ISSN 0006-3509, Biophysics, 2020, Vol. 65, No. 6, pp. 948–950. © Pleiades Publishing, Inc., 2020.
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CELL BIOPHYSICS

Changes of the Biophysical Properties of Blood Corpuscles from the Elderly under Mechanical Stress in vitro

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Received March 5, 2020; revised April 21, 2020; accepted May 20, 2020

Abstract—Features of biophysical properties in blood cells from the elderly under mechanical stress applied in vitro have been studied. This stress was reported to trigger the signaling pathway engaged by purinergic receptor activation. The stiffness of the cell surfaces of red blood cells, granulocytes, lymphocytes, and platelets decreased, and the surface potential became more positive. Our findings add to the knowledge of the effect of mechanical deformation on leukocytes and platelets, which are the key regulators of homeostatic processes in the microvasculature, and on red blood cells involved in the regulation of vascular tone in arterioles and tissue oxygenation in the elderly.

Keywords: mechanical stress, purinergic signaling system, surface potential, Young's modulus

DOI: 10.1134/S0006350920060196

In the greatest majority of cases, the aging of the body is accompanied by the development of cardiovascular diseases. These diseases are caused mainly by molecular and cellular changes in the endothelium [1] and by changing rheological indices of blood [2]. These disturbances may be associated with changes in the functioning of purinergic receptors in blood corpuscles and endothelium cells. Several studies provide evidence for a significant role of purinergic signaling in the development of various cardiovascular conditions: myocardium infarction, cardiac failure, hypertension, stroke, and clotting [3]. In addition, elderly and senile people are at greater risk of infectious diseases, caused primarily by weakening of the immune system. The purinergic regulation of immunocompetent cells mediating inflammatory responses in the body plays a significant role in mechanisms of the senescence of the immune system [4].

It has been shown that erythrocytes that are undergoing mechanical deformation release ATP molecules [5], thereby triggering paracrine and autocrine signaling pathways [6]. In the intercellular space, the ATP molecules are degraded to ADP, AMP, and adenosine [7], which are agonists of the wide range of purinergic receptors present on the surface of blood corpuscles.

Therefore, this study was dedicated to the biophysical features of the response of blood corpuscles from elderly people to mechanical stress in vitro.

Abbreviation: AFM, atomic force microscopy.

MATERIALS AND METHODS

Experiments were conducted with blood samples from elderly volunteers (60 to 75 years, $n = 30$), who were undergoing standard medical examinations in the regional hospital of Belgorod. Samples were taken from the cubital vein by skilled personnel.

Mechanical stress was modeled in vitro as described in [8]. The model provides conditions close to the physiological parameters of microvasculature [9]. Whole blood (0.5 mL) was loaded into a disposable tuberculin syringe and air bubbles were carefully removed. The cell suspension was passed through a 25-mm disposable needle. For the injection, the syringe was installed vertically and a 1-kg weight was placed onto the upper end of the syringe plunger. The blood was sprayed into 2-mL centrifuge tubes. The distance from the needle tip to the bottom was 15 mm, and the tangential stress of the wall was within 6600 dyn/cm^2. The mean velocity in the needle reached 5100 cm/s and the travel time of corpuscles was 0.6 ms. Hence, cells were exposed to relatively high tension within a short time. Experimental blood samples were exposed to mechanical stress and control ones remained intact.

Concentrations of high-energy compounds in mechanical stress in vitro were assayed colorimetrically [10] on a KFK-3 photometer (Sergiev Posad Optical-Mechanical Plant, Russia). The concentrations were calculated from the difference between the optical densities in the tube where phosphate bonds were hydrolyzed and in the control tube, which was not subjected to hydrolysis. The standard curve was

Table 1. The surface potential (mV) of blood corpuscles

Cell population	Control	Experiment
Erythrocytes	−15.2 ± 0.8	−10.7 ± 1.2*
Granulocytes	−25.8 ± 1.2	−17.5 ± 0.6*
Lymphocytes	−39.6 ± 0.2	−28.7 ± 0.3*
Platelets	−16.2 ± 1.3	−7.4 ± 1.1*

* Differences are significant according to the Student's t test at $p < 0.05$.

Table 2. The Young modulus (nN) of blood corpuscles

Cell population	Control, mV	Experiment, mV
Erythrocytes	4.2 ± 0.01	3.3 ± 0.01*
Granulocytes	6.3 ± 0.01	5.1 ± 0.01*
Lymphocytes	4.7 ± 0.02	3.6 ± 0.01*
Platelets	4.5 ± 0.03	2.7 ± 0.02*

* Differences are significant according to the Student's t test at $p < 0.05$.

drawn by using solutions with phosphate concentrations (State Standard Sample 77912000) from 50 to 500 μg/mL at 50-μg/mL intervals. The measurements were done in triplicate for each sample.

Whole blood was separated into erythrocyte, leukocyte, and platelet concentrates by centrifuging at 1500 rpm for 5 min. Plasma enriched with platelets was collected and additionally centrifuged at 2500 rpm for 15 min; in this way, a pure platelet suspension was obtained. The leukocyte ring was collected into a separate tube. The leukocyte suspension was separated into granulocytes and lymphocytes with an EasySep Magnet and an EasySep/EasySep Direct Human Total Lymphocyte Isolation Kit (StemCell Technologies, United States).

