MORPHOFUNCTIONAL STATE OF CRYOPRESERVED BLOOD CELLS AT MODERATE LOW TEMPERATURE

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Cryoprotectants enable the long-term storage of biomaterials. Despite progress in cryopreservation, there are a number of problems associated with damage to the cell membranes that result from insufficient efficacy and toxicity of some components. In this regard, it is important to develop non-toxic cryopreservation agents performing well at low temperature. The study was aimed to assess morphofunctional features of blood cells in the lactulose-based cryopreservation agent considering the effects of moderate low temperature (–40 °C). Blood cells (leukocytes, erythrocytes, platelets) collected from 30 conditionally healthy female voluntary donors aged 18–23 years were assessed. The complete blood count test was performed using the Gemalight 1270 automated hematology analyzer. Computerized cytomorphometric assessement was performed using the MECOS-C2 hardware and software complex. The study results showed morphological and functional integrity of blood cells after the 24 h storage at the temperature of–40 °C when added the lactulose-based cryopreservation agent developed: erythrocytes — 85.3 ± 0.30% ($p < 0.05$), platelets – 75 ± 0.71% ($p < 0.05$), leukocytes – 90.1 ± 0.91% ($p < 0.05$) of the values reported before freezing. The findings demonstrate the potential of using lactulose as a non-toxic component of cryopreservation systems, which will expand the range of cryopreservation agents used and make it possible to analyze morphofunctional parameters of frozen whole blood samples when conducting large-scale studies.

Keywords: cryopreservation, erythrocytes, leukocytes, platelets, lactulose, moderate low temperature

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МОРФОФУНКЦИОНАЛЬНОЕ СОСТОЯНИЕ КРИОКОНСЕРВИРОВАННЫХ ФОРМЕННЫХ ЭЛЕМЕНТОВ КРОВИ ПРИ УМЕРЕННО НИЗКИХ ТЕМПЕРАТУРАХ

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Криопротекторы позволяют долгосрочно хранить биоматериалы. Несмотря на имеющиеся успехи в криоконсервации, существует ряд проблем, связанных с разрушением клеточных оболочек, из-за недостаточной эффективности и токсичности некоторых компонентов. В связи с этим, важное значение имеет разработка нетоксичных криоконсервантов, эффективно работающих при низких температурах. Целью работы было оценить морфофункциональные особенности форменных элементов крови в криоконсерванте с лактулозой с учетом воздействия умеренно низкой температуры (–40 °C). Были исследованы форменные элементы крови (лейкоциты, эритроциты, тромбоциты), полученные от 30 условно здоровых добровольцев-доноров женского пола в возрасте 18–23 лет. Общий анализ крови выполняли на автоматическом гематологическом анализаторе «Гемалайт 1270». Компьютерное цитоморфометрическое исследование проводили на аппаратно-программном комплексе «МЕКОС-Ц2». По результатам исследования установлена морфологическая и функциональная сохранность форменных элементов крови после одних суток хранения при температуре –40 °C при добавлении разработанного криоконсерванта с лактулозой: для эритроцитов — 85,3 ± 0,30% ($p < 0,05$), для тромбоцитов — 75 ± 0,71% ($p < 0,05$), для лейкоцитов — 90,1 ± 0,91% ($p < 0,05$) от значений, зарегистрированных до замораживания. Результаты демонстрируют потенциал использования лактулозы в качестве нетоксичного компонента для криоконсервирующих систем, что расширит спектр применяемых криоконсервантов и позволит проводить анализ морфофункциональных параметров образцов замороженной цельной крови при крупномасштабных исследованиях.

Ключевые слова: криоконсервирование, эритроциты, лейкоциты, тромбоциты, лактулоза, умеренно низкая температура

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Optimization of biomaterial cryopreservation methods enabling the prolonged preservation of their functional and phenotypic features is one of the priorities and promising directions of modern biology, as well as experimental and clinical medicine. Biomaterial cryopreservation methods should have low toxicity and support the cellular homeostatic mechanisms. A significant slowdown in cell metabolism begins at the temperature of –70 °C, therefore, the liquid nitrogen cryopreservation is used. However, this requires bulky, expensive equipment, regular liquid nitrogen supply, which affects the material storage cost. Furthermore, such preservation type contributes to the thrombogenicity changes [1], therefore, effective cryopreservation agents ensuring maximum integrity of the preserved biological entity biological properties are in demand.

