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The effect of intermittent cold exposure on the level of reactive oxygen species production in erythrocytes of rats with induced polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is a common endocrine disorder in women of reproductive age, often associated with systemic oxidative stress as a key pathogenic factor. This underscores the need to identify modulatory influences on the body's redox balance. Intermittent cold exposure is one such factor, showing antioxidant effects through activation of adaptive mechanisms.

The study used blood samples from 40 female rats (28 days old), randomly divided into four equal groups. Group 1 was the intact control. Group 2 was exposed to intermittent cold for 4 hours daily over 25 days. In Group 3, PCOS was induced by subcutaneous dehydroepiandrosterone (DHEA) administration, causing a hyperandrogenic state and characteristic ovarian changes. Group 4 received both DHEA and cold exposure to assess their combined effect. For analysis, 100 µl of venous blood was mixed with 1 ml of 0.9% NaCl and centrifuged (5 min, 1000 rpm). After plasma removal, erythrocytes were washed twice with 1 and 0.9% NaCl (1 ml per 10 µl of cells). Then, 2 µl of erythrocytes were incubated with 10 µM H_2DCFDA for 30 minutes in the dark. This probe is oxidized by reactive oxygen species (ROS) to fluorescent DCF. After incubation, cells were washed, resuspended, and analyzed using the FL1 channel of a BD FACSCantoII flow cytometer (BD Biosciences, USA). Fluorescence intensity was assessed by median and interquartile range [25; 75%], and statistical analysis was performed.

The intensity of DCF fluorescence in erythrocytes was 42.77 [40.89; 44.80] arbitrary units (a.u.) in Group 1, 41.52 [39.75; 42.58] a.u. in Group 2, 66.08 [63.46; 68.33] a.u. in Group 3, and 42.69 [39.79; 44.45] a.u. in Group 4. Subsequent statistical analysis revealed no significant difference between Groups 1 and 2 ($p > 0.05$). However, a significant increase in fluorescence intensity was observed in rats with induced PCOS (group 3) compared to the control Groups ($p < 0.05$), indicating excessive production of ROS in erythrocytes. At the same time, in Group 4, the fluorescence intensity was significantly lower than in Group 3 ($p < 0.05$) and did not differ significantly from that in Groups 1 and 2 ($p > 0.05$), suggesting normalization of ROS levels under the combined effect of DHEA and intermittent cold exposure.

The experiment demonstrated that induced PCOS was accompanied by excessive generation of ROS in erythrocytes, indicating a disruption of redox homeostasis and the development of oxidative stress — one of the key pathogenic mechanisms of the syndrome. Intermittent cold exposure reduces ROS production in rats with hormonally induced pathology to control levels, demonstrating its potential effectiveness in correcting oxidative imbalance in PCOS.

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Study of the permeability of the plasma membrane of human erythrocytes for DMSO molecules in the presence of PVA of various concentrations

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Today, the development and implementation of effective methods for cryopreservation and long-term storage of human erythrocytes has a significant impact on the blood supply system, especially for addressing the shortage of rare blood types and providing medical institutions with significant volumes of erythrocytes during emergencies.

The cryoprotectants, among which DMSO is currently the most widely used, contribute to the preservation of the structural integrity and biological activity of erythrocytes. To increase the efficiency of cryopreservation, water-soluble polymers, namely polyvinyl alcohol (PVA), are added as a component to cryoprotectant solutions. PVA is a biocompatible, non-toxic, impermeable cryoprotectant that helps suppress the process of recrystallization of ice crystals during thawing of biological objects. (Liang Y, 2024, Colton JK., 2024, Melissa JMD, 2017).

The aim of the work was to determine the passive permeability coefficients of the human erythrocyte membrane for DMSO molecules in the presence of PVA (m.w. 9 kDa) of various concentrations and to conduct a morphological analysis of the state of the cells.

The study used erythrocyte concentrate obtained from human donor blood, prepared with "Glugitsir" hemopreservative at the Kharkiv Regional Blood Service Center, which was stored for no more than 48 hours at a temperature of (4 ± ± 2) °C. The 1M aqueous solutions of DMSO with the addition of 0.1, 0.2, 0.5 and 1% aqueous solutions of PVA were studied. The passive permeability coefficients of human erythrocyte membranes for DMSO molecules in hypotonic solutions in water in the presence of PVA of various concentrations were determined by the small-angle light scattering method. Morphological state of cells under the influence of PVA was studied by the immersion method of microscopic observation in a confocal laser scanning microscope "LSM S10 META Carl Zeiss" (Carl Zeiss, Germany).

It has been shown that the permeability coefficient of erythrocyte membranes for DMSO molecules significantly increases in a 1M aqueous solution of DMSO with 0.1% PVA relative to such a solution without PVA. As the concentration of PVA in the solution increases, the permeability coefficient slowly decreases. The increase in the permeability coefficient of erythrocyte membranes in the solution in the presence of 0.1% PVA can be attributed to a change in the membrane structure, which allows DMSO molecules to penetrate the erythrocyte more quickly. At the same time, after exposure of erythrocytes to 0.1% PVA solution, cell transformation into spherocytes is observed in almost 100% of cases. Thus, the presence of PVA at a concentration of 0.1% contributes to an increase in the permeability of the human erythrocyte membrane and a decrease in the total time of the cryopreservation procedure.