat studies show excellent results of using tacrolimus [13] for prevention of rejection compared to cyclosporins [14]. Tacrolimus also proved effective in the rabbit study [15], and, according to the findings, it effectively prevented rejection in pigs for 12 months [16]. Cyclosporine monotherapy was effective in the studies involving sheep [17]. These data combined show that monotherapy with cyclosporins or tacrolimus is effective in rodents and large domestic animals.

Organ transplantation is a complex surgical problem, the main aspect of which is restoration of graft perfusion through establishing arterial inflow and venous outflow [2, 15]. When performing organ transplantation, blood flow restoration results from performing anastomosis of the main artery responsible for blood supply to the donated organ and the large main blood vessel of the recipient; venous outflow is restored in the same way [18]. In this regard many authors report the development of complications associated with abnormal venous outflow after transplantation. These data have provided the basis for identification of several major ways of blood flow restoration in the transplant, however, the optimal method has not been defined [7, 11].

When preparing for the first living donor UT in human, the surgical technique for retrieval of uterine arteries and veins in patients who underwent surgery due to uterine cancer and cervical cancer and lymphadenectomy was tested [19]. Later the results of this study provided the basis for the development of surgical protocol for the living donor UT in humans [20].

The study was based on the hypothesis that it was possible not to use uterine veins in the transplantation method since ovarian veins were enough. The other papers report the use of both groups of veins during transplantation [21].

All the researchers point out that the use of uterine arteries is associated with a number of complexities.

It is difficult to retrieve uterine veins when retrieving organ from a living donor (it is difficult to separate these from the ureter which is located in close proximity in terms of anatomy).

Uterine veins are intricately woven highly branched thin blood vessels that present a challenge when performing surgery, as a result, the surgical procedure is very time-consuming.

In case of multiorgan donation from a deceased donor the uterus is not a priority (it is retrieved last, if necessary), therefore it is difficult to retrieve the kidney: the kidney must be retrieved with the ureter of maximum length, that is why the uterus would be unusable (adjacent uterine blood vessels would be destroyed).

To date, there is no approved protocol for surgical treatment of AUFI by uterus transplantation in the Russian Federation. The study was aimed to review the results of the studies of uterus transplantation in model animals (rabbits) reported in the world literature.

METHODS

The study was conducted in 2021 by the research team of the Federal Siberian Research Clinical Center of the Federal Medical Biological Agency of the Russian Federation. Laboratory animals were used: 20 female rabbits of the Chinchilla breed with the body weight of 1800–2000 g and the confirmed normal reproductive function (parous rabbits).

The animals underwent a two-week quarantine in the vivarium according to the animal housing requirements before the experiment, they were given specialized wafers supplemented with vegetables, cereals, hay, and dairy products. The following light/dark cycle was used: 12 : 12 h, light since 08:00 am. There was one rabbit per cage. The animals' health status was assessed daily, and the data were recorded in the measurement diary.

Blood flow parameters, vascular resistance index, and blood pressure were measured by Doppler ultrasound (Hitachi Aloka ProSound Alpha 6; Japan). All the experiments were recorded, photographic evidence was provided. Statistical data processing involved the use of the standard algorithm of statistical procedures, and the methods of statistical analysis were applied in accordance with the studied trait type and the number of comparison groups. The Shapiro–Wilk test was used to assess the distribution of quantitative indicators. In case of non-normal distribution the descriptive statistics was presented as median and percentiles. The Kruskal–Wallis test was used for multiple comparisons to assess the significance of differences, while the Mann–Whitney U test was used for pairwise comparison.

Table 1. Anatomic and hemodynamic parameters of the rabbit female reproductive blood vessels

Parameter		Value
Weight		3700 g
Body length		37 cm
Right uterine artery length		52 mm
Left uterine artery length		54 mm
Right ovarian artery length		43 mm
Left ovarian artery length		45 mm
Doppler parameters		
Right uterine artery	V_{min}	3,1
	V_{max}	29,7
	PI	3,92
	RI	0,89
Left uterine artery	V_{min}	3,3
	V_{max}	27,8
	PI	3,89
	RI	0,88
Right ovarian artery	V_{min}	1,42
	V_{max}	22,78
	PI	4,02
	RI	0,77
Left ovarian artery	V_{min}	1,53
	V_{max}	23,62
	PI	3,97
	RI	0,69

In case of normal distribution the descriptive statistics was presented as mean and standard error of the mean. Significance of differences between the normally distributed indicators in the studied groups was assessed using the Student's *t*-test.

Program of experimental studies

The experiment involved 20 model laboratory animals (rabbits), divided into four study groups:

– group 1 (n = 5) — the animals underwent ligation of the uterine veins;

– group 2 (n = 5) — the animals underwent ligation of the ovarian veins;

– group 3, controls (n = 5) — intact animals;

– group 4, controls (n = 5) — the animals underwent ligation of the uterine and ovarian veins.

The animal experiments were carried out in three phases. Various vessels that supplied blood to the uterus were ligated, a total of two blood vessel groups: the ovarian artery and ovarian veins, the uterine artery and uterine veins. When the uterine veins were ligated and the ovarian venous outflow was preserved only, the animals were monitored for two weeks in order to make sure that they had no dystrophy of the organ.

Experiment 1. Anatomical evaluation of the vascular territories (inflow and outflow vessels) of female reproductive organs

Dissection of blood vessels of the uterus, ovaries, and vagina was performed with subsequent labeling of the vessels to determine the vessel length and the sites of anastomosis with the main arteries and veins, reveal collateral circulation, and identify shunts. After the blood vessel retrieval, the vessel length and diameter were measured; Doppler ultrasound was used to assess blood flow parameters, vascular resistance index, and blood pressure.

Experiment 2. Assessment of the compensatory capabilities of various venous vascular territories responsible for drainage of reproductive organs

At this stage ligation of various main blood vessels providing blood inflow and outflow from reproductive organs was performed with subsequent immediate and delayed assessement of their impact on blood flow and functional state of the reproductive organs.

The animal was followed-up for 14 days during the experiment. After 14 days, midline incision was performed again in order to revise pelvic organs and the site of previous surgery. Blood flow parameters were measured by Doppler ultrasound in the pelvic blood vessels and the uterus once again.

Experiment 3. Uterus transplantantation involving the use of ovarian veins as the only venous outflow tract

The uterus was separated from the parametrial tissue, the urinary bladder and the colon were separated from the uterus, and the ligaments of those were transected. At the next stage vagina was transected. Then ligation and transection of the uterine veins was performed that was followed by clipping of the iliac arteries and transection of those at the sites where the uterine arteries arised. After that the donor animals were withdrawn from the experiment by intravenous administration of the lethal dose of magnesium sulfate. Cannulation of the uterine arteries and ovarian veins of the transplant and the transplant perfusion using the Custodiol solution (at least 200 mL, in accordance

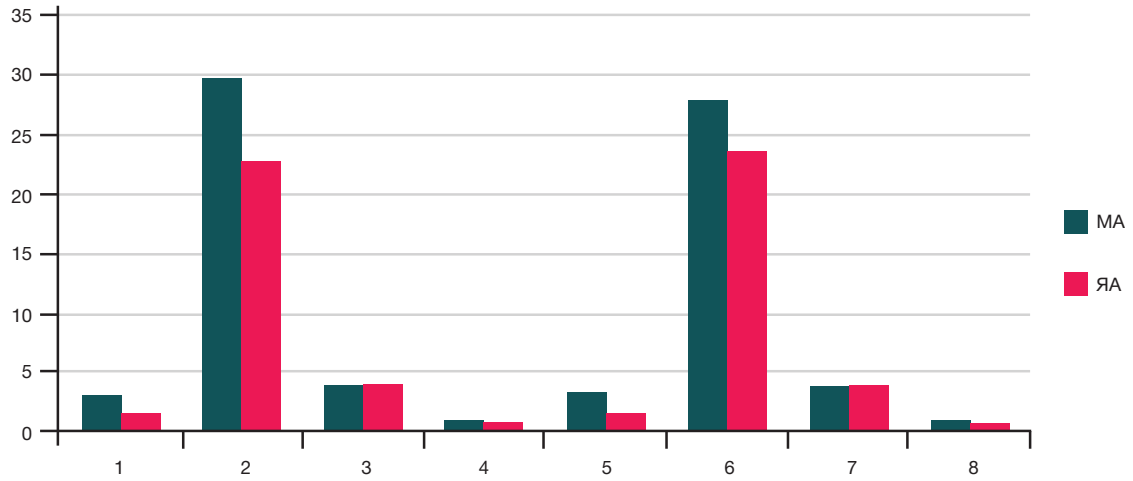

Fig. Doppler parameters of animal 1

with the transplant size) combined with heparin solution (1 : 5000) were performed. After adequate washing (i.e. until there were no blood components in the washing fluid) the transplant vascular pedicles were prepared for transplantation.

After 14 days, midline incision was performed again in order to revise pelvic organs, the site of previous surgery, the transplant condition, and anastomoses. Blood flow parameters were measured by Doppler ultrasound in the pelvic blood vessels and the transplanted uterus once again.

RESULTS

The data obtained by assessing the anatomic parameters and hemodynamic indicators of blood flow in the reproductive organs of female rabbits by Doppler ultrasound are provided in Table 1.

Comparative analysis of the parameters of anatomic and substitutional curculation was performed for each of 20 animals. Consider these with an example of animal 1 (Figure).

The data provided in Figure show that the differences in V_{min} values between the uterine and ovarian arteries can be explained by the differences between the anatomic vessel diameters. Both the length and the diameter of these blood vessels vary considerably (the uterine artery has a much larger diameter) and, since the V_{min} value represents blood flow during diastole, it is obvious that blood flow is greated in the vessel with the larger diameter, and the differences between blood vessels may be considerable. That is why it should be expected that the V_{min} value of the uterine artery would be significantly higher than that of the ovarian artery. The measurements performed in 20 animals confirm that it is 2–2.5 times higher.

Comparative analysis of blood flow parameters was performed in all experimental animals.

The V_{max} value represents blood flow during systole, it is slightly higher in the uterine value than in the ovarian artery. This can also be explained by anatomic features. The aorta is divided into two common iliac arteries, which, in turn, are divided into two external and internal iliac arteries. The uterine artery arises from the internal iliac artery, while the ovarian artery arises directly from the aorta.

In contrast to V_{min}, the differences between the V_{max} values of the uterine and ovarian arteries should be insignificant (15–30%). This was confirmed by measuring the Doppler parameters in all 20 animals. In animal 6, minimum blood flow velocity in the left UA was more than two times higher than in the OA (227%). In animal 7, minimum blood flow velocity in the left UA was twice as high as in the left OA, the same differences were observed in the right arteries. The differences between V_{max} values were insignificant: 26% for the left ovarian arteries, 22% for the right arteries.

Statistical processing of the results using the Student's *t*-test is provided in Table 2.

A total of 20 surgeries were performed in model animals (rabbits). Parameters of blood inflow/outflow were defined in each animals for the following pairs: right and left uterine arteries, right and left ovarian arteries (Table 2). No pairs showed significant differences between the total blood flow values.

DISCUSSION

The criteria of successful transplantation and uterus removal must be defined prior to transplantation. The uterus transplant failure is determined by the following charateristics: no return of menstruation within certain period after transplantation, no pregnancy regardless of multiple embryo transfer procedures, uterine atrophy. The time period for these outcomes has not been defined, however, it must be determined prior to transplantation, since the long-term use of immunosuppressive drugs poses a risk to the recipient. The measures to counter functional failure are as follows: assessment of the transplanted uterus by echography with subsequent prescription of immunosuppressors. No immunosuppression was used during our study.

The uterus transplantation clinical use in humans requires a thorough discussion of issues related to reproductive ethics. Medical, ethical, social, and religious background is different in the countries that have performed such surgical procedures, such as Saudi Arabia, Turkey, and Sweden, that is why transplantation of the uterus should be thoroughly discussed taking into account all the provisions. It is important to consider the question, whether uterus transplantation is socially significant, i.e. whether the method of uterus transplantation satisfies the need of patients with uterine factor infertility for childbirth. The uterus transplantation method contributes to improving the methods for treatment of such patients and probably improves their health and quality of life. Based on the urgent issues in the countries where surrogacy is not allowed, the surgical procedure is not unnecessary or excessive. However, a handful of gynecologists and a much smaller number of patients and people in society are aware of the issues of uterus transplantation, that is why it is important for the surgical procedure to be widely perceived by the public. The large-scale public opinion polls aimed at studying the

Table 2. Student's t-test for the comparison group

Comparison groups	*p*
Right uterine artery V_{min}	< 0,001
Right ovarian artery V_{min}	
Right uterine artery V_{max}	< 0,001
Right ovarian artery V_{max}	
Right uterine artery PI	0,032
Right ovarian artery PI	
Right uterine artery RI	0,003
Right ovarian artery RI	
Left uterine artery V_{min}	< 0,001
Left ovarian artery V_{min}	
Left uterine artery V_{max}	< 0,001
Left ovarian artery V_{max}	
Left uterine artery PI	0,491
Left ovarian artery PI	
Left uterine artery RI	0,01
Left ovarian artery RI	

Note: *p* — significance level of the differences

social needs and attitude towards surgical procedure are the next step.

CONCLUSIONS

The main idea of the study is that it is possible to artificially change the nature of blood flow in the implanted organ during the uterus transplantation: the proposed method is aimed at avoiding the time-consuming and potentially dangerous surgical procedure involving restoration of blood outflow through the uterine artery in the recipient. Such an approach makes it possible to develop a new protocol for treatment and surgery that would significantly reduce the risk of postoperative complications.

References

1. Brännström M, et al. Livebirth after uterus transplantation. The Lancet. 2015; 385 (9968): 607–16.
2. Brännström M, et al. Uterus transplantation: animal research and human possibilities. Fertility and Sterility. 2012; 97 (6): 1269–76.
3. Brinsden P. Gestational surrogacy. Human Reproduction Update. 2003; 9 (5): 483–91.
4. Chan Y, et al. Reproductive outcomes in women with congenital uterine anomalies: a systematic review. Ultrasound in Obstetrics & Gynecology. 2011; 38 (4): 371–82.
5. El-Akouri R. Successful uterine transplantation in the mouse: pregnancy and post–natal development of offspring. Human Reproduction. 2003; 18 (10): 2018–23.
6. McCulloch P, et al. No surgical innovation without evaluation: the IDEAL recommendations. The Lancet. 2009; 374 (9695): 1105–12.
7. Adachi M, et al. Evaluation of allowable time and histopathological changes in warm ischemia of the uterus in cynomolgus monkey as a model for uterus transplantation. Acta Obstetricia et Gynecologica Scandinavica. 2016; 95 (9): 991–8.
8. Akhi S, et al. Uterine rejection after allogeneic uterus transplantation in the rat is effectively suppressed by tacrolimus. Fertility and Sterility. 2013; 99 (3): 862–70.
9. Díaz-García C, et al. The effect of warm ischemia at uterus transplantation in a rat model. Acta Obstetricia et Gynecologica Scandinavica. 2012; 92 (2): 152–9.
10. Kisu I, et al. A new surgical technique of uterine auto–transplantation in cynomolgus monkey: preliminary report about two cases. Archives of Gynecology and Obstetrics. 2011; 285 (1): 129–37.
11. Enskog A, et al. Uterus transplantation in the baboon: methodology and long–term function after auto–transplantation. Human Reproduction. 2010; 25 (8): 1980–7.
12. El-Akouri R. Pregnancy in transplanted mouse uterus after long–term cold ischaemic preservation. Human Reproduction. 2003; 18 (10): 2024–30.
13. Groth K, et al. Effects of immunosuppression by cyclosporine A on allogenic uterine transplant in the rat. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2012; 163 (1): 97–103.
14. Tricard J, et al. Uterus tolerance to extended cold ischemic storage after auto–transplantation in ewes. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2017; 214: 162–7.
15. Wranning C, et al. Uterus transplantation in the rat: Model development, surgical learning and morphological evaluation of healing. Acta Obstetricia et Gynecologica Scandinavica. 2008; 87 (11): 1239–47.
16. Avison D, et al. Heterotopic Uterus Transplantation in a Swine Model. Transplantation. 2009; 88 (4): 465–9.
17. Gauthier T, et al. Uterine allotransplantation in ewes using an aortocava patch. Human Reproduction. 2011; 26 (11): 3028–36.
18. Gonzalez–Pinto I, et al. Uterus Transplantation Model in Sheep With Heterotopic Whole Graft and Aorta and Cava Anastomoses. Transplantation Proceedings. 2013; 45 (5): 1802–4.
19. Gauthier T, et al. Uterus retrieval process from brain dead donors. Fertility and Sterility. 2014; 102 (2): 476–82.
20. Johannesson L, et al. Vascular Pedicle Lengths After Hysterectomy. Obstetrics & Gynecology. 2012; 119 (6): 1219–25.
21. Fronek J, et al. Human Uterus Transplantation from Living and Deceased Donors: The Interim Results of the First 10 Cases of the Czech Trial. Journal of Clinical Medicine. 2021; 10 (4): 586.

Литература

1. Brännström M, et al. Livebirth after uterus transplantation. The Lancet. 2015; 385 (9968): 607–16.
2. Brännström M, et al. Uterus transplantation: animal research and human possibilities. Fertility and Sterility. 2012; 97 (6): 1269–76.
3. Brinsden P. Gestational surrogacy. Human Reproduction Update. 2003; 9 (5): 483–91.
4. Chan Y, et al. Reproductive outcomes in women with congenital uterine anomalies: a systematic review. Ultrasound in Obstetrics & Gynecology. 2011; 38 (4): 371–82.
5. El-Akouri R. Successful uterine transplantation in the mouse: pregnancy and post–natal development of offspring. Human Reproduction. 2003; 18 (10): 2018–23.
6. McCulloch P, et al. No surgical innovation without evaluation: the IDEAL recommendations. The Lancet. 2009; 374 (9695): 1105–12.
7. Adachi M, et al. Evaluation of allowable time and histopathological changes in warm ischemia of the uterus in cynomolgus monkey as a model for uterus transplantation. Acta Obstetricia et Gynecologica Scandinavica. 2016; 95 (9): 991–8.
8. Akhi S, et al. Uterine rejection after allogeneic uterus transplantation in the rat is effectively suppressed by tacrolimus. Fertility and Sterility. 2013; 99 (3): 862–70.
9. Díaz-García C, et al. The effect of warm ischemia at uterus transplantation in a rat model. Acta Obstetricia et Gynecologica Scandinavica. 2012; 92 (2): 152–9.
10. Kisu I, et al. A new surgical technique of uterine auto–transplantation in cynomolgus monkey: preliminary report about two cases. Archives of Gynecology and Obstetrics. 2011; 285 (1): 129–37.
11. Enskog A, et al. Uterus transplantation in the baboon: methodology and long–term function after auto–transplantation. Human Reproduction. 2010; 25 (8): 1980–7.
12. El-Akouri R. Pregnancy in transplanted mouse uterus after long–term cold ischaemic preservation. Human Reproduction. 2003; 18 (10): 2024–30.
13. Groth K, et al. Effects of immunosuppression by cyclosporine A on allogenic uterine transplant in the rat. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2012; 163 (1): 97–103.
14. Tricard J, et al. Uterus tolerance to extended cold ischemic storage after auto–transplantation in ewes. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2017; 214: 162–7.
15. Wranning C, et al. Uterus transplantation in the rat: Model development, surgical learning and morphological evaluation of healing. Acta Obstetricia et Gynecologica Scandinavica. 2008; 87 (11): 1239–47.
16. Avison D, et al. Heterotopic Uterus Transplantation in a Swine Model. Transplantation. 2009; 88 (4): 465–9.
17. Gauthier T, et al. Uterine allotransplantation in ewes using an aortocava patch. Human Reproduction. 2011; 26 (11): 3028–36.
18. Gonzalez–Pinto I, et al. Uterus Transplantation Model in Sheep With Heterotopic Whole Graft and Aorta and Cava Anastomoses. Transplantation Proceedings. 2013; 45 (5): 1802–4.
19. Gauthier T, et al. Uterus retrieval process from brain dead donors. Fertility and Sterility. 2014; 102 (2): 476–82.
20. Johannesson L, et al. Vascular Pedicle Lengths After Hysterectomy. Obstetrics & Gynecology. 2012; 119 (6): 1219–25.
21. Fronek J, et al. Human Uterus Transplantation from Living and Deceased Donors: The Interim Results of the First 10 Cases of the Czech Trial. Journal of Clinical Medicine. 2021; 10 (4): 586.

MOLECULAR MODELING AND EXPERIMENTAL CONFIRMATION OF THE SEARCH FOR AGENTS MITIGATING TOXIC ACTION OF HYDROGEN SULFIDE

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Mathematical modeling is a promising method enabling *in silico* calculations with subsequent suggestion of cell membrane protective agents used to reduce the consequences of exposure to hydrogen sulfide-containing gas in emergency situations. This study aimed to investigate the nature of interaction of hydrogen sulfide (H_2S) and N-Acetyl-L-Cycteine (NAC) with the components of cell membranes. We built a mathematical model of interatomic interactions of cell membrane components with H_2S and NAC (two separate models), then made the quantum-chemical calculations using our proprietary technique and set up GAMESS Z-matrices reflecting type and position of atoms in the molecules. The structure of the molecules was optimized with the help of MOPAC package built into ChemOffice. Lecithin-based liposomes in a sulfide solution (with Na_2S being the donor of H and HS ions) were used as an experimental model of the biological membrane. Redox potential in mV was the comparison parameter in assessment of interaction of the H_2S system components and NAC with phospholipid. The results include patterns showing the phospholipid reactive centers blocked by NAC under toxic exposure to H_2S. Liposomal models of cell membranes were formed and redox parameters measured. Biological experiment confirmed the acceptable accuracy of the designed method of calculation of intermolecular interactions when used as a basis for further selection of agents capable of adjusting toxic doses of hydrogen sulfide. Membrane models of H_2S interaction with protein and lecithin were visualized *in silico* and *in vitro*. The possibility of using NAC as an H_2S inhibitor has been confirmed.

Keywords: mathematical modeling, hydrogen sulfide, phospholipid, lecithin, liposomes, redox potential

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МОЛЕКУЛЯРНОЕ МОДЕЛИРОВАНИЕ И ЭКСПЕРИМЕНТАЛЬНОЕ ПОДТВЕРЖДЕНИЕ ПОИСКА СРЕДСТВ КОРРЕКЦИИ ТОКСИЧЕСКОГО ВОЗДЕЙСТВИЯ СЕРОВОДОРОДА

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В качестве меры по снижению последствий воздействия на организм сероводородсодержащего газа в результате аварийных ситуаций перспективно использовать математическое моделирование, позволяющее *in silico* рассчитать и предложить средства защиты клеточных мембран. Целью работы было оценить характер взаимодействия сероводорода (H_2S) и ацетилцистеина (АЦЦ) с компонентами клеточных мембран. С помощью математического моделирования межатомных взаимодействий компонентов клеточной мембраны сначала с H_2S, а затем с АЦЦ осуществлены квантово-химические расчеты с использованием авторской методики. Созданы z-матрицы программного комплекса Gamess — метода PM3, отражающие тип и положение атома в молекуле. Оптимизацию структуры молекулы производили с помощью Морас, встроенного в ChemOffice. В качестве экспериментальной модели биологической мембраны использовали липосомы на лецитиновой основе в сульфидном растворе (Na_2S — донор H- и HS-ионов). Параметром сравнения взаимодействия компонентов системы H_2S и АЦЦ с фосфолипидом послужил окислительно-восстановительный потенциал (Redox-потенциал), выраженный в мВ. Представлены схемы, иллюстрирующие блокированные АЦЦ реактивные центры фосфолипида в условиях токсического воздействия H_2S. Сформированы липосомные модели клеточных мембран и замерены Redox-показатели. Биологический эксперимент подтвердил приемлемость авторской методики расчета межмолекулярного взаимодействия в качестве базиса для дальнейшего подбора средств коррекции токсических доз сероводорода. Визуализированы мембранные модели взаимодействия H_2S с белком и лецитином *in silico* и *in vitro*. Подтверждена возможность применения АЦЦ в качестве ингибитора H_2S.

Ключевые слова: математическое моделирование, сероводород, фосфолипид, лецитин, липосомы, редокс-потенциал

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In the era of industrialization, hazardous emissions from plants and factories are a possibility. For example, at hydrogen sulfide-containing (H_2S) gas production and processing facilities, well equipment depressurization may lead to accidents involving combustion or spread of gas fluid without combustion. Today, it is necessary to be prepared for such accidents with gas. Among other things, violations of the production technology, like overspeeding during drilling of gas-saturated strata, make them more probable [1–6].