The best results are achieved when using the combined cryopreservation agents containing both penetrating and non-penetrating cryoprotectants. However, despite progress in cryopreservation, there are a number of problems associated with damage to the cell membranes that result from insufficient efficacy [2] and toxicity of some components [3–5]. It is reasonable to add lipids, proteins and carbohydrates as the components of natural origin [6–10], as well as polyhydric alcohols [11] in order to reduce toxic effects of the cryopreservation agent components, including when freezing human venous blood [12]. Trehalose has a pronounced protective effect on the cell membranes [13–16]; a synergistic effect has been confirmed for trehalose used in combination with dimethyl sulfoxide (DMSO) [17].

The search for new non-toxic components, such as lactulose, represents one of the cryopreservation improvement directions. No data on the toxic, teratogenic or mutagenic effects were obtained when conducting animal experiments and human clinical trials [18]. Furthermore, protective effects of lactulose improving the lactic acid bacterial culture survival rate during freezing have been reported. Adding up to 3% lactulose to the mixture containing the lactic acid bacteria contributed to the increase in the number of viable bacterial cells when freezing the products to the temperature of –18 °C [19]. There is evidence that lactulose used in combination with lecithin has a cryoprotective effect on probiotics at the temperature of –20 °C [20]. Thus, lactulose can be used in practice as a non-toxic cryopreservation agent component to preserve biological entities during freezing.

Fresh whole blood samples are most preferable for analysis, however, the disadvantages of handling whole blood include the need for rapid analysis after biosample collection and the limited number of the repeated tests that can be conducted without collecting extra blood [21]. The experience of capillary blood cryopreservation in the field with subsequent cytometry analysis due to the need for immediate sample testing have been reported [22], however, there are no similar data on cryopreservation of venous blood. Moreover, preservation of the peripheral blood hematopoietic stem cells as a procedure for treatment of hematological, oncological and autoimmune diseases is relevant [23].

The study was aimed to assess morphofunctional features of blood cells in the lactulose-based cryopreservation agent considering the effects of moderate low temperature (–40 °C).

METHODS

Inclusion criteria: conditionally healthy female donors aged 18–23 years in the first phase of their menstrual cycle, a total of 30 individuals with no exacerbation of chronic disorders. The research object was peripheral venous blood stabilized with K3 EDTA (ethylenediaminetetraacetic acid) in vitro.

Samples of peripheral venous blood collected from voluntary donors were divided into three groups. The control group included 10 blood samples, in which blood cell characteristics and parameters were assessed at the temperature of +20 ± 1.0 °C. Experimental group 1 included 10 blood samples supplemented with the cryopreservation agent. These samples were assessed after 4 h at the temperature of +20 ± 1.0 °C. Experimental group 2 included 10 blood samples supplemented with the cryopreservation agent; the samples were put into a state of cold anabiosis for 24 h at the temperature of–40 °C. Then the samples were thawed to assess blood cell characteristics and parameters at the temperature of +20 ± 1.0 °C.

Isotonic salines were used to prepare the model cryopreservation agent. Glycerin and DMSO were used as the cryocomponents penetrating into the cell, while the non-penetrating component was represented by the lactulose disaccharide. The ultimate composition of the model cryopreservation agent had the following component ratios (% v/v): glycerin (AR, Russia) — 20, DMSO (CP, Russia) — 10, lactulose (Lactisan manufactured according to TU 9229-004-53757476–04; Russia) — 2.5, sodium chloride (AR, Russia) — 0.25, sodium phosphate, dibasic (AR, Russia) — 0.25, water for injection (Dalhimfarm; Russia) — to 100%.

