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MODIFICATION OF ERYTHROCYTE MEMBRANE PROTEINS BY POLYETHYLENE GLYCOL 1500

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The aim of the work was to study the effect of polyethylene glycol 1500 on the Ca^{2+}-ATPase activity and changes in CD44 surface marker expression in human erythrocyte membranes. Determination of the Ca^{2+}-ATPase activity was carried out in sealed erythrocyte ghosts by the level of accumulation of inorganic phosphorus. Changes in the expression of CD44 and the amount of $CD44^{+}$-erythrocytes were evaluated by flow cytometry. There was revealed an inhibition of Ca^{2+}-ATPase activity as well as a decrease in the level of CD44 expression and in the $CD44^{+}$-cell amount, reflecting rather complex rearrangements in the membrane-cytoskeleton complex of erythrocytes under the influence of PEG-1500. Effect of PEG-1500 on the surface marker CD44 could be mediated by modification of proteins of membrane-cytoskeleton complex, as evidenced by increased loss of the CD44 in erythrocyte membranes after application of protein cross-linking reagent diamide. Reducing the Ca^{2+}-ATPase activity might contribute to the increase in intracellular Ca^{2+} level and thus led to a modification in interactions of integral proteins with cytoskeletal components that eventually could result in membrane vesiculation and decrease in expression of the CD44 marker, which is dynamically linked to the cytoskeleton.

Key words: Ca^{2+}-ATPase, CD44, polyethylene glycol 1500, erythrocyte.

Polyethylene glycols (PEG) of various molecular weights are low toxic biocompatible polymers widely used in biology, medicine and pharmaceuticals [1]. Typically, PEG is used as a precipitating agent for the isolation and purification of proteins, nucleic acids and viruses. Fusogenic properties of the polymer provide a preparation of hybridoma cell cultures by the fusion of the two cell types. Surface modification by covalently attached PEG is the basis of the camouflage biotechnology, designed to reduce the immunogenicity of molecules and cells in order to extend their survival *in vivo*. The process of pegylation enhances the solubility of macromolecules in water and improves biocompatibility, that is important for drug development. In combination with other components the PEG is used to form hydrogels, which allow a controlled release of therapeutic agents, moreover they are also applied in regenerative medicine and some medical devices.

PEG has also demonstrated cryoprotective properties in respect of certain cell types, providing strong protection against effects of extreme environmental factors associated with freezing and thawing processes [2, 3]. Such factors include [4]: the formation of crystals, the growth of salt concentration in the supercooled liquid, increasing the osmotic pressure, phase transitions and lateral separation of lipids in membrane systems, dehydration of macromolecules. PEG of m.w. 1500 (PEG-1500) does not cross the plasma membrane, that is referred to the exocellular type of cryoprotectants, which cause considerable interest among researchers [2–6], because on their base washing-free methods of cell cryopreservation can be developed. PEG-1500 provides effective protection of the erythrocytes during freezing down to ultralow temperatures (–196 °C) [2]. However, the transfer of cryopreserved erythrocytes to physiological conditions reveals latent membrane injuries that may be caused by a modification of the structural and functional state of the individual components of the membrane under the influence of PEG-1500. Taking into account the prospects of PEG-1500

using for erythrocyte cryopreservation and establishment of blood banks, the research of membrane modification in the cryoprotectant presence may be the key condition to understanding and leveling of the adverse effects of the polymer interaction with cells by adjusting the processing conditions of cells with cryoprotective medium or introducing additional components into it.

