NATIONAL ACADEMY OF SCIENCES OF UKRAINE
PALLADIN INSTITUTE OF BIOCHEMISTRY

THE UKRAINIAN BIOCHEMICAL JOURNAL

Volume 90, Special Issue, 2018 **Kyiv**

FEBS3+ Meeting – XIth Parnas Conference – Young Scientists Forum "Biochemistry and Molecular Biology for Innovative Medicine"

Contents

Pre-Conference Workshop 4
Plenary lectures 5
Key-note lectures 6
Young Scientists' Talks
Symposium I. Intracellular signaling 9
Symposium II. The biochemistry of the cell 12
Symposium III. Cell signaling in cancer 15
Symposium IV. Ligand-receptor binding in cell functions 18
Symposium V. From cell to organism 21
Symposium VI. Molecular biology of bacteria and viruses 24
Symposium VII. Metabolic engineering in the construction of the advanced biotechnological producers 27
Symposium VIII. Studying biology in single-molecule resolution 30
Symposium IX. Biochemical mechanisms in disease 33

© Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, 2018

ANALYSIS OF APOPTOSIS/NECROSIS STAGES OF CORD BLOOD NUCLEATED CELLS AFTER CRYOPRESERVATION WITH DMSO AND GLUTATHIONE

O. Ye. MAKASHOVA, O. L. ZUBOVA, P. M. ZUBOV, O. O. MYKHAILOVA, L. A. BABIJCHUK

Institute for Problems of Cryobiology and Cryomedicine, National Academy of Sciences of Ukraine, Kharkiv; e-mail: olena.makashova@gmail.com

Cord blood nucleated cells (CBNCs) were subjected to physico-chemical stresses during cryopreservation, which causes the development of apoptotic processes both immediately after freeze-thawing and delayed in time. Antioxidants are largely able to prevent the development of pathological processes. In connection with this, we assessed the development of CBNCs apoptosis/necrosis traits immediately after cryopreservation in presence of DMSO and glutathione, as well as after cell transfer under the conditions close to physiological ones.

The CBNCs fractions isolated by polyglycine were cryopreserved in solutions containing DMSO (5, 7.5 and 10%) and glutathione (1 and 3 mM). They were frozen at a rate of 1-3 °C per min to -80 °C, followed by immersion in liquid nitrogen. The apoptosis/necrosis stages of cells were determined by cytofluorimetry using combinations AnnexinV and 7-AAD. Transfusion modeling was performed by moving a portion of frozen-thawed cells to Hanks solution (37 °C, 1 hour).

After cryopreservation with DMSO the different stages of apoptosis/necrosis covered up to 30% of CBNCs, with predominance of necrosis (AnnexinV$^-$7AAD$^+$). In the samples frozen with 7.5% DMSO and 1 or 3 mM glutathione, the number of necrotic cells was lower in 2 and 2.5 times respectively, and the number of intact cells was 11-12% higher than without antioxidant.

Incubation of cells in Hanks solution for 1 h led to an increase in AnnexinV$^-$7AAD$^+$-cells among all the samples by 2 times compared with those obtained immediately after freeze-thawing. The number of intact cells (AnnexinV$^-$/7AAD$^-$) in all experimental groups was lower by 10-30% than immediately after freeze-warming. The largest number of intact cells was observed in samples frozen with 7.5 and 10% DMSO. In samples cryopreserved with glutathione adding, the amount of AnnexinV$^-$7AAD$^+$-cells was lower by 18-32%, and AnnexinV$^-$7AAD$^-$-cells increased by 17-22%. Introduction of glutathione into the cryoprotective solution 5% DMSO allows preserving up to 62% of AnnexinV$^-$/7AAD$^-$-cells after 1 h incubation in the Hanks solution. These results exceed the analogous indices after application of DMSO at 7.5 and 10% concentrations without antioxidant.

Adding glutathione to a cryoprotective solution can prevent the development of apoptotic processes in the cells and may be a prerequisite for the development of cryoprotective media with a lower content of DMSO.