The cryopreservation agent solution was autoclaved (without DMSO) at 1.2 atm for 30 min. The resulting solution was stored in a refrigerator at +2 – +4 °C. DMSO was sterilized using the sterilizing filtration unit and stored in the sterile tubes at the temperature of –10 °C. The DMSO solution was added to the ready-made sterile cryopreservation agent immediately before freezing blood samples.

Cryopreservation agent was added to the samples of the experimental groups 1 and 2 to the blood : cryopreservation agent ratio of 2 : 1 (v/v) using a dispenser. The tubes were sealed with plugs, and the content was mixed for 10 min. Later (after 4 h) samples of the experimental group 1 were assessed at the temperature of +20 ± 1.0 °C. Samples of the experimental group 2 were put in the freezer of the electric deep-freeze refrigerator with the temperature of –20 ± 1.0 °C into the refrigerant in the form of solution (38–45% v/v of 96% (v/v) ethanol) with the cold adaptation temperature of –26 — –30 °C; the volume of sample to be frozen was 10% of the refrigerant volume, the samples were kept in it for 30 min and transferred to the electric deep-freeze refrigerator chamber with the temperature of –40 ± 1.0 °C for 24 h for final freezing and storage. After that the samples were thawed in the UT-4334 water bath (ULAB; Russia) while rocking (2–3 times/s) in the manual mode at the temperature of +40 ± 1 °C for 1 min. Then blood cell characteristics and parameters were assessed at the temperature of +20 ± 1.0 °C. The computerized cytomorphometric assessement of blood cells was performed using the MECOS-C2 hardware and software complex (Medical Computer Systems; Russia). The Gemalight 1270 automated hematology analyzer (Dixion; Russia) was used to conduct in vitro diagnostic testing of blood in laboratory settings. To determine red blood counts (RBC), cells were enumerated in the blood cell suspension with the sample diluted to the ratio of 1 : 40,000. The hematology analyzer was used to determine 21 laboratory indicators reflecting the state of the white blood cell, red blood cell, and platelet components.

The results obtained were processed using the IBM SPSS Statistic 23.0 software package (IBM Corp., Armonk, NY; USA). Distributions of the studied indicator values were assessed using the Shapiro–Wilk test. When the indicator value distribution was normal, significance of intergroup differences was assessed using the nonparametric Student's t-test for independent samples; the Mann–Whitney U test was used when the distribution of indicator values was non-normal. The mean (X), error of the mean (м), and standard deviation (δ) were calculated for the normally distributed indicators. The intergroup differences were considered significant at the error probability (p) ≤ 0.05 (5% probability).

RESULTS

The analysis of white blood cell indicators of blood in the control group was performed before adding the cryopreservation agent. In the experimental group 1, it was conducted 4 h after adding

Table 1. White blood cell indicators of CBC in the studied groups (X ± м; *p*)

CBC indicators	Control group (*n* = 10)	Experimental group 1 +20 ± 1.0 °C (*n* = 10)	Experimental group 2 –40 ± 1.0 °C (*n* = 10)	Significance of differences, *p*
WBC, ×10⁹/l	5.60 ± 0.92	4.55 ± 0.74*	4.14 ± 0.85**	*p* < 0.01
Lym, %	38.31 ± 1.35	25.70 ± 1.87*	11.9 ± 1.10**	*p* < 0.01
Gran, %	56.20 ± 3.67	51.10 ± 2.05*	45.3 ± 2.71**	*p* < 0.01
Mid, %	6.20 ± 0.32	10.20 ± 1.21	10.32 ± 1.27	*p* ≥ 0.2
Integrity, %	100	81 ± 0.89*	90.1 ± 0.91**	*p* < 0.01

Note: * — a significant difference between the control group and the experimental group 1 (*p* < 0.01); ** — a significant difference between the control group and the experimental group 2 (*p* < 0.01)

the cryopreservation agent at the temperature of +20 ± 1.0 °C. In the experimental group 2, it was performed after thawing blood samples with the cryopreservation agent following the 24 h incubation at the temperature of –40 ± 1.0 °C (Table 1).

The analysis of white blood cell indicators in the experimental groups 1 and 2 revealed a downward trend of these indicators, however, the proportion and percentage were still within the reference ranges.