It is known that the stability of erythrocytes largely depends on the state of membrane proteins associated by point contacts to cytoskeletal components into an integrated protein network. One of these integral proteins involved in forming connections with the cytoskeleton is a surface marker CD44 [7]. It can be assumed that the effect of PEG-1500, communicating only with the outer surface of the membrane, on integral proteins is stipulated by disturbance of their interactions with cytoskeleton proteins. Application protein-linking reagents, in particular diamide, would assist in clarifying the involvement of cytoskeletal proteins to a change in the integral protein CD44 expression under the influence of PEG-1500. It is known that the diamide causes oxidation of sulfhydryl groups and the formation of disulfide bridges between proteins [8]. In erythrocytes the diamide induces crosslinking of mainly spectrin polypeptides, which are the main component of the erythrocyte cytoskeleton, and, to a lesser extent, the main component of integral membrane proteins — band 3 [9]. Restraining the dynamics of protein-protein interactions in membrane-cytoskeleton complex in such a manner, it is possible to evaluate the role of this structure in changing of the CD44 characteristics in erythrocytes under the influence of the cryoprotectant. It should also consider the fact that protein-protein interactions in membrane-cytoskeleton complex of erythrocytes are substantially dependent on intracellular Ca^{2+} level, which is regulated by the only one element of active transport of this cation in erythrocytes: plasma membrane Ca^{2+}-ATPase.

The aim of the work was to study the effect of PEG-1500 on Ca^{2+}-ATPase activity and an integral membrane protein CD44, involved in forming of contacts with erythrocyte cytoskeleton. Application of diamide, restraining the dynamics of protein-protein interactions, will help to clarify the role of cytoskeletal proteins in the alteration of CD44 expression in PEG-1500 presence.

Materials and Methods

In the study the following reagents were used: disodium ATP, Tris, Hepes, EGTA, diamide (Sigma, USA), $CaCl_2$, $MgCl_2$, PEG-1500 (Fluka, USA), bovine serum albumin (BSA) (PAA Laboratories GmbH, Austria), KCl, NaCl, glucose, other reagents of Russian and Ukrainian production (chemically pure or extra-pure grades) and CD44-FITC (BD Biosciences) — monoclonal fluorophore-conjugated antibodies having the name, which is one-to-one to the identified surface marker.

The objects of the study were donor's blood erythrocytes, obtained from the Blood Center (Kharkiv), with a shelf life not more than 5 days at 3–5 °C. Erythrocytes were washed from plasma and leukocyte components with salt medium A (150 mM NaCl, 10 mM Tris-HCl, pH 7,4), as described previously [10].

The erythrocyte membranes (ghosts) were isolated by the method [11]. The ghosts were sealed in the medium, used for determining of an enzymatic activity of Ca^{2+}-ATPase [12], which included 135 mM KCl, 10 mM Tris, 10 mM HEPES (pH 7.4), 0.037 mM $MgCl_2$, 4 mM ATP, 1 mM EGTA, 1.5 mM PMSF, and necessary amounts of $CaCl_2$, added at the rate of free $[Ca_i^{2+}]$ at the level of $2–4\times10^{-6}$ M (the medium B). For the calculation of free Ca^{2+} concentration the software MAXChelator (http://www.standford.edu/~cpatton/maxc.html) was used. The produced ghosts divided into two equal parts and incubated in media with or without $CaCl_2$ (only in the presence of EGTA) at 37 °C. After sealing stage the restored cells were pelleted and washed with a saline medium A. Before the beginning of the experiment the aliquots of ghosts from each sample were taken to determine the base level of inorganic phosphate (P_i) and protein concentration. Restored cells (100 μl) were incubated in solutions of PEG-1500 [5–30% (0,033–0,2 M)], prepared on the basis of the medium A, for 20 min at 22 °C. The medium A was used as a control when assessing the Ca^{2+}-ATPase activity. The changes in the Ca^{2+}-ATPase activity were assessed by the difference in the accumulation of P_i in Ca^{2+}-containing and Ca^{2+}-free media after subtracting baseline values P_i. Enzymatic reaction was stopped by adding cold TCA solution (5% final concentration). Protein was precipitated by centrifugation. The P_i in the supernatant was examined by the method [13]. Protein concentration was determined by the method of Bradford [14].

