MEMBRANE POTENTIAL OF RED BLLOD CELLS UNDER TYPE 1 DIABETES IN HUMANS

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Introduction. Membrane potential (also transmembrane potential or membrane voltage) is the difference in electric potential between the interior and the exterior of a biological cell [1]. Circulating red cells are one of the most peroxidation-susceptible biological tissues and they often serve as a model target tissue for studies on pathophysiology of oxidative damage [2]. The oxidative damage of cells destroys the normal packing of lipid bilayer and changes the properties of cell proteins. It has been postulated that the oxygen free radicals participate in cell damage in various tissues in diabetes mellitus [3]. Recently, an increased susceptibility to peroxidation of low-density lipoproteins and red blood cell membranes from diabetic patients has been shown, which can be responsible for the development of the vascular complications in diabetes mellitus [4]. Alterations in cell membrane potential upon oxidative stress may represent an early stage in the evolution of oxidative injury and may precede the formation of membrane discontinuities. Therefore, the aim of this work was to evaluate the changes of the membrane potential of human red blood cells subjected to oxidative stress in vitro and affected by diabetes.

The aim of the study was to show the effect of diabetes on the red blood cell membranes of people with type 1 diabetes.

Research method. Blood samples from type I diabetic patients were obtained from the Diabetological clinic of Grodno Medical University Hospital (Grodno). Blood from healthy donors was purchased from the Central Blood Bank in Grodno. Blood from diabetic patients was taken into 3% sodium citrate after overnight fast before morning insulin. After removing plasma and the leukocyte layer, erythrocytes were washed 3 times with cold (4 °C) phosphate-buffered saline (PBS: 0.15 mol/L NaC1, 1.9 mmol/L NaH2PO4, 8.0 mmol/L Na2HPO4, pH 7.4). Erythrocytes were used immediately after isolation.

The membrane potential was measured using an optical molecular probe (DISC3[5]), which responds to membrane potential of the cell. This method is well described in the literature and commonly used [5]. Briefly, before or after oxidative stress, erythrocytes were suspended at a hematocrit of 0.2% in buffered saline, containing 10 mmol/L Tris-HC1, pH 7.4 and 150 mmol/L (KC1 + NaC1) with K^+ concentrations in the range between 50 mmol/L to 140 mmol/L. To all suspensions of cells, fluorescent dye was added to give a final concentration of 2.10^{-5} mol/L. After the time required to level off the fluorescence intensity of the dye, valinomycin at a concentration of 10^{-6} mol\L was added to the samples and the fluorescent intensities were measured before and after valinomycin addition, and plotted as $(I_o - I_{VAI})/I_O$ us $\log_2$ of the corresponding external K^+ concentration. The intersection point of the curve with the abscissa corresponds to K^+_{out} (i.e. the external potassium

concentration), where no change in fluorescent intensity occurs upon addition of valinomycin. Valinomycin is an ionophore known to induce a marked K^+ permeability through the membranes. In low potassium media, it induces the hyperpolarization of red blood cells, whereas the depolarization of the membrane occurs in high-potassium media. The membrane potential was calculated using Nernst equation:

$$E_m = \frac{RT}{F} \times \frac{K^+_{out}}{K^+_{in}}$$

where: E is the membrane potential; R is the gas constant; F is the Faraday's constant; K^+_{out} is the external potassium concentration for which no change of fluorescence was observed after the addition of valinomycin, and Kin is the cellular potassium concentration for erythrocytes. The value of 150 mmol/L was used as the internal K concentration in red blood cells. DiSC [5] and valinomycin were added as concentrated ethologic solutions. The final ethanol concentration in the samples did not exceed 0.4 %. Fluorescence was excited at 625 nm and registered at 660 nm with a Perkin-Elmer LS-5B spectrofluorometer.

Result and discussion. Membrane potential is the difference in electric potential between the interior and the exterior of a biological cell [6]. Its magnitude reflects the steady-state activities of ions in both media in relation to the passive conductances and possible pumps for the individual ions. Changes in membrane potential can give information about the changes of the basic function and properties of the membrane.

The magnitude of the membrane potential of red blood cells is different from that of almost any other cell types in being small: (-10 to -15) mV [1]. The reported values for resting cells vary from -50 to -80 mV. Circulating red cells are one of the most peroxidation-susceptible biological tissues and they often serve as a model target tissue for studies on pathophysiology of oxidative damage [2]. The oxidative damage of cells destroys the normal packing of lipid bilayer and changes the properties of cell proteins. It has been postulated that the oxygen free radicals participate in cell damage in various tissues in diabetes mellitus [3]. In our experiments the values of [K+]OUT obtained from the plots (I0-Ival)/I0 vs log2 K+ served to calculate the membrane potential according to the Nernst equation for the control red blood cells, value of (-11.0 ±1.7) mV was obtained (n=17), whereas erythrocyte membranes in diabetes mellitus type I were hyperpolarized, showing the membrane potential equal to (-14.8 ±1.9) mV (n=16). This difference is statistically significant: $p < 0.01$.

We observed the hyperpolarization of red blood cell membrane of diabetic patients due to changes in cell membrane ion permeability. Simultaneously we observed considerable accumulation of the products of lipid peroxidation in the membranes or human erythrocytes during diabetes. We suggested that the observed changes in red blood cell during diabetes are the result of constant oxidative stress and influence on blood rheology during diabetes.

Conclusions. The value of the membrane electrochemical potential of red blood cells of healthy donors was obtained (-11.0 ±1.7) mV (n=17), whereas

erythrocyte membranes in diabetes mellitus type I patients were hyperpolarized, showing the membrane potential equal to (-14.8 ±1.9) mV (n=16). This difference is due to constant oxidative stress in red blood cells during diabetes.

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MELATONIN DIMINISHES OXIDATIVE STRESS IN PLASMA AFTER LOW DOSE LIPOPOLYSACCHARIDE EXPOSURE IN MICE

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Introduction. The principal components of the outer membrane of Gram-negative bacteria are lipopolysaccharides (LPS), also termed endotoxins, which initiate inflammatory-induced immune responses (Guha and Mackman, 2001). LPS induces inflammatory cytokine release, including interleukin IL-6, IL-1β, and tumor necrosis factor α *via* toll-like receptor 4 binding, activating the signalling pathways of mitogen-activated protein kinases (MAPKs), including extracellular signal-related kinase 1/2, p38MAPK, c-Jun NH_2-terminal kinase and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), have been demonstrated to be involved in LPS-induced inflammatory responses (Chan and Riches, 2001; Qi et al., 2013; Cochet and Peri, 2017).

The spectrum of physiological effects caused by melatonin is quite wide (Melchiorri et al. 1995; Escames et al. 1997; von Gall et al. 2002; Kurhaluk and Tkachenko, 2020; Kurhaluk et al., 2020, 2021). The action of this substance is observed at the systemic, tissue, cellular and subcellular levels (Saravanan et al. 2007). Melatonin reduces both basal and bacterial LPS-induced lipid peroxidation in vitro, as was shown by Sewerynek et al. (1995). Melatonin inhibited LPS-induced inflammation and oxidative stress in cultured mouse mammary tissue. It might contribute to mastitis therapy while treating antibiotic resistance (Shao et al., 2015;