The white blood cell computerized cytomorphometry data analysis was performed before adding the cryopreservation agent in the control group, after adding the cryopreservation agent and 4 h incubation in the experimental group 1 at the temperature of +20 ± 1.0 °C, and after thawing blood samples with the cryopreservation agent with the 24 h incubation in the experimental group 2 at the temperature of –40 ± 1.0 °C (Table 2).

The data obtained show a downward trend of the indicators, however, the values are within the reference range of changes. Furthermore, in the experimental groups 1 and 2, where the cryopreservation agent was added, the indicator values are stable: the total number of nuclei is 1, the total number of nuclear segments is 1, the number of inclusion bodies/holes in the nucleus is 0, and the number of nuclear "tails" is 2.

The analysis of red blood cell indicators was performed before adding the cryopreservation agent in the control group, after adding the cryopreservation agent and 4 h incubation in the experimental group 1 at the temperature of +20 ± 1.0 °C, and after thawing blood samples with the cryopreservation agent with the 24 h incubation in the experimental group 2 at the temperature of –40 ± 1.0 °C (Table 3).

Comparative analysis of complete blood counts in the control group, experimental groups 1 and 2 under exposure to moderate low temperature has revealed a downward trend of the indicators, however, the proportion and percentage are still within the reference ranges of blood cell characteristics.

The red blood cell computerized cytomorphometry data analysis was performed before adding the cryopreservation agent in the control group, after adding the cryopreservation agent and 4 h incubation in the experimental group 1 at the temperature of +20 ± 1.0 °C, and after thawing blood samples with the cryopreservation agent with the 24 h incubation in the experimental group 2 at the temperature of –40 ± 1.0 °C (Table 4).

The results of comparative analysis of the control group and experimental group 1 demonstrate a downward trend of the majority of indicators, however, the values obtained are within the reference ranges of blood cell characteristics. The data obtained by comparative analysis in the experimental group 2 demonstrate a downward trend of all the indicators. Furthermore, the values obtained are within the reference ranges of blood cell characteristics and suggest stability of the samples amidst crioprotectant load.

The analysis of red blood cell indicators was performed before adding the cryopreservation agent in the control group, after adding the cryopreservation agent and 4 h incubation in the experimental group 1 at the temperature of +20 ± 1.0 °C, and after thawing blood samples with the cryopreservation agent with the 24 h incubation in the experimental group 2 at the temperature of –40 ± 1.0 °C (Table 5).

Comparative analysis of complete blood counts in the control group and experimental group 1 at the temperature of +20 ± 1.0 °C has revealed a downward trend of a number of indicators, however, all the values are at the boundary of reference range. The analysis of platelet indicators in the experimental group 2 has revealed a downward trend of the values, however, the proportion and percentage are still within the reference ranges of blood cell characteristics.

The platelet computerized cytomorphometry data analysis was performed before adding the cryopreservation agent in the control group, after adding the cryopreservation agent and 2 h incubation in the experimental group 1 at the temperature

Table 2. Differences in the computerized cytomorphometry data in the studied groups (X ± м; *p*)

Entity properties	Control group (*n* = 10)	Experimental group 1 +20 ± 1.0 °C (*n* = 10)	Experimental group 2 –40 ± 1.0 °C (*n* = 10)	Significance of differences, *p*
Cell area, μm²	70 ± 4.19	59 ± 4.75	70 ± 3.98	*p* ≥ 0.1
Cell shape factor	14.11 ± 1.62	18.01 ± 1.23	17.2 ± 1.45	*p* ≥ 0.1
Cell polarization index	0.16 ± 0.01	0.31 ± 0.01	0.11 ± 0.01	*p* ≥ 0.1
Optical density of cytoplasm	0.66 ± 0.01	0.50 ± 0.01	0.63 ± 0.01	*p* ≥ 0.1
Area of the nucleus, μm²	52 ± 3.85	40 ± 3.21*	32 ± 2.24**	*p* < 0.01
Nucleus shape factor	14.3 ± 2.1	13.1 ± 2.88	13.9 ± 2.88	*p* ≥ 0.05
Polarization of the nucleus	0.06 ± 0.001	0.02 ± 0.001*	0.16 ± 0.01**	*p* < 0.01
Nuclear–cytoplasmic ratio	0.74 ± 0.01	0.68 ± 0.01	0.45 ± 0.01	*p* ≥ 0.1
Nuclear complement share	0.04 ± 0.001	0.02 ± 0.001	0.04 ± 0.001	*p* ≥ 0.1