In such cases, hydrogen sulfide is a pathogenic factor that promotes disruption of the energy metabolism and boosts processes associated with the activity of free radicals in cells [7, 8]. The results of the said disruption and processes are damaged membranes and energy deficiency. Subsequently, on the one hand, the level of macroerg compounds goes down and content of Ca^{2+} ions in the cells goes up, and, on the other hand, the level of adenosine triphosphoric acid (ATP) decreases, which promotes shutdown of ion pumps and prevents ingress of Ca^{2+} ions from the intercellular medium, activation of membrane-binding phospholipases, hydrolysis of some phospholipids and reinforcement of permeability of the membranes [9, 10].

It can be assumed that development of various pathological conditions and diseases is associated with molecular changes in cell plasmalemmas. Being a target for H_2S, membranes are involved in the pathological process that activates universal cell damage mechanisms associated with increased oxidation with free radicals and disruption of ion homeostasis [11–13].

In the recent years, active investigation of the molecular basis behind cell damage has boosted interest in the features of biological functioning of phospholipids [14, 15]. In biomembranes, lipid component is a functionally active matrix that integrates external influences and launches cellular control programs. There are three classes of membrane lipids: neutral lipids (30%), glycolipids (10%) and phospholipids (60%), the most widely represented class. The chemical energy of oxidized substrates or ATP is converted into electrical energy, namely, into a transmembrane difference in electrical potentials or into the energy of difference between concentration of substances contained in solutions separated by the membrane, and vice versa. Membranes can convert one form of energy into another. Retention of enzymes in mitochondria and oxidative phosphorylation, enzyme activity and cell sensitivity to hormonal and nervous regulation, as well as spatial identity depend on the state of the lipid component of the membrane [16–19].

The complexity of identification of the role of individual molecular mechanisms in realization of the processes that destroy membranes results from the closeness of their relationship. In this connection, deriving generalized descriptions of the cumulative patterns of cellular systems' reactions to pathological impulses of various origins not only promises better understanding of the general biological laws and how they function, but also allows re-evaluation of the methodology enabling adjustment thereof.

It is impossible to understand the actual behavior of a molecule without knowing its structure. Any change in the set of coordinates of the nuclei, as infinitesimal as it may be, builds a new geometry. The spatial structure of a molecule is not an inherent characteristic property of the system. Still, molecular structure allows pinpointing a certain temporary position of the molecule and grants understanding of the concept of its change. Mathematical modeling enables sequential investigation of various external factors. To determine the equilibrium spatial structure of the molecules, it is necessary to optimize the mutual arrangement of their atoms. Optimization of geometry means a search for atomic coordinates at which the system has the lowest energy value [20–22]. As a result of optimization, individual groups of atoms of a molecular system change their spatial position to a more favorable one in relation to neighboring atoms. Parametric method 3 (PM3) is one of the most consistent methods, carefully calibrated for a wide range of compounds, including organic and inorganic molecules of atoms of the main subgroups and hydrogen-bonded systems. It conveys the structure, thermodynamics, dipole moments, ionization potentials, vibrational frequencies well. In case of simple organic compounds, it approaches the density functional theory (DFT) in accuracy but surpasses it in performance by dozens of times. The average error in determining the enthalpy of a formation is about 5 kcal/mol. The advantages of other semi-empirical methods over PM3 are mainly seen in evaluation of electronic, magnetic resonance parameters and electronic excitation spectra [21, 22].

From the chemical point of view, intermolecular electron transfer is a reduction/oxidation reaction, or RedOx. The processes of electron transfer in mitochondrial membranes form the physicochemical basis for the mechanisms of energy storing in a cell. Currently, it is assumed that the transfer of electrons between the cell's liquid phase components and intracellular proteins regulates the activity of cell proteins. Development of many diseases is associated with the increased levels of oxidants in the body; the list of such disorders includes atherosclerosis, liver cirrhosis, cataracts, arthritis, coronary heart disease, bronchial asthma, hepatitis, and diabetes. Albert Szent-Gy–rgyi, a Nobel Prize winner, wrote: "Balance between electron donors and acceptors with different biopotentials is one of the main parameters of life..." In the course of a redox reaction, the reduced form of one redox pair (reductant) donates electrons to the oxidized form (oxidizer) of another pair [23–25]. H_2S is a known active ion exchange participant capable of binding copper atoms in cytochrome oxidase and thereby blocking the transfer of electrons from this mitochondrial respiratory chain enzyme to oxygen. Since body fluids are open systems constantly exchanging electrons with the environment, we discretized possible cross-reactions and used the liposomal membrane model in an aqueous medium.

The purpose of this work is to compare the degree of intermolecular interaction of two-component systems with a biomembrane (liposomal model thereof) in the reaction with hydrogen sulfide and acetylcysteine with the aim of adjusting the membrane's redox potential.

METHODS

Mathematical calculations were performed in GAMESS using the PM3 method (GBASIS=PM3), the gradient norm accuracy was up to the fourth decimal place inclusive (OPTTOL=1.0E-4). The results enabled building the z-matrix, the configuration of which is quite close to the point of global minimum of the potential energy surface. The calculation yielded the gradient value of 0.0000327 kcal/mol/angstrom, which makes the computational capabilities of GAMESS rather high compared to ChemOffice. The total energy value for the resulting configuration of molecules is calculated in atomic energy units and given in kJ/mol.

We use three criteria to increase the accuracy of the adsorption interactions model: the magnitude of charge transfer (Δq), the distance between atoms (Å) and thermal effects of the adsorption complexes (ΔE_{ads}).

Protein and phospholipid components were chosen as components of the cell membrane. The structure of the protein

Table 1. Values of energy characteristics and bond lengths in the adsorption complexes of interaction of a pentapeptide molecule in vapors with hydrogen sulfide and ACC, results of application of PM3

Pentapeptide +	By hydrogen (...) bond between the indicated atoms	Bond length, Å	Charge transfer amount Δq, e	Energy characteristic ΔEads, KJ/mol
Hydrogen sulfide	$H_{61}...S_{77}$	2.582	135	–5.833
	$H_{61}...N_{26}$	1.007	211	–13.448
	$H_{78}...S_{77}$	1.293	–0.0167	–16.101
	$H_{79}...S_{77}$	1.291	–0.0182	–3.469
ACC	$H_{66}...S_{7}$	2.62	96	–38.38
	$H_{60}...S_{7}$	2.73	28	–36.89
	$H_{71}...S_{7}$	2.53	96	–34.46
	$H_{65}...S_{7}$	1.75	985	–34.39

was a simple pentapeptide resulting from the calculation of the optimal equilibrium of the system from the viewpoint of the minimum total atomic energy. Given that phospholipid molecules can move from one side of the membrane to another, the in vitro model excludes variation in the biological response to pathogenic factors and minimizes the asymmetry of phospholipids in the membrane. As the base of liposomes, we used lecithin that was two-thirds phospholipids.

The liposomes were prepared as described below.

We prepared the sulfide solution within the first stage of hydrolysis as per the following molecular equation:

$$Na_2S + HOH <=> NaHS + NaOH,$$

and the complete ionic equation for this reaction was as follows:

$$2Na^+ + S^{2-} + HOH <=> Na^+ + HS^- + Na^+ + OH^-.$$

We stopped the reactions before the second stage of hydrolysis since that would imply formation of volatile forms of hydrogen sulfide, which is toxic. Another reason behind this decision is the specific dosage approach adopted for this experiment.

We used a weighing unit (Conzept; Italy) with a weighing accuracy of 0.1 mg, a redox potential meter (in millivolts, mV) (Russia), a magnetic stirrer, chemical beakers and a cylinder (graduated, with a volume of 100 ml), pipettes, automatic dispensers, an ultrasonic disperser (China), powdered sodium sulfide, a fume hood.

The prepared solution of sodium sulfide (10%) had the sulfide concentration of 88 mg/l. Twelve grams of Na_2S were mixed with 80 ml of distilled water (introduced thereto). We determined the exposure factoring in achievement of a stable change of the parameter at a constant level. The measurements were taken thrice, with subsequent derivation of the average values.

The liposome solution was prepared from soy lecithin (Protein company; Russia) based on isopropanol at the ratio of 30:70. Having added a 0.1% solution of methylene blue (for better visualization), we treated the solution with ultrasonic (US) waves for 10 minutes, and then added the liposome solution to the sulfide solution at the ratio of 1:1. The saturated solution of lecithin (1 g + 25 ml of isopropanol) was supplemented with 10 ml of ACC solution (10 mg/ml) and then exposed to ultrasonic waves. The redox potential was measured in solutions of liposomes with lecithin and ACC before and after immersion in a sulfide medium 15 minutes after preparation of the media.

Abnormalities of distribution were detected with the help of the Kolmogorov–Smirnov and Shapiro–Wilk tests. Numerical values are given as median and P5-, P95-percentiles. The differences were considered significant at $p \leq 0.05$. For the analysis, we used descriptive statistical methods of Statistica 11.0 (StatSoft; USA).

RESULTS

Modeling the hydrogen sulfide's effect on a biological membrane includes generation of a mathematical model and a visual structure of the elements of the membrane and the toxicant. The plan implies a search for the sites of sorbate molecule that play a part in interactions resulting in the release of the greatest amount of energy. The energy of the activated complex should be at the minimum level possible. With the given structures, the interactions occur mainly in the amino group (NH_2), sulfide (SH), carbonyl (CO), hydroxyl (OH), and methyl (CH_3) groups, and may also involve hydrogen atoms of the benzene ring. Hydrogen sulfide can be bound both with hydrogen in amino and sulfide groups and with sulfur in amino, sulfide and hydroxyl groups. The strongest bonds include a hydrogen atom of the protein's SH group.

The tables below present the results of calculations of energy and geometric properties of the most significant molecular adsorption complexes (AC) with pentapeptide (Table 1) and lecithin (Table 2). The investigated amino acid sequence in the protein peptide was as follows: cysteine–phenylalanine–alanine–cysteine–tyrosine.

The tables show active sites of hydrogen bond formation under the influence of ACC and hydrogen sulfide on the components of the cell membrane. Transfer of the charge confirms that the interaction takes place, and the magnitude of the charge transfered indicates the direction in which the electrons move between the atoms of the interacting substances. The sign of the charge reflects the molecule acting on the membrane (H_2S or ACC). Positive sign means the charge is redistributed from hydrogen in the substance to the more electronegative atoms of the membrane (Fig. 1). Negative sign indicates the electrons move from the membrane's hydrogen to the "implanted" substances. The strongest interactions are observed between the lecithin's $H_{35}...S_7$ and ACC, in contrast to the hydrogen bonds of protein molecules and ACC (Fig. 2). Participation of proton of the ACC's SH group in formation of the hydrogen bond translates into greater density of electrons of the sulfur atom, which boosts its nucleophilic properties. Active SH group in the molecule of ACC conditions the properties of this substance. The length of the formed bond is most typical for this type of interaction, and the adsorption energy value reflects the robustness of the adsorption complexes. In this case, lecithin is the preferred target (Fig. 3).

Any change of interatomic positions affects the molecule's geometry as a whole, and hence alters the properties of the

Table 2. Values of energy characteristics and bond lengths in the adsorption complexes of interaction of a lecithin molecule in vapors with hydrogen sulfide and ACC, results of application of PM3

Lecithin +	By hydrogen (...) bond between the indicated atoms	Bond length, Å	Charge transfer amount Δq, e	Energy characteristic ΔEads, KJ/mol
ACC	$H_{35}...S_7$	3.04	–0.0136	–74.75
	$O_{14}...H_{12}$	1.8	–0.0209	–65.13
	$H_{25}...S_7$	3.05	221	–57.48
	$H_{29}...S_7$	2.57	–0.0311	–56.87
Hydrogen sulfide	$H_{33}...S_2$	2.62	172	–51.52
	$O_{14}...H_1$	1.76	–0.0429	–49.7
	$H_{41}...S_2$	2.43	294	–48.85
	$H_{35}...S_1$	3.03	293	–46.85

system, but a structured calculation allows registering the molecule's position in time and helps understand the principle of changes as they are shaped by external factors. As a result, the following tasks become more simple: establishing individual geometric positions of the molecules relative to each other; learning the peculiarities of the effect produced by the attacking substance; mapping active atoms of the membrane components; analyzing and showing the competitive replaceability of the antidote.

As shown, interactions of hydrogen sulfide and ACC have a lower minimum of the adsorption energy when reacting with lecithin than with peptide. This means we characterize such interactions as structures with more stable positions from the energy viewpoint. Therefore, the in vitro model is the lipid component of the membrane, and the liposomes formed have the diameter of 36.92 [27.98; 54.39] μm (Fig. 4).

The redox potential of the sulfide solution was –718 [699; 723] mV. This is an extremely low value, which indicates the strength of reduction capabilities of the hydrogen sulfide ions. The redox potential of the saturated lecithin solution was –77 [–72; –81] mV. After ultrasonic treatment and preparation of the liposome solution with ACC, the redox potential was –54 [–41; –59] mV. The redox potential of the final solution of sulfides + lecithin + ACC has grown to –122 [–120; –131] mV.

Thus, introduction of liposomes with ACC (acting as an oxidizer) restored the redox potential. Consequently, when interacting with hydrogen sulfide ions, active SH groups of the ACC give the solution properties of a buffer.

DISCUSSION

Currently, researchers rely on quantum chemical calculations when studying models of systems of molecular complexes. Their works include models of complexes consisting of two or more molecules that were described (with underlying tasks executed manually) earlier. One of such works presented a model of interaction in a three component system of oil, water and demulsifier [26]. The inclusion of water as one of the molecular system's components can only be justified if the purpose is to investigate the details of the demulsification process in a specific case of a possibility randomly set by the user. An alternative approach is accounting for solvation [27], a feature available in any quantum chemistry program, like GAMESS. The research efforts in this field target specific problems and mainly focus on determining the 3D structure of the molecules, distribution of charge and electron density of each atom of a molecule, total energy of the molecules, energies and heat accompanying formation of molecules, energies of electrons, energies of nuclei and dipole moments of atoms that make up the molecule [28, 29].

This study focuses on a systematic consideration of the possible adsorption complexes emerging as the molecules form a hydrogen bond between them. The focus enabled sequential investigation of the process of immersion of membrane's components in a hydrogen sulfide environment with subsequent evaluation of introduction of ACC as a competitor for hydrogen sulfide. With the help of quantum chemical modeling, we established the key values of energy peculiar to the formation of reacting molecules and their systems. To establish the stable positions of adsorption complexes we have additionally determined and calculated three criteria for each of them: the adsorption energy, the charge transfered from one molecule to another and the size of the formed hydrogen bond. Having eliminated the structures that did not meet the criteria, we learned the signatures of active atoms on the molecular surfaces specific to this kind of interactions [21].

We prepared the liposomes using generally recognized methods [16–18]. Isopropyl alcohol was used as a solvent, as it is less toxic (3600 – 5740 mg/kg) compared to other organic solvents (xylene, chloroform) [30] and more economically

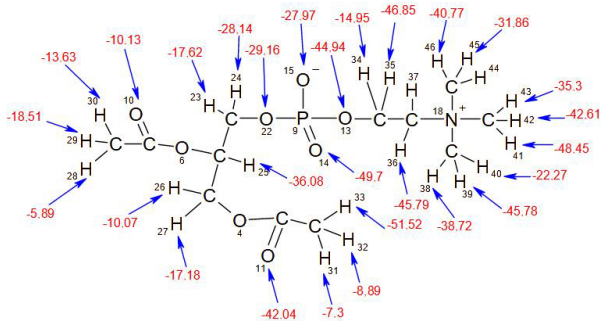

Fig. 1. Active sites signature pattern example, interaction of lecithin and hydrogen sulfide

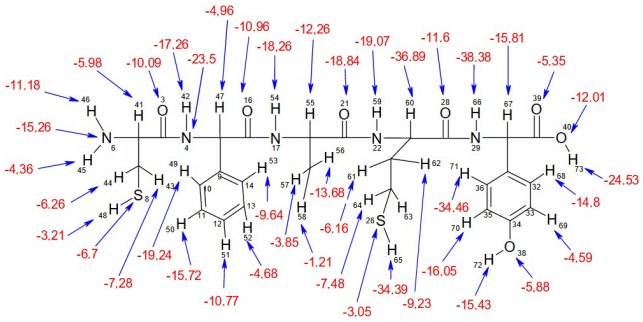

Fig. 2. Active sites signature pattern example, interaction of pentapeptide and ACC

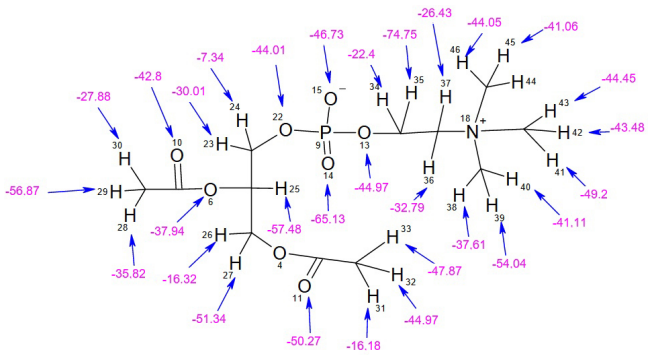

Fig. 3. Active sites signature pattern example, interaction of lecithin and ACC

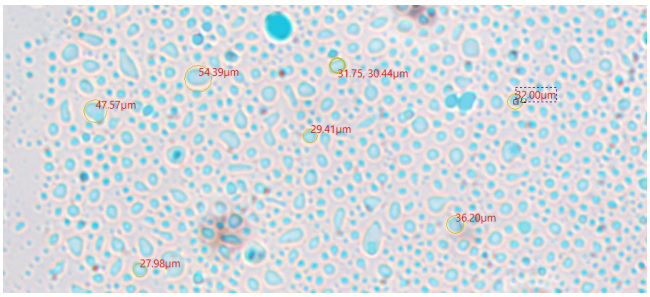

Fig. 4. Liposomes stained with methylene blue (magnification ×20)

sound compared to, for example, ethyl alcohol. We used a flow of gaseous nitrogen instead of a rotary alcohol evaporator, which greatly simplified the experiment.

CONCLUSIONS

In general, the suggested *in silico* method of mathematical modeling of action of toxic blockers on a cell membrane model can complement *in vitro* experiments. The results of this study allow a detailed description of the mechanism of events occurring on the cell membrane's surface. Further research will help improve understanding of the structure and properties of potential antidotes for a number of cytotoxic substances. Our data indicate that reaction sites for this type of interactions may form, and they predominantly appear on the atoms represented in the interaction signatures (Fig. 1–3). The studied interaction between molecular components of biomembranes — protein and phospholipid, on the one hand, and hydrogen sulfide and ACC molecules "implanted" onto them, on the other hand, — is a good starting point for further investigations of the processes occurring on the surface of the cell membrane. Thus, it can be stated that lecithin is the most preferable target for an experimental study of the membrane component. Calculations of the energies of interaction of adsorption complexes and the redox potential of the systems show that lecithin combines more optimally with ACC compared to a combination with hydrogen sulfide. The oxidative environment created through addition of liposomes with ACC to a sulfide solution indicates that, from the energy viewpoint, the interaction of lecithin with ACC is better than with sulfide ions. Competitive hydrogen bonds between phospholipid and ACC with hydrogen sulfide in the background support further experiments. For example, a model of liposomes in a plasma solution will yield a better understanding and grounds for subsequent investigation of the interaction between molecules of ACC and hydrogen sulfide at a more complex organ level of biosystem organization.

References

1. Bubnova ES, Kapustina MV. Gidrologo-gidroximicheskie usloviya v pridonnom sloe Gdan'skoj vpadiny Baltijskogo morya v 2003–2018 godax. Izvestiya KGTU. 2019; 55: 47–58. Russian.
2. Kolesnikova EEh, Golovina IV. Aktivnost' oksidoreduktaz v oksifil'nyx tkanyax morskogo ersha Scorpaena porcus Linnaeus, 1758 pri kratkosrochnoj serovodorodnoj nagruzke. Zhurnal ehvolyucionnoj bioximii i fiziologii. 2020; 56 (5): 399–410. DOI: 10.31857/S0044452920050046. Russian.
3. Velikorodov AV, Tyrkov AG, Kovalev VB, Serebryakov OI, Nosachev SB. Ehkologicheskaya ocenka atmosfernogo vozduxa v Nizhnem Povolzh'e. Geologiya, geografiya i global'naya ehnergiya. 2018; 1 (68): 103–9. Russian.
4. Grant RH, Boehm MT, Hagevoort GR. Emissions of hydrogen sulfide from a western open-lot dairy. J Environ Qual. 2022; 51 (4): 622–31. DOI: 10.1002/jeq2.20360. Epub 2022 May 26. PMID: 35446998.
5. Chiappe C, Pomelli CS. Hydrogen Sulfide and Ionic Liquids: Absorption, Separation, and Oxidation. Top Curr Chem (Cham). 2017; 375 (3): 52. DOI: 10.1007/s41061-017-0140-9. PMID: 28447285.
6. El Hachem K, Kang M. Methane and hydrogen sulfide emissions from abandoned, active, and marginally producing oil and gas wells in Ontario, Canada. Sci Total Environ. 2022; 823: 153491. DOI: 10.1016/j.scitotenv.2022.153491. PMID: 35124029.
7. Golubkina EV, Dyukareva OS, Trizno NN, Udochkina LA, Trizno MN. Vliyanie processov peroksidacii v trombocitax na sistemu gemostaza pri vozdejstvii serovodorodsoderzhashhego gaza. Zhurn mediko-biol issl. 2019; 7 (1): 40–48. Russian.
8. Malone Rubright SL, Pearce LL, Peterson J. Environmental toxicology of hydrogen sulfide. Nitric Oxide. 2017; 1 (71): 1–13. DOI: 10.1016/j.niox.2017.09.011. PMID: 29017846; PMCID: PMC5777517.
9. Rozhkova IS, Tyoplyj DL, Fel'dman BV. Analiz morfofiziologicheskix izmenenij timusa pri xronicheskoj intoksikacii i vvedenii antioksidantov. Astraxanskij medicinskij zhurnal. 2015; 10 (4): 73–78. Russian.
10. Ehsaulova TA, Bazaeva OV, Zyazina EN, Zyazin SN, Ponomaryova AA. Vliyanie serovodorodsoderzhashhego gaza na sostoyanie zdorov'ya naseleniya. Sovremennaya nauka: aktual'nye problemy teorii i praktiki. Seriya: Estestvennye i texnicheskie nauki. 2020; 1: 225–9. Russian.
11. Azatyan SG, Mazhitova MV. Perspektivy primeneniya ehkstrakta rastenij roda Astragalus pri serdechno-sosudistyx patologiyax. Prikaspijskij vestnik mediciny i farmacii. 2022; 3 (1): 6–14. DOI 10.48612/agmu/2022.3.1.6.14. Russian.
12. Sentyurova LG, Galimzyanov XM, Sherysheva YuV, Xuzhaxmetova LK, Berlyakova EM. Biologicheskie ritmy organizma mlekopitayushhix i cheloveka. Astraxanskij medicinskij zhurnal. 2018; 13 (2): 54–64. DOI 10.17021/2018.13.2.54.64. Russian.
13. Veremchuk LV, Tsarouhas K, Vitkina TI, Mineeva EE, Gvozdenko TA, Antonyuk MV, et al. Impact evaluation of environmental factors on respiratory function of asthma patients living in urban territory. Environ Pollut. 2018; 235: 489–96. DOI: 10.1016/j.envpol.2017.12.122. PMID: 29324378.
14. Yamamoto T, Endo J, Kataoka M, Matsuhashi T, Katsumata Y, Shirakawa K, et al. Decrease in membrane phospholipids unsaturation correlates with myocardial diastolic dysfunction. PLoS One. 2018; 13 (12): e0208396. DOI: 10.1371/journal.pone.0208396.
15. Manni MM, Tiberti ML, Pagnotta S, Barelli H, Gautier R, Antonny B. Acyl chain asymmetry and polyunsaturation of brain phospholipids facilitate membrane vesiculation without leakage. Elife. 2018; 7: e34394. DOI: 10.7554/eLife.34394.
16. Amézqueta S, Fernández-Pumarega A, Farré S, Luna D, Fuguet E,

