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БІОЛОГІЯ ЛЮДИНИ ТА МЕДИЦИНА XXI: СУЧАСНИЙ СТАН, ПРОБЛЕМИ ТА ПЕРСПЕКТИВИ

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СЕКЦІЯ VI.
НАНОМЕДИЦИНА, БІОТЕХНОЛОГІЇ, БІОМЕДИЧНА ТА БІОІНЖЕНЕРІЯ

DISTURBANCES OF THE MEMBRANE ASYMMETRY AND MECHANICAL STABILITY OF HUMAN ERYTHROCYTES UPON CRYOPRESERVATION WITH POLYETHYLENE GLYCOL

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Introduction. Donated blood is widely used in medicine to restore oxygen supply to tissues in patients with different pathologies. Storing cells at ultra-low temperatures (cryopreservation) allows creating significant blood reserves and solve the problem of blood shortage in emergency cases. Endo- and exocellular cryoprotective agents (CPA) are used to protect cells against stress factors accompanying freeze-thawing. Exocellular substances do not penetrate the membrane, and their removal out of the cell suspension becomes a dispensable step due to a gradual decrease in their concentration in the bloodstream that provides an advantage of exocellular CPA over endocellular ones. However, it has not yet been possible to develop methods for erythrocyte cryopreservation with exocellular CPA,

which would provide an acceptable level of cell viability under physiological conditions. One of the promising substances as endocellular CPA can be polyethylene glycol with a molecular weight of 1500 (PEG) [1].

The aim of the study was to examine the effect of freeze-thawing of human erythrocytes under the PEG protection on the asymmetric distribution of lipids in the membrane and cell mechanical stability.

Methods. Erythrocytes, washed from plasma and white blood cells, were joined with PEG solution (30% PEG, 150 mM NaCl, 10 mM Tris-HCl (pH 7.4)) in a ratio of 1: 1 by volume. The cells were frozen by immersion in liquid nitrogen (–196°C), the samples were thawed at 42–44°C. The externalization of phosphatidylserine to the membrane surface was assessed by flow cytometry on the basis of the cell binding of annexin V conjugated to a fluorescent label FITC (annexin V-FITC). The mechanical stability of cryopreserved erythrocytes was determined according to the previously described method [2] by their hemolysis caused by moving plastic beads.

Results. Phospholipids of the plasma membrane of erythrocytes are unevenly distributed between the outer and inner layers of the lipid bilayer [4]. Most of phosphatidylcholine and sphingomyelin are located in the outer monolayer, while phosphatidylethanolamine and phosphatidylserine (PS) are almost completely concentrated on the cytoplasmic side of the membrane. It was found that in the membrane of erythrocytes cryopreserved under the PEG-1500 protection, the amount of cells binding annexin V-FITC can reach 30% (23.5 ± 5.4)% after an hour of incubation of frozen-thawed cells at 37°C in the presence of CPA. The reasons for the transmembrane redistribution of PS can be associated with an increase in the level of cytosolic Ca^{2+} due to osmotic shrinkage of cells in a hypertonic environment and inhibition of the Ca^{2+}-ATPase activity [3], which entail the activation of scramblase, an enzyme that destroys lipid asymmetry. In a certain extent the disturbance of the membrane lipid structure of cryopreserved erythrocytes reflects the possibility of the formation of defects in the plasma membrane. In addition, externalization of PS serves as a signal for the activation of macrophages with the subsequent removal of such cells from the body. Obviously, such disturbances can negatively affect cryopreserved erythrocytes in the bloodstream after transfusion.

As shown by the analysis of hemolytic injuries of cells under mechanical stress, the resistance of erythrocytes cryopreserved with PEG decreases related to the control parameters. This indicates sublethal injuries of membranes upon freeze-thawing, as well as negative consequences of the development of biochemical reactions activated by an increase in the concentration of intracellular Ca^{2+}, induced by inhibition of the Ca^{2+}-ATPase activity [3]. In particular, the disruption of the structure of the lipid bilayer caused by the redistribution of lipids in the membrane can contribute to changes in the mechanical properties of erythrocytes cryopreserved with PEG.

Conclusions. The disturbances in the asymmetric distribution of PS and decrease in the cell mechanical stability are revealed in erythrocytes cryopreserved under the PEG protection. Analysis of the reasons for the development of these processes can contribute to the improvement of methods of erythrocytes cryopreservation under the PEG protection and increasing in the cell stability upon cell freeze-thawing with exocellular CPAs.

References:

1. Babijchuk LA, Zemlianskykh NG. (2001) Optimization and advantages of washing-out method of erythrocyte cryopreservation with PEO-1500. *Problems of Cryobiology,* (1), 35-41.
2. Zemlianskykh NG. (2018) The Effects of cryoprotective substances on the mechanical stability and geometric parameters of human erythrocytes. *Biophysics*, 63 (1), 66–76.
3. Zemlianskykh NG, Babiychuk LA. (2017) The changes in erythrocyte Ca^{2+}-ATPase activity induced by PEG-1500 and low temperatures. *Cell and Tissue Biol*ogy, 11 (2), 104–110.