Effect of PEG-1500 on the erythrocytes membrane surface marker CD44 was evaluated similarly to the previously described procedures [10], by incubating the cells for 20 hrs in solution of 30% PEG-1500, prepared on the base of Ringer medium with adding 5 mM glucose, 125 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 32 mM HEPES (pH 7.4). As a control in this series of experiments the Ringer-glucose medium was used. A part of the samples before exposure to PEG-1500 was incubated in the presence of 2.5 mM diamide at 37 °C for 1 hr in Ringer medium. The erythrocyte CD44 parameters were determined by flow cytometry (FACS Calibur, Becton Dickenson, USA). In each measurement 30,000 events were counted. Data were analyzed using WinMDI 2.8 software.

Our findings were statistically analyzed using the software package Statgraphics plus 2.1. Data are presented as M ± SE (mean value ± standard error). Statistical significance of differences between experimental groups was evaluated using Fisher's rank test for multiple comparison of sample with the least significant difference. Each series comprised at least 8 experiments.

Results and Discussion

Assessment of Ca^{2+}-ATPase activity in erythrocytes in the presence of PEG-1500 showed that the adding even a small amount of the cryoprotectant into a medium led to inhibition of Ca^{2+}-ATPase activity (Fig. 1).

In the presence of significant concentrations of polymer (20–30%), which are capable of exerting a pronounced cryoprotective effect, there was observed a decrease in the rate of P_i accumulation approximately 3 times. Evidently, the inhibition of Ca^{2+}-ATPase activity in erythrocytes, exposed to hypertonic medium of PEG-1500, will entail an increase in intracellular Ca^{2+} and can affect the protein-protein interactions in membrane-cytoskeleton complex.

In the study of changes in surface marker CD44 in erythrocytes under the influence of the cryoprotectant by flow cytometry we assessed two parameters: composition of cell suspensions characterized by the ratio of $CD44^+$- and $CD44^-$-erythrocytes (for convenience the percentage of only $CD44^+$-erythrocytes was assessed), and level of surface marker expression for $CD44^+$-erythrocytes, i.e. the amount or density of CD44 molecules in membranes of individual cells.

The characteristic of the marker CD44 expression in cells is the median of the distribution histograms showing a value, relative to which, cells in the suspension are divided into two equal size parts. Since only a part of erythrocytes in suspension binds the label CD44-FITC, it was necessary to assess the distribution of native erythrocytes, which were not incubated with CD44-FITC, within FL1 fluorescence channel to clearly identify the area of $CD44^-$-erythrocytes (negative control). Cutting off $CD44^-$-cells (Fig. 2, curve *1*), we identified the marker margins, corresponding to $CD44^+$-erythrocyte area (Fig. 2, curve *2*).

In general, in native suspension about 80% (79,3±4,9%) of the erythrocytes were CD44-positive with an average median of the histogram distribution of 13.3±1.3 arbitrary units (a. u.) of fluorescence intensity (FI).

In the samples of native erythrocytes the restraining of dynamics of protein-protein interactions in membrane-cytoskeleton complex, caused by diamide-induced cross-links, entails a decrease in the expression level of CD44 (9.6 ± 0.5 a. u. IF) without significant changes (75,9 ± 4,1) in the amount of $CDD44^+$-cells (Fig. 2, curve *3*).

Exposure of erythrocytes in the medium containing 30% PEG-1500 (Fig. 3, curve *2*) was accompanied by a decrease in CD44 expression (9.1 ± 1.6 a. u. IF) in membranes and reducing of $CD44^+$-erythrocyte amount (41.9 ± 4.8%) in the range of the marker standard margins M1 with simultaneous appearance of a small amount of cells (2.3 ± 1.0%) with CD44 expression above the control values (median value 157.3 ± 15.1 a.u. IF) corresponding to the margins M2 in histograms. Pretreatment of cells with diamide exerted more significant impact on CD44 in erythrocytes, exposed to 30% cryoprotectant solution (Fig. 3, curve *3*) as compared to the control. Amount of $CD44^+$-cells decreased to 22.5 ± 5.3, and the surface marker expression level was only 5.7 ± 0.4 a. u. IF. However, pretreatment of cells with diamide prevented the development of processes in membrane, resulting in the formation of the erythrocyte subpopulation with higher expression level of CD44, corresponding to the margins M2.