Rosés M. Lecithin liposomes and microemulsions as new chromatographic phases. J Chromatogr A. 2020; 1611: 460596. DOI: 10.1016/j.chroma.2019.460596.
17. Urabe G, Shimada M, Ogata T, Katsuki S. Pulsed Electric Fields Promote Liposome Buddings. Bioelectricity. 2021; 3 (1): 68–76. DOI: 10.1089/bioe.2020.0016.
18. Dyrda G, Boniewska-Bernacka E, Man D, Barchiewicz K, Słota R. The effect of organic solvents on selected microorganisms and model liposome membrane. Mol Biol Rep. 2019; 46 (3): 3225–232. DOI: 10.1007/s11033-019-04782-y.
19. Arai K, Sato Y, Nakajima I, Saito M, Sasaki M, Kanamori A, Iwaoka M. Glutathione peroxidase-like functions of 1,2-diselenane-4,5-diol and its amphiphilic derivatives: Switchable catalytic cycles depending on peroxide substrates. Bioorg Med Chem. 2021; 29: 115866. DOI: 10.1016/j.bmc.2020.115866.
20. Zharkix LI, Golubkina EV, Trizno NN, avtory; FGBOU VO «Astraxanskij GMU» Minzdrava RF, patentoobladatel'. Sposob profilaktiki intoksikacii organizma zhivotnyx pri ostrom otravlenii serovodorodsoderzhashhim gazom v ehksperimente. 2020. Patent RF # 2739256 C1, 22.12.2020. Russian.
21. Smirnova YuA, Golovackaya LI. Svidetel'stvo o gosudarstvennoj registracii programmy dlya EhVM # 2022614450 Rossijskaya Federaciya. PROGRAMMA DLYa EhVM "TFinG": # 2022613564: zayavl. 15.03.2022: opubl. 22.03.2022; zayavitel' Federal'noe gosudarstvennoe byudzhetnoe obrazovatel'noe uchrezhdenie vysshego obrazovaniya «Volzhskij gosudarstvennyj universitet vodnogo transporta». Russian.
22. Smirnova YuA. Golovackaya LI. Razrabotka algoritma i metoda transformacii zapisi atomno-molekulyarnyx sistem. Prikaspijskij zhurnal: upravlenie i vysokie texnologii. 2022; 2 (58): 61–67. Russian.
23. Cao JT, Fu YZ, Wang YL, Zhang HD, Liu XM, Ren SW, Liu YM. Liposome-assisted chemical redox cycling strategy for advanced signal amplification: A proof-of-concept toward sensitive electrochemiluminescence immunoassay. Biosens Bioelectron. 2022; 214: 114514. DOI: 10.1016/j.bios.2022.114514.
24. Li M, Wang S, Xu J, Xu S, Liu H. PH/Redox-Controlled Interaction between Lipid Membranes and Peptide Derivatives with a "Helmet". J Phys Chem B. 2019; 123 (31): 6784–91. DOI: 10.1021/acs.jpcb.9b05367. PMID: 31306021.
25. He W, Du Y, Zhou W, Yao C, Li X. Redox-sensitive dimeric camptothecin phosphatidylcholines-based liposomes for improved anticancer efficacy. Nanomedicine (Lond). 2019; 14 (23): 3057–74. Available from: https://doi.org/10.2217/nnm-2019-0261.
26. Malova VO, Zolotaryova NV. Modelirovanie mezhmolekulyarnyx kontaktov organicheskix kislot s pripoverxnostnym sloem vodoneftyanoj ehmul'sii. Fundamental'nye i prikladnye problemy polucheniya novyx materialov: issledovaniya, innovacii i texnologii: Materialy XVI Mezhdunarodnoj nauchno-prakticheskoj konferencii, Astraxan', 26–28 aprelya 2022 g. Pod obshhej redakciej L.A. Dzhigola. Astraxan': Astraxanskij gosudarstvennyj universitet, 2022; c. 101–104. Russian.
27. Magdalinova NA, Ivanova LV, Klyuev MV. Vliyanie rastvoritelya v sinteze geksagidrofenoksazinov: kvantovo-ximicheskij podxod. Nauchno-issledovatel'skaya deyatel'nost' v klassicheskom universitete: tradicii i innovacii: Materialy Mezhdunarodnogo nauchno-prakticheskogo festivalya, Ivanovo, 19–29 aprelya 2022 goda. Ivanovo: Ivanovskij gosudarstvennyj universitet, 2022; c. 105–112. Russian.
28. Raximov FF, Bekov US. Kvantovo-ximicheskie raschyoty zaryadov kremniorganicheskix soedinenij — kak osnova ustojchivosti promezhutochnogo i perexodnogo sostoyanij. Universum: ximiya i biologiya. 2022; 5–2 (95): 47–50. Russian.
29. Petrushenko IK. Kvantovo-ximicheskoe modelirovanie adsorbcii vodoroda v porax: issledovanie metodami DFT, SAPT0 i IGM. Izvestiya vuzov. Prikladnaya ximiya i biotexnologiya. 2022; 12 (3): 363–72. DOI: 10.21285/2227-2925-2022-12-3-363-372. Russian.
30. National Center for Biotechnology Information. PubChem Compound Summary for CID 3776, Isopropyl Alcohol. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Isopropyl-Alcohol. Accessed Jan. 23, 2023.

Литература

1. Бубнова Е. С., Капустина М. В. Гидролого-гидрохимические условия в придонном слое Гданьской впадины Балтийского моря в 2003–2018 годах. Известия КГТУ. 2019; 55: 47–58.
2. Колесникова Е. Э., Головина И. В. Активность оксидоредуктаз в оксифильных тканях морского ерша Scorpaena porcus Linnaeus, 1758 при краткосрочной сероводородной нагрузке. Журнал эволюционной биохимии и физиологии. 2020; 56 (5): 399–410. DOI: 10.31857/S0044452920050046.
3. Великородов А. В., Тырков А. Г., Ковалев В. Б., Серебряков О. И., Носачев С. Б. Экологическая оценка атмосферного воздуха в Нижнем Поволжье. Геология, география и глобальная энергия. 2018; 1 (68): 103–9.
4. Grant RH, Boehm MT, Hagevoort GR. Emissions of hydrogen sulfide from a western open-lot dairy. J Environ Qual. 2022; 51 (4): 622–31. DOI: 10.1002/jeq2.20360. Epub 2022 May 26. PMID: 35446998.
5. Chiappe C, Pomelli CS. Hydrogen Sulfide and Ionic Liquids: Absorption, Separation, and Oxidation. Top Curr Chem (Cham). 2017; 375 (3): 52. DOI: 10.1007/s41061-017-0140-9. PMID: 28447285.
6. El Hachem K, Kang M. Methane and hydrogen sulfide emissions from abandoned, active, and marginally producing oil and gas wells in Ontario, Canada. Sci Total Environ. 2022; 823: 153491. DOI: 10.1016/j.scitotenv.2022.153491. PMID: 35124029.
7. Голубкина Е. В., Дюкарева О. С., Тризно Н. Н., Удочкина Л. А., Тризно М. Н. Влияние процессов пероксидации в тромбоцитах на систему гемостаза при воздействии сероводородсодержащего газа. Журн. медико-биол. иссл. 2019; 7 (1): 40–48.
8. Malone Rubright SL, Pearce LL, Peterson J. Environmental toxicology of hydrogen sulfide. Nitric Oxide. 2017; 1 (71): 1–13. DOI: 10.1016/j.niox.2017.09.011. PMID: 29017846; PMCID: PMC5777517.
9. Рожкова И. С., Тёплый Д. Л., Фельдман Б. В. Анализ морфофизиологических изменений тимуса при хронической интоксикации и введении антиоксидантов. Астраханский медицинский журнал. 2015; 10 (4): 73–78.
10. Эсаулова Т. А., Базаева О. В., Зязина Е. Н., Зязин С. Н., Пономарёва А. А. Влияние сероводородсодержащего газа на состояние здоровья населения. Современная наука: актуальные проблемы теории и практики. Серия: Естественные и технические науки. 2020; 1: 225–9.
11. Азатян С. Г., Мажитова М. В. Перспективы применения экстракта растений рода Astragalus при сердечно-сосудистых патологиях. Прикаспийский вестник медицины и фармации. 2022; 3 (1): 6–14. DOI 10.48612/agmu/2022.3.1.6.14.
12. Сентюрова Л. Г., Галимзянов Х. М., Шерышева Ю. В., Хужахметова Л. К., Берлякова Е. М. Биологические ритмы организма млекопитающих и человека. Астраханский медицинский журнал. 2018; 13 (2): 54–64. DOI 10.17021/2018.13.2.54.64.
13. Veremchuk LV, Tsarouhas K, Vitkina TI, Mineeva EE, Gvozdenko TA, Antonyuk MV, et al. Impact evaluation of environmental factors on respiratory function of asthma patients living in urban territory. Environ Pollut. 2018; 235: 489–96. DOI: 10.1016/j.envpol.2017.12.122. PMID: 29324378.
14. Yamamoto T, Endo J, Kataoka M, Matsuhashi T, Katsumata Y, Shirakawa K, et al. Decrease in membrane phospholipids unsaturation correlates with myocardial diastolic dysfunction. PLoS One. 2018; 13 (12): e0208396. DOI: 10.1371/journal.pone.0208396.
15. Manni MM, Tiberti ML, Pagnotta S, Barelli H, Gautier R, Antonny B. Acyl chain asymmetry and polyunsaturation of brain phospholipids facilitate membrane vesiculation without leakage.

Elife. 2018; 7: e34394. DOI: 10.7554/eLife.34394.
16. Amézqueta S, Fernández-Pumarega A, Farré S, Luna D, Fuguet E, Rosés M. Lecithin liposomes and microemulsions as new chromatographic phases. J Chromatogr A. 2020; 1611: 460596. DOI: 10.1016/j.chroma.2019.460596.
17. Urabe G, Shimada M, Ogata T, Katsuki S. Pulsed Electric Fields Promote Liposome Buddings. Bioelectricity. 2021; 3 (1): 68–76. DOI: 10.1089/bioe.2020.0016.
18. Dyrda G, Boniewska-Bernacka E, Man D, Barchiewicz K, Słota R. The effect of organic solvents on selected microorganisms and model liposome membrane. Mol Biol Rep. 2019; 46 (3): 3225–232. DOI: 10.1007/s11033-019-04782-y.
19. Arai K, Sato Y, Nakajima I, Saito M, Sasaki M, Kanamori A, Iwaoka M. Glutathione peroxidase-like functions of 1,2-diselenane-4,5-diol and its amphiphilic derivatives: Switchable catalytic cycles depending on peroxide substrates. Bioorg Med Chem. 2021; 29: 115866. DOI: 10.1016/j.bmc.2020.115866.
20. Жарких Л. И., Голубкина Е. В., Тризно Н. Н., авторы; ФГБОУ ВО «Астраханский ГМУ» Минздрава РФ, патентообладатель. Способ профилактики интоксикации организма животных при остром отравлении сероводородсодержащим газом в эксперименте. 2020. Патент РФ № 2739256 C1, 22.12.2020.
21. Смирнова Ю. А., Головацкая Л. И. Свидетельство о государственной регистрации программы для ЭВМ № 2022614450 Российская Федерация. ПРОГРАММА ДЛЯ ЭВМ "TFinG": № 2022613564: заявл. 15.03.2022: опубл. 22.03.2022; заявитель Федеральное государственное бюджетное образовательное учреждение высшего образования «Волжский государственный университет водного транспорта».
22. Смирнова, Ю. А. Головацкая Л. И. Разработка алгоритма и метода трансформации записи атомно-молекулярных систем. Прикаспийский журнал: управление и высокие технологии. 2022; 2 (58): 61–67.
23. Cao JT, Fu YZ, Wang YL, Zhang HD, Liu XM, Ren SW, Liu YM. Liposome-assisted chemical redox cycling strategy for advanced signal amplification: A proof-of-concept toward sensitive electrochemiluminescence immunoassay. Biosens Bioelectron. 2022; 214: 114514. DOI: 10.1016/j.bios.2022.114514.
24. Li M, Wang S, Xu J, Xu S, Liu H. PH/Redox-Controlled Interaction between Lipid Membranes and Peptide Derivatives with a "Helmet". J Phys Chem B. 2019; 123 (31): 6784–91. DOI: 10.1021/acs.jpcb.9b05367. PMID: 31306021.
25. He W, Du Y, Zhou W, Yao C, Li X. Redox-sensitive dimeric camptothecin phosphatidylcholines-based liposomes for improved anticancer efficacy. Nanomedicine (Lond). 2019; 14 (23): 3057–74. Available from: https://doi.org/10.2217/nnm-2019-0261.
26. Малова В. О., Золотарёва Н. В. Моделирование межмолекулярных контактов органических кислот с приповерхностным слоем водонефтяной эмульсии. Фундаментальные и прикладные проблемы получения новых материалов: исследования, инновации и технологии: Материалы XVI Международной научно-практической конференции, Астрахань, 26–28 апреля 2022 г. Под общей редакцией Л.А. Джигола. Астрахань: Астраханский государственный университет, 2022; с. 101–104.
27. Магдалинова Н. А., Иванова Л. В., Клюев М. В. Влияние растворителя в синтезе гексагидрофеноксазинов: квантово-химический подход. Научно-исследовательская деятельность в классическом университете: традиции и инновации: Материалы Международного научно-практического фестиваля, Иваново, 19–29 апреля 2022 года. Иваново: Ивановский государственный университет, 2022; с. 105–112.
28. Рахимов Ф. Ф., Беков У. С. Квантово-химические расчеты зарядов кремниорганических соединений — как основа устойчивости промежуточного и переходного состояний. Universum: химия и биология. 2022; 5–2 (95): 47–50.
29. Петрушенко И. К. Квантово-химическое моделирование адсорбции водорода в порах: исследование методами DFT, SAPT0 и IGM. Известия вузов. Прикладная химия и биотехнология. 2022; 12 (3): 363–72. DOI: 10.21285/2227-2925-2022-12-3-363-372.
30. National Center for Biotechnology Information. PubChem Compound Summary for CID 3776, Isopropyl Alcohol. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Isopropyl-Alcohol. Accessed Jan. 23, 2023.

ASSESSMENT OF INDIVIDUAL HEMATOPOIETIC STEM CELL RESPONSE TO GAMMA EXPOSURE USING HUMANIZED MICE

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Assessment of individual responses of cells, tissues and the whole body to radiation exposure is an important challenge for radiobiology and radiation safety. The study was aimed to develop the method for estimation of the human hematopietic stem cell (HSC) individual response in the humanized mouse model. The cord blood or peripheral blood HSCs were administered to the NOD SCID immunodeficient mice. The number of maturing HSCs ($CD34^+$ cells) and mature $CD45^+$ leukocytes was assesed after the acute gamma exposure to the doses of 0.5 Gy, 1 Gy, and 1.5 Gy, along with the HSC share among all $CD45^{low/+}$ cells within three days (period of maximum mortality) and 14 days (period of active restoration) after exposure. The relationship between the indicato values and the exposure dose was calculated by regression analysis. There was exponential relationship between the human HSC survival rate in humanized mice and the dose on day three after exposure ($R^2 = 0.93$; $F = 211$; $p < 0.01$), while the relationship between the number of HSCs and the dose on day 14 after exposure was linear ($R^2 = 0.65$; $F = 12.9$; $p = 0.01$). The *C*14/3 coefficient calculated as a ratio of the HSC share among all human $CD45^{low/+}$ cells on day 14 after exposure to the same parameter on day three after exposure was proposed as an indicator of HSC mortality and HSC number restoration. *C*14/3 negatively correlated with the exposure dose ($R^2 = 0.57$; $F = 13.3$; $p = 0.004$), it was higher in radioresistant mice and the model of cysteamine-induced radioresistance in humanized mice. The model mice humanized using the peripheral blood HSCs can be used to assess individual HSC response to acute external gamma exposure based on *C*14/3 and the data on the HSC survival and restoration.

Keywords: hematopoietic stem cells, acute radiation syndrome, humanized mice, individual radiosensitivity

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Compliance with the ethical standards: the study was approved by the Ethics Committee of the Urals Research Center for Radiation Medicine (protocol № 2 of 27 June 2022). Cord blood sampling was performed after obtaining the informed consent from donors at the Chelyabinsk Regional Perinatal Center. Peripheral blood samples were provided by the Blood Transfusion Station of FMBA of Russia (Chelyabinsk) in accordance with the Decree of the Government of the Russian Federation № 331 of 12 April 2013. Animals were handled in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (1986, Strasbourg), Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

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ОЦЕНКА ПЕРСОНИФИЦИРОВАННОЙ РЕАКЦИИ ГЕМОПОЭТИЧЕСКИХ СТВОЛОВЫХ КЛЕТОК НА ГАММА-ОБЛУЧЕНИЕ С ИСПОЛЬЗОВАНИЕМ ГУМАНИЗИРОВАННЫХ МЫШЕЙ

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Оценка персонифицированной реакции клеток, тканей и организма на радиационное воздействие является важной проблемой радиобиологии и радиационной защиты. Целью исследования было разработать метод оценки персонифицированной реакции гемопоэтических стволовых клеток (ГСК) человека в модели гуманизированных мышей. ГСК пуповинной или периферической крови вводили иммунодефицитным мышам NOD SCID. После острого внешнего гамма-облучения в дозах 0,5 Гр, 1 Гр и 1,5 Гр оценивали число ГСК ($CD34^+$-клеток), созревающих и зрелых лейкоцитарных клеток $CD45^+$, долю ГСК среди всех $CD45^{low/+}$-клеток через трое суток (период максимальной гибели) и 14 суток (период активного восстановления) после облучения. Методом регрессионного анализа рассчитывали зависимость показателей от дозы облучения. Описана экспоненциальная зависимость выживаемости ГСК человека у гуманизированных мышей через трое суток после облучения ($R^2 = 0,93$; $F = 211$; $p < 0,01$), линейная зависимость от дозы количества ГСК на 14-е сутки после облучения ($R^2 = 0,65$; $F = 12,9$; $p = 0,01$). В качестве показателя, отражающего гибель ГСК и восстановление их числа и функциональной активности, предложен коэффициент *K*14/3, равный отношению доли ГСК среди всех $CD45^{low/+}$-клеток человека на 14-е сутки после облучения к этому параметру на третьи сутки. *K*14/3 имел обратную зависимость от дозы облучения ($R^2 = 0,57$; $F = 13,3$; $p = 0,004$), был выше у радиорезистентных мышей и в модели индуцированной цистеамином радиорезистентности у гуманизированных мышей. Модель мышей, гуманизированных ГСК периферической крови, может быть использована для оценки персонифицированных реакций ГСК на острое внешнее гамма-облучение на основе применения показателя *K*14/3, данных о выживаемости и восстановлении ГСК.

Ключевые слова: гемопоэтические стволовые клетки, острый радиационный синдром, гуманизированные мыши, индивидуальная радиочувствительность

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Developing the approaches to estimation of individual radiosensitivity remains a priority of human radiation biology due to rapid development of nuclear technologies, nuclear medicine technologies and the perspectives of long-term interplanetary spaceflights [1, 2]. Assessment of individual response to exposure is necessary to identify the groups with high risk of exposure effects. This would be useful for both selection of staff to work with the sources of ionizing radiation and cosmonauts, and personalization of the risk of health effects of human exposure to radiation.

The phenomenon of radiosensitivity is assessed by the researchers at various levels, from radiosensitivity of the whole body manifested in survival or death after exposure, to radiosensitivity of tissues and cells and their susceptibility to the long-term adverse exposure effects [1–3]. However, there is no uniform system of determining individual radiosensitivity in humans. Intensive research focused on studying molecular genetic, immunological, hematological and other markers, the personalized predictors of the tissue response and the long-term effects of both acute and chronic exposure to radiation, is under way [1, 2, 4, 5].

Red bone marrow is one of the most radiosensitive tissues in the human body. The red bone marrow resistance to radiation exposure depends on the DNA damage repair, cell repopulation in the tissue on the accounts of proliferating revenant cells, the ability of the tissue to form the functional reserve of cells, etc. [1]. In the bone marrow form of acute radiation syndrome, the effects on the body (the chance of death) result largely from the hematopoietic stem cell (HSC) survival rate and the kinetics of the revenant cell populations [6, 7]. Ionizing radiation has a suppressive effect on the HSC proliferative and regenerative potential. Furthermore, the issue of the relationship between the HSC response to exposure and individual radiosensitivity remains unresolved.

The use of humanized mice for assessment of individual human HSC response to ionizing radiation is promising [8, 9]. The following types of response to radiation exposure have been reported in the model mice humanized by transplantation of human HSCs: enhanced γH2AX foci, increased expression of the *p16INK4a* gene, loss of HSC capability of repopulation after transplantation to secondary recipients, and reduced differentiation repertoir [10]. Similar processes that are typical for the HSC natural ageing [11–13] are also observed in cases of human or animal external exposure [14]. In particular, clonal expansion of hematopoietic cells was reported in exposed animals [15] and astronauts who experienced high levels of radiation exposure during spaceflights [16].

The above defines the relevance and validity of such task as the development of methods for estimation of the HSC individual response to exposure and its relation to radiosensitivity.

The study was aimed to develop the technology for estimation of the human HSC individual response to exposure based on the HSC xenotransplantation to immunodeficient mice in order to determine radiosensitivity in terms of the hematopoietic tissue response to acute radiation exposure.

METHODS

The NOD SCID immunodeficient mice (breeding nursery of the SPF vivarium, Institute of Cytology and Genetics, Siberian Branch of Russian Academy of Sciences; Novosibirsk, Russia) were used to obtain humanized mice. Animals were housed in the specific pathogen free (SPF) environment with a temperature of 22 ± 2 °C and humidity of 50–60% and fed with the autoclaved SPF pellets for stock mice. They were given free access to food and water, the 12-hour light cycle was used. Cervical dislocation was used to withdraw animals from the experiment.

Animals were administered peripheral blood and cord blood HSCs [17]. HSCs were obtained from the cord blood samples collected in the Regional Perinatal Center and from the donor peripheral blood product, the buffy coat (Blood Transfusion Station of FMBA of Russia; Chelyabinsk, Russia). HSCs were isolated from blood by innumomagnetic separation using the EasySep Human Cord Blood CD34 Positive Selection Kit II (Stem Cell Technologies; Canada). HSCs were identified as the $CD45^{low}CD34^{+}$ cells.

The mice received intravenous lateral tail vein injections of the cord blood HSCs after acute exposure to the external gamma dose of 2.5 Gy. The HSC engraftment in the murine bone marrow and the development of human lymphogranulopoiesis occurred within nine weeks. Equal amounts of HSCs collected from each cord blood donor were administered to at least three mice (the number of administered HSCs was 30,000–200,000 cells per animal). One mouse was left as a control, while the other two were exposed to the doses of 0.5, 1.0, and 1.5 Gy nine weeks after the HSC transplantation (three HSC donors per dose). The number of human and murine cells was measured in the femurs of the control mouse and other mice three days and 14 days after exposure.

HSCs isolated from peripheral blood were administered to mice by intraosseous tibia injection after applying isoflurane anesthesia. Equal amounts of HSCs collected from each peripheral blood donor were administered to at least four mice (the number of administered HSCs was 30,000–115,000 cells per animal). Two animals that received cells isolated from one donor were exposed before HSC administration (non-exposed human cells), and two mice that received cells isolated from the same donor were exposed after HSC administration (exposed human cells). The same exposure doses, 0.5, 1.0, and 1.5 Gy, were used (three donors per dose). Human and murine cells were enumerated three days and 14 days after exposure (one mouse with non-exposed human HSCs and another one with exposed cells for each timepoint).

The exposure was provided using the IGUR-1M radiobiological research gamma-unit (Quantum; Russia). The unit was equipped with the sources of ^{137}Cs, the dose rate was 0.91 Gy/min, and the gamma field non-uniformity did not exceed 10%.

The number of murine $CD45^{+}$ cells (stained with the PE-conjugated rat anti-CD45 monoclonal antibody, clone 30-F11; BD Pharmingen, USA), human $CD45^{+}$ leukocytes (stained with the FITC-conjugated mouse anti-CD45 monoclonal antibody, clone HI30; Stem Cell Technologies, Canada), and $CD45^{low}CD34^{+}$ human stem cells (stained with the APC-conjugated mouse anti-CD34 monoclonal antibody, clone 581; Stem Cell Technologies, Canada) in the bone marrow of mice was measured by flow cytometry. The Accuri C6 cytometer was used for measurement (BD Biosciences; USA). We calculated the number of cells per milliliter of suspension containing cells isolated from one bone.

