EFFECT OF THE MODEL OF MECHANIAL STRESS (IN VITRO) ON THE BIOPHYSICAL PROPERTIES OF BIOMEMBRANE OF GRANULOCYTES

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Abstract

In the microvasculature the granulocytes function under conditions of force impact from the shifting layers of the moving plasma while the erythrocytes and endothelium cells excrete ATP molecules in the intercellular space acting as para- and autocrine regulators of interactions between the blood cells. It is possible to study interactions between granulocytes and erythrocytes thought purinergic signalling in small vessels under conditions close to physiological ones using the model of mechanical "stress" *in vitro.* The functional activity of granulocytes is determined by such properties of plasmalemma as stiffness and potential of a surface as well as the force of intercellular adhesion. In connection with the foregoing, the goal of the work was to investigate the biophysical properties of granulocytes' plasmalemma in the condition of mechanical "stress" *in vitro.* Atomic force microscopy methods were used in the semi-contact scanning and force spectroscopy mode to achieve this goal. In the condition of mechanical "stress" was found an increase in the ATP concentration by 147% as compared with the control. At the same time, the biophysical properties of the surface of blood cells changed: the potential of surface and stiffness of granulocytes decreased by 32% and 15% respectively, while the negative charge of the erythrocyte's surface increased by 112% which contributed to increase the adhesion force in the system "erythrocyte-granulocyte" by 55% as compared with the control. Thus, the obtained data allows summarized that the ATP molecule excreting by erythrocytes in the condition of mechanical "stress" is an auto- and paracrine regulator of biophysical properties of erythrocytes' and granulocytes' plasmalemma that have an important impact in the study of the mechanisms of intercellular interactions in the microvasculature and searching the pharmacological regulators of vascular tone.

Keywords: granulocytes, erythrocytes, stiffness of cell's surface, surface's potential, adhesion force, ATP, mechanical stress.

INTRODUCTION

Normally, granulocytes function under the conditions of mechanical "stress" in microvasculature. Mechanical "stress" is the force exerted by the shifting layers of the moving plasma. It is known that in response to mechanical "stress" erythrocytes and endothelial cells excrete ATP molecules into the intercellular space [1], which trigger a whole cascade of intercellular interactions by activating purinergic receptors localized on granulocyte membranes involved in signal transduction mechanisms [2]. The physiological activity of granulocytes and their direct participation in intercellular communication mostly depends on the properties of the plasmalemma and the competence of their receptor apparatus. When performing the main immunocompetent and regulatory functions, the key property that allows granulocytes to implement their physiological functions is such surface characteristics as rigidity, surface potential and intercellular adhesion strength. Considering the abovementioned, the aim of the work was to study the biophysical properties of the granulocyte plasmalemma under the conditions of mechanical "stress" in vitro..

MATHERIALS AND METHODS

The experiments were performed with the blood of healthy people, volunteers (n = 20), who underwent medical examination at the regional clinical hospital in Belgorod. Blood sampling was carried out from the cubital vein with the participation of specialized medical personnel. The studies were carried out in

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compliance with the requirements of the Declaration of Helsinki, informed consent from all subjects of the experiment was obtained in accordance with the recommendations (Declaration on the ethical principles of medical research in which people participate, adopted by the 52nd General Assembly of the World Medical Association, Edinburgh, Scotland, October, 2000). Each blood sample was divided into two, one experimental was subjected to mechanical stress, the second was left intact and used as a control. To simulate the conditions of shear deformation of membranes, an in vitro mechanical stress model was used according to the method described in (Migliorini et al., 2002). In control and experimental samples, leukocytes were separated by centrifugation at 1500 rpm for 5 minutes.

In order to assess the concentration of ATP produced by erythrocytes during mechanical stress in vitro, ATP was measured in the test samples by the colorimetric method (TL & GV, 1988), using a photoelectric photometer KFK-3. The ATP concentration was calculated from the difference in optical densities between the test tube in which the hydrolysis of phosphate bonds was carried out and the sample without the hydrolysis of phosphate bonds, using a calibration graph. A calibration graph was constructed using a phosphate ion solution (GSO 7791-2000) at the concentrations from 50 to 500 µg/ml with a step of 50 µg/ml. ATP measurement was performed in triplicate for each sample.

The biophysical properties of the granulocyte cell surface were studied using INTEGRA VITA atomic force microscope (AFM) (the configuration based on the inverted optical microscope Olympus IX-71; the manufacturer NT MDT, Zelenograd, 2009). The electrical properties of erythrocytes and granulocyte membranes were assessed by measuring the surface potential (SP) in the Kelvin probe mode by AFM. The cell suspension for the surface potential (SP) measuring and the procedure for SP measuring were carried out according to the method described in (EA & MYu, 2014). The cantilevers with a conductive titanium coating of the NSG03/TiN series (Nanoworld, USA) were used for work. 20 erythrocytes and granulocytes were scanned from each sample; the resulting scans were processed using the Nova program (NT-MDT, Russia).

