*Palladin Institute of Biochemistry of NAS of Ukraine*
*Ukrainian Biochemical Society*
*Council of Young Scientists of the Division of Biochemistry,*
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# JOINT MEETING OF THE 25th ANNUAL CONFERENCE "MODERN ASPECTS OF BIOCHEMISTRY AND BIOTECHNOLOGY" & 2nd CONFERENCE FOR YOUNG SCIENTISTS OF THE DIVISION OF BIOCHEMISTRY, PHYSIOLOGY AND MOLECULAR BIOLOGY NATIONAL ACADEMY OF SCIENCES OF UKRAINE

**6-9 June 2017, Kyiv, Ukraine**

**Sections:**

1. Biomaterials
2. Bioactive Compounds
3. Cancerogenesis
4. Gene Expression
5. Metabolites and Correction of Metabolic Processes
6. Proteomics and Protein Functions
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# ACCUMULATION OF ROS IN THE PRESENCE OF EXOGENOUS $H_2O_2$ IN HUMAN CORD BLOOD NUCLEATED CELLS DURING CRYOPRESERVATION

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Reactive oxygen species (ROS) are constantly generated under normal conditions in cellular metabolism. However, the cell has an extensive direct (interception) or indirect (oxidative damage reversal) antioxidant defense system for ROS inactivation. When the level of ROS exceeds the cell defense mechanisms and redox homeostasis is altered, the state of oxidative stress develops. In this state an accumulation of ROS high concentrations is observed and it can cause peroxidation of lipids, oxidation of proteins, damage to nucleic acids, enzyme inhibition, activation of programmed cell death pathway and ultimately leading to death of the cells.

Cryopreservation of cord blood (CB) units is the only way for long-term storage of hematopoietic stem cells (HSC) that is widely and successfully used in clinical practice for the treatment of many diseases. It is important to understand that homeostasis disruption can occur at each stage of cryopreservation technology: isolation of total nucleated cells (TNCs), which contain HSC, TNCs treatment with CPA and, especially, at freeze-thawing stage.

Thus, in this study, we assessed the redox state of TNCs at each stage of cryopreservation technology by monitoring of the intracellular ROS in the presence or absence of exogenous $H_2O_2$.

Dextran-isolated TNCs were cryopreserved with 5% DMSO. For redox state assessment, TNCs were incubated in the presence of 0.01 or 0.025 mM $H_2O_2$. Intracellular content of ROS was evaluated by flow cytometry (FACS Calibur, BD, USA) using DCFH2-DA in terms of geometric mean fluorescence intensity (MFI).

It was shown that after 15 min of incubation of whole CB and dextran-isolated TNCs with different concentration of exogenous $H_2O_2$ the MFI was reduced to the control level in non-treated samples. Further incubation with $H_2O_2$ led to more pronounced rise in MFI versus the control: 1.8 times for CB and 3.0 times for isolated TNCs. For DMSO-treated TNCs, MFI decreasing was less pronounced in first 15 min and did not reach the control remaining 3-times higher level regardless of $H_2O_2$ concentration. Continued incubation resulted in a 4-fold increase in the MFI versus the control level.

For freeze-thawing samples, a decrease in MFI index in first 15 min after $H_2O_2$ adding was not observed. Moreover, the prolonged incubation with hydrogen peroxide led to MFI decline: from 3.5 to 1.9-fold for 0.01 mM $H_2O_2$ and from 6.5 to 3.0-fold for 0.025 mM $H_2O_2$. This kind of cell response can indicate a delayed recovery of antioxidant defense system after cryopreservation. However, in the dynamics up to 45 min, the MFI index decreased to the control level in samples without exogenous $H_2O_2$ and became comparable with MFI index in the whole CB.

Thus, cryopreservation did not result in a significant redox homeostasis disruption in CB TNCs and activity of antioxidant system remained at the level close to the control in the whole CB.