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EFFECT OF FREEZING ON ANTI-INFLAMMATORY EFFICIENCY OF HUMAN PLACENTAL EXTRACTS

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Introduction: It is known that placental tissues contain a large number of biologically active substances that determines a wide use of placental extracts in medicine. Due to its high anti-inflammatory efficacy, the extracts are used in the treatment of wounds of various origins, pressure ulcers, inflammatory processes of the oral cavity, etc [1, 2]. Placenta rapidly loses its unique properties during hypothermic storage. Low-temperature storage of placenta allows to ensure the availability of raw materials necessary for the preparation of extracts at any convenient time. In this work the influence of freezing on an anti-inflammatory efficiency of human placental extracts (HPE) was investigated.

An increase in platelet aggregation and a decrease in erythrocyte membrane resistance are the inflammation biomarkers in human body [3-5]. Therefore researchers often evaluate an anti-inflammatory effect of drugs of various origins *in vitro* using these biomarkers. The aim of the research was to assess the effect of freezing on the ability of placenta extracts to reduce the induced aggregation of platelets and to increase the thermal stability of red blood cells.

Materials and methods. Placental specimens were frozen in plastic bags either down to – 196 °C (in liquid nitrogen) or down to –20 °C (in a freezer), then thawed in a water bath at 37 °C. Water-salt extracts were prepared by the standard method [1-3]. Fractions of HPE were obtained using gel chromatography.

The ADP-induced platelet aggregation in platelet rich plasma (PRP) was investigated in this work [3]. To study the effect of HPE on this index, PRP was incubated for 15 minutes with HPE and its fractions at 37 °C. The percentage of platelet aggregation was estimated by the change in the light scattering level at 650 nm spectrophotometrically.

To study the thermohemolysis, the washed red blood cells were resuspended with phosphate- buffered saline solution in a 1:1 ratio, incubated with HPE and fractions of different molecular weights for one hour. Then the erythrocytes were washed from HPE and fractions, resuspended and exposed of hyperthermia (20 min in an adjustable water thermostat at 55°C). Afterwards, the hemolysis was evaluated by measuring of the optical density in supernatant at a wavelength of 540 nm spectrophotometrically.

The temperature-dependent dynamics of the erythrocyte cytosol status was studied using the hydrophilic spin probe TEMPON (2,2,6,6-tetramethyl-4-oxo-piperidine-1-oxyls) in combination with a pericidal broadening agent potassium. After erythrocyte suspension heating in spectrometer's resonator, there were also recorded the spectra at a fixed temperature of 55°C for 25-30 min every 5 min. Erythrocytes thermostability was evaluated by the rate of amplitude decrease of the mid-field component of TEMPON spectrum. The EPR spectra were recorded with EPR with a thermostatic device.

Results: The HPE were shown to modify the behavior of structural-dynamic state of erythrocyte cytosol within the range of + (40 ÷ 50) °C, and erythrocyte thermal stability, by reducing of the rate of barrier properties disturbance in erythrocyte membrane at + 55°C. Placental freezing down to -20°C and 196°C did not affect the HPE ability to modify the high-temperature changes in erythrocytes.

The HPE was found to reduce the ADP-induced platelet aggregation and the level of thermal hemolysis by (20.4 ± 2.1)% and (18.7 ± 1.9)%, respectively. The fractions obtained from HPE efficiently reduced the platelet aggregation and thermal hemolysis of erythrocytes. The highest percentage of inhibition of platelet aggregation was found in the high molecular weight fraction (32.3 ± 3.5)%. Fractions of <4 kDa and 12-20 kDa are efficient in decreasing the thermal hemolysis of erythrocytes and platelet aggregation.

Placental cooling both down to -20 and -196°C and following thawing did not lead to loss of the capability its extracts to reduce an induced platelet aggregation and thermohemolysis of red blood cells. Extract fractions from the frozen placenta preserved the ability to inhibit the platelet aggregation and thermal hemolysis of erythrocytes. After placental cool-

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ing down to - 20 °C, the percentage of inhibition of thermal hemolysis by fractions of HPE <4 kDa and 12-20 kDa was (29.4 ± 3.1)% and (26 ± 2.8)%, respectively. The percentage of inhibition of platelet aggregation in this case was (43.8 ± 4.5)% and (38.7 ± 4.1)%, respectively.

Thus, the placental freezing did not result in a significant decrease in anti-aggregation efficiency of the extracts and extract fractions and their ability to increase the thermal stability of red blood cells. According to the presented *in vitro* model for assessing an anti-inflammatory effect, using the presented biomarkers, it can be concluded that the anti-inflammatory properties of the extracts are preserved after placental freezing. A high efficiency of fractions with weight <4 kDa and 12-20 kDa was demonstrated.

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