The survival rate of exposed cells was calculated as a ratio of the number of exposed cells measured within three days and 14 days to the number of cells of the same donor in the control non-exposed mouse (for cord blood HSCs) or as a ratio of the number of exposed cells to the number of non-exposed cells of the same donor measured in the same timepoint after exposure (for peripheral blood HSCs). The HSC share was calculated as a percentage of the total number of human $CD45^{low}/CD45^{+}$ cells for each humanized animal. We

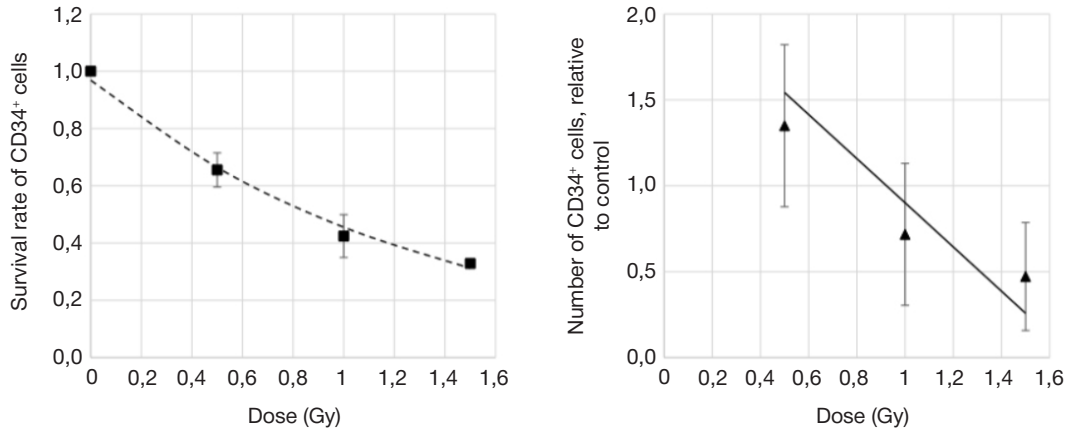

Fig. 1. Survival rate of the CD45^{low}CD34^{+} human cells in the model mice humanized with the cord blood HSCs as a function of the acute external gamma exposure dose. **A**. HSC survival rate three days after exposure. **B**. HSC number relative to the non-exposed control 14 days after exposure

also determined a coefficient that was calculated as a ratio of the HSC share on day 14 after exposure to the HSC share on day three after exposure (*C*14/3), since it was previously shown that this indicator was associated with the animal survival rate and cell repopulation in the bone marrow of non-humanized mice in in vivo studies [18].

Two experiments were conducted to assess the *C*14/3 coefficient prognostic properties in order to define the human HSC individual response to radiation exposure and the relationship between this indicator and radiosensitivity. The first experiment involved measuring the *C*14/3 coefficient for the fraction of murine CD117^{+} hematopoietic stem cells after exposure to the dose of 1 Gy in animals of two lines with different radiosensitivity: the radiosensitive NOD SCID line ($LD_{50/30}$ = 3.5 Gy) and the relatively radioresistant C57Bl/6 line ($LD_{50/30}$ = 6.0 Gy) [18].

In the second experiment, HSCs obtained from three cord blood donors were used for humanization in five humanized mice instead of three. These three additional humanized animals were administered the drug with a known radioprotective effect, mercaptoethylamine (cysteamine) at a dose of 200 mg/kg of body weight (Serva; USA), by intraperitoneal route 30 min before the exposure. The animals that received cells from two donors were exposed to a dose of 0.5 Gy, while the animals that received cells from the third donor were exposed to a dose of 1 Gy. We compared *C*14/3 coefficients in humanized mice not administered cysteamine and mice with increased radioresistance due to cysteamine.

The mean and standard error of the mean were calculated for the studied parameters. Regression analysis was performed using Microsoft Office Excel (Microsoft; USA) to reveal the relationship between the studied indicators and the dose. Student's *t*-test was used to compare regression coefficients. The results were considered significant at the probability that the null hypothesis was true ($p < 0.05$).

RESULTS

After administration of the cord blood HSCs to mice with severe combined immunodeficiency (NOD SCID) human cells colonize the murine bone marrow and form a self-sustaining pool of HSCs and a pool of maturing cells, mostly lymphocytes/granulocytes [17, 19]. This model makes it possible to study the radiation-induced apoptosis and repopulation of human stem cells, as well as their potential for maintaining the pool of maturing human cells *in vivo* in the humanized animal model.

Our experiments have shown that the humanized mice exposure results in the dose-dependent decrease in the survival rate of the CD45^{low}CD34^{+} human cells three days after exposure (Fig. 1A). Exponential dose dependence is observed (R^2 = 0.67; F = 38.65; $p < 0.001$).

On day 14 after exposure the number of HSCs in exposed mice increased. In different donors, it could exceed or fall below the baseline reference levels that corresponded to no exposure (Fig. 1B). No significant correlation between the exposure dose and the decrease in the number of HSCs was observed on day 14 after exposure (R^2 = 0.34; F = 4.01; p = 0.079 for linear relationship).

After exposure the share of stem cells among all human cells in the bone marrow of humanized mice decreased compared to baseline (25 ± 8%). There was a linear relationship between the dose within the range of 0.5–1.5 Gy and the *C*14/3 coefficient calculated as the ratio of the share of stem cells on day 14 after exposure to the share of stem cells on day three after exposure (Fig. 2). This indicator decreased with increasing dose (R^2 = 0.57; F = 13.26; p = 0.004). The indicator reflects the stem cells' survival rate, their regeneration 14 days after exposure, and ability to produce differentiating cells.

The experiment involving the use of radioprotective drug, cysteamine, showed that the humanized mice that were protected with radioprotector had a higher *C*14/3 coefficient than the non-protected mice exposed to the same dose (Fig. 3). Thus, the increased coefficient indicates higher radioresistance.

The same pattern was revealed when comparing *C*14/3 coefficients in mice of the lines with different radiosensitivity

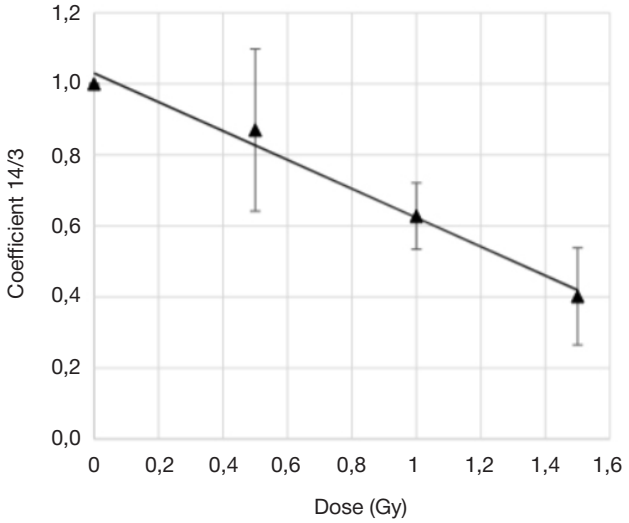

Fig. 2. Relationship between the *C*14/3 coefficient and the dose for CD45^{low}CD34^{+} human cells in the model mice humanized with the cord blood HSCs

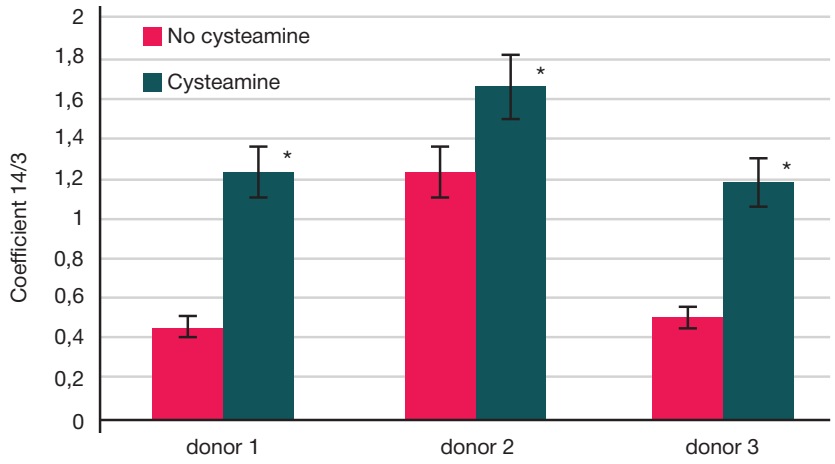

Fig. 3. *C*14/3 coefficient for the CD45^{low}CD34^{+} human cells in the model mice humanized with the cord blood HSCs isolated from three donors. Responses of human hematopoietic cells in humanized mice to radiation exposure in the groups administered (induced radioresistance) and not administered cysteamine. * — significant differences between groups, $p < 0.05$

(Fig. 4). Thus, *C*14/3 coefficient in radiosensitive NOD SCID mice ($LD_{50/30}$ = 3.5 Gy, 95% CI 3.4–3.8 Gy) exposed to a dose of 1 Gy was 0.23 ± 0.06, which represented a significant difference (t = 3.9; p = 0.003) from the *C*14/3 coefficient (0.98 ± 0.18) of the C57Bl/6 mice with normal radiosensitivity ($LD_{50/30}$ = 6.0 Gy, 95% CI 5.8–6.2 Gy). This proves that the coefficient can reflect the differences in hematopoietic responses between mice with different radiosensitivity in vivo.

After administration of peripheral blood HSCs of adult humans, HSCs are unable to function in the bone marrow of mice for a long time, but can maintain the HSC pool and produce maturing cells for at least 14 days [17]. Just as in the model mice humanized with the cord blood HSCs, there is exponential relationship between the human HSC survival rate and the external gamma exposure dose on day three after exposure (R^2 = 0.93; F = 211; $p < 0.001$; Fig. 5A).

On day 14 after exposure, the number of HSCs increased and exceeded the control level (no exposure) in the animals exposed to a dose of 0.5 Gy; in the animals exposed to doses of 1.0 and 1.5 Gy, the number of cells remained low compared to the number of HSCs observed on day 14 in the non-exposed animals (Fig. 5B). The linear model of the relationship between the dose and the number of HSCs relative to the non-exposed control 14 days after exposure was described (R^2 = 0.65; F = 12.90; p = 0.009).

It is important to note that the models describing dose dependence of the cell survival rate that were obtained for the cord blood and peripheral blood HSCs were similar: no significant differences were revealed when comparing the coefficients (t = 1.18; p = 0.24) and the constant terms (t = 0.15; p = 0.88) of the equations that related the HSC survival rate and the dose on day three after exposure or when comparing the slope (t = 0.19; p = 0.85) and the constant terms (t = 0.34; p = 0.74) of the equations that related the relative cell number and the dose on day 14 after exposure.

Just like in the model mice humanized with the cord blood HSCs, *C*14/3 coefficient in the short-term model of mouse humanization with the peripheral blood HSCs was a function of dose (R^2 = 0.45; F = 5.67; p = 0.048) and reflected individual characteristics of the HSC donors (Fig. 6).

DISCUSSION

Assessment of individual radiosensitivity in terms of both severity of early tissue reactions and the risk of delayed exposure effects is necessary to identify the groups with high risk of adverse exposure effects (in case of high radiosensitivity) and to select staff (individuals with high radioresistance).

Individual response to radiation exposure can be measured at different levels of organization of the organism based on estimation of various final effects of exposure, such as death of the organism, cancer, non-cancerous disorders, tissue reactions after exposure, chromosomal aberrations, and molecular alterations [2, 3]. Not all studies succeed in determining the relationship between the molecular and cellular responses *in vitro* and the severity of tissue reactions to acute exposure [4, 20, 21].

It seems that clonogenic cell survival can be considered as a good predictor of radiosensitivity [4]. HSCs can be considered as a representational model for assessment of the hematopoietic system radiosensitivity. Obtaining humanized mice may be a promising method for assessment of the HSC individual response [8, 9], since these humanized mice may be considered as "avatars" reflecting the entire range of features of the cellular response inherent to the donor of cells. Such an approach is being developed within the framework of introducing personalized care for cancer patients and studying individual features of the immune system [22–25].

When human HSCs are transplanted to mice with severe combined immunodeficiency previously exposed to sublethal radiation dose, human cells can colonize the niches in the animal bone marrow that have become vacant after exposure and exist in the body of the mouse for a long time (2–12 months after administration). Moreover, HSCs not only maintain their

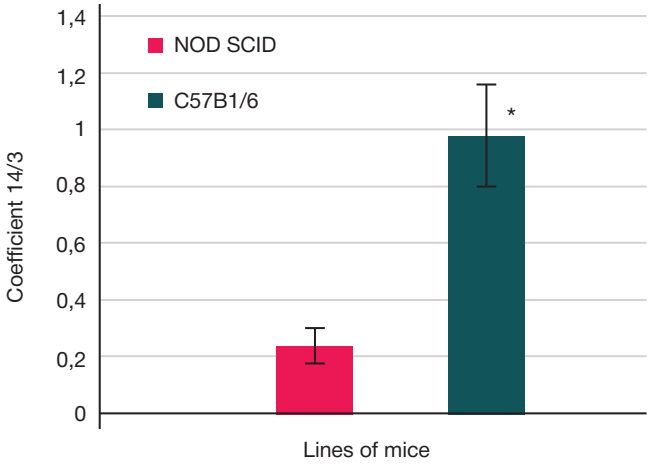

Fig. 4. *C*14/3 coefficient for hematopoietic CD117^{+} cells of the lines of mice with high (NOD SCID) and normal (C57Bl/6) radiosensitivity. * — significant differences between groups, $p < 0.05$

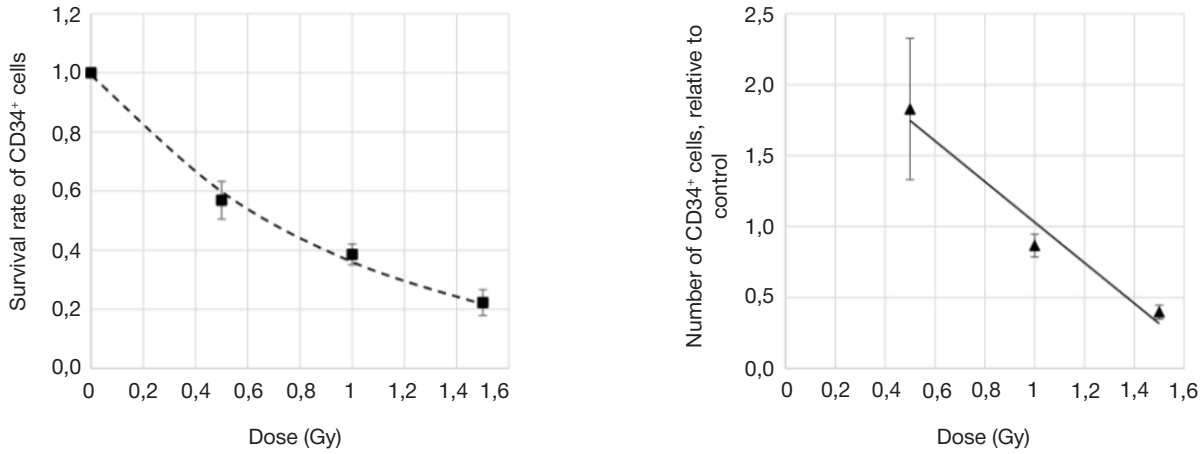

Fig. 5. Survival rate of the CD45^{low}CD34^{+} human cells in the model mice humanized with the peripheral blood HSCs as a function of the acute external gamma exposure dose. **A**. HSC survival rate three days after exposure. **B**. HSC number relative to the non-exposed control 14 days after exposure

pool of multipotent stem cells, but also give rise to the mature and functionally active blood cells, mostly myeloid cells.

The mice humanized by adminisration of the cord blood HSCs can be used for development of the acute radiation syndrome model, since these mice provide an opportunity to assess the effects of exposure on the HSC mortality, subsequent repopulation, and functional activity. Our study shows the the HSC survival rate in the short term after exposure (within three days) and the number of HSCs 14 days after exposure (during the period of restoring the population) depend on the dose in case of exposure to the doses of 0.5–1.5 Gy.

The *C*14/3 coefficient proposed by the authors that characterizes the change in the HSC share relative to other human CD45$^{low/+}$ cells within 14 days after exposure is considered as an integrated indicator that reflects the HSC survival, subsequent restoration, and functional activity. The earlier studies show the relationship between this indicator and survival rates in mice of various lines, i.e. the association with radiosensitivity at the organism level [18]. The negative relationship between the *C*14/3 coefficient and the exposure dose has been revealed in the model mice humanized with the cord blood HSCs. This indicates the possibility of using this parameter for assessment of the severity of the tissue response to ionizing radiation. The experiments that involve comparison of these indicators in the organisms that obviously have different radiosensitivity (NOD SCID and C57Bl/6 mice, humanized mice administered or not administered radioprotector) have revealed unidirectional differences: the coefficient was higher in more radioresistant subjects. The association of higher *C*14/3 with the lower doses and higher radioresistance may be due to the more effective HSC repopulation after exposure, higher proliferative potential, and more effective restoration of the stem cell pool before triggering proliferation and maturation of the CD45^{+} cells.

When administering peripheral blood HSCs to mice with immunodeficiency, we have failed to obtain a long-lasting pool of stem cells in the animal bone marrow [17], however, simultaneous use of radiation exposure during transplantation of human cells and enumeration of HSCs and maturing hematopoietic cells within 14 days after transplantation is possible. The study shows that the dose dependence of the HSC survival rate and the HSC number 14 days after exposure is the same in the humanized mouse models with the sustained cord blood and peripheral blood HSCs. Therefore, the model obtained using peripheral blood HSCs that does not involve prolonged repopulation can be also used for simulation of acute radiation syndrome, as well as for assessment of the radiation-induced cell death and cell restoration. The calculated *C*14/3 coefficient has also shown negative correlation with the dose in the model obtained using peripheral blood HSCs.

It is important to note the fact that the HSC survival on day three after exposure in different donors of both cord blood and peripheral blood has shown much smaller individual differences than the number of HSCs that has raised within 14 days, the number of maturing CD45^{+} cells, and, consequently, the share of HSCs among all human cells and the *C*14/3 coefficient. It is clear that individual characteristics are less likely to affect the differences in the radiation-induced cell death and are more likely to affect the possibility of repair, subsequent restoration of the stem cell pool, and the HSC clonogenic activity.

The largest individual differences were observed in the humanized mice exposed to a dose of 0.5 Gy. Such exposure level may be recommended for assessment of individual HSC response that indicates individual radiosensitivity in the humanized mouse model obtained using peripheral blood HSCs.

CONCLUSIONS

The model humanized with human HSCs isolated from cord blood and peripheral blood can be used to assess the HSC response to radiation exposure in vivo. The *C*14/3 coefficient calculated as a ratio of the HSC share among all human CD45$^{low/+}$ cells on day 14 after exposure to the same parameter on day three after exposure is the most effective integrated indicator that reflects the HSC radiation-induced death, restoration of the HSC number, and HSC functional activity. This coefficient can be used to estimate the human HSC individual response to radiation exposure.

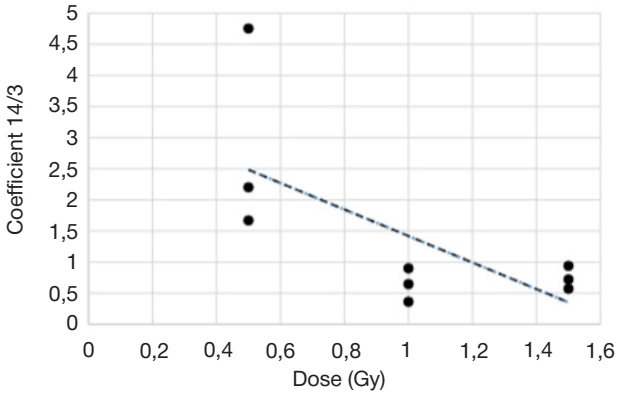

Fig. 6. Relationship between the *C*14/3 coefficient and the dose for CD45^{low}CD34^{+} human cells in the model mice humanized with the peripheral blood HSCs isolated from adult humans

References

1. Xaitov RM, Akleev AV, Kofiadi IA. Individual'naya radiochuvstvitel'nost' i immunitet: nacional'noe rukovodstvo. Chelyabinsk: Kniga, 2018; 216 s. Russian.
2. Applegate KE, Rühm W, Wojcik A, Bourguignon M, Brenner A, Hamasaki K, et al Individual response of humans to ionising radiation: governing factors and importance for radiological protection. Radiat Environ Biophys. 2020; 59 (2): 185–209. DOI: 10.1007/s00411-020-00837-y. PMID: 32146555.
3. Human Radiosensitivity. Report of the independent Advisory Group on Ionising Radiation Documents of the Health Protection Agency. London, 2013; 164 p.
4. Ferlazzo ML, Bourguignon M, Foray N. Functional Assays for Individual Radiosensitivity: A Critical Review. Semin Radiat Oncol. 2017; 27 (4): 310–5. DOI: 10.1016/j.semradonc.2017.04.003. PMID: 28865513.
5. Kogarko IN, Akleev AV, Petushkova VV, Nejfax EA, Kogarko BS, Ktitorova OV, i dr. Adaptivnyj otvet kak kriterij ocenki individual'noj radiochuvstvitel'nosti cheloveka. Radiaciya i risk (Byulleten' NREhR). 2022; 1. Dostupno po ssylke: https://cyberleninka.ru/article/n/adaptivnyy-otvet-kak-kriteriy-otsenki-individualnoy-radiochuvstvitelnosti-cheloveka-obzor (data obrashheniya: 08.11.2022). Russian.
6. Fliedner TM, Graessle DH. Hematopoietic cell renewal systems: mechanisms of coping and failing after chronic exposure to ionizing radiation. Radiat Environ Biophys. 2008; 47: 63–69. DOI: org/10.1007/s00411-007-0148-6.
7. Shao L, Luo Y, Zhou D. Hematopoietic stem cell injury induced by ionizing radiation. Antioxid Redox Signal. 2014; 20 (9): 1447–62. DOI:10.1089/ars.2013.5635.
8. Fujiwara S. Humanized mice: a brief overview on their diverse applications in biomedical research. J Cell Physiol. 2018; 233: 2889–901.
9. Ito R, Takahashi T, Ito M. Humanized mouse models: Application to human diseases. J Cell Physiol. 2018; 233 (5): 3723–8.
10. Wang C, Oshima M, Sashida G, et al. Non-lethal ionizing radiation promotes aging-like phenotypic changes of human hematopoietic stem and progenitor cells in humanized mice. PLoS ONE. 2015; 10 (7): e0132041. DOI: org/10.1371/journal.pone.0132041.
11. Zhang L, Mack R, Breslin P, Zhang J. Molecular and cellular mechanisms of aging in hematopoietic stem cells and their niches. J Hematol Oncol. 2020; 13 (1): 157. DOI: 10.1186/s13045-020-00994-z. PMID: 33228751; PMCID: PMC7686726.
12. de Haan G, Lazare SS. Aging of hematopoietic stem cells. Blood. 2018; 131 (5): 479–87. DOI: 10.1182/blood-2017-06-746412. PMID: 29141947.
13. Mejia-Ramirez E, Florian MC. Understanding intrinsic hematopoietic stem cell aging. Haematologica. 2020; 105 (1): 22–37. DOI: 10.3324/haematol.2018.211342. PMID: 31806687; PMCID: PMC6939535.
14. Shao L, Luo Y, Zhou D. Hematopoietic stem cell injury induced by ionizing radiation. Antioxid Redox Signal. 2014; 20 (9): 1447–62. DOI: 10.1089/ars.2013.5635. PMID: 24124731; PMCID: PMC3936513.
15. Yoshida K, Satoh Y, Uchimura A, Misumi M, Kyoizumi S, Taga M, et al. Massive expansion of multiple clones in the mouse hematopoietic system long after whole-body X-irradiation. Sci Rep. 2022; 12 (1): 17276. DOI: 10.1038/s41598-022-21621-6. PMID: 36241679; PMCID: PMC9568546.
16. Brojakowska A, Kour A, Thel MC, et al. Retrospective analysis of somatic mutations and clonal hematopoiesis in astronauts. Commun Biol. 2022; 5: 828. Available from: https://doi.org/10.1038/s42003-022-03777-z.
17. Atamanyuk NI, Pryaxin EA, Andreev SS, Aldibekova AE, Tryapicyna GA, Shaposhnikova IA, i dr. Ispol'zovanie ksenotransplantacii gemopoehticheskix kletok cheloveka, vydelennyx iz perifericheskoj krovi vzroslyx lyudej i pupovinnoj krovi, immunodeficitnym mysham dlya izucheniya dejstviya ioniziruyushhego izlucheniya. Voprosy radiacionnoj bezopasnosti. 2021; 1: 72–83. Russian.
18. Atamanyuk NI, Styazhkina EV, Obvinceva NA, Tryapicyna GA, Shaposhnikova IA, Andreev SS, i dr. Kinetika gibeli i vosstanovleniya kletok kostnogo mozga u myshej dvux linij s raznoj radiochuvstvitel'nost'yu posle ostrogo γ-oblucheniya. Voprosy radiacionnoj bezopasnosti. 2021; 4: 62–72. Russian.
19. Biancotti J-C, Town T. Increasing hematopoietic stem cell yield to develop mice with human immune systems. BioMed Research International. 2013; 11. DOI: 10.1155/2013/740892.
20. Brzozowska K, Pinkawa M, Eble MJ, et all. In vivo versus in vitro individual radiosensitivity analysed in healthy donors and in prostate cancer patients with and without severe side effects after radiotherapy. Int J Radiat Biol. 2012; 88: 405–13.
21. Kišonas J, Venius J, Sevriukova O, Grybauskas M, Dabkevičienė D, Burneckis A, et al. Individual Radiosensitivity as a Risk Factor for the Radiation-Induced Acute Radiodermatitis. Life. 2022; 12 (1): 20. Available from: https://doi.org/10.3390/life12010020.
22. Yip H, Haupt C, Maresh G, Zhang X, Li L. Humanized mice for immune checkpoint blockade in human solid tumors. Am J Clin Exp Urol. 2019; 7 (5): 313–20. PMID: 31763362; PMCID: PMC6872471.
23. Golebiewska A, Hau AC, Oudin A, Stieber D, Yabo YA, Baus V, et al. Patient-derived organoids and orthotopic xenografts of primary and recurrent gliomas represent relevant patient avatars for precision oncology. Acta Neuropathol. 2020; 140 (6): 919–49. DOI: 10.1007/s00401-020-02226-7. PMID: 33009951; PMCID: PMC7666297.
24. Nilsson JA, Olofsson Bagge R, Ny L. Mouse avatars take off as cancer models. Nature. 2018; 562 (7726): 192. DOI: 10.1038/d41586-018-06982-1. PMID: 30305752.
25. Rottstegge M, Tipton T, Oestereich L, Ruibal P, Nelson EV, Olal C, et al. Avatar Mice Underscore the Role of the T Cell-Dendritic Cell Crosstalk in Ebola Virus Disease and Reveal Mechanisms of Protection in Survivors. J Virol. 2022; 96 (18): e0057422. DOI: 10.1128/jvi.00574-22. PMID: 36073921; PM.