Thus, an exposure of erythrocytes in 30% PEG-1500 causes a change in the Ca^{2+}-ATPase activity and affects the characteristics of the integral protein associated with cytoskeleton and simultaneously presenting the surface marker CD44. Using diamide, which forms disulfide cross-links between the major proteins of membrane-cytoskeleton complex,

confirms, as exemplified by CD44, the ability of PEG-1500 to change the structural and functional properties of membranes via modifying of regulatory effects of the cytoskeletal proteins.

A model of reconstructed erythrocytes provides the possibility to assess the Ca^{2+}-ATPase activity affected by exocellular compound under the conditions allowing it to prevent a direct contact with cytoplasmic domains of an enzyme. Effects of organic compounds, many of those as know to exhibit cryoprotective properties, on the Ca^{2+}-ATPase activity were investigated in various biological models. In particular, the stimulation of enzymatic activity of isolated Ca^{2+}-ATPase derived from erythrocyte membranes was demonstrated in presence of PEG, dimethyl sulfoxide (DMSO), glycerol, ethylene glycol [15]. However, the observed effect is rather associated with the characteristics of model system than reflects the influence of these substances on the Ca^{2+}-ATPase activity in a cell, because the stimulation of its activity was due to the recovery of hydrophobic interactions within membrane domains of isolated enzyme under the influence of amphiphilic substances [15]. It was shown that in various experimental models trends of changing in the Ca^{2+}-ATPase activity under the influence of organic solvents could be disagreed. In particular, despite the fact that DMSO stimulates the Ca^{2+}-ATPase activity of isolated purified enzyme [15], it does not change the basic Ca^{2+}-ATPase activity in membranes of erythrocyte ghosts and inhibits calmodulin-stimulated enzyme activity [16]. The effect of glycerol on the Ca^{2+}-ATPase in the sealed ghosts and saponin-permeabilized erythrocytes were also different [17]. Since the ability of Ca^{2+}-pump to transport ions Ca^{2+} is primarily determined by its catalytic activity, one can expect an increase in the level of intracellular Ca^{2+} in the erythrocytes incubated in PEG-containing solutions. Growing of Ca^{2+} level in the PEG presence is promoted via water loss by cells under the influence of osmotic gradient due to a high concentration of the non-penetrating across membrane polymer. Ions Ca^{2+}, functioning as a second messenger, undoubtedly play a role in cell survival under stress conditions.

Increasing in the Ca^{2+} level, which has been observed even in the presence of an isotonic solution of PEG-1500 [18], should affect the structural state of membrane components, and changes in a surface marker expression can be resulted from occurring structural rearrangements caused by stress factors. Whereas the cryoprotective agent is referred as "compatible cosolvents" and it is capable to maintain the structure of protein macromolecules, membranes and cells in a state close to native without causing denaturation or impaired structural order [19–21], but it has been still able to induce structural and functional changes in erythrocytes, which during a prolonged exposure resulted in lowering of $CD44^+$-cells amount and marker expression level. The most likely cause of the mentioned changes in erythrocytes in cryoprotectant presence is the loss of membrane material in the form of vesicles with the capture of molecules of the surface marker CD44 in their composition. For erythrocyte membranes the vesiculation is a recognized

