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Розробка протоколів спостереження та збереження мезенхімальних стовбурових клітин для подальшого клінічного дослідження

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Development of Protocols for Monitoring and Preservation of Mesenchymal Stem Cells for Further Clinical Examination

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Currently, mesenchymal stem cells (MSCs) are widely used to treat an increasing number of diseases. Examples of effective use of MSCs include oncohematological, metabolic and neurological diseases, disorders of the musculoskeletal system, circulatory system, congenital malformations.

The MSCs were isolated from human bone marrow (hBM) samples according to the method described by R. Ian Freshney, 2007. Cultivation of MSCs was realized in a complete nutrient medium (CNM) IMDM (Sigma, USA), under standard conditions at 37°C, 5% CO_2, 95% humidity. The MSC receptor profile was determined with FACSAria III flow cytometer (BD, USA). The expression levels of receptors specific to MSCs was analysed. Cells were incubated with monoclonal antibodies produced by BD Stemflow, USA: CD105-PerCP-Cy5.5; CD90-FITC; CD73-APC; CD44-PE. After that the MSCs suspensions were prepared for cryopreservation by adding to the cell samples a cooled vitrification medium (VM). The VM contained 20% albumin 20% v/v, 40% glucose 5% v/v; 7% DMSO v/v and 6% HES 10% v/v. Then the cryotubes with MSCs were subjected to different types of freezing and storage: 1) slow uncontrolled freezing at –80°C, storage at –80°C; 2) slow controlled cooling with 1°C/min rate down to –180°C by means of the BIOFREEZE BV45 freezer, (Consarctic GmbH, Germany), storage at –170°C; 3) rapid uncontrolled cooling in supercooled liquid nitrogen at –220°C for 20 min, storage at –196°C in liquid nitrogen. After freezing/thawing cycle, the MSCs viability was assessed by flow cytometry using a method for determining the 7AAD fluorescent dye incorporation.

As a result, after culturing for 4 weeks in the above CNM, the MSCs samples, before being placed for storage, had the following characteristics: 63.3% of $CD90^+$ cells, 62.2% – $CD105^+$, 82.7% – $CD73^+$, the percentage of living cells was 98%. The percentage of living cells post-thaw was determined in all the samples. In those, subjected to slow freezing down to –80°C, the percentage of living cells was 69.2%. In the specimens cooled slowly down to –180°C, this made 78.2%. The highest percentage of living cells (82.2%) was found in those samples, which were rapidly frozen in a supercooled liquid nitrogen at –220°C.

The best result was achieved using the protocol of rapid freezing down to –220°C, storage at –170°C and rapid thawing.

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Вплив кріоконсервованих мультипотентних мезенхімальних стромальних клітин на регуляцію імунозапального процесу при ад'ювантному артриті

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Influence of Cryopreserved Multipotent Mesenchymal Stromal Cells on Regulation of Immune Inflammation in Adjuvant Arthritis

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Rheumatoid arthritis is an autoimmune disease of multifactorial origin, in the initiation and maintenance of which a wide range of cellular populations of organism is involved. The results of experimental and clinical studies in recent years suggest that therapy with cryopreserved multipotent mesenchymal stromal cells (CrMMSCs) is a modern approach to stimulate regenerative processes and restore the immune system in pathologies of various genesis.

The aim of this study was to evaluate the influence of generalized and local administration of CrMMSCs from cartilage tissue on the indices of the immuno-inflammatory process in adjuvant arthritis (AA).

A model of AA in male rats was induced by subplantar administration of a complete Freund's adjuvant. On day 7 of modeling, animals were administered with: saline (control group); CrMMSCs from cartilage tissue locally or generalized (experimental groups). On day 21 content of C-reactive protein (CRP) and rheumatoid factor (RF) were measured in serum, and content of $CD3^+$, $CD4^+$, $CD8^+$ cells and immuno-regulatory index ($CD4^+/CD8^+$) were evaluated in the spleen. The results were processed with Kruskal-Wallis analysis and the Newman-Keuls method for multiple comparisons.

In the control group (AA) the inflammatory process was pronounced, as evidenced by a significant increase in serological markers (CRP and RF in 5.1 and 3.5 times, respectively) and a decrease in cellular immunity (2.0-fold for $CD3^+$, 2.1-fold for $CD4^+$, 1.8-fold for $CD8^+$ cell content and 1.3-fold for immuno-regulatory index) related to intact animals. Both local and generalized administration of CrMMSCs to animals with AA helped to reduce the content of CRP respectively by 1.4- and 1.9-fold and RF by 1.7- and 2.1-fold, compared to the control. Content of T cells in the spleen of animals with local administration of CrMMSCs was higher than the corresponding values in the control group, namely 1.7-fold for $CD3^+$, 1.8-fold for $CD4^+$, 1.4 fold for $CD8^+$, and the immunoregulatory index was increased by 1.2 times. After generalized cell administration the content of lymphocyte subpopulation ($CD3^+$, $CD4^+$, $CD8^+$) in the spleen and $CD4^+/CD8^+$ ratio were normalized.

The analysis of the obtained results showed that local and generalized administration of cartilage-derived CrMMSCs to animals with AA led to the correction of the immuno-inflammatory process. This data can be used to substantiate and develop the methods of arthritis therapy in clinical practice.