Note: * — a significant difference between the control group and the experimental group 1 (*p* < 0.01); ** — a significant difference between the control group and the experimental group 2 (*p* < 0.01)

Table 3. Red blood cell indicators of CBC in the studied groups (X ± м; *p*)

CBC indicators	Control group (*n* = 10)	Experimental group 1 +20 ± 1,0 °C (*n* = 10)	Experimental group 2 –40 ± 1,0 °C (*n* = 10)	Significance of differences, *p*
RBC, ×10^{12}/l	4.62 ± 0.23	4.08 ± 0.21	3.5 ± 0.55	$p \geq 0.05$
HGB, g/l	131 ± 5.27	119.25 ± 5.48*	101.3 ± 4.73**	$p \geq 0.1$
MCV, fl	86.3 ± 4.32	84.3 ± 4.14	80.6 ± 3.38	$p \geq 0.1$
HCT, %	39.3 ± 1.58	35.4 ± 1.49	30.4 ± 1.63	$p \geq 0.2$
MCH, pg	28.5 ± 1.37	26.2 ± 1.45	21.1 ± 1.52	$p \geq 0.05$
MCHC, g/l	336 ± 29.72	311 ± 23.46	266 ± 14.42	$p \geq 0.5$
RDW, %	12.2 ± 1.67	11.7 ± 1.48	10.3 ± 1.25	$p \geq 0.5$
Integrity, %	100	89 ± 0.20*	85.3 ± 0.30**	$p < 0.01$

Note: * — a significant difference between the control group and the experimental group 1 ($p < 0.01$); ** — a significant difference between the control group and the experimental group 2 ($p < 0.01$)

of +20 ± 1.0 °C, and after thawing blood samples with the cryopreservation agent with the 24 h incubation in the experimental group 2 at the temperature of –40 ± 1.0 °C (Table 6).

The analysis of data of the control group and experimental group 1 at the temperature of +20 ± 1.0 °C demonstrates a downward trend of the majority of indicators, however, the values obtained are within the reference ranges of blood cell characteristics. The data of comparative analysis in the experimental group 2 demonstrate a downward trend of the indicators, being within the reference ranges of blood cell characteristics.

DISCUSSION

The results of comparative analysis of the white blood cell indicators in the control group and experimental group 1, where the cryopreservation agent was added, showed a downward trend of the majority of indicators, however, all values were within the reference ranges. Such indicators, as the "total number of nuclei — 1", "total number of nuclear segments — 1", "number of inclusion bodies/holes in the nucleus — 0", "number of nuclear "tails" — 2" were the same in the control group and the experimental group 1. The values obtained were used as a reference for the indicators of frozen samples. The analysis of white blood cell computerized cytomorphometry data before and after adding the cryopreservation agent with freezing to –40 °C showed that the indicators of the experimental group 2 were the same as in the control group: the total number of nuclei was 1, the total number of nuclear segments was 1, the number of inclusion bodies/holes in the nucleus was 0, and the number of nuclear "tails" was 2. The analysis of complete blood count indicators and the whole blood leukocyte morphometry characteristics in the control group and experimental groups revealed a downward trend of the indicator values, however, the data obtained were within the permissible range of blood cell characteristics. Thus, in the control group WBC × 10^9/L were 5.60 ± 0.92 ($p < 0.01$), in the experimental group 1 these were 4.55 ± 0.74 ($p < 0.01$), and in the experimental group 2 these were 4.14 ± 0.85 ($p < 0.01$). The leukocyte morphometry indicators, such as the cell area measured in μm^2, were 70 ± 4.19 ($p \geq 0.1$) in the control group, 59 ± 4.75 ($p \geq 0.1$) in the experimental group 1, and 70 ± 3.98 ($p \geq 0.1$) in the experimental group 2.