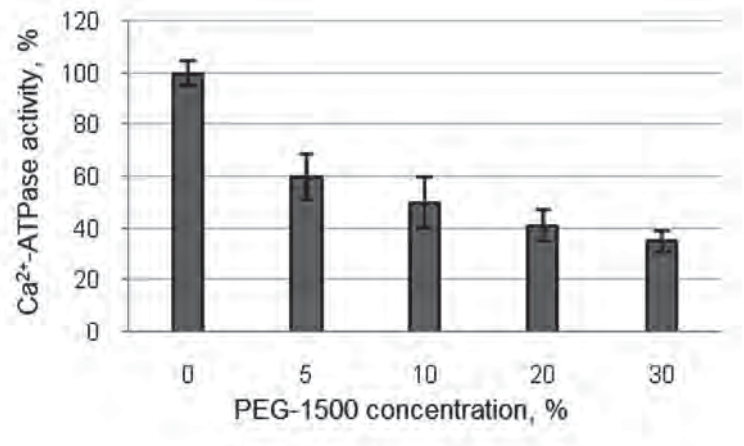

Fig. 1. **Change in Ca^{2+}-ATPase activity in the sealed erythrocytes depending on the PEG-1500 concentration**
All the experimental values significantly different from control with a significance level $P < 0.001$

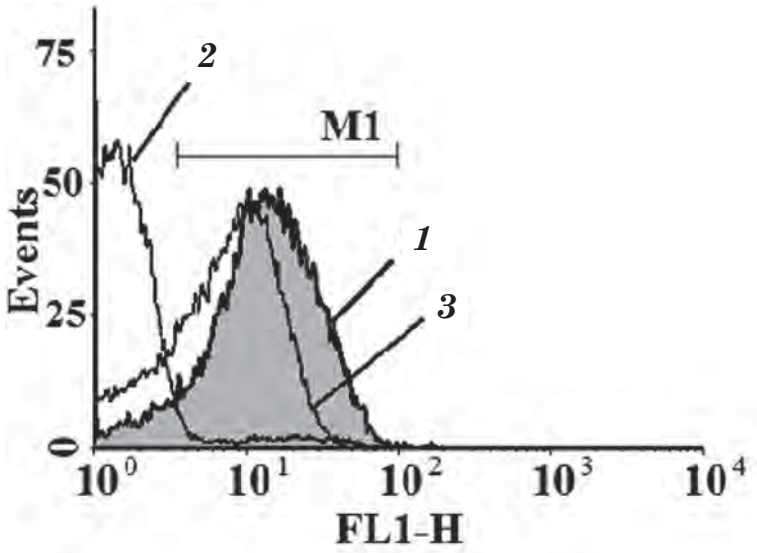

Fig 2. **Distribution histogram of native erythrocytes labeled with CD44-FITC after exposure to the Ringer medium:**

1 (here and in Fig. 3) — distribution histogram of the native erythrocytes labeled with CD44-FITC (M1 specifies the area of $CD44^+$-erythrocytes);

2 — distribution histogram of the native erythrocytes not labeled with CD44-FITC (negative control);

3 — distribution histogram of the native erythrocytes, pretreated with diamide

The data are of a typical experiment. The X axis is value of fluorescence intensity of cells in FL-1 channel (a.u. IF FL1), represented by logarithmic scale values. The Y axis is the number of calculable events in normalized form

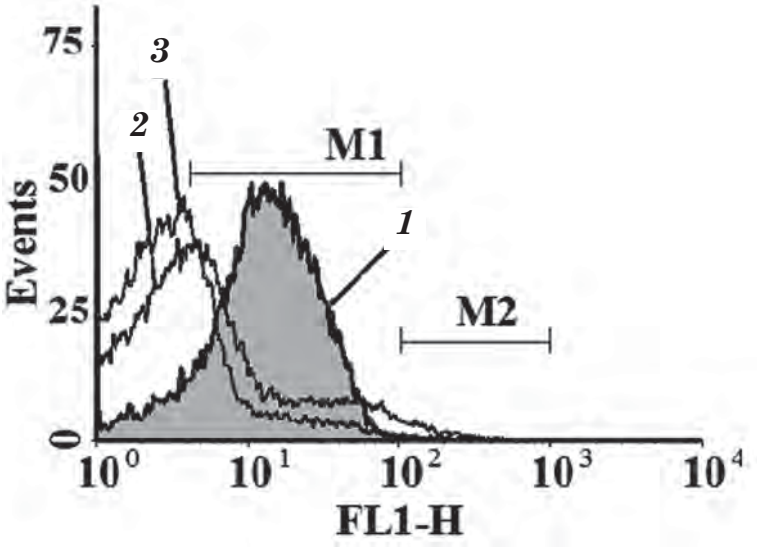

Fig. 3. **Distribution histogram of erythrocytes labeled with CD44-FITC after exposure to 30% solution of PEG-1500:**