The measurement of intercellular adhesion forces was carried out on an AFM in force spectroscopy mode. A biosensor chip was constructed for this based on native erythrocyte and the tipless CSG11 (USA) according to the method developed by the team of authors (Skorkina, Shamray, & Sladkova, 2018). The choice of an erythrocyte as a biosensor is based on the idea that the population of erythrocytes in the microvasculature is the most numerous and actively interacts with other blood cells; moreover, the cell surface of an erythrocyte carries many antigenic determinants that can be presented for recognition by leukocytes upon contact with plasmalemmas. The force of intercellular adhesion was measured in the "erythrocyte-granulocyte" system, recording the force curves from the surface of 20 cells, thus, 400 measurements were made in the study. The adhesion forces were calculated using the Nova software, according to Hooke's law:

$$F = k \times \Delta Height \quad (1)$$

where F is the adhesion force, nN;

k — cantilever stiffness, N/m;

Δ Height - change of the scanner static tube length in the direction Z, nm.

Cell surface stiffness was assessed by numerical data on Young's modulus. The method for Young's modulus record is based on measuring the degree of the sample surface deformation when it interacts with the tip of the AFM probe (Alonso & Goldmann, 2003). We used modified AFM probes made by a team of authors on the basis of polymer microspheres with the curvature radius of 5 µm (Ellsworth et al., 2009). Young's modulus was measured by NTEGRA Vita AFM in the power spectroscopy mode, according to the algorithm described in (Skorkina, Fedorova, Muravyov, & Sladkova, 2012). 20 cells were scanned from each sample. Thus, the total sample of the cell population in the blood of healthy people was 400 cells in each experimental and control samples.

The results of experimental studies were processed by the methods of variation statistics. The significance of differences between the control and experimental samples was determined using the Student's t test at $p<0.05$ in the case of a normal distribution of the trait and the Mann-Whitney U test at $p<0.05$ for nonparametric data. The work presents the average values (M) and the values of the statistical error of the mean (m).

RESULTS

Under the influence of mechanical stress in vitro, the concentration of ATP increased by 147.7% ($p<0.05$) in the blood of the experimental group as compared to the control (table).

Table 1: ATP concentration in blood and biophysical properties of granulocyte cell surface

Values	Control	Experiment
ATP concentration in blood, µg/ml	63.9 ± 5.4	158.3 ± 7.3**
Adhesion force in "erythrocyte-granulocyte" system, nN	34.1 ± 0.2	52.7±0.5*
Young's modulus of granulocytes, µPa	4.16 ± 0.01	3.53 ± 0.02**
Erythrocyte surface potential, mV	-13.36 ± 7.0	-28.48 ± 4.9**

Granulocyte surface potential, mV	-38.5 ± 1.4	-29.08 ± 1.2**

*Note: *statistically significant differences between the indicators according to Mann-Whitney U-test (p<0.05).*
***statistically significant differences between indicators according to Student's test (p <0.05).*

Young's modulus of granulocytes decreased by 15% (p<0.05) in experimental blood samples as compared to the control. Under mechanical stress, when erythrocytes excrete ATP molecules into the intercellular space, both para- and autocrine effects of the ATP molecule were revealed, in particular, the surface potential of granulocytes decreased by 32% (p<0.05), and the charge of the cell surface of erythrocytes increased by 112% (p <0.05%) as compared to the control. More negatively charged surface of erythrocytes began to adhere more actively to the positively charged surface of granulocytes. Thus, the adhesion force increase by 55% (p<0.05) was established in the "erythrocyte-granulocyte" system as compared with the control.