Литература

1. Хаитов Р. М., Аклеев А. В., Кофиади И. А. Индивидуальная радиочувствительность и иммунитет: национальное руководство. Челябинск: Книга, 2018; 216 с.
2. Applegate KE, Rühm W, Wojcik A, Bourguignon M, Brenner A, Hamasaki K, et al Individual response of humans to ionising radiation: governing factors and importance for radiological protection. Radiat Environ Biophys. 2020; 59 (2): 185–209. DOI: 10.1007/s00411-020-00837-y. PMID: 32146555.
3. Human Radiosensitivity. Report of the independent Advisory Group on Ionising Radiation Documents of the Health Protection Agency. London, 2013; 164 p.
4. Ferlazzo ML, Bourguignon M, Foray N. Functional Assays for Individual Radiosensitivity: A Critical Review. Semin Radiat Oncol. 2017; 27 (4): 310–5. DOI: 10.1016/j.semradonc.2017.04.003. PMID: 28865513.
5. Когарко И. Н., Аклеев А. В., Петушкова В. В., Нейфах Е. А., Когарко Б. С., Ктиторова О. В., и др. Адаптивный ответ как критерий оценки индивидуальной радиочувствительности человека. Радиация и риск (Бюллетень НРЭР). 2022; 1. Доступно по ссылке: https://cyberleninka.ru/article/n/adaptivnyy-otvet-kak-kriteriy-otsenki-individualnoy-radiochuvstvitelnosti-cheloveka-obzor (дата обращения: 08.11.2022).
6. Fliedner TM, Graessle DH. Hematopoietic cell renewal systems: mechanisms of coping and failing after chronic exposure to ionizing radiation. Radiat Environ Biophys. 2008; 47: 63–69. DOI: org/10.1007/s00411-007-0148-6.
7. Shao L, Luo Y, Zhou D. Hematopoietic stem cell injury induced by ionizing radiation. Antioxid Redox Signal. 2014; 20 (9): 1447–62. DOI:10.1089/ars.2013.5635.

8. Fujiwara S. Humanized mice: a brief overview on their diverse applications in biomedical research. J Cell Physiol. 2018; 233: 2889–901.
9. Ito R, Takahashi T, Ito M. Humanized mouse models: Application to human diseases. J Cell Physiol. 2018; 233 (5): 3723–8.
10. Wang C, Oshima M, Sashida G, et al. Non-lethal ionizing radiation promotes aging-like phenotypic changes of human hematopoietic stem and progenitor cells in humanized mice. PLoS ONE. 2015; 10 (7): e0132041. DOI: org/10.1371/journal.pone.0132041.
11. Zhang L, Mack R, Breslin P, Zhang J. Molecular and cellular mechanisms of aging in hematopoietic stem cells and their niches. J Hematol Oncol. 2020; 13 (1): 157. DOI: 10.1186/s13045-020-00994-z. PMID: 33228751; PMCID: PMC7686726.
12. de Haan G, Lazare SS. Aging of hematopoietic stem cells. Blood. 2018; 131 (5): 479–87. DOI: 10.1182/blood-2017-06-746412. PMID: 29141947.
13. Mejia-Ramirez E, Florian MC. Understanding intrinsic hematopoietic stem cell aging. Haematologica. 2020; 105 (1): 22–37. DOI: 10.3324/haematol.2018.211342. PMID: 31806687; PMCID: PMC6939535.
14. Shao L, Luo Y, Zhou D. Hematopoietic stem cell injury induced by ionizing radiation. Antioxid Redox Signal. 2014; 20 (9): 1447–62. DOI: 10.1089/ars.2013.5635. PMID: 24124731; PMCID: PMC3936513.
15. Yoshida K, Satoh Y, Uchimura A, Misumi M, Kyoizumi S, Taga M, et al. Massive expansion of multiple clones in the mouse hematopoietic system long after whole-body X-irradiation. Sci Rep. 2022; 12 (1): 17276. DOI: 10.1038/s41598-022-21621-6. PMID: 36241679; PMCID: PMC9568546.
16. Brojakowska A, Kour A, Thel MC, et al. Retrospective analysis of somatic mutations and clonal hematopoiesis in astronauts. Commun Biol. 2022; 5: 828. Available from: https://doi.org/10.1038/s42003-022-03777-z.
17. Атаманюк Н. И., Пряхин Е. А., Андреев С. С., Алдибекова А. Е., Тряпицына Г. А., Шапошникова И. А., и др. Использование ксенотрансплантации гемопоэтических клеток человека, выделенных из периферической крови взрослых людей и пуповинной крови, иммунодефицитным мышам для изучения действия ионизирующего излучения. Вопросы радиационной безопасности. 2021; 1: 72–83.
18. Атаманюк Н. И., Стяжкина Е. В., Обвинцева Н. А., Тряпицына Г. А., Шапошникова И. А., Андреев С. С., и др. Кинетика гибели и восстановления клеток костного мозга у мышей двух линий с разной радиочувствительностью после острого γ-облучения. Вопросы радиационной безопасности. 2021; 4: 62–72.
19. Biancotti J-C, Town T. Increasing hematopoietic stem cell yield to develop mice with human immune systems. BioMed Research International. 2013; 11. DOI: 10.1155/2013/740892.
20. Brzozowska K, Pinkawa M, Eble MJ, et all. In vivo versus in vitro individual radiosensitivity analysed in healthy donors and in prostate cancer patients with and without severe side effects after radiotherapy. Int J Radiat Biol. 2012; 88: 405–13.
21. Kišonas J, Venius J, Sevriukova O, Grybauskas M, Dabkevičienė D, Burneckis A, et al. Individual Radiosensitivity as a Risk Factor for the Radiation-Induced Acute Radiodermatitis. Life. 2022; 12 (1): 20. Available from: https://doi.org/10.3390/life12010020.
22. Yip H, Haupt C, Maresh G, Zhang X, Li L. Humanized mice for immune checkpoint blockade in human solid tumors. Am J Clin Exp Urol. 2019; 7 (5): 313–20. PMID: 31763362; PMCID: PMC6872471.
23. Golebiewska A, Hau AC, Oudin A, Stieber D, Yabo YA, Baus V, et al. Patient-derived organoids and orthotopic xenografts of primary and recurrent gliomas represent relevant patient avatars for precision oncology. Acta Neuropathol. 2020; 140 (6): 919–49. DOI: 10.1007/s00401-020-02226-7. PMID: 33009951; PMCID: PMC7666297.
24. Nilsson JA, Olofsson Bagge R, Ny L. Mouse avatars take off as cancer models. Nature. 2018; 562 (7726): 192. DOI: 10.1038/d41586-018-06982-1. PMID: 30305752.
25. Rottstegge M, Tipton T, Oestereich L, Ruibal P, Nelson EV, Olal C, et al. Avatar Mice Underscore the Role of the T Cell-Dendritic Cell Crosstalk in Ebola Virus Disease and Reveal Mechanisms of Protection in Survivors. J Virol. 2022; 96 (18): e0057422. DOI: 10.1128/jvi.00574-22. PMID: 36073921; PM.

RESULTS OF EPIDEMIOLOGICAL SURVEILLANCE FOR COVID-19 AMONG STUDENTS AND TEACHING STAFF OF THE UNIVERSITY

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Organization of training in the context of COVID-19 pandemic demanded the development and implementation of active epidemiological surveillance for acute respiratory infections in students and teaching staff of the Medical University. The study was aimed to identify the features of the COVID-19 epidemic process among students and teaching staff in 2020–2022. The analysis of COVID-19 incidence among students and teaching staff in the academic years 2020–2021 and 2021–2022 was carried out. The study was conducted on 6293 students enrolled in the academic year 2020–2021, 6148 students enrolled in the academic year 2021–2022, and 772 teaching staff members. In the academic year 2020–2021, COVID-19 was detected in 681 students, among whom the cumulative incidence (CI) was 10.83 (95% CI: 10.08-11.61) per 100 students, and 79 teaching staff members, among whom the CI was 10.23 (95% CI: 8.09–12.37); in the academic year 2021–2022 infection was detected in 690 students, the CI was 11.44 (95% CI: 10.64–12.24) per 100 students, and 75 teaching staff members, the CI was 9.71 (95% CI: 7.62%–11.80%). In 26.3% affected individuals, COVID-19 was detected when contacting the University outpatient clinic. The incidence among students living in the dormitories did not exceed that among students living in private apartments ($p = 0.36$), and no outbreaks were reported. There was a strong positive correlation between the incidence among residents of St. Petersburg and the incidence among students ($r = 0.77$). Over the entire period, probable setting of transmission was determined in 39.9% of infected individuals, contact most often (15.2%) occurred when working in the health care facilities. The incidence of novel coronavirus infection (COVID-19) among students and teaching staff members in the academic years 2020–2021 and 2021–2022 is directly related to their involvement in the COVID-19 epidemic process in St. Petersburg.

Keywords: novel coronavirus infection, preventive measures, educational process, health care facility, epidemiological surveillance

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Compliance with ethical standards: the study was approved by the Ethics Committee of Mechnikov North-Western State Medical University (protocol № 7 of 07 October 2020). The informed consent was submitted by all study participants. the study was approved by the Ethics Committee of Mechnikov North-Western State Medical University (protocol № 7 of 07 October 2020). The informed consent was submitted by all study participants.

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РЕЗУЛЬТАТЫ ЭПИДЕМИОЛОГИЧЕСКОГО НАДЗОРА ЗА COVID-19 СРЕДИ ОБУЧАЮЩИХСЯ И ПРОФЕССОРСКО-ПРЕПОДАВАТЕЛЬСКОГО СОСТАВА УНИВЕРСИТЕТА

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Организация обучения в условиях пандемии COVID-19 потребовала разработки и внедрения активного эпидемиологического надзора за острыми респираторными заболеваниями среди обучающихся и профессорско-преподавательского состава (ППС) медицинского университета. Целью работы было выявить особенности эпидемического процесса COVID-19 среди обучающихся и ППС университета в 2020–2022 гг. Проведен анализ заболеваемости COVID-19 среди обучающихся и ППС за 2020–2021 и 2021–2022 учебные годы. Под наблюдением находились 6293 обучающихся в 2020–2021 учебном году и 6148 в 2021–2022 учебном году, ППС — 772 человека. В 2020–2021 учебном году COVID-19 выявлен у 681 обучающегося, кумулятивная инцидентность (КИ) 10,83 (95% ДИ 10,08–11,61) на 100 обучающихся и 79 человек ППС — КИ 10,23 (95% ДИ 8,09–12,37), в 2021–2022 учебном году — у 690 обучающихся, КИ 11,44 (95% ДИ 10,64–12,24) на 100 обучающихся и 75 человек ППС — КИ 9,71 (95% ДИ 7,62%–11,80%). У 26,3% заболевших COVID-19 инфекция была выявлена при обращении в поликлинику Университета. Заболеваемость обучающихся, проживающих в общежитиях, не превышала заболеваемость среди тех, кто проживал на частных адресах ($p = 0,36$), также не было зарегистрировано вспышек. Найдена сильная положительная связь между заболеваемостью жителей Санкт-Петербурга и заболеваемостью обучающихся ($r = 0,77$). За весь период вероятное место заражения установлено у 39,9% заболевших, наиболее часто (15,2%) — в медицинской организации по месту работы. Заболеваемость новой коронавирусной инфекцией (COVID-19) среди обучающихся и профессорско-преподавательского состава за 2020–2021 и 2021–2022 учебные годы напрямую обусловлена их вовлечением в эпидемический процесс COVID-19 в Санкт-Петербурге.

Ключевые слова: новая коронавирусная инфекция, профилактические мероприятия, учебный процесс, медицинская организация, эпидемиологический надзор

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This paper is a continuation of a series of articles on prevention of the novel coronavirus infection spread among students and teaching staff of the Mechnikov North-Western State Medical University(hereinafter University) [1, 2].

During the COVID-19 pandemic many higher education institutions were forced to switch to distance learning. However, medical education cannot be effective without practical skills. That is why it was decided to conduct workshop-type classes and practical classes in the face-to-face format in the University's classrooms since September 2020 in case the classes involved developing practical skills. This resulted in the need to develop and implement a number of preventive and anti-epidemic measures taking into account the fact that medical students were engaged in providing care to COVID-19 patients in both outpatient and inpatient settings. Despite the fact that there are many publications discussing the COVID-19 rate among students, all these publications are based on the results of the questionnaire surveys of students, not on the objective morbidity data.

The study was aimed to identify the features of the COVID-19 novel coronavirus infection epidemic process among students and teaching staff of the University in the academic years 2020–2021 and 2021–2022 based on the implemented epidemiological surveillance.

METHODS

Active epidemiological surveillance for acute respiratory infections in students and teaching staff was developed and implemented in the University in order to carry out and adjust anti-epidemic measures in the context of face-to-face training during the ongoing COVID-19 pandemic. The detailed scheme of epidemiological surveillance was reported earlier [1].

Analysis of morbidity was performed every week to ensure the timely adjustment of anti-epidemic measures.

The measures recommended by Rospotrebnadzor [3] and some additional measures were implemented to prevent the spread of novel coronavirus infection:

– outreach activities (movies, video lectures, posters, newsletters);

– active detection of individuals showing signs of acute respiratory infection (ARI);

– setting up an isolation ward for admission and assessment of students and employees with symptoms of ARI in the University outpatient clinic;

– switching individuals with symptoms of ARI and confirmed COVID-19 or exposed people to distance learning;

– withdrawing individuals with symptoms of ARI and confirmed COVID-19 or contact persons from the dormitory;

The incidence of COVID-19 for the mentioned above academic years (between September 1 and June 30) was assessed. All students and teachers were included in the study. During the studied period, a total of 4879 students were followed-up in the academic year 2020–2021 and 4703 were followed-up in 2021–2022; 1414 residents were followed-up in the academic year 2020–2021 and 1445 were followed-up in 2021–2022; a total of 772 teaching staff members were followed-up. The number of students of different fields by year is provided in Table.

The disease was detected when contacting local medical institution, ambulance or University outpatient clinic. Clinical diagnosis and laboratory confirmation were provided in accordance with the version of regulatory documents that was valid at the time of seeking medical care [4]. Cumulative incidence of COVID-19 (the ratio of the number of diagnosed cases in the studied group to the total number of individuals in the group within the studied period multiplied by 100) among students of various faculties and teaching staff and its monthly trends were calculated. The Pearson correlation coefficient for the relationship between the weekly trends in the number of cases diagnosed among residents of St. Petersburg and University students ® was calculated.

All the infected people were interviewed in order to reveal probable places of infection and contact persons. The place of infection was considered to be determined, when the student reported the contact with the confirmed case of COVID-19 within 14 days since the emergence of symptoms in the years 2020–2021 and within 7 days in the year 2022. Cumulative incidence of COVID-19 among students enrolled in different semesters was calculated based on the probable setting of transmission infection. The structure of junior and senior students of various faculties and residents (share of all disease cases) was also calculated based on the probable place of infection.

The 95% confidence intervals were calculated using the Wilson score. The differences were considered significant when *p*-value was below 0.05.

RESULTS

A total of 1371 students and 155 teaching staff members were diagnosed with COVID-19 during the studied period. Furthermore, in the academic year 2020–2021, COVID-19 was detected in 681 students, the cumulative incidence (CI) was 10.83 (95% CI: 10.08–11.61) per 100 students, and 79 teaching staff members, the CI was 10.23 (95% CI: 8.09–12.37); in the academic year 2021–2022, infection was detected in 690 students, the CI was 11.44 (95% CI: 10.64–12.24)

Table. Number of students of different fields by year

Year	Medical specialty (faculty)							
	Nursing care		General medicine		Preventive medicine		Dentistry	
	Academic year							
	2020–2021	2021–2022	2020–2021	2021–2022	2020–2021	2021–2022	2020–2021	2021–2022
1	19	13	695	786	156	156	95	70
2	10	11	718	586	160	125	90	66
3	8	7	581	607	142	140	72	56
4		8	556	505	127	114	66	61
5			581	535	116	123	72	58
6			480	563	135	113		
Total	37	39	3611	3582	836	771	395	311

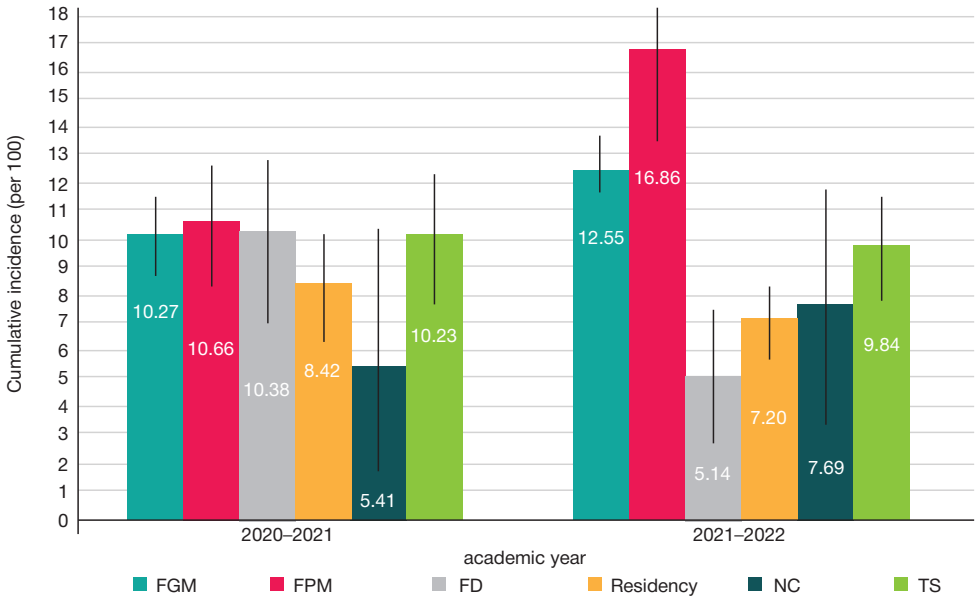

Fig. 1. Cumulative incidence of COVID-19 among students and teaching staff in 2020–2021 and 2021–2022. FGM — Faculty of General Medicine, FPM — Faculty of Preventive Medicine, FD — Faculty of Dentistry, NC — Nursing Care, TS — teaching staff

per 100 students, and 75 teaching staff members, the CI was 9.71 (95% CI: 7.62–11.80%).

It should be noted that almost every fifth COVID-19 case (19%) was revealed in the University outpatient clinic, where the isolation ward for admission and assessment of students and employees with symptoms of ARI was set up. A total of 1058 contacted the clinic, of them the diagnosis of COVID-19 was confirmed in 278 individuals (26.3%). Among those who contacted the clinic, 487 students (46%) lived in the University dormitories, and COVID-19 was detected in 124 (25.4%) of them. The diagnosis was confirmed by PCR within 12 h after seeking medical care. Information was immediately sent to the coordinator of anti-epidemic measures, to head of the service for accommodation and socio-household arrangements, and the deputy deans of the faculties. This enabled earlier isolation of infected individuals and timely implementation of anti-epidemic measures. The incidence among students living in the dormitories reported over the entire research period did not exceed that among students living in private apartments, it was 19.1 and 18.9 per 100 students, respectively. No outbreaks among students living in the dormitories were reported.

In the year 2020–2021, the incidence among students of different faculties, residents, and teaching stuff was at the same level. In 2021–2022, the highest incidence was revealed in students of the Faculty of Preventive Medicine due to the greatest engagement in the outbreak caused by the SARS-CoV-2 Omicron strain (Fig. 1, 2).

The highest incidence was observed in senior students (years 4–6) in 2021–2022, it was 12.60 per 100 students (95% CI: 11.24–14.09). The incidence in junior students (years 1–3) was 11.52 per 100 students (95% CI: 10.33–12.76). In 2020–2021 these indicators were lower: 9.67 (95% CI: 8.61–10.84; p = 0.03) in junior students and 9.28 (95% CI: 8.12–10.59; p = 0.0006) in senior students.