2 — distribution histogram of the erythrocytes exposed in presence of 30% PEG-1500;

3 — distribution histogram of the erythrocytes exposed in presence of 30% PEG-1500, which have been pretreated with diamide

The X axis is value of fluorescence intensity of cells in FL-1 channel (a.u. IF FL1) represented by logarithmic scale values. The Y axis is the number of calculable events in normalized form (the data of a typical experiment)

mechanism of cell response to either stress or aging [22–24]. The changes, affecting CD44, may depend on structural modification of lipid bilayer and proteins of the membrane-cytoskeleton complex. In particular, the lipid packing mismatch in neighboring areas, caused for example by phase transitions in specific membrane areas, affected by dehydration processes, are able to induce the formation of vesicles [25]. Furthermore, the CD44 molecules are known to exist in the membrane as individual molecules or a part of the intricate protein complexes. In particular, CD44 may be associated with the cytoskeleton network via ankyrin and protein 4.1 [26]. These links are dynamic and may vary in different parts of membrane. Non-associated with the cytoskeleton the CD44 molecules can be captured easier into the vesicles forming on the erythrocyte membranes under stress. It should be noted that changes in the intracellular Ca^{2+} level play a key role in initiation of these processes, since Ca^{2+} and calmodulin decrease the affinity of interaction between the protein 4.1 and CD44 [26], whereby the latter becomes a free membrane protein or gets able to form a link with ankyrin. Expected redistribution of interaction within network of membrane-cytoskeleton protein complex involving CD44, ankyrin, protein 4.1 and band 3 in the presence of cryoprotectants can affect the physical properties of membrane.

Important information to understand the cryoprotectant mechanism actions on erythrocyte membrane can be provided by an evidence on the specificity of changes in the marker CD44 expression under the effect of a 30% PEG-1500 solution, which is characterized by the appearance of cells with higher expression of the marker CD44 (M2). This subpopulation emergence upon the absence of capability for protein synthesis in erythrocytes can be associated with structural changes in membranes wherein there is partial or complete fusion of membrane systems, that leads to the enrichment of individual cells with the marker. It is known that PEG contributes to an aggregation and fusion of cells [27, 28]. However, PEG-1500 has been previously shown [29] does not cause a fusion of erythrocytes. For this purpose, PEG-6000 is used at a concentration of about 40%, furthermore cell fusion requires additional conditions, one of those is cellular rehydration [30], not presented in our experiments. Nevertheless, in the erythrocyte membranes on the contact with high PEG-1500 concentrations the structural changes can be initiated that eventually lead to the formation of sufficient area with a modified structure that allows fusion of individual membranes. In the described experimental conditions, fusion of vesicles, carrying the marker, with the cells seems the most probable.