DISCUSSION

The conditions of mechanical "stress" in vitro were simulated in the performed study - the force effect from the shifting layers of the moving plasma. This model was chosen to create the conditions that are as close as possible to the physiological parameters of the microvasculature (Migliorini et al., 2002). After the conducted experiments, it was found that the concentration of ATP in blood samples subjected to mechanical "stress" increased by almost 2.5 times. The data obtained confirm the fact of ATP molecule excretion into the intercellular space by erythrocytes in response to the force from the shifting plasma layers (Sprague et al., 2007). Consequently, the conditions (in vitro) for ATP release were created similar to physiological ones in experimental blood samples, which made it possible to study the auto- and paracrine effects of the ATP molecule on red blood cells and granulocytes. In particular, the paracrine effects of endogenous ATP were observed in the change of granulocyte biophysical properties - the decrease of plasmalemma rigidity and surface potential. Changes in the properties of granulocyte surface are probably mediated through the receptors of the purinergic signaling system, which trigger a whole cascade of signaling reactions through the mechanism of autocrine feedback According to the literature, a signaling role is assigned to the ATP molecule in the activation of the cell surface by P2X receptors of the 3-family (P2X1-P2X7) of activated ATP ligand-ion channels (Campwala & Fountain, 2013). The dominant subtypes of receptors of the purinergic signaling system on the plasmalemma of granulocytes are P2Y1, 2, 4, 6, 11 (Ellsworth et al., 2009). A number of studies have shown that ATP stimulates neutrophil chemotaxis (W. G. Junger, 2008; Verghese, Kneisler, & Boucheron, 1996). The released ATP is metabolized to adenosine on the cell surface and activates the P2Y and A3 receptors, which are necessary for cell orientation and movement in response to chemotactic stimuli (Chen et al., 2010). At the same time, a purinergic signaling cascade is implemented in neutrophils, which is associated with the intracellular Ca2+ level increase(Meshki, Tuluc, Bredetean, Garcia, & Kunapuli, 2006). It is possible that the launch of the purinergic intracellular signaling pathway leads to the experimentally observed decrease in rigidity and the loss of some negative charge on the cell surface of granulocytes. From the point of view of the functional activity of granulocyte evaluation, such a change in the biophysical properties of the plasmalemma is quite objective, since softer cells in the microvasculature migrate more easily and adhere more strongly to neighboring cells, which ensures that they perform direct physiological functions.

The autocrine effects of purinergic signaling were established in the performed experiment, which were associated with the negative charge increase of the erythrocyte membrane under the influence of mechanical stress by almost 2 times as compared with the control. Based on the literature data, we believe that in this case the decay products of the ATP molecule (in the form of ADP or adenosine) affect the charge of the cell surface. Now, it has been proven that there are no specific P receptors for the ATP molecule directly on the erythrocyte membrane (Erlinge & Burnstock, 2008), however, the receptors for ADP (ADP-P2Y13) (Wang et al., 2005) and adenosine (adenosine-A2B) (Oonishi, Sakashita, & Uyesaka, 1997). have been identified. The increase of the negative charge of the erythrocyte surface, from the point of view of blood flow through the microcirculatory bed, can be regarded as a positive physiological effect that prevents erythrocytes from sticking together and prevents the formation of aggregates in the narrow lumen of capillaries. At the same time, an important point in this study is the established increase of adhesion strength between erythrocytes and granulocytes, which confirms the important physiological role of erythrocytes in adhesive function of leukocyte enhancement. According to the Lattica-Bolcmana approach in the "virtual blood vessel" it is shown that the direct interaction between erythrocytes and leukocytes (flowing of leukocytes around erythrocytes) is necessary to increase the tangential force and torque, which promotes the rolling of leukocytes along the surface of the endothelial wall in the microvasculature (Pafundo, Alvarez, Krumschnabel, & Schwarzbaum, 2010). Consequently, during mechanical deformation of red cells in microvessels, they perform the regulatory function, triggering a cascade of signaling processes aimed at changing the biophysical properties of the plasma membrane of granulocytes - reduction of rigidity and the cell surface charge, which ensures the adhesive property increase of granulocytes and their physiological functions.

CONCLUSION

Thus, under the conditions of mechanical deformation stimulation of blood cells (the model of mechanical stress in vitro), they proved the increase of ATP concentration in the blood and the change in the biophysical properties of the plasma membrane of blood cells. Under the influence of mechanical stress, the rigidity and charge of the cell surface of granulocytes decreased, at the same time the surface of red blood cells acquired a more pronounced negative charge, which ultimately contributed to the adhesion force increase in the "erythrocyte-granulocyte" system. The data obtained indicate the leading regulatory role of the ATP molecule, which acts as a paracrine regulator of the biophysical properties of the plasma membrane of granulocytes under the conditions of blood cell mechanical deformation stimulation. The obtained experimental data are important for the study of intercellular interaction mechanisms in the microvasculature and can be taken into account during the search and development of pharmacological regulators of vascular tone.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

Alonso, J. L., & Goldmann, W. H. (2003). Feeling the forces: atomic force microscopy in cell biology. *Life sciences, 72*(23), 2553-2560. doi:https://doi.org/10.1016/S0024-3205(03)00165-6

Campwala, H., & Fountain, S. J. (2013). Constitutive and agonist stimulated ATP secretion in leukocytes. *Communicative & integrative biology, 6*(3), 4567-4575. doi:https://doi.org/10.4161/cib.23631

Chen, Y., Yao, Y., Sumi, Y., Li, A., To, U. K., Elkhal, A., . . . Hauser, C. (2010). Purinergic signaling: a fundamental mechanism in neutrophil activation. *Science signaling, 3*(125), ra45-ra45. doi:https://doi.org/10.1126/scisignal.2000549

Cowen, D. S., Lazarus, H. M., Shurin, S. B., Stoll, S. E., & Dubyak, G. R. (1989). Extracellular adenosine triphosphate activates calcium mobilization in human phagocytic leukocytes and neutrophil/monocyte progenitor cells. *The Journal of clinical investigation, 83*(5), 1651-1660. doi:https://doi.org/10.1172/JCI114064

EA, S., & MYu, S. (2014). Estimation of surface potential of lymphocytes from patients with leukemia using Kelvin probe mode. Biophysics, . *59*(3), 310-313.