The COVID-19 incidence peaks were revealed in autumn 2020 and winter 2022. In autumn 2020, a quarter of all disease cases detected among students resulted from exposure in health care facilities related to the students' and residents' work in the COVID centers. Furthermore, multiple cases of non-compliance with the self-isolation regime after the emergence of ARI symptoms were revealed. When the SARS-CoV-2 Delta strain prevailed, the highest incidence was observed in

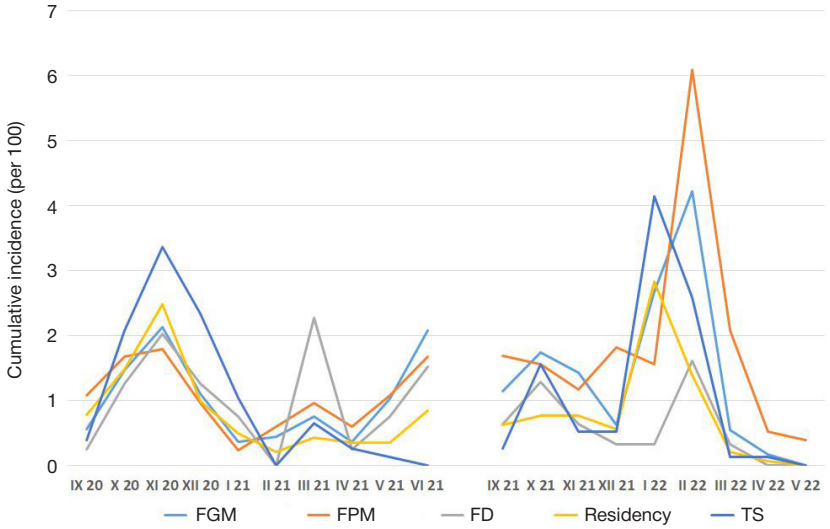

Fig. 2. Monthly trends in cumulative incidence of COVID-19 among students and teaching staff in 2020–2021 and 2021–2022. FGM — Faculty of General Medicine, FPM — Faculty of Preventive Medicine, FD — Faculty of Dentistry, TS — teaching staff

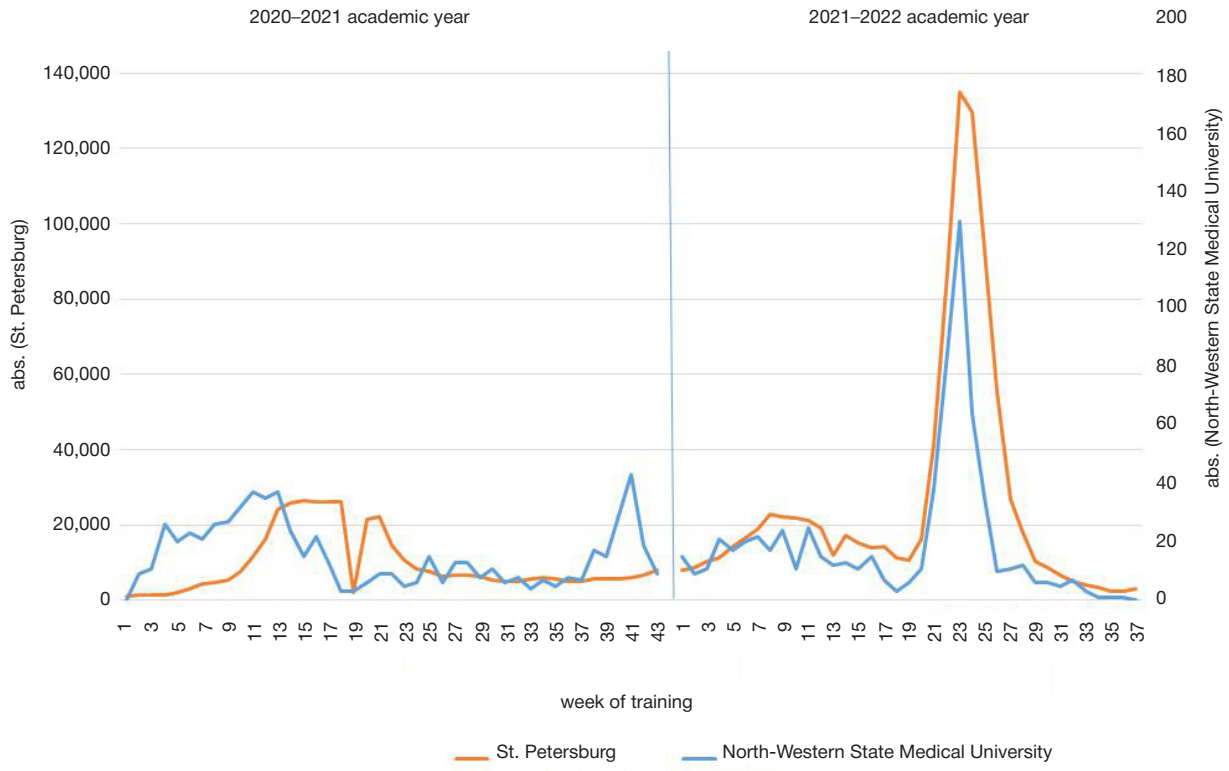

Fig. 3. Trends in the number of COVID-19 cases revealed among the University students and teaching staff and the residents of St. Petersburg

the teaching staff members, which was probably due to the age-related factor. The rise of incidence during weeks 40–41 (May 31–June 13, 2021) was caused by the students' non-compliance with the self-isolation regime in cases of infection during the end-of-semester examinations: 70% of infected students having symptoms of ARI continued attending the University. Furthermore, testing students for SARS-CoV-2 performed prior to summer internship revealed 30% of the total number of cases [1]. In winter 2022, when the SARS-CoV-2 Omicron strain prevailed, the teaching staff and residents were maximally involved as early as January 2022, while students were involved in February, which was due to student holidays.

The COVID-19 epidemic process among students and teaching staff members in the University depended on the epidemic process among residents of St. Petersburg: a strong positive correlation was revealed (correlation coefficient $r = 0.77$) (Fig. 3).

Re-infection was detected in 58 students (4.3% of the total number of infected individuals). The peak number of re-infection was revealed during the spread of SARS-CoV-2 Omicron strain in February 2022.

We managed to determine the probable setting of transmission infection in 39.9% of affected individuals over the entire period. The students most often reported contacting persons with confirmed COVID-19 at work or during their internship in the health care facilities (15.2% of affected individuals), 12.1% were contact in the group during face-to-face training, contact in the family at home was reported by 6.6%, contact in the dormitory was mentioned by 2.2%, 2.5% reported contact in other circumstances, and 1.3% noted they were contact to multiple sources of infection. The incidence among students who contacted persons with confirmed COVID-19 over the entire observation period was 8.8 per 100 students, while the incidence among students had contact to undefined sources of infection was 13.3 per 100 students. However, the incidence rates in individuals with various types of contact were different in the epidemic process intensity and were higher during the incidence peaks compared to periods with lower incidence (47.8 and 28.9%, respectively; $p << 0.01$) mostly due to infection resulting from exposure at work or during internship in the medical institution or University. Multiple contact, i.e. contact to multiple sources of infection during the incubation period, were reported in 2021–2022 (Fig. 4).

The infected senior students of the Faculties of General Medicine and Preventive Medicine and residents most often reported contacting persons with confirmed COVID-19 at work or during internship in the health care facilities ($p << 0.01$). This is not surprising, since it is senior students and residents who work and do internships in health care facilities. Infection due to contact in the University prevailed among junior students ($p = 0.0004$). The largest share of contacts with persons having confirmed COVID-19 under different circumstances outside the University was reported by students of the Faculty of Dentistry (Fig. 5).

DISCUSSION

The risk of infection increases during face-to-face training at universities. Thus, a dramatic increase in the COVID-19 incidence among students was observed in the USA in early 2020–2021. The survey performed by New York Times in more than 1600 colleges revealed more than 26,000 COVID-19 cases in more than 750 colleges across the country by August 26, more than 51,000 cases in more than 1020 US colleges by September 3, and more than 130,000 cases in 1300 colleges by September 25 [5]. In the other university 528 students (24.1%) out of 2187 were diagnosed with COVID-19 during the fall semester 2020, which was 8 times higher compared to the values obtained during our study [6]. The SARS-CoV-2 seroprevalence in 2905 students of five universities in the UK was 17.8% (95% CI: 16.5–19.3) in December 2020, it was within the range of 7.6–29.7% [7]. The incidence among students of one more university in 2020–2021 was 15.7 per 100 students [8].

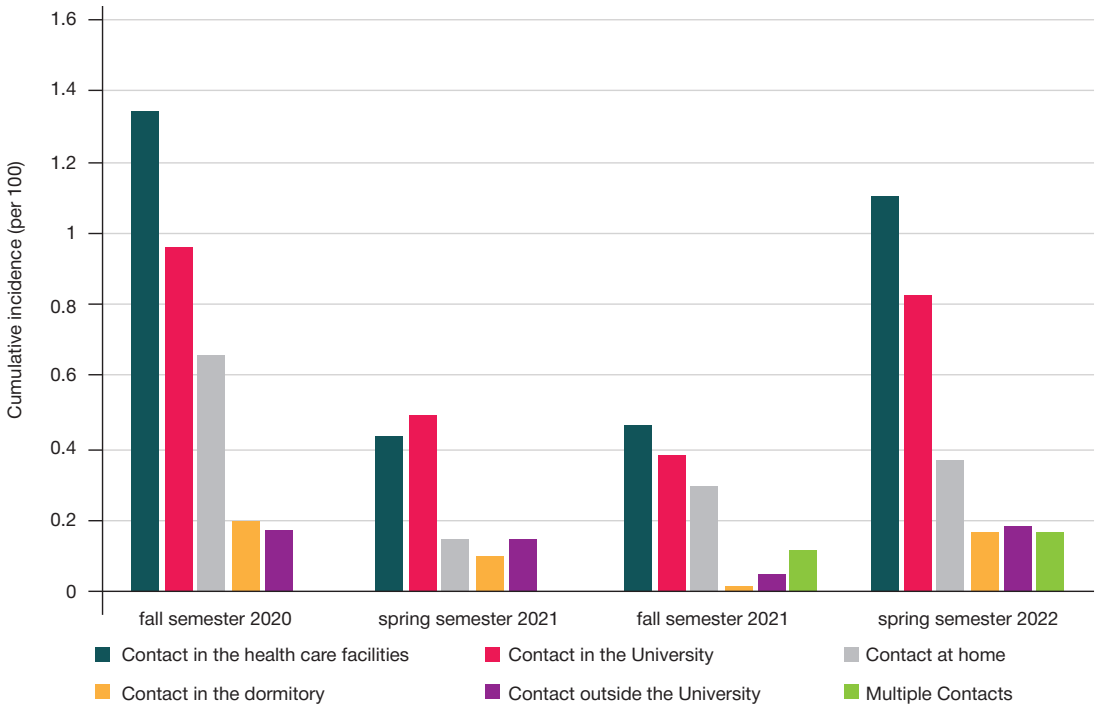

Fig. 4. Cumulative incidence of COVID-19 by probable setting of transmission infection among students enrolled in different semesters

In our study, the rapid growth of incidence in early 2020–2021 was also observed. The analysis of the causes of COVID-19 spread in September 2020 showed that students who attended the University while having ARI symptoms were the main cause. Thus, in September 2020, 25.4% of infected individuals came to the university on the day of symptom onset and 32.7% of individuals with COVID-19 continued attending face-to-face classes for more than one day after the disease onset. The studies conducted in other Russian universities have also shown that about a quarter of students having symptoms of COVID-19 do not seek medical care [9, 10]. This is usually due to the fear of making up missed classes. University administration decided to switch the infected individuals to distance learning, i.e. the classes were not marked as missed and the infected students did not have to make up any classes. Furthermore, a poster was created with a message not to attend classes after the emergence of ARI signs. Such posters were stationed at the entrance to each department. These measures reduced twice the attendance rate of individuals showing ARI symptoms, which made it possible to reduce the incidence rate when used along with implementation of other preventive and anti-epidemic measures. Rapid detection and isolation of infected people and exposed individuals along with strict compliance

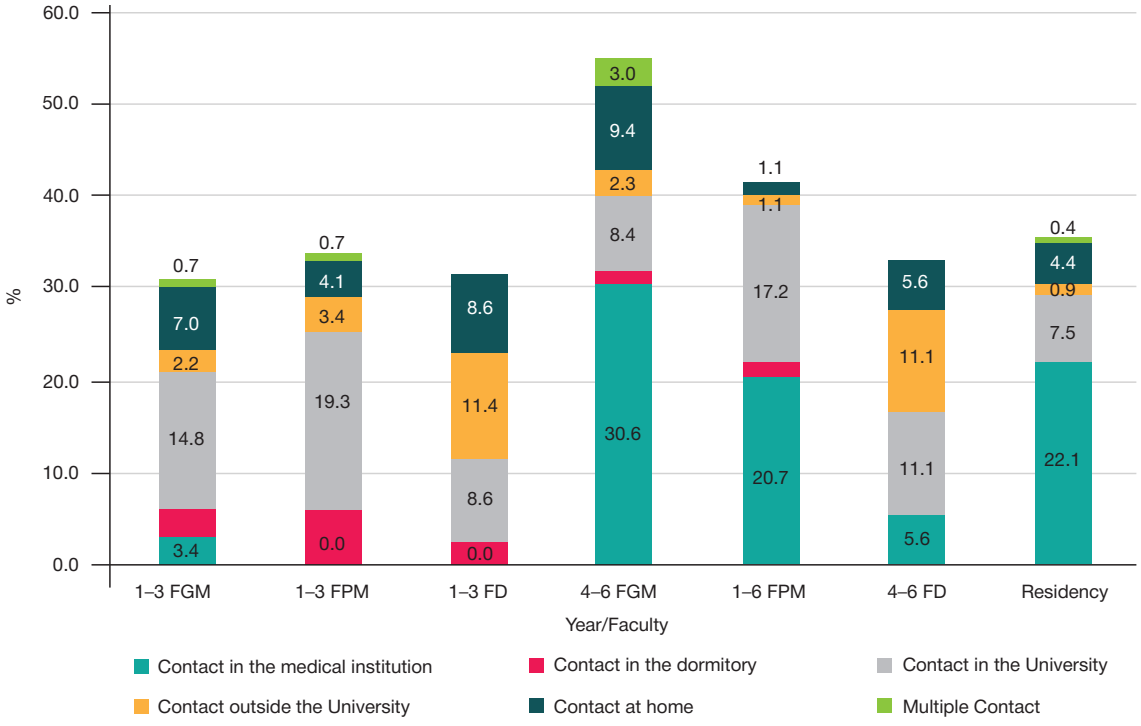

Fig. 5. Probable setting of transmission infection (share of all disease cases) of junior and senior students of various faculties and residents

with the face mask requirements are the key measures to control the spread of coronavirus infection.

During the pandemic (September–June 2020–2021 and 2021–2022), the incidence among the University students and teaching staff generally resulted from their involvement in the COVID-19 epidemic process in St. Petersburg, however, it was lower compared to that observed in the aggregate population of St. Petersburg. More than 1,350,000 COVID-19 cases were reported in St. Petersburg over the studied period, and the incidence that constituted 25 per 100 residents was higher than the incidence reported in the University. The highest incidence values were reported during the period of peak incidence in St. Petersburg. The relationships between the incidence rates of students and residents of settlements were described by some other researchers [11]. For example, the lower number of COVID-19 cases was registered among residents of Pennsylvania, who were not students, than among students [12].

Working in health care facilities was the main risk factor of morbidity in students. Active questioning of infected individuals made it possible to define the probable setting of transmission infection in more than a third of cases. Almost a half of them were contact when working in the health care facilities , which to a great extent determined the incidence among senior students and residents. Thus, the incidence among students working in health care facilities of Barnaul exceeded the regional average by 4.7 times. The share of infected 4–6-year students was 75.3% [13]. The students of Smolensk medical university also noted that health care facilities were among the major probable setting of transmission infection [14]. This is due to the clear COVID-19 status of patients in health care facilities and, probably, to the higher risk of infection in the context of medical care. Predominance of infection cases among junior University students is probably due to less knowledge and low adherence to preventive measures [15, 16].

The implemented measures made it possible to prevent high incidence among the University teaching staff members, which was extremely important, since many of them had some risk factors of severe COVID-19. The cumulative incidence of COVID-19 among teaching staff and students was 19.9 and 22.3 per 100, respectively.

The mass media of the Russian Federation repeatedly reported the COVID-19 outbreaks in the student dormitories. Thus, information about at least 15 outbreaks with the total number of affected individuals of at least 324 (4–79) was published on the open online resources. One of the studies showed that students living in the room together had a twice as much chance to become infected with COVID-19 than those who lived alone [5]. Active detection and isolation of infected and exposed individuals made it possible to avoid outbreaks among students living in the dormitories. The low percentage of re-infection cases that were observed only during the spread of the SARS-CoV-2 Omicron strain should be noted.

CONCLUSIONS

The incidence of novel coronavirus infection (COVID-19) among students and teaching staff members in the academic years 2020–2021 and 2021–2022 is directly related to their involvement in the COVID-19 epidemic process in St. Petersburg. The measures developed and implemented in the University in order to control the spread of novel coronavirus infection made it possible to prevent outbreaks among students and teaching staff and achieve the lower levels compared to overall population of St. Petersburg despite the face-to-face learning format. The incidence among students living in the dormitories over the entire period did not exceed that among individuals living in private apartments due to the development and implementation of active epidemiological surveillance for acute respiratory infections. No outbreaks among students living in the dormitories were detected, while mass media repeatedly reported COVID-19 outbreaks in the dormitories of other universities. Infection most often occurred upon exposure to the source of infection when working in the health care facilities. The experience of the University can be used in the future in case of new challenges related to the spread of infections.

References

1. Sayganov SA, Lyubimova AV, Meltser AV, Lopatin ZV, Kuznetsova OYu, Kovaleva OV, i dr. Ehpidemiologicheskie osobennosti rasprostraneniya novoj koronavirusnoj infekcii COVID-19 sredi obuchayushhixsya universiteta v 2020–2021 uchebnom godu. Profilakticheskaya i klinicheskaya medicina. 2021; 81 (4): 10–19. Russian.
2. Sayganov SA, Meltser AV, Lyubimova AV, Kuznetsova OYu., Zuyeva LP, Aslanov BI, i dr. Opyt organizacii mer po predotvrashheniyu rasprostraneniya novoj koronavirusnoj infekcii sredi obuchayushhixsya obrazovatel'noj organizacii, prozhivayushhix v obshhezhitiyax. Profilakticheskaya i klinicheskaya medicina. 2020; 76 (3): 4–11. Russian.
3. MR 3.1/2.1.0205-20. 3.1. Profilaktika infekcionnyx boleznej. 2.1. Kommunal'naya gigiena. Rekomendacii po profilaktike novoj koronavirusnoj infekcii (COVID-19) v obrazovatel'nyx organizaciyax vysshego obrazovaniya. Metodicheskie rekomendacii (utv. Glavnym gosudarstvennym sanitarnym vrachom RF 29.07.2020) (red. ot 11.02.2021). MR 3.1./2.1.02.05.20. Metodicheskie rekomendacii. Russian.
4. Vremennye metodicheskie rekomendacii ministerstva zdravooxraneniya Rossijskoj Federacii «Profilaktika, diagnostika i lechenie novoj koronavirusnoj infekcii (COVID 19)» versii 8.1-15 [Internet] Citirovano 21 yanvarya 2022 [okolo 248 str.] Dostupno po ssylke: https://xn--80aesfpebagmfblc0a.xn--p1ai/info/ofdoc/who/. Russian.
5. Watson S, Hubler S, Ivory D, Gebeloff R. A new front in America's pandemic: college towns.New York Times. [Internet] September 6, 2020. Updated September 25, 2020. Accessed January 21, 2022. [about 3 p.]. Available from: https://www.nytimes.com/interactive/2020/us/covid-college-cases-tracker.html.
6. Bigouette JP, Ford L, Segaloff HE, Langolf K, Kahrs J, Zochert T, et al. Association of Shared Living Spaces and COVID-19 in University Students, Wisconsin, USA, 2020. Emerg Infect Dis. 2021; 27 (11): 2882–6.
7. Vusirikala A, Whitaker H, Jones S, Tessier E, Borrow R, Linley E, et al. Seroprevalence of SARS-CoV-2 antibodies in university students: Cross-sectional study. England. J Infect. 2021; 83 (1); 104–11.
8. Cass AL, Slining MM, Carson C, Cassidy J, Epright MC, Gilchrist AE, et. al. Risk Management of COVID-19 in the Residential Educational Setting: Lessons Learned and Implications for Moving Forward. Int J Environ Res Public Health. 2021; 18 (18): 9743.
9. Lelenkova LYu, Nozhkina NV. Ocenka informirovannosti i otnosheniya studentov-medikov k ehpidemicheskoj situacii i vakcinacii protiv COVID-19. V sbornike: VI Mezhdunarodnaya (76 Vserossijskaya) nauchno-prakticheskaya konferenciya «Aktual'nye voprosy sovremennoj medicinskoj nauki i zdravooxraneniya». 2021; 783–8. Russian.
10. Nemchaninova DA, Marchenko DD. Osobennosti zabolevaemosti

COVID-19 sredi studentov medicinskogo vuza. FORCIPE. 2022; 5 (53): 568. Russian.
11. Pollock BH, Kilpatrick AM, Eisenman DP, Elton KL, Rutherford GW, Boden-Albala BM, et al. Safe reopening of college campuses during COVID-19: The University of California experience in Fall 2020. PLoS One. 2021; 16 (11): 0258738. PubMed PMID: 34735480.
12. Bharti N, Lambert B, Exten C, Faust C, Ferrari M, Robinson A. Large university with high COVID-19 incidence is not associated with excess cases in non-student population. Sci Rep. 2022; 12 (1): 3313.
13. Suvorova SM, Shirokostup SV, Lukyanenko NV. Analiz zabolevaemosti novoj koronavirusnoj infekciej COVID-19 sredi studentov medicinskogo vuza. V sbornike: V. G. Akimkin, redaktor. Molekulyarnaya diagnostika i biobezopasnost'-2021. COVID-19: ehpidemiologiya, diagnostika, profilaktika: sbornik tezisov Onlajn-kongressa s mezhdunarodnym uchastiem (28–29 aprelya 2021 g., Moskva). M.: FBUN CNII Ehpidemiologii Rospotrebnadzora, 2021; 48 s. Russian.
14. Chubanov VA, Zakharov YeA, Zaboyenkova YeA. Koronavirusnaya infekciya u studentov Smolenskogo gosudarstvennogo medicinskogo universiteta. Smolenskij medicinskij al'manax. 2022; (3): 153–57. Russian.
15. Delova LA. Soblyudenie mer profilaktiki COVID-19 studentami medicinskix special'nostej: po materialam sociologicheskogo oprosa. V sbornike: Innovacionnye issledovaniya: teoreticheskie osnovy i prakticheskoe primenenie: sbornik statej Nacional'noj (Vserossijskoj) nauchno-prakticheskoj konferencii, Saratov, 22 iyulya 2021 goda. Ufa: «OMEGA SAJNS», 2021; 296–301. Russian.
16. Wassif GO, El Din DAG. Relationship between knowledge, attitude, and practice of COVID-19 precautionary measures and the frequency of infection among medical students at an Egyptian University. PLoS One. 2022; 17 (9): 0274473. PubMed PMID: 36121862.

Литература

1. Сайганов С. А., Любимова А. В., Мельцер А. В., Лопатин З. В., Кузнецова О. Ю., Ковалева О. В. и др. Эпидемиологические особенности распространения новой коронавирусной инфекции COVID-19 среди обучающихся университета в 2020–2021 учебном году. Профилактическая и клиническая медицина. 2021; 81 (4): 10–19.
2. Сайганов С. А., Мельцер А. В., Любимова А. В., Кузнецова О. Ю., Зуева Л. П., Асланов Б. И. и др. Опыт организации мер по предотвращению распространения новой коронавирусной инфекции среди обучающихся образовательной организации, проживающих в общежитиях. Профилактическая и клиническая медицина. 2020; 76 (3): 4–11.
3. МР 3.1/2.1.0205-20. 3.1. Профилактика инфекционных болезней. 2.1. Коммунальная гигиена. Рекомендации по профилактике новой коронавирусной инфекции (COVID-19) в образовательных организациях высшего образования. Методические рекомендации (утв. Главным государственным санитарным врачом РФ 29.07.2020) (ред. от 11.02.2021). МР 3.1./2.1.02.05.20. Методические рекомендации.
4. Временные методические рекомендации Министерства здравоохранения Российской Федерации «Профилактика, диагностика и лечение новой коронавирусной инфекции (COVID 19)» версии 8.1-15. Цитировано 21 января 2022. Доступно по ссылке: https://xn--80aesfpebagmfblc0a.xn--p1ai/info/ofdoc/who/.
5. Watson S, Hubler S, Ivory D, Gebeloff R. A new front in America's pandemic: college towns.New York Times. [Internet] September 6, 2020. Updated September 25, 2020. Accessed January 21, 2022. [about 3 p.]. Available from: https://www.nytimes.com/interactive/2020/us/covid-college-cases-tracker.html.
6. Bigouette JP, Ford L, Segaloff HE, Langolf K, Kahrs J, Zochert T, et al. Association of Shared Living Spaces and COVID-19 in University Students, Wisconsin, USA, 2020. Emerg Infect Dis. 2021; 27 (11): 2882–6.
7. Vusirikala A, Whitaker H, Jones S, Tessier E, Borrow R, Linley E, et al. Seroprevalence of SARS-CoV-2 antibodies in university students: Cross-sectional study. England. J Infect. 2021; 83 (1); 104–11.
8. Cass AL, Slining MM, Carson C, Cassidy J, Epright MC, Gilchrist AE, et. al. Risk Management of COVID-19 in the Residential Educational Setting: Lessons Learned and Implications for Moving Forward. Int J Environ Res Public Health. 2021; 18 (18): 9743.
9. Леленкова Л. Ю., Ножкина Н. В. Оценка информированности и отношения студентов-медиков к эпидемической ситуации и вакцинации против COVID-19. В сборнике: VI Международная (76 Всероссийская) научно-практическая конференция «Актуальные вопросы современной медицинской науки и здравоохранения». 2021; 783–8.
10. Немчанинова Д. А., Марченко Д. Д. Особенности заболеваемости COVID-19 среди студентов медицинского вуза. FORCIPE. 2022; 5 (53): 568.
11. Pollock BH, Kilpatrick AM, Eisenman DP, Elton KL, Rutherford GW, Boden-Albala BM, et al. Safe reopening of college campuses during COVID-19: The University of California experience in Fall 2020. PLoS One. 2021; 16 (11): 0258738. PubMed PMID: 34735480.
12. Bharti N, Lambert B, Exten C, Faust C, Ferrari M, Robinson A. Large university with high COVID-19 incidence is not associated with excess cases in non-student population. Sci Rep. 2022; 12 (1): 3313.
13. Суворова С. М., Широкоступ С. В., Лукьяненко Н. В. Анализ заболеваемости новой коронавирусной инфекцией COVID-19 среди студентов медицинского вуза. В сборнике: В. Г. Акимкин, редактор. Молекулярная диагностика и биобезопасность-2021. COVID-19: эпидемиология, диагностика, профилактика: сборник тезисов Онлайн-конгресса с международным участием (28–29 апреля 2021 г., Москва). М.: ФБУН ЦНИИ Эпидемиологии Роспотребнадзора, 2021; 48 с.
14. Чубанов В. А., Захаров Е. А., Забоенкова Е. А. Коронавирусная инфекция у студентов Смоленского государственного медицинского университета. Смоленский медицинский альманах. 2022; (3): 153–57.
15. Делова Л. А. Соблюдение мер профилактики COVID-19 студентами медицинских специальностей: по материалам социологического опроса. В сборнике: Инновационные исследования: теоретические основы и практическое применение: сборник статей Национальной (Всероссийской) научно-практической конференции, Саратов, 22 июля 2021 года. Уфа: «ОМЕГА САЙНС», 2021; 296–301.
16. Wassif GO, El Din DAG. Relationship between knowledge, attitude, and practice of COVID-19 precautionary measures and the frequency of infection among medical students at an Egyptian University. PLoS One. 2022; 17 (9): 0274473. PubMed PMID: 36121862.

