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Influence of low temperature storage on biological activity of a low molecular fraction (below 5 kD) from human cord blood in experiments *in vitro*

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Nowadays, the investigation and preservation of the biological activity of bio-stimulant drugs, produced based on the low molecular weight components of human cord blood is still relevant. Previously, we have shown that the low molecular (below 5 kD) calf cord blood fraction has a significant stimulating effect on human blood phagocytic cells *in vitro*. Furthermore, the determination of neutrophil phagocytic ability under the influence of the fraction was quite effective test for the analysis of its biological activity. In this regard, this test has been used for determining the biological activity of the low molecular (5 kD) human cord blood fraction (HCBF) after low temperature storage. HCBF was obtained from the whole human cord blood by ultrafiltration, and then lyophilized and stored at -80°C and -196°C within 12 months. Nucleated cell suspension was isolated by differential centrifugation of the whole human donor blood. To estimate phagocytic activity the one-day inactivated culture *Staphylococcus aureus* strain No 209 as a phagocytosis item was used. HCBF was added to a leukocyte suspension before *Staphylococcus aureus* addition. Phagocytic Index (PI)-percentage of phagocyting neutrophils and Phagocytic Number (PN)-the average quantity of bacteria per one neutrophil characterizing intake capacity of cells were registered after 45 or 120 minutes incubation at +37°C. Index of Phagocytosis Completeness (IPC) characterizing digestion activity of neutrophils was calculated as the following fraction: PN after 45 minutes incubation/PN after 120 minutes incubation. The results showed that both freshly isolated HCBF and HCBF stored within 12 months at -80°C and -196°C had stimulating effect on neutrophil phagocytic activity and neutrophil digestive absorbing capacity, but did not significantly affect the PI of neutrophils. Incubation of neutrophils with HCBF after storage within 12 months at -80°C resulted in a reduction of PN by 17.81% and IPC by 9.93% than the values after incubation with HCBF stored at -196°C. PN after incubation of neutrophils with HCBF stored at -196°C was same as the index after incubation with freshly isolated HCBF. Thus, the longer preservation of biological activity of the human cord blood fraction (below 5 kD) without additional preservatives and stabilizers is possible at -196°C.

Biography

Moiseyeva N M has completed her PhD and Post-doctoral studies from Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of the Ukraine. She is currently a Senior Scientist of the Department of Biochemistry of Cold Adaptation. Her research interests include Cryobiology, Drug Discovery and Biochemistry. She deals with investigating of the regenerative activity and cryopreservation methods of the low molecular human cord blood fraction. She has published more than 40 papers in reputed journals and 4 patents.

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