Ellsworth, M. L., Ellis, C. G., Goldman, D., Stephenson, A. H., Dietrich, H. H., & Sprague, R. S. (2009). Erythrocytes: oxygen sensors and modulators of vascular tone. *Physiology, 24*(2), 107-116. doi:https://doi.org/10.1152/physiol.00038.2008

Erlinge, D., & Burnstock, G. (2008). P2 receptors in cardiovascular regulation and disease. *Purinergic signalling, 4*(1), 1-20. doi:https://doi.org/10.1007/s11302-007-9078-7

Junger, W. G. (2008). Purinergic regulation of neutrophil chemotaxis. *Cellular and Molecular Life Sciences, 65*(16), 2528-2540. doi:https://doi.org/10.1007/s00018-008-8095-1

Junger, W. G. (2011). Immune cell regulation by autocrine purinergic signalling. *Nature Reviews Immunology, 11*(3), 201-212. doi:https://doi.org/10.1038/nri2938

Meshki, J., Tuluc, F., Bredetean, O., Garcia, A., & Kunapuli, S. P. (2006). Signaling pathways downstream of P2 receptors in human neutrophils. *Purinergic signalling, 2*(3), 537-544. doi:https://doi.org/10.1007/s11302-006-9007-1

Migliorini, C., Qian, Y., Chen, H., Brown, E. B., Jain, R. K., & Munn, L. L. (2002). Red blood cells augment leukocyte rolling in a virtual blood vessel. *Biophysical journal, 83*(4), 1834-1841. doi:https://doi.org/10.1016/S0006-3495(02)73948-9

Oonishi, T., Sakashita, K., & Uyesaka, N. (1997). Regulation of red blood cell filterability by Ca2+ influx and cAMP-mediated signaling pathways. *American Journal of Physiology-Cell Physiology, 273*(6), C1828-C1834. doi:https://doi.org/10.1152/ajpcell.1997.273.6.C1828

Pafundo, D. E., Alvarez, C. L., Krumschnabel, G., & Schwarzbaum, P. J. (2010). A volume regulatory response can be triggered by nucleosides in human erythrocytes, a perfect osmometer no longer. *Journal of Biological Chemistry, 285*(9), 6134-6144. doi:https://doi.org/10.1074/jbc.M109.078246

Skorkina, M. Y., Fedorova, M. Z., Muravyov, A. V., & Sladkova, E. A. (2012). The use of nanomechanic sensor for studies of morphofunctional properties of lymphocytes from healthy donors and patients with chronic lymphoblastic leukemia. *Bulletin of Experimental Biology and Medicine, 154*(1), 163-166. doi:https://doi.org/10.1007/s10517-012-1899-x

Skorkina, M. Y., Shamray, E. A., & Sladkova, E. A. (2018). Measuring of Adhesion Force in the Cell—Cell System Based on Atomic Force Microscopy Technology. *Bulletin of Experimental Biology and Medicine, 164*(4), 528-529. doi:https://doi.org/10.1007/s10517-018-4026-9

Sprague, R. S., Stephenson, A. H., & Ellsworth, M. L. (2007). Red not dead: signaling in and from erythrocytes. *Trends in Endocrinology & Metabolism, 18*(9), 350-355. doi:https://doi.org/10.1016/j.tem.2007.08.008

TL, A., & GV, R. (1988). Biological chemistry: practice guide. Moscow: Graduate School: . 115-117.

Verghese, M. W., Kneisler, T. B., & Boucheron, J. A. (1996). P2U agonists induce chemotaxis and actin polymerization in human neutrophils and differentiated HL60 cells. *Journal of Biological Chemistry, 271*(26), 15597-15601. doi:https://doi.org/10.1074/jbc.271.26.15597

Wang, L., Olivecrona, G. r., Götberg, M., Olsson, M. L., Winzell, M. S. r., & Erlinge, D. (2005). ADP acting on P2Y13 receptors is a negative feedback pathway for ATP release from human red blood cells. *Circulation research, 96*(2), 189-196. doi:https://doi.org/10.1161/01.RES.0000153670.07559.E4