Ukrainian Society of Cell Biology

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11-13 October 2015

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ESTIMATION OF ANTIOXIDANT PROPERTIES OF N-ACETYL-L-CYSTEINE DURING CORD BLOOD NUCLEATED CELLS CRYOPRESERVATION WITH DMSO

Babiychuk L.O., Zubova O.L., Makashova O.E., Zubov P.M.
Institute for Problems of Cryobiology and Cryomedicine, National Academy of Sciences of Ukraine, Pereyaslavskaya Street, 23, 61015 Kharkiv, Ukraine.
Email address: pmzubov@mail.ru

Increasing frequency of clinical application of cord blood (CB) medicinal products stipulated necessity to accumulate large amount of their doses. The solution of this problem is only possible if the cells are stored in frozen state at the temperature of liquid nitrogen. However, freezing and thawing processes can lead to the development of metabolic disorders in cells. One of the reasons of such disorders may be associated with the accumulation of high concentrations of reactive oxygen species (ROS). Prevention of the production of ROS may be due to adding of antioxidants into cryoprotective medium, particularly N-acetyl-L-cysteine (AC).

The purpose of this work is the assessment of the influence of different concentrations of N-acetyl-L-cysteine on the amount of cord blood nucleated cells (NC) with excessive level of ROS, as well as their safety and viability before and after cryopreservation with DMSO.

For the study the human CB after natural labors was used. CB NCs fraction was isolated by sedimentation using 6% polyglucin with molecular weight 60000. Isolated cells were treated with 25% DMSO to a final concentration of 7.5% in the sample. Samples were cryopreserved in «Cryoson» freezer (Germany) with the cooling rate of 1-3^{0}C/min up to -80^{0}C, followed by immersion in liquid nitrogen (-196^{0}C). Antioxidant N-acetyl-L-cysteine («Sigma») was used in concentrations of 5; 10; 15 and 30 mM. The cell amount was counted in the Goryaev chamber according to standard techniques. NCs viability was evaluated by flow cytometry (FACS Calibur, Becton Dickinson, USA) using the monoclonal antibody CD45FITC and fluorescent DNA stain 7-AAD (BD) according to ISHAGE protocol. The amount of cells with excessive ROS content was determined by accumulation of 2',7'- dichlorofluorescein diacetate (DCF) inside cells and evaluated with flow cytometry.

Estimation of CB NCs amount with excessive ROS content before and after cryopreservation showed that the addition of DMSO shifts prooxidant-antioxidant balance in cells and characterizes by the development of stress reaction such as the formation of excess ROS (10,3 ± 1,8% of cells). In consequence of freezing and thawing an increase in ROS formation was more pronounced in CB NCs (20,6 ± 2,1) as compared to those revealed before the cryopreservation. Safety and viability indexes upon the freezing of cell suspension with 7.5% DMSO were 78,0 ± 2,1% and 78,9 ± 3,2%, respectively.

Adding of AC into the cryoprotective medium contributed to reducing in intracellular ROS content inside cryopreserved cells as compared with data without adding antioxidant. The least CB NCs amount with excessive ROS content was revealed upon cell cryopreservation in cryoprotective medium containing 10 or 15 mM AC (not more than 9% of the cells). It should also be noted that under such conditions the CB NCs safety and viability indexes were higher by 7-10% as compared with samples without adding AC.

Thus, N-acetyl-L-cysteine can promote antioxidant processes in cells upon cryopreservation and contributes to the prevention of developing of oxidative stress, reduction of ROS in cells and, consequently, improves their safety and viability.