CLINICAL AND VIROLOGICAL CHARACTERISTICS OF CHRONIC HEPATITIS B AND RESPONSE TO ANTIVIRAL THERAPY

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Chronic hepatitis B (CHB) is a common infectious disease that represents one of the main causes of liver cirrhosis (LC) and hepatocellular carcinoma (HCC). CHB is still difficult to treat due to the lack of drugs that completely eliminate hepatitis B virus (HBV) from hepatocytes. The study was aimed to describe the CHB clinical and laboratory features, assess the efficiency of antiviral therapy and identify the factors associated with the response to antiviral therapy. The results of clinical and laboratory assessment, instrumental examination, serological and molecular testing of the patients (n = 201) followed up between 2007–2021 in the Viral Hepatitis Diagnosis and Treatment Center at the Clinical Hospital No. 85 of FMBA of Russia were assessed based on primary sources. Most of the patients in the group were males (56.7%); the HBeAg-negative patients predominated (93%). LC was diagnosed in nine patients (4.5%), among them one patient had HCC. The HBV D genotype was determined in 95.4% of cases, A genotype in 3.1% of cases, and C genotype in 1.5% of cases. After a year of treatment with the nucleos(t)ide analogues (entecavir or tenofovir) 88% of patients showed no viremia and their biochemical parameters were back to normal (88%). The overall seroconversion rate was 41.7% for HBeAg and 3% for HBsAg. Thus, high rates of virological response and enzyme activity normalization were obtained. Low baseline viremia level is an independent prognostic factor of achieving a virological response. The HBsAg level in the end of therapy makes it possible to predict relapse after the treatment cessation.

Keywords: chronic hepatitis B, antiviral therapy, prognostic factors

Author contribution: Nguyen Thi-Hanh — sample collection, data analysis, manuscript writing; Melnikova LI — sample collection, data analysis; Ilchenko LYu — study design, data analysis, manuscript editing; Kyuregyan KK — literature review; Gordeychuk IV — data analysis; Bondarenko NL — editing and approval of the final version of the article.

Compliance with ethical standards: the study was approved by the Ethics Committee of Pirogov Russian National Research Medical University (protocol № 213 of 13 December 2021).

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КЛИНИКО-ВИРУСОЛОГИЧЕСКАЯ ХАРАКТЕРИСТИКА ХРОНИЧЕСКОГО ГЕПАТИТА В И ОТВЕТ НА ПРОТИВОВИРУСНУЮ ТЕРАПИЮ

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Хронический гепатит В (ХГВ) — широко распространенное инфекционное заболевание, одна из основных причин цирроза печени (ЦП) и гепатоцеллюлярной карциномы (ГЦК). Лечение ХГВ до сих пор затруднено из-за отсутствия препаратов, полностью элиминирующих вирус гепатита В (HBV) из гепатоцита. Целью работы было описать клинико-лабораторные особенности ХГВ, оценить эффективность противовирусной терапии и выявить факторы, ассоциированные с ответом на нее. На основании первичной документации проведена оценка результатов клинико-лабораторного и инструментального обследования, а также данных серологических и молекулярно-биологических методов исследований пациентов (n = 201), наблюдавшихся в период 2007–2021 гг. в Центре диагностики и лечения хронических вирусных гепатитов КБ № 85 ФМБА России. Большинство пациентов в группе — мужчины (56,7%); преобладали HBeAg-негативные больные (93%). У девяти (4,5%) пациентов диагностирован ЦП, у одного из них — ГЦК. Генотип D HBV установлен в 95,4% случаев, А — в 3,1% и С — в 1,5%. После года терапии аналогами нуклеоз(т)идов (энтекавир или тенофовир) у 88% пациентов отсутствовала виремия, нормализовались биохимические показатели (88%). Общий уровень сероконверсии по HBeAg составил 41,7% и по HBsAg — 3%. Таким образом, получены высокая частота достижения вирусологического ответа и нормализация активности ферментов. Низкий исходный уровень виремии является независимым прогностическим фактором для достижения вирусологического ответа. Уровень HBsAg в конце терапии позволяет прогнозировать рецидив после окончания лечения.

Ключевые слова: хронический гепатит В, противовирусная терапия, прогностические факторы

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Chronic hepatitis B (CHB) which is widespread throughout the world represents a serious global public health issue. According to the World Health Organization, there are 296 million people with CHB all over the world, up to 1.5 million new cases of infection are reported annually. In 2019, a total of 820,000 died, mostly from such complications, as liver cirrhosis (LC) and hepatocellular carcinoma (HCC) [1].

In the Russian Federation (RF) the CHB incidence rate stabilized at around 14.0–16.0 per 100,000 population in 2000–2009. There had been a decreasing trend of CHB incidence since 2010. In 2020, the incidence rate was 4.4 per 100,000 population, which was three times lower than in 2010 (13.3 per 100,000 population). This was probably due to active immunization of the population. However, the incidence of CHB in some regions of the RF remains high. Thus, in 2019, the CHB incidence in St. Petersburg was 44.0 per 100,000 population, while it was 54.3 per 100,000 population in the Republic of Tuva, 25.0 per 100,000 population in the Sakha Republic (Yakutia), and 13.0 per 100,000 population in Moscow. The prevalence of CHB in some regions is close to 1000 per 100,000 population, i.e. it constitutes about 1% of the total population [2–4].

The chronic infection caused by hepatitis B virus (HBV) is a dynamic process that reflects interaction between HBV replication and the patient's immune response. Five phases are conventionally distinguished in the natural course of chronic HBV infection based on the presence of HBeAg, HBV DNA levels, alanine aminotransferase (ALT) levels, and the absence or presence of the hepatic inflammation components [5]. Despite the variability in the course of chronic HBV infection, one third of patients eventually develop LC and 5–10% of patients develop HCC [6].

Antiviral therapy (AVT) slows down the disease progression, thereby reducing morbidity and mortality. However, regardless of the advances in therapy, recovery from CHB remains a challenging task, since antiviral drugs that are currently used in actual practice make it possible to achieve clinical remission, but do not eliminate HBV. Improving the survival rate by preventing the disease progression, LC decompensation, and HCC development is a final goal of the CHB treatment.

The study was aimed to describe the CHB clinical and laboratory features, assess the efficiency of AVT and identify the factors associated with the response to AVT.

METHODS

Primary sources (medical records) were analyzed in the Viral Hepatitis Diagnosis and Treatment Center. Among 989 medical records a total of 224 records of all patients infected with HBV who had been followed up between January 2007 and December 2021 were selected to provide the basis for the database.

The results of clinical and laboratory assessment, serological and molecular testing, and instrumental examination were analyzed based on the primary sources.

Inclusion criteria for the retrospective observational study: male and female HBsAg-positive patients; age 18–75 years; availability of the informed consent.

Exclusion criteria: patients having incomplete records; patients with human immunodeficiency virus (HIV), hepatitis C virus (HCV), hepatitis D virus (HDV) co-infection; no informed consent available.

A total of 23 patients were excluded due to non-compliance with the inclusion criteria; the clinical group included 201 people. The follow-up period was 1–15 years. The vast majority of patients (75.1%) were followed up during the first three years, a quarter of patients (23.4%) were followed up for 3–10 years, and three patients (1.5%) were followed up for more than 10 years.

All the patients attached to the medical institutions of FMBA of Russia underwent a comprehensive examination that included analysis of complaints and the disease history along with physical examination when contacting the Center. The following data were recorded when performing examination: gender, age (at the time of the first visit), the date when HBsAg were first detected, and duration of HBV infection. Laboratory and instrumental tests were performed in accordance with the clinical guidelines [2, 7]. The complete blood count (red blood cells, hemoglobin, platelets, white blood cells), biochemical profile (total protein, albumin, cholesterol, ALT, aspartate aminotransferase (AST), total bilirubin (TB), direct bilirubin, alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT)), coagulation profile (partial thromboplastin time (PTT), international normalized ratio (INR), fibrinogen, prothrombin index (PTI), prothrombin time) tests were performed with the analyzers used in the laboratory of the Clinical Hospital № 85 of FMBA of Russia.

Serological markers of HBV infection (HBsAg), antibodies against HBsAg (anti-HBs), antibodies of the immunoglobulin G and M classes against the hepatitis B core antigen (anti-HBcore IgG, anti-HBcore IgM), HBeAg, antibodies against HBeAg (anti-HBe) were defined. The levels of HBsAg and anti-HBs were estimated by ensyme immunoassay (EIA). The HBV DNA was detected by polymerase chain reaction (PCR; sensitivity of the method was at least 50 IU/mL). The HBV genotypes were determined by the PCR amplification and sequencing of the viral genome fragment encoding the small surface protein (HBsAg).

All patients underwent hepatobiliary and spleen ultrasonography (AIXPLORER; France) and esophagogastoduodenoscopy (EGD) according to the indications (OLYMPUS GIF-E3; Japan). The liver stiffness was measured using the Fibroscan 502 Touch system (Echosens; France) according to the standard procedure. The fibrosis stage was determined in accordance with the METAVIR scoring system [8].

The virological response (VR) during treatment with the nucleos(t)ide analogues (NAs) was defined as achieving undetectable viremia (HBV DNA < 50 IU/mL), while VR during treatment with pegylated interferon — (PEG-IFN-α) for 12 months was defined as HBV DNA level < 2000 IU/mL; sustained virological response (SVR) was defined as the serum HBV DNA level < 2000 IU/mL 12 months after the end of therapy. Biochemical response (BR) was characterized by normal ALT activity (< 40 U/L).

The following was diagnosed based on the asessment performed during the first visit: four (2%) patients had HBeAg(+) chronic HBV infection, 10 (5%) – HBeAg(+) CHB, 37 (18.4%) – HBeAg(-) CHB, and 150 (74.6%) – HBeAg(-) chronic HBV infection (inactive HBsAg carriers).

Progression of infection was detected in 31 patients during the follow-up in the Center (within 1–10 years after the first visit): HBeAg(+) CHB in three cases and HBeAg(–) CHB in 19 cases. Furthermore, nine HBeAg(–) patients (4.5%) developed LC, among them one patient developed HCC. Probably, this was due to no AVT.

Statistical analysis

Statistical processing was performed using the SPSS 25.0 software package (SPSS: An IBM Company; USA).

Table 1. Comparative characteristics of HBeAg(+) and HBeAg(–) patients

Parameters	HBeAg(–), *n* = 187	HBeAg(+), *n* = 14	*p**
Gender Males Females	 103 (55.1%) 84 (44.9%)	 11 (78.6%) 3 (21.4%)	0.152
Age, years	50.0 [36.0–58.0]	28.5 [20.5–45.5]	0.001
Red blood cells, 10^{12}/L	4.7 [4.3–5.0]	4.8 [4.5–5.1]	0.488
Hemoglobin, g/L	144.0 [135.0–152.0]	147.5 [138.0–155.0]	0.268
White blood cells, 10^9/L	5.9 [4.9–6.8]	5.7 [4.3–6.3]	0.329
Platelets, 10^9/L	222.0 [194.0–256.0]	224.0 [201.0–267.0]	0.683
Total cholesterol, mmol/L	4.98 [4.3–5.8]	4.78 [4.1–5.2]	0.149
Total bilirubin, μmol/L	14.0 [10.25–18.9]	12.7 [9.1–18.0]	0.39
GGT, U/L	22.2 [15.9–35.1]	24.3 [18.6–43.0]	0.307
ALT, U/L > 40.0 U/L	24.0 [18.0–36.0] 37 (19.8%)	57.6 [34.9–78.0] 10 (71.4%)	0.001 <0.001"
AST, U/L > 40.0 U/L	23.8 [19.9–34.0] 32 (17.1%)	35.3 [30.3–46.0] 6 (42.9%)	0.020 0.029
Fibrosis, kPa	5.4 [4.5–7.2]	5.7 [5.4–6.9]	0.427
HBV DNA, $\log_{10}$ IU/mL	3.4 [1.0–4.1]	7.5 [3.2–7.8]	0.001
HBsAg, $\log_{10}$ IU/mL	3.4 [2.2–3.7]	4.1 [2.1–4.6]	0.259
Disease duration, years	5.0 [1.0–11.0]	5.0 [1.0–7.0]	0.726

Note: the data are presented as ME [25th and 75th percentiles] or *n*/*N* (%); **p* — significance level.

Quantitative indicators were presented as median (ME) [25th and 75th percentiles], and the qualitative data were presented as percentage. The chi-squared test and the Fisher's exact test were used to compare qualitative clinical data between groups, while the numerical data were compared using the Mann–Whitney *U* test. Logistic regression was used to assess the factors related to undetectable HBV DNA levels. The cumulative rates of virologic relapse were estimated by the Kaplan–Meier method and compared using the log-rank test. The Cox regression analysis was used to assess the relationship between the risk factors and the virologic relapse. Log transformation was applied to the HBV DNA and HBsAg levels. A *p*-value lower than 0.05 was considered statistically significant.

RESULTS

Characteristics of patients

A total of 201 patients were enrolled (114 males and 87 females, the male-to-female ratio was 1.3 : 1.0). The patients' median age at the time of the first visit was 50.0 [33.5–58.0] years and the median disease duration was 5.0 [1.0–11.0] years. The majority of patients had minimal clinical manifestations: fatique and the right upper quadrant pain. The patients were divided into two groups in accordance with their baseline HBeAg status: HBeAg positive (HBeAg(+)) and HBeAg negative (HBeAg(–)). The HBeAg(–) patients predominated (187/201; 93%). The patients' demographic and clinical characteristics are provided in Table 1.

The median age of the HBeAg(–) patients was higher than that of the HBeAg(+) patients: 50.0 [36.0–58.0] years and 28.5 [20.5–45.5] years, respectively; $p = 0.001$. The HBeAg(+) patients showed higher median ALT activity than the HBeAg(–) patients (57.6 U/L and 24.0 U/L, respectively; $p = 0.001$). Furthermore, elevated ALT was reported in 19.8% of the HBeAg(–) patients and 71.4% of the HBeAg(+) patients ($p < 0.001$).

Among 37 HBeAg(–) patients (19.8%), ALT activity was three times higher than the upper limit of normal (ULN) in 28 cases, 3–5 times higher than the ULN in four cases, up to 5–10 times higher than the ULN in four cases, and more than 10 times higher than the ULN in one patient. A total of 10 HBeAg(+) patients (71.4%) with elevated ALT were reported (ALT was three times higher than the ULN in eight of them and five times higher than the ULN in in two of them).

Likewise, the median AST level was higher in the HBeAg(+) patients than in the HBeAg(–) patients (35.3 U/L and 23.8 U/L, respectively; $p = 0.020$). The rate of the HBeAg(+) patients with elevated AST was 42.9% compared to the HBeAg(–) patients (17.1%) at $p = 0.029$.

Assessment of liver fibrosis by transient elastography was performed in 151/201 patients (75.1%). The body mass index did not exceed 25 kg/m^2. The following fibrosis stages were determined at the time of the first visit: F0/F1/F2 in 133/151 cases (88.1%), F3/F4 in 18/151 cases (11.9%). The HBV DNA levels were defined in 194 patients: these were 7.5 [3.2–7.8] log IU/mL in the group of HBeAg(+) patients and 3.4 [1.0–4,1] log IU/mL in the group of HBeAg(–) patients ($p = 0.001$).

The HBV genotype was studied in 65/201 patients (32.3%). Predominance of D genotype (62/65 (95.4%)) over the A (2/65 (3.1%)) and C (1/65 (1.5%)) genotypes was noted. HBV genotyping was performed in two HBeAg(+) patients, the HBV A and C genotypes were determined.

Table 2. Antiviral drugs used for treatment of CHB (n = 66)

Drugs	HBeAg(–) patients	HBeAg(+) patients
ETV	37	6
TDF	1	3
TBV*	10	2
LAM*	2	0
PEG-IFN-α-2a	0	1
ETV, TDF	3	0
TBV**, ETV	1	0

Note: ETV — entecavir; TBV — telbivudine; TDF — tenofovir disoproxil fumarate; LAM — lamivudine; PEG-IFN-α-2a — pegylated interferon α-2a; * — AVT in 2009–2011; ** — TBV for 12 months in 2009–2010, then ETV.

Efficacy of AVT for CHB

Currently, AVT for CHB approved in the RF involves the use of the nucleos(t)ide analogues (NAs) and pegylated interferons — (PEG-IFN-α). The NAs that are registered in Russia and are preferred for treatment of CHB include the drugs showing high antiviral activity: entecavir (ETV), tenofovir disoproxil fumarate (TDF), and tenofovir alafenamide (TAF). Since the patients followed up in the Center in 2007–2021 were enrolled, some patients received lamivudine (LAM) or telbivudine (TBV) during the first years of follow-up.

A total of 66 patients (32.8%) in the studied group who bought the drugs themselves received AVT. ETV was most often used by both HBeAg(–) and HBeAg(+) patients: 43/66 (65.2%) received this drug only, while the others (23) received other antiviral medications (Table 2).

A total of 65 patients were prescribed NAs. Among them 61 patients received only one drug, the treatment regimen was changed in four HBeAg(–) patients (antiviral drug was replaced by another one), that is why the total number of observations was 69 (58 HBeAg(–) and 11 HBeAg(+) patients). Three patients received ETV for 21–36 months, then switched to TDF, one patient received TBV for 12 months, then switched to ETV (Table 3).

The HBeAg(+) patients were younger and had higher ALT, AST, and viremia compared to the HBeAg(–) patients (Table 3). The median duration of therapy was 12.0 [11.0–30.0] months. The results of AVT with NAs (ETV, TDF, TBV, LAM) are provided in Table 4.

AVT efficiency in the HBeAg(+) patients

No viremia after 24, 48, 96 weeks of taking ETV was reported in 3/6, 4/5, and 1/1 patients, respectively. In patients who received TDF, VR was achieved after 24, 48, 96 weeks in 0/3, 1/3, and 1/3 patients, respectively. Two patients had been taking TBV for about two years; no viremia was detected in one of them after 24 and 48 weeks.

SVR after discontinuation of treatment was achieved in 3/3 patients. Among them one patient received TBV and the others received ETV.

The HBeAg seroconversion was reported in 4/11 patients (36.4%): it was associated with ETV therapy (two cases) or with TDF and TBV therapy (single cases).

When taking NAs, 2/11 HBeAg(+) patients (18.2%) who received ETV showed the HBsAg clearance, anti-HBs were found in 1/11 patient (9.1%). The HBsAg seroconversion was reported in this patient 27 months after the ETV discontinuation.

ALT activity back to normal after 24, 48, 96 weeks of taking ETV was reported in 1/6, 3/5, and 1/1 patients, respectively. As for patients who received TDF, no BR was observed after 24 and 48 weeks of therapy; one patient out of three showed BR after 96 weeks. ALT activity back to normal was observed in one patient treated with TBV out of two after 24 weeks, such ALT activity persisted at week 48 of therapy.

Only one HBeAg(+) patient had been taking PEG-IFN-α-2a in a dose of 180 mg/week for 48 weeks; HBsAg and HBeAg seroconversion together with undetectable HBV DNA were reported in the end of therapy.

AVT efficiency in the HBeAg(–) patients

After 24, 48, 96 weeks of taking ETV no HBV DNA was reported in 78%, 92.1%, and 94.1% of patients, respectively. In patients who received TDF, VR was achieved after 24, 48, 96 weeks in 3/4, 4/4, and 4/4 cases, respectively. A total of 11 patients had been taking TBV for about two years; VR was reported after 24

Table 3. Comparative characteristics of HBeAg(+) and HBeAg(–) patients who received NAs

Parameters	HBeAg(–) patients, n = 58	HBeAg(+) patients, n = 11	p*
Gender Males Females	 28/58 (48.3%) 30/58 (51.7%)	 8/11 (72.7%) 3/11 (27.3%)	0.137
Age, years	48.0 [32.0–57.0]	30.0 [25.0–52.0]	0.028
ALT, U/L > 40.0 U/L	27.7 [18.1–48.4] 20/58 (34.5%)	60.6 [43.3–90.4] 9/11 (81.8%)	0.006 0.006
AST, U/L > 40.0 U/L	25.3 [19.1–43.4] 15/58 (25.9%)	44.8 [28.2–70.0] 6/11 (54.5%)	0.016 0.078
Platelets, 10^9/L > 180 × 10^9/L	227.5 [179.0–269.0] 43/58 (74.1%)	228.0 [201.0–255.0] 11/11 (100.0%)	0.670 0.105
Fibrosis, kPa	6.6 [5.3–10.4]	6.1 [5.4–7.6]	0.649
HBV DNA, $\log_{10}$ IU/mL	4.0 [3.3–4.8]	7.0 [3.6–8.0]	0.016
HBsAg, $\log_{10}$ IU/mL	3.3 [3.0–3.8]	4.3 [3.8–4.5]	0.105

Note: the data are presented as ME [25th and 75th percentiles] or n/N (%); *p — significance level.

Table 4. Comparative efficiency of antiviral therapy with NAs

Virological response	ETV		TDF		TBV		LAM
	HBeAg(–) patients	HBeAg(+) patients	HBeAg(–) patients	HBeAg(+) patients	HBeAg(–) patients	HBeAg(+) patients	HBeAg(–) patients
Virological response							
24 weeks	32/41 (78.0%)	3/6 (50.0%)	3/4 (75.0%)	0/3 (0.0%)	9/11 (81.8%)	1/2 (50.0%)	2/2 (100.0%)
48 weeks	35/38 (92.1%)	4/5 (80.0%)	4/4 (100.0%)	1/3 (33.3%)	9/10 (90.0%)	1/2 (50.0%)	2/2 (100.0%)
96 weeks	16/17 (94.1%)	1/1 (100.0%)	4/4 (100.0%)	1/3 (33.3%)			
Biochemical response							
24 weeks	39/41 (95.1%)	1/6 (16.7%)	3/4 (75.0%)	0/3 (0.0%)	11/11 (100.0%	1/2 (50.0%)	2/2 (100.0%)
48 weeks	37/38 (97.4%)	3/5 (60.0%)	4/4 (100.0%)	0/3 (0.0%)	10/10 (100.0%)	1/2 (50.0%)	2/2 (100.0%)
96 weeks	16/17 (94.1%)	1/1 (100.0%)	4/4 (100.0%)	1/3 (33.3%)			

Note: the data are presented as *n/N* (%).

and 48 weeks in 9/11 and 9/10 patients. VR was achieved after 24 and 48 weeks of taking LAM in 2/2 patients.

SVR was achieved in 11/24 patients (45.8%). Among them three patients received TBV and eight patients received ETV. Virological relapse was reported in 13/24 patients (54.2%) after discontinuation of treatment with NAs, the median time was 6.0 [6.0–11.0] months.

No HBsAg clearance was reported in any of the HBeAg(–) patients who had been taking NAs.

ALT activity back to normal after 24, 48, 96 weeks of taking ETV was reported in 95.1%, 97.4%, and 94.1% of patients, respectively. As for patients who received TDF, BR was achieved after 24, 48, 96 weeks in 3/4, 4/4, and 4/4 patients, respectively, while in patients treated with TBV and LAM it was achieved after 24 and 48 weeks.

