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ASSESSMENT OF EFFICIENCY OF CRYOPROTECTANT MIXTURES CONTAINING VARIOUS ANTIOXIDANTS

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The use of cord blood (CB) hematopoietic progenitor cells (HPCs) is firmly established in practical medicine as an effective method for treating diseases of various genesis. This has led to the development of protocols of low-temperature storage and the cryobanks network. DMSO at 7.5-10% concentrations is the most common cryoprotectant for cryopreservation of CB nucleated cells (NCs) including HPCs. However besides cytoprotective effects, DMSO may also cause the accumulation of reactive oxygen species (ROS) during cell cryopreservation with following initiation of apoptosis and cell death. Accordingly, the promising approaches to the cryopreservation may be supplementation of medium with antioxidants which are able to "trap" free radicals and reduce the intensity of free radical oxidation production at all stages of freezing and thawing. Among these antioxidants glutathione (GSH), N-acetyl-L-cysteine (NAC) and ascorbic acid (AA) can be used.

The purpose of the study was assessment of cell viability and quantification of CB NCs with ROS excess compared to control after their cryopreservation in medium containing DMSO and different antioxidants.

In the work for CB NCs' cryopreservation we used 7.5% DMSO and different antioxidants (Sigma-Aldrich (USA)) at their final concentrations as follows: AA - 0.1 mM; NAC - 10 mM; GSH - 1 mM. The viability and the amount of cells with ROS excess were determined with flow cytometry (FACS Calibur (BD, USA)) using 7AAD and DCF fluorescent dyes, respectively.

Analysis of the NCs/HPCs viability showed that after cryopreservation under DMSO protection without antioxidants this index decreased by 30/35%, respectively. This may be due partly to ROS production, as the level of cells with ROS excess increased by 6-8% during freezing.

Assessment of viable CB NCs/HPCs after cryopreservation with DMSO and antioxidants showed that the supplementing 7.5% DMSO solution with 0.1 mmol AA ensured getting of viable NCs/HPCs up to 73/77%, respectively. When NAC was used at 10 mM concentration this index was up to 69/78%, while in the sample without the addition of antioxidants were obtained 57/65% of viable NCs/HPCs, respectively. Determining the amount of viable NCs/HPCs which were cryopreserved in samples with 7.5% DMSO and 1 mM GSH after thawing showed an increase of this index by 20/21%.

Quantification of cells with ROS excess indicated that in the group with AA application this index did not differ from the similar group when the antioxidant was not added. In samples cryopreserved with a 7.5% DMSO supplemented with the antioxidants NAC (10 mM) or GSH (1 mM), this index decreased by 12-13% compared to data when any antioxidant was not added.

Thus, the results indicated that antioxidants such as NAC and GSH contributed to an increase in their viability and decrease in the level of cells with ROS excess, while AA did not show antioxidant effect during the NCs cryopreservation. Cryoprotective solutions containing GSH at a concentration of 1 mM and 7.5% DMSO were the most effective.