The results of comparative analysis of the red blood cell computerized cytomorphometry data in the control group and the experimental groups 1 and 2, where the cryopreservation agent was added, showed a downward trend of indicators, however, all the values were within the reference ranges. The analysis of the red blood cell computerized cytomorphometry data in the experimental group 2 with the cryopreservation agent added at the freezing temperature of –40 °C showed a downward trend, however, the results obtained suggested stability of samples and were within the reference ranges of blood cell characteristics. Thus, in the control group RBC × 10^{12}/L were 4.62 ± 0.23 ($p \geq 0.05$), in the experimental group 1 these were 4.08 ± 0.21 ($p \geq 0.05$), and in the experimental group 2 these were 3.5 ± 0.55 ($p \geq 0.05$). The erythrocyte morphometry indicators, such as the cell area measured in μm^2, were 46.6 ± 4.12 ($p < 0.01$) in the control group, 53.3 ± 4.68 ($p < 0.01$) in the experimental group 1, and 41.1 ± 2.42 ($p < 0.01$) in the experimental group 2, respectively.

According to the comparative analysis results, the majority of platelet indicators in the control group and the experimental group 1, where the cryopreservation agent was added,

Table 4. Differences in the erythrocyte computerized cytomorphometry data in the studied groups (X ± м; *p*)

Entity properties	Control group (*n* = 10)	Experimental group 1 +20 ± 1,0 °C (*n* = 10)	Experimental group 2 –40 ± 1,0 °C (*n* = 10)	Significance of differences, *p*
Cell area, μm^2	46.6 ± 4.12	53.3 ± 4.68*	41.1 ± 2.42**	$p < 0.01$
Average diameter, μm	6.4 ± 1.14	7.8 ± 1.17	6.85 ± 1.16	$p \geq 0.1$
Shape factor	13.5 ± 1.2	13.6 ± 1.2	12.7 ± 1.2	$p \geq 0.1$
Polarization	0.096 ± 1.001	0.083 ± 0.001*	0.048 ± 0.001**	$p < 0.01$
Integrated optical density (Red), μm^2	16.4 ± 2.78	18.1 ± 2.91	16.2 ± 2.74	$p \geq 0.05$
Integrated optical density (Green), μm^2	20.3 ± 2.24	21.3 ± 2.31	19.8 ± 2.13	$p \geq 0.1$
Integrated optical density (Blue), μm^2	11.28 ± 2.11	12.95 ± 2.17	11.75 ± 2.15	$p \geq 0.1$

Note: * — a significant difference between the control group and the experimental group 1 ($p < 0.01$); ** — a significant difference between the control group and the experimental group 2 ($p < 0.01$)

Table 5. Platelet indicators of CBC in the studied groups (X ± м; *p*)

CBC indicators	Control group (*n* = 10)	Experimental group 1 +20 ± 1,0 °C (*n* = 10)	Experimental group 2 –40 ± 1,0 °C (*n* = 10)	Significance of differences, *p*
PLT, ×10^9/l	230.8 ± 5.78	198.6 ± 5.36*	150.1 ± 4.71**	$p < 0.01$
MPV, fl	8.24 ± 1.22	7.56 ± 1.24	7.04 ± 1.17	$p \geq 0.1$
PCT, %	2.23 ± 0.31	2.1 ± 0.24	1.97 ± 0.71	$p \geq 0.1$
P-LCR, %	17.38 ± 2.72	21.24 ± 2.61	19.17 ± 2.45	$p \geq 0.05$
P-LCC, ×10^9/l	41.61 ± 3.12	50.8 ± 3.32	46.14 ± 3.74	$p \geq 0.05$
Integrity, %	100	86.2 ± 0.31*	75 ± 0.71**	$p < 0.01$

Note: * — a significant difference between the control group and the experimental group 1 ($p < 0.01$); ** — a significant difference between the control group and the experimental group 2 ($p < 0.01$)

Table 6. Platelet computerized cytomorphometry indicators in the studied groups (X ± м; *p*)

