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Оцінка ефективності застосування кріоконсервованих мультипотентних мезенхімальних стромальних клітин із жирової тканини при терапії ад'ювантного артриту

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Assessment of Effectiveness of Cryopreserved Multipotent Mesenchymal Stromal Cells from Adipose Tissue in Adjuvant Arthritis Treatment

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The accumulation of knowledge on the biology of multipotent mesenchymal stromal cells (MMSCs) provides new insights into their potential clinical application, especially for the treatment of arthritis. MMSCs have trophic, immune suppressive, regenerative potentials, due to those they can affect the course of chronic degenerative disorders, prevent cartilage degradation and help suppress the local inflammation and tissue damage in inflammatory autoimmune diseases and, in particular, in rheumatoid arthritis.

The aim of this research was to evaluate the effectiveness of cryopreserved multipotent mesenchymal stromal cells from adipose tissue (cMMSCsAT) when treating the adjuvant arthritis in rats.

The adjuvant arthritis (AA) in male rats ($n = 25$, weight 250 ± 10 g) was simulated by subplantar administration of a complete Freund's adjuvant (SantaCruz, USA) at a dose of 0.25 ml. The cMMSCsAT were cryopreserved under the protection of 10% DMSO and 20% fetal bovine serum with a cooling rate of 1 deg / min to –80°C, followed by an immersion in liquid nitrogen. On day 7 of the AA modeling the animals were injected into the tail vein: control group – with a saline; experimental group – with the cMMSCsAT at a dose of 0.25×10^6 cells / ml. A group of intact animals was also formed as a control. On day 28, the erythrocyte sedimentation rate (ESR), leukocyte content, total protein, and antioxidant defense system (ADS) activity were determined in blood. The arthritis index (AI) was defined as the ratio of the experimental paw joint circumference to the index of the control joint of the same animal. Student's t-test with the Excel program was used for statistical processing of the results.

The development of inflammation in animals of the control group was manifested in the joint edema and hyperemia (increase in AI by 1.5 times on day 28), which persisted throughout the experiment. In the blood of animals with AA there was determined an increased content of leukocytes (1.5 times), ESR (3.5 times), total protein (1.2 times) relative to the corresponding indices in intact animals. In the animals with the introduced cMMSCsAT the development of joint edema tended to decrease (AI was 1.24 times lower than in the control group on day 28) but did not reach the normal levels. In animals of the experimental group, the content of leukocytes did not differ significantly from the corresponding index in intact animals, ESR and total protein levels exceeded the norm by 1.17 and 1.15 times, respectively. The activity of the ADS system in the blood of animals of the control and experimental groups was 2.3 and 1.4 times lower than for intact animals.

The analysis of the findings showed a tendency to normalization of AI and blood parameters in animals with intravenous administration of cMMSCsAT under conditions of experimental adjuvant arthritis.

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Кріоконсервування бруньок винограду

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Cryopreservation of Grape Buds

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The easiest way for a long-term storage of most plant genetic resources is the seed cryopreservation. However for vegetatively propagated plants, the characteristics of the mother plant are virtually not kept in posterity, while seed propagation. Thus, the most proper and modern approach for a long-term storage of vine is cryopreservation of annual cuttings, buds and meristems. Dormant grape buds are large and complex object for cryopreservation. They contain a large number of different channels and cavities, filled with air at atmospheric pressure. The heterogeneity in morphology and physiology of buds' tissues may limit their successful cryopreservation. The task of the efficient bud saturation with cryoprotectant may be solved by using the vacuum to speed up the chemicals' infiltration into various plant tissues.

The purpose of the study was to compare the efficiency of the vacuum infiltration vitrification (VIV) method and the traditional passive saturation of isolated dormant grape buds with PVS 2.

Annual vine single-bud cuttings of Russian Concord, Riparia X Rupestris and Zagadka varieties were collected in autumn and winter. Dormant grape buds were saturated with PVS 2 cryoprotective medium by using the traditional passive saturation via incubation in cyoprotectant at normal atmospheric pressure for 60 min, and vacuum infiltration at 40 kPa pressure for 15 min. The saturation efficiency was evaluated with the low-temperature differential scanning calorimetry by the changes in the phase transition temperatures, enthalpy of water crystallization, and glass transition. Post-cryo integrity of bud meristematic parts was evaluated by staining with triphenyl tetrazole chloride solution. The viability of grape buds was assessed visually by the time of bud swelling.

After VIV saturation the intensity of glass transition in buds was twice increased, the melting enthalpy of bound water augmented by 2.9 times, the melting temperature reduced by 4 degrees as compared to passive saturation. The VIV enabled 60% level of grape bud post-cryo integrity for Russian Concord and Riparia X Rupestris varieties and 80% for Zagadka one. The grape bud viability was 30% for Russian Concord, 40% for Zagadka, 0% for Riparia X Rupestris varieties. No viable buds among all the studied grape varieties after cryopreservation with the traditional passive saturation were observed.

Thus the use of VIV method reduces the incubation time of grape buds in cryoprotective medium and increases the cryoprotectant concentration in buds' tissues that allows improving their integrity and viability after cryopreservation.