The crosslinking of proteins with diamide can, to a certain extent, serve as confirmation of the PEG-1500 effect on the surface marker CD44 by modifying the bonds between proteins of membrane-cytoskeleton complex in the cryoprotectant presence. Assuming that the cryoprotectant affects not only the lipid bilayer, but also a system of protein-protein interactions and thereby control changes in the CD44 parameters, it can be expected that the modification of proteins with diamide will entail either slowing or intensifying loss of the CD44 marker by cells. As shown by experiments, previous oxidation of proteins by diamide really leads to a significant intensification of the CD44 loss in erythrocytes exposed to PEG-1500, compared to the similar variants of the control samples. It was also noted that the treatment with diamide prevented the formation of erythrocyte subpopulation with higher level of CD44 expression in the presence of 30% PEG-1500 solution. This is obviously related to the fact that the proteins “cross-linked” with diamide impede the formation of protein-free areas in the membrane, indispensable for membrane fusion. In other words, the diamide stabilizes the lipid bilayer through control of its structure by “crosslinked proteins” and complicates the PEG-induced fusion of membrane. Meanwhile the loss of CD44 in erythrocytes, treated by diamide, only amplified under these conditions, confirming the cryoprotectant effect on the surface marker via its impact on proteins of membrane-cytoskeleton complex. The inhibitory effect of PEG-1500 on Ca^{2+}-ATPase activity and a decrease in CD44 expression in erythrocytes may be stipulated by physicochemical properties of the polymer solution. It is known that PEG causes a change in surface potential [31] and dielectric constant of the solution [32], exhibits amphiphilic (hydrophilic and hydrophobic) properties as it is dissolved not only in water but also in some non-polar organic solvents [33, 34]. In aqueous solutions the PEG binds 2–3 water molecules

per monomer unit [33] that determines its dehydrating effect against various macromolecules and membranes. Further, the PEG molecules can be distributed at the air-water interface forming stable monolayers [35, 36]. Surface-active properties of the polymer, its dehydrating action and changing in the polarity in the presence of PEG may have some influence on membrane surface [37] that may cause a decrease in membrane fluidity [38]. It has been shown that the adding of PEG modifies the surface potential of lipid monolayers [33] and causes a shift of temperature of the phase lipid transition [39]. It has also been found that PEG molecules induce dehydration of multilamellar lipid vesicles that is accompanied by a reduction in effective size of the polar headgroups of the membrane phospholipids and enhanced van der Waals interactions between the acyl chains of the matrix lipid [40, 41]. These structural changes are typically compensated by an increased thickness of the membrane and decrease in the mobility of lipid molecules that leads to segregation of different types of membrane lipids. Probably similar changes may affect some areas of erythrocyte membranes and exert a certain negative impact on the stability of membranes in the presence of PEG-1500 under stressful conditions of cryopreservation.

The observed changes in Ca^{2+}-ATPase activity in erythrocytes under the influence of PEG-1500 does not give a comprehensive view on its mechanism of inhibition and requires further assessment of its functioning characteristics, in particular the analysis of Ca^{2+}-transporting capacity in presence of PEG-1500 in the medium. In future such a study could potentially give a clearer insight in the state and role of Ca^{2+}-regulating constituents in changes of structural and functional state of the cells at using of exocellular cryoprotectants and low temperature effects. Especially important issue is the assessment of changes in the level of intracellular Ca^{2+}, which can be both a regulator of the structural state of membrane and trigger of apoptosis under stressful conditions. Effect of PEG-1500 on the surface marker CD44 apparently is implemented in various ways, and the change in the level of intracellular Ca^{2+}, which determines the modification of protein-protein interactions in the membrane-cytoskeleton complex, plays definitely an important role. The consequence of the modification of the structural state of the membrane components in the presence of PEG-1500 may be resulted in changing the erythrocyte stability under stress conditions.

Thus, the experiments revealed a decrease in the Ca^{2+}-ATPase activity, as well as a reduction in the expression of surface markers CD44 and $CD44^{+}$-cell amount on the exposure of erythrocytes in the presence of PEG-1500. A decrease in the Ca^{2+}-ATPase activity may be caused by a modification of the membrane structure under the influence of physical and chemical properties of 30% PEG-1500 solution. The consequence of reducing of the Ca^{2+}-ATPase activity is rising intracellular Ca^{2+} level that can lead to a modification of the interactions between integral proteins and cytoskeleton components and, eventually, contribute to membrane vesiculation with an inclusion into them integral proteins not associated with the cytoskeleton, thereby reducing the expression of surface marker CD44. The appearance of a small amount of erythrocytes with the higher expression of CD44 indicates structural changes in the membrane, whereat a partial or a complete fusion of the membranes may take place (probably, fusion of cells with vesicles carrying the marker). The treatment with diamide impedes the formation of the erythrocyte subpopulation with higher level of the CD44 expression in the presence of PEG-1500, apparently, due to the fact that proteins “cross-linked” with diamide inhibit the formation of protein-free areas in the membranes indispensible for membrane fusion. Despite the fact that the diamide stabilized the lipid bilayer affecting its structure by “cross-linked proteins” and complicated the fusion of membrane constituents the loss of CD44 in erythrocytes, treated with diamide, only amplified. And this loss can serve as a strong argument in the evidence of the cryoprotectant effect on the surface marker via its action on proteins of membrane-cytoskeleton complex.