Entity properties	Control group (*n* = 10)	Experimental group 1 +20 ± 1,0 °C (*n* = 10)	Experimental group 2 –40 ± 1,0 °C (*n* = 10)	Significance of differences, *p*
Area, μm^2	7.7 ± 0.21	7.5 ± 0.36	7.2 ± 0.31	$p \geq 0.1$
Min. diameter, μm	2.85 ± 0.14	2.63 ± 0.16	2.31 ± 0.24	$p \geq 0.1$
Max. diameter, μm	4.03 ± 0.24	3.94 ± 0.32	3.74 ± 0.36	$p \geq 0.1$
Average diameter, μm	3.44 ± 0.13	3.29 ± 0.16*	3.03 ± 0.21**	$p < 0.01$
Shape factor	12.9 ± 2.18	12.3 ± 1.21	12.1 ± 1.74	$p \geq 0.05$

Note: * — a significant difference between the control group and the experimental group 1 ($p < 0.01$); ** — a significant difference between the control group and the experimental group 2 ($p < 0.01$)

showed a downward trend at room temperature, however, all the values were were within the reference ranges of blood cell characteristics. The analysis of platelet indicators in the experimental group 2, where the cryopreservation agent was added, with incubation at the temperature of –40 °C showed a downward trend of the proportion and percentage of indicators, however, the values obtained were within the reference ranges of blood cell characteristics. Thus, in the control group PLT × 10^9/L were 230.8 ± 5.78 ($p < 0.01$), in the experimental group 1 these were 198.6 ± 5.36 ($p < 0.01$), and in the experimental group 2 these were 150.1 ± 4.71 ($p < 0.01$). The platelet morphometry indicators, such as the cell area measured in μm^2, were 7.7 ± 0.21 ($p \geq 0.1$) in the control group, 7.5 ± 0.36 ($p \geq 0.1$) in the experimental group 1, and 7.2 ± 0.31 ($p \geq 0.1$) in the experimental group 2, respectively.

The analysis of studies focused on the search for new effective cryopreservation agents has shown that there are a large number of studies focused on cryopreservation of erythrocytes rather than platelets or leukocytes. When the Cryosin solution was used as a cryopreservation agent, the erythrocyte integrity rate was 83.8 ± 4.09% [24]. The following data were obtained when assessing viability of the nucleated cells contained in the leukocyte concentrates in the phases of cell acquisition, freezing and thawing: much more cells remained viable after washing off DMSO than without washing — 94.4% vs. 86.7% [25]. When the donor blood platelets protected with the combined cryopreservation agent were frozen, their functional activity was maintained within the range of 63.5–88.8% [26]. Thus, the data obtained during our study are comparable with the literature data on the analysis of integrity of certain blood cells in the course of cryopreservation.

The lactulose-based cryopreservation agent developed is effective under conditions of freezing to –40 °C and affordable (all the components are produced in the Russian Federation), which expands the range of the cryopreservation agents used and enables the analysis of morphofunctional parameters of frozen whole blood samples within the framework of large-scale studies conducted in emergency situations, in the aftermath of accidents of natural and human-induced origin, under conditions of terrorist attacks, armed conflicts, biomaterial storage in the long expeditions and remote areas. Moreover, the personalized approach to blood component transfusion in the cases of emergency need for transfusion of one's own cryopreserved whole blood aimed to reduce the risk of the influence of foreign complexes on the recipient's body is promising. The data provided suggest the need for further investigation of the effects of lactulose used as the cryocomponent on the integrity of biological entities.

CONCLUSIONS

Processing of the data obtained revealed morphological and functional integrity of blood cells in the lactulose-based cryopreservation agent developed after freezing for 24 h at the temperature of –40 °C: erythrocytes — 85.3 ± 0.30% ($p < 0.05$), platelets — 75 ± 0.71% ($p < 0.05$), leukocytes — 90.1 ± 0.91% ($p < 0.05$) of the values reported before freezing. In the light of current studies focused on the search for new effective cryopreservation agents, lactulose can be used as a non-toxic component when developing cryocompositions for preservation of biological entities during freezing.

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