The biophysical properties of blood corpuscles were examined with an INTEGRA Vita atomic force microscope (an assembly based on the Olympus IX71 inverted optical microscope, NT-MDT Company, Zelenograd, Russia). The electrical properties of the erythrocyte, granulocyte, lymphocyte, and platelet membranes were assessed by measuring the surface potentials with the AFM in the Kelvin probe mode. The preparation of the cell suspension for measuring the surface potential and the measurement followed the method reported in [11]. Use was made of cantilevers with conducting titanium coating of the NSG03/TiN series (Nanoworld, United States). Twenty cells of each population were scanned in each sample, and the scans were processed with Nova software (NT MDT, Russia).

Cell surface stiffness was assessed from the Young modulus numerical data. The method for recording Young modulus involves the measurement of sample surface deformation in its interaction with the AFM probe tip. We employed modified AFM probes made by us from polymeric microspheres with the corner radius 5 μm [12]. The Young modulus was measured with the AFM INTEGRA VITA in the force spectroscopy mode as described in [13]. Twenty cells of each population were scanned in each sample.

The experimental results were processed by variation statistics methods. The significance of differences between experimental and control samples was assessed by the Student's t test at $p < 0.05$ and the normal distribution. The mean values M and standard errors of the mean m are presented.

RESULTS AND DISCUSSION

The concentration of high-energy compounds in blood in experiments with modeled shearing deformation of blood cells was 0.021 ± 0.001 μmol/L, 2.6 times higher than the control value (0.008 ± 0.001).

Mechanical stress was shown to alter the biophysical properties of blood corpuscles. In particular, the surface potentials of erythrocytes, granulocytes, lymphocytes, and platelets became more positive. As indicated in Table 1, it increased with reference to the control by 30, 32.2, 27.5, and 54.3%, respectively ($p < 0.05$).

Mechanical stress decreased the Young modulus of blood corpuscles from elderly people: by 22% in erythrocytes ($p < 0.05$), 19% in granulocytes ($p < 0.05$), 23% in lymphocytes ($p < 0.05$), and 40% in platelets (($p < 0.05$) compared to the control values (Table 2).

Our experiments reproduced the in vitro mechanical stress conditions that had been reported to approximate the physiological parameters of the microcirculatory bloodstream and favor the activation of purinergic signaling in blood corpuscles [8, 9, 14, 15]. The 2.6-fold increase in the concentration of high-energy compounds noted in blood samples exposed to mechanical stress may be related to the excretion of ATP molecules to the intercellular space by erythrocytes in response to the force action of shearing plasma layers [1].

The effect of mechanical stress manifested itself as changes in biochemical properties of blood corpuscles from the peripheral blood of elderly people. In particular, the increase in membrane charge and lower stiffness of erythrocytes may be associated with the effects of high-energy compounds, that is, ATP and its derivatives, on P2Y13 receptors on the cell surface mediated by changes in the action of ion channels selective for Ca^{2+} [16]. The activation of these receptors on the erythrocyte membrane may be accompanied by changes not only in their functions but also in cell morphology, ion composition in the cytoplasm, and the state of cytoskeletal proteins [17]. A similar mechanism involving the effect of high-energy compounds

may alter the biophysical parameters of lymphocytes. The lymphocyte surface houses receptors of the P2X family, which are membrane ion channels [18] that control the cytosolic Ca^{2+} concentration [19].

Elevated concentrations of high-energy molecules were also accompanied by lower stiffness and a higher surface potential of granulocyte plasmalemma. It had been shown that the presence of receptors for purines (of which P2Y1, P2Y2, P2Y4, P2Y6, and P2Y11 are predominant [20]) on the granulocyte surface was essential for cell orientation and movement in response to chemotactic stimuli [19, 21]. The detection of the increase in the platelet surface charge, probably by means of binding of high-energy compound metabolites to P2Y receptors of platelets [20], is an important point in our study. Platelets are known to be involved in the formation of neutrophil extracellular traps. An increase in the surface potential of both granulocytes and platelets may deter the interaction between their surfaces, thereby weakening the inflammatory response of immunocompetent cells in the blood of the elderly.

Thus, our model study of mechanical stress in vitro has demonstrated an elevation in the concentration of high-energy compounds in blood from elderly people. Along with it, mechanical stress results in a decrease in the Young modulus and increase in plasmalemma charge in all of the four populations of blood corpuscles. The experimental results contribute substantially to the knowledge of the effect of mechanical deformation on leukocytes and platelets, which are the key regulator of homeostasis in the vasculature, and on erythrocytes, which are involved in the regulation of rheological parameters and oxygen supply to tissues. Further study of blood corpuscle morphology and functional parameters under shear deformation of membranes may help us with the correction of pathological conditions (immune dysfunction, microcirculation disturbance, and progression of cardiovascular diseases) that accompany aging.

FUNDING

This work was supported by the Russian Science Foundation, Studies initiated by young scientists program 2018–2020 (agreement 18-75-00041).

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interests

The authors declare that they have no conflict of interest.

Statement on the Welfare of Animals

All manipulations with humans met the ethical standards of the Declaration of Helsinki, 1964, as amended by the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000. Informed consent was obtained from each participant of the study.

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Translated by V. Gulevich