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МОДИФІКАЦІЯ МЕМБРАННИХ ПРОТЕЇНІВ ЕРИТРОЦИТІВ ПОЛІЕТИЛЕНГЛІКОЛЕМ 1500

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Метою роботи було вивчення впливу поліетиленгліколю (ПЕГ-1500) на активність Ca^{2+}-АТРази і зміну експресії поверхневого маркера CD44 у мембранах еритроцитів людини. Визначення активності Ca^{2+}-АТРази виконували в замкнутих тінях еритроцитів за рівнем накопичення неорганічного фосфору. Зміну експресії CD44 і кількості $CD44^+$-еритроцитів оцінювали методом проточної цитометрії. Встановлено інгібування активності Ca^{2+}-АТРази, а також зниження рівня експресії CD44 і зменшення кількості $CD44^+$-клітин, що відображає складні перебудови у мембранно-цитоскелетному комплексі еритроцитів під впливом ПЕГ-1500. Вплив ПЕГ-1500 на поверхневий маркер CD44 може бути опосередкований модифікацією протеїнів мембранно-цитоскелетного комплексу, про що свідчить посилення втрати CD44 мембранами в еритроцитах після застосування протеїнзшивного реагента діаміду. Зниження активності Ca^{2+}-АТРази може сприяти підвищенню рівня внутрішньоклітинного Ca^{2+} і призвести до модифікації взаємодій інтегральних протеїнів з компонентами цитоскелета, унаслідок чого може відбуватись везикуляція мембран і зниження експресії маркера CD44, який динамічно пов'язаний із цитоскелетом.

Ключові слова: Ca^{2+}-АТРаза, CD44, поліетиленгліколь, еритроцити.

МОДИФИКАЦИЯ МЕМБРАННЫХ ПРОТЕИНОВ ЭРИТРОЦИТОВ ПОЛИЭТИЛЕНГЛИКОЛЕМ 1500

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Целью работы было изучение влияния полиэтиленгликоля (ПЭГ-1500) на активность Ca^{2+}-АТРазы и изменение экспрессии поверхностного маркера CD44 в мембранах эритроцитов человека. Определение активности Ca^{2+}-АТРазы проводили в замкнутых тенях эритроцитов по уровню накопления неорганического фосфора. Изменение экспрессии CD44 и количества $CD44^+$-эритроцитов оценивали методом проточной цитометрии. Установлено ингибирование активности Ca^{2+}-АТРазы, а также снижение уровня экспрессии CD44 и уменьшение количества $CD44^+$-клеток, что отражает сложные перестройки в мембранно-цитоскелетном комплексе эритроцитов под влиянием ПЭГ-1500. Влияние ПЭГ-1500 на поверхностный маркер CD44 может быть опосредовано модификацией протеинов мембранно-цитоскелетного комплекса, о чем свидетельствует усиление потери CD44 мембранами в эритроцитах после применения протеинсшивающего реагента диамида. Снижение активности Ca^{2+}-АТРазы может способствовать повышению уровня внутриклеточного Ca^{2+} и привести к модификации взаимодействий интегральных протеинов с компонентами цитоскелета, вследствие чего может происходить везикуляция мембран и снижение экспрессии маркера CD44, который динамически связан с цитоскелетом.

Ключевые слова: Ca^{2+}-АТРаза, CD44, полиэтиленгликоль, эритроциты.