Thus, in patients who received NAs with a high barrier to drug resistance (ETV and TDF), VR was achieved after 24 and 48 weeks of treatment in 70.4 and 88.0%, while BR was achieved in 79.6 and 88.0%, respectively. It was shown that the rate of achieving VR and BR after 24 and 48 weeks of treatment with NAs was higher in the HBeAg(–) patients than in the HBeAg(+) patients, however, no differences were observed after 96 weeks of taking NAs (Table 5). After discontinuation of treatment with NAs, SVR was achieved in 14/27 patients (51.9%). When comparing the rates of achieving SVR, no significant differences were revealed between the HBeAg(+) and HBeAg(–) patients ($p = 0.222$). The HBeAg seroconversion was achieved in five cases (41.7%): after treatment with PEG-IFN-α (one case), ETV (two cases), TDF (one case), and TBV (one case). The HBsAg clearance was observed in three patients (4.5%): the patient who received PEG-IFN-α and two patients who received ETV. The HBsAg seroconversion was reported in two cases (3.0%) of treatment with PEG-IFN-α and ETV.

Factors affecting the ARV efficiency

Factors predictive of virological response

The univariate and multivariate logistic regression models were used to analyze the factors associated with VR at week 48 of the NA therapy. The univariate regression analysis identified the following factors associated with undetectable HBV DNA levels after 48 weeks of therapy: HBeAg status ($p = 0.011$); HBV DNA ($p = 0.001$) and ALT ($p = 0.042$) levels. The multivariate regression analysis showed that the baseline HBV DNA level (relative risk (RR) 0.411; 95% confidence interval (CI) 0.211–0.800; $p = 0.009$) was an independent prognostic factor of aviremia (Table 6).

HBsAg as a predictor of SVR after discontinuation of NA therapy

After discontinuation of NA therapy in 27 patients who had achieved VR, virological relapse was reported in 13/27 individuals (48.1%). The cumulative rate of virological relapse 6, 12, 24, and 36 months after discontinuation of NAs reached 25.9%, 40.7%, 44.4%, and 48.1%, respectively. Most cases of virological relapse were detected during the first 12 months of follow-up (11/13; 84.6%). The Cox regression analysis taking into account gender, age, HBV DNA levels before treatment, HBeAg status, and the HBsAg levels in the end of therapy showed that higher HBsAg levels in the end of therapy were

Table 5. Comparative efficiency of antiviral therapy with NAs in the HBeAg(+) and HBeAg(–) patients

Virological response	HBeAg(–) patients	HBeAg(+) patients	*p**
24 weeks	46/58 (79.3%)	4/11 (36.4%)	0.007
48 weeks	50/54 (92.6%)	6/10 (60.0%)	0.016
96 weeks	20/21 (95.2%)	2/4 (50.0%)	0.057
24 weeks	55/58 (94.8%)	2/11 (18.2%)	< 0.001
48 weeks	53/54 (98.1%)	4/10 (40.0%)	< 0.001
96 weeks	20/21 (95.2%)	2/4 (50.0%)	0.057
SVR	3/3 (100.0%)	11/24 (45.8%)	0.222

Note: the data are presented as *n/N* (%); **p* — significance level.

Table 6. Univariate and multivariate analysis of the raw factors associated with VR after 48 weeks of therapy with the nucleos(t)ide analogues

Indicators	Univariate analysis			Multivariate analysis		
	OP	95% CI	*p**	OP	95% CI	*p**
Gender (female)	0.931	0.212–4.097	0.925			
Age (increment 1 year)	1	0.954–1.049	0.988			
HBeAg(+) status	0.12	0.024–0.609	0.011	0.248	0.027–2.249	0.215
HBV DNA (increment 1 $\log_{10}$ IU/mL)	0.336	0.180–0.627	0.001	0.411	0.211–0.800	0.009
ALT (increment 1 U/L)	0.993	0.986–1.000	0.042	0.996	0.986–1.005	0.392
AST (increment 1 U/L)	0.991	0.980–1.001	0.083			
Platelets (less than 180 × 10^9/L)	0.818	0.146–4.582	0.819			
Liver fibrosis (F3/F4)	0.485	0.072–3.290	0.459			

Note: **p* — significance level.

predictive of virological relapse after discontinuation of NAs (RR: 3.909; 95% CI: 1.729–8.835; $p = 0.001$).

The patients with SVR had lower HBsAg levels in the end of therapy than the patients with virological relapse (1.9 [1.4–2.6] and 3.5 [3.3–4.0] log10 IU/mL, respectively; $p < 0.001$).

The patients were divided into three groups based on the HBsAg levels in the end of therapy:

Group 1: HBsAg < 100 IU/mL ($n = 8$);

Group 2: HBsAg — 100–1000 IU/mL ($n = 6$);

Group 3: HBsAg > 1000 IU/mL ($n = 13$).

No virological relapse was observed in patients of group 1, however, it was observed in 33.3% of group 2 (2/6) and 84.6% of group 3 (11/13). Significant intergroup differences in the virological relapse rate were revealed (log rank X2 = 12,280; $p = 0.02$). The HBsAg level < 100 IU/mL in the end of therapy was a significant predictor of SVR after discontinuation of NAs.

DISCUSSION

The clinical presentation of chronic HBV infection is characterized by the long term mildly symptomatic or asymptomatic disease with rare exacerbations or no exacerbations at all. However, the main danger related to this infection is the high risk of LC and HCC that reaches 8–20% within 5 years after the diagnosis in individuals with chronic HBV infection [2]. Our study explains the CHB course in patients who have been followed up in the Center for 1–15 years. The majority of patients had minimal clinical manifestations: fatique and the right upper quadrant pain predominated. However, among them LC was diagnosed in nine patients (4.5%), one of these patients developed HCC.

Currently, the HBeAg(–) form of this infection predominates in many countries of the world, including the RF. According to the reference center of surveillance for viral hepatitis of the Central Research Institute of Epidemiology of Rospotrebnadzor, in 2015 the share of HBeAg(–) patients was 90%. Our study yielded the same result: the HBeAg(–) patients constututed 93%. Predominance of the HBV D genotype (95.4%) was detected that was in line with the data of other studies focused on the HBV genotype distribution in Russia [7, 9].

The group of the HBeAg(+) patients is represented by younger individuals with the higher viremia and higher rate of hyperenzymemia compared to the HBeAg(–) patients.

Suppression of virus replication is an important goal of AVT and the basic premise of the CHB progression prevention. Currently, NAs are used for treatment of CHB in the world, including the RF, due to their high antiviral activity, low rate of side effects and the ease of use (1 tablet per day). Furthermore, NAs with a high barrier to drug resistance (ETV and TDF) are the top-priority drugs to be used for AVT.

The data obtained confirm high efficiency of the ETV and TDF therapy in patients with CHB. Aviremia after 48 weeks of treatment with ETV or TDF was achieved in 88% of cases. This, some papers report aviremia in 89.4% [10] and 88% [11] of patients who received ETV for a year. The other authors also observed aviremia in 86.2% of patients after a year of treatment with TDF [12].

Our findings showed the differences in achieving aviremia between the HBeAg(–) and HBeAg(+) patients. The patients with the HBeAg(–) CHB had a higher rate of VR after 24 and 48 weeks of treatment with NAs than the HBeAg(+) patients. Earlier it was shown that no HBV DNA was detected within the year of treatment in 75% of the HBeAg(+) patients and 99% of the HBeAg(–) patients who received ETV [10]. However, no differences in VR between patients of the Center were observed when performing treatment with NAs for 96 weeks.

Achieving BR defined as ALT activity back to normal can be considered as the desired therapy outcome. In the analyzed group of patients, BR was reported in 88% of cases after 48 weeks of treatment with ETV or TDF. The other researchers also demonstrate high rate of the ALT activity normalization during treatment with ETV and TDF [13–15]. Furthermore, we have shown the differences in the BR rate between the HBeAg(–) and HBeAg(+) patients after 24 and 48 weeks of treatment with NAs, which is consistent with the data provided by other authors [12, 16, 17]. It has been shown that the presence of HBeAg before treatment can be predictive of the ALT normalization failure [18].

The HBsAg clearance with or without seroconversion is considered an optimal treatment outcome and recovery from CHB. In the analyzed group, the HBsAg clearance was observed only in three HBeAg(+) patients (4.5%): in one patient who received PEG-IFN-α and two patients who received ETV. The HBsAg seroconversion was reported in two cases (3%).

The literature data suggest the higher rate of HBsAg clearance in patients with the HBeAg(+) hepatitis B. Thus, when performing treatment with TDF for 48 weeks, the HBsAg clearance after seven years was observed in 3.2% and 11.8% of patients with HBeAg(+) CHB and in 0% and 0.3% of patients with HBeAg(–) CHB, respectively [12, 19]. In general, very few (about 1%) of HBeAg(–) patients achieved the HBsAg clearance, even in case of the long term NA therapy (> 5 years) [11, 20]. The HBsAg clearance and seroconversion are more often reported in the HBeAg(+) CHB patients: 5–10% of the long term treatment cases [21, 22].

As for patients of the Center, the lower baseline HBV DNA level was an independent factor associated with aviremia after 48 weeks of treatment with NAs, which was consistent with the data reported by other authors [10]. They showed that the baseline HBV DNA level ≤7.6 $\log_{10}$ copies/mL was an independent prognostic factor of developing VR by year three of treatment. Similar results were obtained in a number of studies [23–25].

The virological relapse after the NA therapy discontinuation was observed in 13/27 patients of the Center (48.1%), while the higher HBsAg level in the end of therapy was predictive of virological relapse after the NA discontinuation. It has been shown that high baseline HBV DNA level and high HBsAg level in the end of treatment are the independent predictors of virological relapse [26]. In the analyzed group, no virological relapse was observed in patients with the HBsAg level in the end of therapy of less than 100 IU/mL, in contrast to patients with the levels of 100–1000 IU/mL (33.3%) and more than 1000 IU/mL (86,4%), respectively.

The recent systematic review that includes 11 studies involving 1716 patients suggests that the HBsAg levels in the end of therapy of less than 100 IU/mL are optimal for discontinuation of NAs and reduce the risk of virological relapse 12 months after the therapy cessation or later [27].

CONCLUSIONS

Chronic infection caused by HBV is a slowly progressive disease with the typical asimptomatic or mildly symptomatic course, however, the risk of developing LC and HCC is relatively high. The timely AVT is the only way to prevent these complications. AVT with NAs ensure high rates of virus replication suppression and ALT normalization. Low baseline level of viral load is an independent prognostic factor of achieving VR. The HBsAg level in the end of therapy is useful for predicting the HBV infection relapse after the treatment cessation.

References

1. World Health Organization. Hepatitis B Fact Sheet. July 27, 2020.
2. Ministerstvo zdravooxraneniya Rossijskoj Federacii. Xronicheskij virusnyj gepatit B (XVGV) u vzroslyx. 2019. Russian.
3. Pokrovskij VI, Totolyan AA, Ehsaulenko EV, Suxoruk AA. Virusnye gepatity v Rossijskoj Federacii: Analiticheskij obzor. 11 vypusk. SPb.: FBUN NIIEhM imeni Pastera, 2018. Russian.
4. O sostoyanii sanitarno-ehpidemiologicheskogo blagopoluchiya naseleniya v Rossijskoj Federacii v 2020 godu: Federal'naya sluzhba po nadzoru v sfere zashhity prav potrebitelej i blagopoluchiya cheloveka. 2021. Russian.
5. European Association For The Study Of The Liver. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. Journal of hepatology. 2017; 67 (2): 370–98.
6. Torresi J, Locarnini S. Antiviral chemotherapy for the treatment of hepatitis B virus infections. Gastroenterology. 2000; 118 (2): S83–S103.
7. Ivashkin VT, Yushhuk ND, Maevskaya MV, Znojko OO, Dudina KR, Karetkina GN, i dr. Klinicheskie rekomendacii Rossijskoj gastroehnterologicheskoj associacii i Rossijskogo obshhestva po izucheniyu pecheni po diagnostike i lecheniyu vzroslyx bol'nyx gepatitom B. Rossijskij zhurnal gastroehnterologii, gepatologii, koloproktologii. 2014; 3: 58–88. Russian.
8. Panevkina S, Abduraxmanov D, Ibragimov Eh, Rozina T, Nikulkina E, Tanashhuk E, i dr. Ocenka fibroza pecheni pri dlitel'noj terapii xronicheskogo gepatita V nukleozidnymi i nukleotidnymi analogami. Terapiya. 2021; 7 (6): 24–31. Russian.
9. Orlov SG, Myazin AE, Chulanov VP, redaktory. Rasprostranennost' genotipov virusa gepatita V sredi lic, xronicheski inficirovannyx virusom gepatita V v Moskve i Moskovskoj oblasti. Materialy Rossijskoj nauchno-prakticheskoj konferencii «Genodiagnostika infekcionnyx boleznej» 25–27 oktyabrya 2005 g. Sosnovka, Novosibirskaya obl. 2005; 56 s. Russian.
10. Ono A, Suzuki F, Kawamura Y, Sezaki H, Hosaka T, Akuta N, et al. Long-term continuous entecavir therapy in nucleos (t) ide-naïve chronic hepatitis B patients. Journal of hepatology. 2012; 57 (3): 508–14.
11. Luo J, Li X, Wu Y, Lin G, Pang Y, Zhang X, et al. Efficacy of entecavir treatment for up to 5 years in nucleos (t) ide-naïve chronic hepatitis B patients in real life. International journal of medical sciences. 2013; 10 (4): 427.
12. Marcellin P, Heathcote EJ, Buti M, Gane E, de Man RA, Krastev Z, et al. Tenofovir disoproxil fumarate versus adefovir dipivoxil for chronic hepatitis B. New England Journal of Medicine. 2008; 359 (23): 2442–55.
13. Yuen M-F, Seto W-K, Fung J, Wong DK-H, Yuen JC-H, Lai C-L. Three years of continuous entecavir therapy in treatment-naive chronic hepatitis B patients: VIRAL suppression, viral resistance, and clinical safety. Official journal of the American College of Gastroenterology| ACG. 2011; 106 (7): 1264–71.
14. Kayaaslan B, Akinci E, Ari A, Tufan ZK, Alpat SN, Gunal O, et al. A long-term multicenter study: Entecavir versus Tenofovir in treatment of nucleos (t) ide analogue-naive chronic hepatitis B patients. Clinics and research in hepatology and gastroenterology. 2018; 42 (1): 40–7.
15. Batirel A, Guclu E, Arslan F, Kocak F, Karabay O, Ozer S, et al. Comparable efficacy of tenofovir versus entecavir and predictors of response in treatment-naïve patients with chronic hepatitis B: a multicenter real-life study. International Journal of Infectious Diseases. 2014; 28: 153–9.
16. Chang T-T, Gish RG, De Man R, Gadano A, Sollano J, Chao Y-C, et al. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. New England Journal of Medicine. 2006; 354 (10): 1001–10.
17. Lai C-L, Shouval D, Lok AS, Chang T-T, Cheinquer H, Goodman Z, et al. Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. New England Journal of Medicine. 2006; 354 (10): 1011–20.
18. Jacobson IM, Washington MK, Buti M, Thompson A, Afdhal N, Flisiak R, et al. Factors associated with persistent increase in level of alanine aminotransferase in patients with chronic hepatitis B receiving oral antiviral therapy. Clinical Gastroenterology and Hepatology. 2017; 15 (7): 1087–94.
19. Buti M, Tsai N, Petersen J, Flisiak R, Gurel S, Krastev Z, et al. Seven-Year Efficacy and Safety of Treatment with Tenofovir Disoproxil Fumarate for Chronic Hepatitis B Virus Infection. Digestive Diseases and Sciences. 2015; 60 (5): 1457–64.
20. Petersen J, Heyne R, Mauss S, Schlaak J, Schiffelholz W, Eisenbach C, et al. Effectiveness and safety of tenofovir disoproxil fumarate in chronic hepatitis B: a 3-year prospective field practice study in Germany. Digestive diseases and sciences. 2016; 61 (10): 3061–71.
21. Gish R, Chang TT, Lai CL, De Man R, Gadano A, Poordad F, et al. Loss of HBsAg antigen during treatment with entecavir or lamivudine in nucleoside-naive HBeAg-positive patients with chronic hepatitis B. Journal of viral hepatitis. 2010; 17 (1): 16–22.
22. Heathcote EJ, Marcellin P, Buti M, Gane E, Robert A, Krastev Z, et al. Three-year efficacy and safety of tenofovir disoproxil fumarate treatment for chronic hepatitis B. Gastroenterology. 2011; 140 (1): 132–43.
23. Park JW, Kwak KM, Kim SE, Jang MK, Suk KT, Kim DJ, et al. Comparison of the long-term efficacy between entecavir and tenofovir in treatment-naïve chronic hepatitis B patients. BMC gastroenterology. 2017; 17 (1): 1–9.
24. Zoutendijk R, Reijnders JG, Brown A, Zoulim F, Mutimer D, Deterding K, et al. Entecavir treatment for chronic hepatitis B:

adaptation is not needed for the majority of naive patients with a partial virological response. Hepatology. 2011; 54 (2): 443–51.
25. Ibragimov EhK, Abduraxmanov DT, Rozina TP, Nikulkina EN, Tanashhuk EL, Odincov AV, et al. Ehffektivnost' i bezopasnost' dlitel'noj terapii xronicheskogo gepatita V nukleozidnymi i nukleotidnymi analogami. Terapevticheskij arxiv. 2019; 91 (2): 40–7.
26. Chen C-H, Hung C-H, Wang J-H, Lu S-N, Hu T-H, Lee C-M. Long-term incidence and predictors of hepatitis B surface antigen loss after discontinuing nucleoside analogues in noncirrhotic chronic hepatitis B patients. Clinical Microbiology and Infection. 2018; 24 (9): 997–1003.
27. Liu J, Li T, Zhang L, Xu A. The role of hepatitis B surface antigen in nucleos (t) ide analogues cessation among asian patients with chronic hepatitis B: a systematic review. Hepatology. 2019; 70 (3): 1045–55.

Литература

1. World Health Organization. Hepatitis B Fact Sheet. July 27, 2020.
2. Министерство здравоохранения Российской Федерации. Хронический вирусный гепатит В (ХВГВ) у взрослых. 2019.
3. Покровский В. И., Тотолян А. А., Эсауленко Е. В., Сухорук А. А. Вирусные гепатиты в Российской Федерации: Аналитический обзор. 11 выпуск. СПб.: ФБУН НИИЭМ имени Пастера, 2018.
4. О состоянии санитарно-эпидемиологического благополучия населения в Российской Федерации в 2020 году: Федеральная служба по надзору в сфере защиты прав потребителей и благополучия человека. 2021.
5. European Association For The Study Of The Liver. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. Journal of hepatology. 2017; 67 (2): 370–98.
6. Torresi J, Locarnini S. Antiviral chemotherapy for the treatment of hepatitis B virus infections. Gastroenterology. 2000; 118 (2): S83–S103.
7. Ивашкин В. Т., Ющук Н. Д., Маевская М. В., Знойко О. О., Дудина К. Р., Кареткина Г. Н., и др. Клинические рекомендации Российской гастроэнтерологической ассоциации и Российского общества по изучению печени по диагностике и лечению взрослых больных гепатитом В. Российский журнал гастроэнтерологии, гепатологии, колопроктологии. 2014; 3: 58–88.
8. Паневкина С., Абдурахманов Д., Ибрагимов Э., Розина Т., Никулкина Е., Танащук Е., и др. Оценка фиброза печени при длительной терапии хронического гепатита В нуклеозидными и нуклеотидными аналогами. Терапия. 2021; 7 (6): 24–31.
9. Орлов С. Г., Мязин А. Е., Чуланов В. П., редакторы. Распространенность генотипов вируса гепатита В среди лиц, хронически инфицированных вирусом гепатита В в Москве и Московской области. Материалы Российской научно-практической конференции «Генодиагностика инфекционных болезней» 25–27 октября 2005 г. Сосновка, Новосибирская обл. 2005; 56 с.
10. Ono A, Suzuki F, Kawamura Y, Sezaki H, Hosaka T, Akuta N, et al. Long-term continuous entecavir therapy in nucleos (t) ide-naïve chronic hepatitis B patients. Journal of hepatology. 2012; 57 (3): 508–14.
11. Luo J, Li X, Wu Y, Lin G, Pang Y, Zhang X, et al. Efficacy of entecavir treatment for up to 5 years in nucleos (t) ide-naïve chronic hepatitis B patients in real life. International journal of medical sciences. 2013; 10 (4): 427.
12. Marcellin P, Heathcote EJ, Buti M, Gane E, de Man RA, Krastev Z, et al. Tenofovir disoproxil fumarate versus adefovir dipivoxil for chronic hepatitis B. New England Journal of Medicine. 2008; 359 (23): 2442–55.
13. Yuen M-F, Seto W-K, Fung J, Wong DK-H, Yuen JC-H, Lai C-L. Three years of continuous entecavir therapy in treatment-naive chronic hepatitis B patients: VIRAL suppression, viral resistance, and clinical safety. Official journal of the American College of Gastroenterology| ACG. 2011; 106 (7): 1264–71.
14. Kayaaslan B, Akinci E, Ari A, Tufan ZK, Alpat SN, Gunal O, et al. A long-term multicenter study: Entecavir versus Tenofovir in treatment of nucleos (t) ide analogue-naive chronic hepatitis B patients. Clinics and research in hepatology and gastroenterology. 2018; 42 (1): 40–7.
15. Batirel A, Guclu E, Arslan F, Kocak F, Karabay O, Ozer S, et al. Comparable efficacy of tenofovir versus entecavir and predictors of response in treatment-naïve patients with chronic hepatitis B: a multicenter real-life study. International Journal of Infectious Diseases. 2014; 28: 153–9.
16. Chang T-T, Gish RG, De Man R, Gadano A, Sollano J, Chao Y-C, et al. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. New England Journal of Medicine. 2006; 354 (10): 1001–10.
17. Lai C-L, Shouval D, Lok AS, Chang T-T, Cheinquer H, Goodman Z, et al. Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. New England Journal of Medicine. 2006; 354 (10): 1011–20.
18. Jacobson IM, Washington MK, Buti M, Thompson A, Afdhal N, Flisiak R, et al. Factors associated with persistent increase in level of alanine aminotransferase in patients with chronic hepatitis B receiving oral antiviral therapy. Clinical Gastroenterology and Hepatology. 2017; 15 (7): 1087–94.
19. Buti M, Tsai N, Petersen J, Flisiak R, Gurel S, Krastev Z, et al. Seven-Year Efficacy and Safety of Treatment with Tenofovir Disoproxil Fumarate for Chronic Hepatitis B Virus Infection. Digestive Diseases and Sciences. 2015; 60 (5): 1457–64.
20. Petersen J, Heyne R, Mauss S, Schlaak J, Schiffelholz W, Eisenbach C, et al. Effectiveness and safety of tenofovir disoproxil fumarate in chronic hepatitis B: a 3-year prospective field practice study in Germany. Digestive diseases and sciences. 2016; 61 (10): 3061–71.
21. Gish R, Chang TT, Lai CL, De Man R, Gadano A, Poordad F, et al. Loss of HBsAg antigen during treatment with entecavir or lamivudine in nucleoside-naive HBeAg-positive patients with chronic hepatitis B. Journal of viral hepatitis. 2010; 17 (1): 16–22.
22. Heathcote EJ, Marcellin P, Buti M, Gane E, Robert A, Krastev Z, et al. Three-year efficacy and safety of tenofovir disoproxil fumarate treatment for chronic hepatitis B. Gastroenterology. 2011; 140 (1): 132–43.
23. Park JW, Kwak KM, Kim SE, Jang MK, Suk KT, Kim DJ, et al. Comparison of the long-term efficacy between entecavir and tenofovir in treatment-naïve chronic hepatitis B patients. BMC gastroenterology. 2017; 17 (1): 1–9.
24. Zoutendijk R, Reijnders JG, Brown A, Zoulim F, Mutimer D, Deterding K, et al. Entecavir treatment for chronic hepatitis B: adaptation is not needed for the majority of naive patients with a partial virological response. Hepatology. 2011; 54 (2): 443–51.
25. Ибрагимов ЭК, Абдурахманов ДТ, Розина ТП, Никулкина ЕН, Танащук ЕЛ, Одинцов АВ, et al. Эффективность и безопасность длительной терапии хронического гепатита В нуклеозидными и нуклеотидными аналогами. Терапевтический архив. 2019; 91 (2): 40–7.
26. Chen C-H, Hung C-H, Wang J-H, Lu S-N, Hu T-H, Lee C-M. Long-term incidence and predictors of hepatitis B surface antigen loss after discontinuing nucleoside analogues in noncirrhotic chronic hepatitis B patients. Clinical Microbiology and Infection. 2018; 24 (9): 997–1003.
27. Liu J, Li T, Zhang L, Xu A. The role of hepatitis B surface antigen in nucleos (t) ide analogues cessation among asian patients with chronic hepatitis B: a systematic review. Hepatology. 2019; 70 (3): 1045–55.