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ASSESSMENT OF APOPTOSIS OR NECROSIS STAGES OF HUMAN CORD BLOOD NUCLEATED CELLS AFTER CRYOPRESERVATION BY DIFFERENT METHODS

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Recently during treatment of many diseases cryopreserved suspension of cord blood nucleated cells (CB NCs, $CD45^+$) has been used as a preparation containing hematopoietic progenitor/stem cells (HPCs, $CD34^+$). Efficiency of their application is mainly determined by the preservation of NCs structural and functional integrity after thawing. In this context, the research aim was to investigate the apoptosis or necrosis stages in various populations of human CB NCs after cryopreservation, depending on the method of whole CB separation, and under the influence of cryoprotectants with different mechanism of action and low temperatures.

The number of cells and their viability (7AAD) was determined by flow cytometry (FACS Calibur, USA) using BD monoclonal antibodies (Becton Dickinson, USA). The apoptosis/necrosis stages were evaluated using simultaneous staining of NCs with 7AAD and Annexin V. NCs were isolated from the whole CB by three different method: in dextran solution, by two-step centrifugation and in ficoll-density solution. Our previous studies revealed that for cryopreservation of NCs, which have been isolated by different methods, the following combinations with cryoprotectants were more effective: cells isolated in dextran solution were frozen with 5% of DMSO; cells isolated by two-step centrifugation were frozen with 10% of PEG-1500; cells isolated in ficoll-density solution were frozen with both 5% of DMSO or 10% of PEG-1500.

The efficiency of the NCs isolation from the whole CB by the two-step centrifugation not involving the use of chemicals and allowed to isolate 90,6±4,3% of NCs and 91,2±5,9% HPCs without loss of their viability (93.2±0.8% and 94.4±1.2% of $7AAD^-$-cells correspondingly) was shown. High efficiency of NCs isolation was also provided by sedimentation in dextran solution: 82.9±1.4% of NCs and 81.3±4.6% HPCs with high level of viability (97.1±0.4% and 98.4±0.8% of $7AAD^-$-cells correspondingly). Ficoll-density solution allowed isolate about half of all NCs from the whole CB.

It was established that NCs isolation in dextran solution and subsequent freezing under 5% DMSO protection, as well as cells isolation by two-step centrifugation and subsequent freezing under 10% of PEG-1500 protection, kept viable more than 80% $CD45^+$- and 90% $CD34^+$-cells. There was shown different sensitivity of NC populations to the damaging effects of cryopreservation factors: lymphocytes and monocytes were the most cryoresistant (more than 95 and 90% viable cells correspondingly). Thus, the main decrease in cells amount and their viability was due to granulocytes.

The analysis of apoptosis or necrosis stages of CB NCs showed that isolation in dextran solution and cryopreservation with 5% DMSO, as well as isolation by two-step centrifugation and cryopreservation with 10% PEG-1500, kept alive the majority of NCs: 79.3±1.2% and 72.9±2.4% of Annexin V^-7AAD^--cells respectively. At the same time, cryopreservation of ficoll-isolated cells, regardless of used cryoprotectant, kept alive (Annexin V^-7AAD^-) about half of all NCs. Analysis of distribution of damaged cells between apoptosis or necrosis stages showed that regardless of the method of cryopreservation cell damage occurred primarily as necrosis pathway. However, a major percentage of injuries in any of the presented cryopreservation technologies accounted for population of granulocytes, but lymphocytes and monocytes were highly cryoresistant (more than 95 and 90% of living cell correspondingly).Thus obtained results indicate that cryopreservation of CB NCs according to our method allows to preserve structural integrity and viability of the cells at a sufficiently high level.