



ABSTRACTS

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RECOVERY OF ERYTHROCYTE OXYGEN TRANSPORT FUNCTION AFTER HYPOTHERMIC STORAGE UNDER THE ACTION OF HUMAN CORD BLOOD LOW-MOLECULAR FRACTION AND THE ACTOVEGIN DRUG

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The cord blood-based drugs are known to increase the energy of human donor blood cells (1). They can be used in transfusiology to restore the functions of erythrocytes after long-term storage or at pathological conditions associated with impaired oxygen transport function (2-3). In this research the effect of low-molecular fraction (below 10 kDa) derived from human cord blood (CBF) and Actovegin on the content of hemoglobin forms in erythrocytes stored in hypothermia was studied. Evaluation of the ratio of hemoglobin forms in erythrocytes stored in hypothermia for 7-21 days, showed that in the control there is a redistribution between the content of oxy-, deoxy- and methemoglobin towards increasing the proportion of the latter two. After incubation in a rehabilitation medium with CBF there was a significant increase in oxyhemoglobin (15-20%) and a parallel decrease in deoxy- and methemoglobin (18-20% and 25-60%, correspondingly). The resulted ratio of the indices was restored to the initial level, as evidenced by an increase in the haemoglobin oxygenation coefficient in an average by 1.5 times.

After using Actovegin as a rehabilitative medium, the same pattern was found. But it should be noted that its effect on the content of deoxyhemoglobin was more pronounced compared to the first series of experiments. Thus, on day 21 of storage, a probable decrease of this index by 30% was recorded. At the same time the content of oxyhemoglobin increased by 19%. At the final term, the hemoglobin oxygenation coefficient increased more than 70%, which exceeded even the native level.

Thus, by incubating erythrocytes stored in hypothermia in a medium with CBF or Actovegin was able to strongly increase the oxyhaemoglobin content. Both rehabilitation media affected similarly the ratio of haemoglobin forms. In general, the findings indicate the normalization of oxygen transport function of erythrocytes and increased blood oxygen capacity.

(1) Gulevsky AK et al. (2015) *Biotechnologia Acta* **6**, 63-70. (2) Henkelman S et al. (2015) *Vox Sang* **108**, 103-112. (3) Cavezzi A et al. (2020) *Clin Pract* **10**, 1271.

protein content in cells was determined by flow cytometry (FACS Calibur, BD, USA). The concentration of IL-10 in HCBL was determined by enzyme-linked immunosorbent assay.

Differences in functional parameters of Treg after cryopreservation and lyophilization of HCBL were found. The amount of Treg decreased by 2.2 fold in cHCBL, while it increased in 1.6 fold in lHCBL compared with their quantity in a fresh preparation, which did not affect the content of IL-10. At the same time, after cryopreservation and lyophilization, an increase in the average fluorescence intensity of the CD25 marker in Treg was noted, which may indicate a higher readiness of cells to implement their immune suppressive potential. In addition, the total fluorescence intensity for the CD25 marker and the Foxp3 protein content were maximal in Treg from lHCBL.

The findings indicate the ability of cryopreservation and lyophilization to stimulate the Treg functional potential, increasing the immune regulatory properties of HCBL. Moreover, after lyophilization such an activity is manifested to a greater extent.

(1) Tsutsayeva AO, Grischenko VI, Kudokotseva OV, Shcheglov AV, Tupchienko GS, Prokopuk OS (inventors); Institute for Problems of Cryobiology and Cryomedicine, assignee. [Method of cryopreservation of hemopoietic cells of cord blood] Ukrainian patent 31847 A 1998 November 5. Ukrainian. (2) Goltsev AM, Taranik GC, Grisha IG, Sokil LV, Bondarovich MO, Ostankov MV, Lutsenko OD, Goltsev KA, Ostankova LV (inventors); Institute for Problems of Cryobiology and Cryomedicine, assignee. [Method of lyophilization of leukoconcentrate of cord blood]. Ukrainian patent 113006, 2017 Jan 10. Ukrainian.

AMPHIPHILES PROTECT HUMAN ERYTHROCYTES UNDER POST-HYPERTONIC SHOCK

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This research is devoted to a comparative study of the effect of amphiphiles, differing by physicochemical properties on the sensitivity of erythrocytes under post-hypertonic shock. Post-hypertonic shock simulates the influence on cells of cryodamage factors, acting at erythrocyte thawing stage, as well as when that of cells, cryopreserved under protection of penetrating cryoprotective agent are transferred into bloodstream (1). Post-hypertonic shock (PHS) of erythrocytes was initiated by transferring cells from dehydration medium (1.65 mol/L NaCl) into rehydration one (0.15 mol/L NaCl) at 0°C. The various classes of amphiphiles (anionic sodium dodecyl sulfate, non-ionic decyl- β ,D-glucopyranoside, and cationic trifluoperazine) were used. Amphiphiles were supplemented into rehydration medium prior to transferring the cell. Amphiphiles were shown to protectively affect erythrocytes in various extents. Under these conditions, decyl- β ,D-glucopyranoside and trifluoperazine were more effective, for which maximum antihemolytic activity are recorded at a level of ~60%, in contrast to sodium dodecyl sulfate, which maximum antihemolytic activity was 2 times lower (~30%). A comparative study of the efficiency of amphiphiles under post-hypertonic shock of erythrocytes showed the differences in the size of concentration plateau and the values of effective concentrations. A narrowing of the plateau was revealed in a series: decyl- β ,D-glucopyranoside > trifluoperazine > sodium dodecyl sulfate. Decyl- β ,D-glucopyranoside characterize the maximum effective concentration (1,000 μ mol/L) and the minimal one for sodium dodecyl sulfate (50 μ mol/L). The obtained results suggest that the effectiveness of amphiphiles under PHS conditions of erythrocytes is associated with their ability to integrate into membrane, possibly, to the defect formation areas. This is accompanied by an increase in membrane surface area and, therefore, in critical hemolytic volume of cells, which allows them to swell to a larger volume in rehydration medium.

(1) Semionova YA et al. (2017) *Problems of Cryobiology and Cryomedicine* 27, 51-60.