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CELL BIOPHYSICS

The Change of Electrical Properties of Blood Corpuscles under in vitro Mechanical Stress

E. A. Sladkova*

Belgorod National Research University, Belgorod, 308015 Russia

**e-mail: sladkova@bsu.edu.ru*

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Abstract—Changes in the electrical properties of blood corpuscles that are experiencing mechanical stress modeled in vitro have been studied. An increase in the concentration of ATP molecules in the intercellular space in response to the mechanical effect of moving plasma layers both in the blood of healthy people and patients with acute lymphoblastic leukemia is demonstrated. The surface potential of red blood cells and platelets becomes more positive both in the blood of healthy people and in leukemia patients. In contrast, the negative charge of lymphocytes in healthy people decreases in response to mechanical stress but increases in the blood of patients with acute lymphoblastic leukemia.

Keywords: mechanical stress, purinergic signaling system, surface potential, lymphocytes, red blood cells, platelets

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Purinergic signaling is a complex system in which ATP and related molecules act as cell-to-cell messengers. When released to the intercellular space, ATP activates specific receptors of the P2 family [1]. Simultaneously, ectonucleotidases convert ATP to dephosphorylated metabolites, including adenosine. The latter, in turn, stimulates P1 receptors. The activity of both receptors affects various cell processes [2]. Four subtypes of P1 (P1R) receptors (A1, A2A, A2B, and A3), eight subtypes of P2Y receptors (P2Y1, 2, 4, 6, 11, 12, 13, and 14), and seven P2X receptors (P2X1 through 7) are known [1]. Generally, all subtypes of P1 and P2 are expressed by immune cells, depending on cell type and differentiation. They play important roles in inflammatory processes.

Recently, a number of studies have been dedicated to the pathophysiological role of purinergic signaling and its therapeutic potential in various diseases [3–5]. There is evidence that various kinds of tumor cells release considerable amounts of ATP in response to mechanical deformation, hypoxia, and some agents, as well as to subsequent necrosis and ischemia [4]. P2X7 receptors have been found in human leukemic lymphocytes. Some data indicate that the expression and function of P2X7 receptors, which may mediate cell death or proliferation depending on the intensity of activation, correlate with the severity of lymphoblastic leukemia [6].

Abbreviation: ALL—acute lymphoblastic leukemia.

It has been shown that erythrocytes that pass through narrow lumina of capillaries and experience mechanical deformation release ATP molecules, which are degraded in the intercellular space within seconds, upon being digested by ectonucleotidases [7]. Their metabolites, ADP and adenosine, interact with A2 family receptors on the erythrocyte surface to further enhance ATP release through pannexin 1 channels [8].

With regard to the fact that mechanical action on blood cells may stimulate the operation of the purinergic signaling system and purine receptors act as ion channels [9, 10], it is pertinent to investigate the electric properties of various cell populations under mechanical stress conditions in the norm and in pathological processes.

The goal of this work is to study changes in electric properties of blood corpuscles in mechanical stress in vitro.

MATERIALS AND METHODS

Peripheral blood samples were taken from 30 adult healthy volunteers and 30 patients with acute lymphoblastic leukemia (ALL). Samples were taken from the cubital vein into disposable sterile vacutainers with anticoagulant EDTA-K2 at the concentration 2.0 mg (0.006843 mol/L) per 1 mL of blood. The procedure was conducted by skilled personnel of the laboratory of the St. Ioasaf Belgorod regional clinical hospital.

Venous blood samples from healthy donors and ALL patients were divided into two portions each. In the experimental portions, purinergic signaling pathways were activated by the mechanical stress model as in [10]. The control portions were left intact.

Blood ATP was assayed colorimetrically [11, 12]. The assay was based on the elimination of two phosphoric acid moieties by brief hydrolysis under acidic conditions. The amount of phosphorus that was unstably bound to ATP in blood samples was assessed from the contents of organic phosphorus before and after the hydrolysis. Whole blood (0.1 mL) was placed in a tube cooled on ice and homogenized with 1 mL of 2.5% trichloroacetic acid for 5 min. One milliliter of normal saline (0.9%) was added and extraction was continued on ice for another 5 min. Half-milliliter portions of the resulting solution were placed into two tubes; 1 mL of 1 M hydrochloric acid was added to the experimental portion and it was placed into a boiling water bath for 10 min to hydrolyze phosphate bonds. The solution was cooled and 1 mL of 1 M sodium hydroxide was added. One milliliter of 1 M sodium hydroxide and one milliliter of 1 M hydrochloric acid were added to the control tube without boiling; 7.5 mL of normal saline was added to each tube. For the qualitative assay, 5 mL of liquid was taken from both tubes into fresh ones. To each portion, 0.5 mL of 1 M ammonium molybdate, 0.5 mL of 1% ascorbic acid, and 2 mL of normal saline were added. The contents were rapidly mixed and left at room temperature for 10 min.

The experimental and control samples were analyzed on a KFK-3 photometer (Russia) vs. normal saline at $\lambda = 670$ nm. ATP concentrations were calculated from the differences in optical density between the control tube (without acidic hydrolysis) and the experimental tube with reference to a standard curve. The standard curve was plotted by using phosphate ion solutions (GSO 77912000) at concentrations from 50 to 500 μg/mL at 50-μg/mL intervals. ATP was measured in three replications for each sample.

Blood corpuscle samples from the experimental and control groups were prepared as follows: to separate corpuscles into erythrocytes, leukocytes, and platelets, experimental and control tubes with blood were centrifuged at 1500 rpm for 15 min. The bottom fraction of plasma and the leukocyte layer were collected into another tube and centrifuged at 1500 rpm for 10 min. The supernatant was discarded. The resulting leukocyte suspension was separated into granulocytes and lymphocytes with an EasySep Magnet and an EasySep/EasySep Direct Human Total Lymphocyte Isolation Kit (StemCell) (Thermo Scientific, United States). Platelet suspensions were prepared as in [13].

Cell surface potentials were measured with an INTEGRA Vita atomic force microscope (assembly on the base of the Olympus IX71 inverted optical microscope, NT-MDT Company, Zelenograd, Russia). No less than 15 cells were scanned from each sample. The scanning was performed by the semicontact Kelvin probe method. Use was made of cantilevers with conducting titanium coating of the NSG03/TiN series (Nanoworld, United States). Cells were suspended, and the surface potentials were measured as in [14]. The resulting images were processed with Nova software (NT-MDT Company) with the Point Instruments tool. For each cell, surface potentials were measured at 20 sites and the mean value was calculated.

The significance of the differences between the experimental and control samples was assessed by the Student's t test at $p < 0.05$ for the normal distribution of data. The mean values (M) and standard errors of the mean (m) are indicated.

RESULTS AND DISCUSSION

Mechanical stress increased the ATP level in the blood of healthy donors by a factor of 2.3 ($p < 0.05$) in comparison to intact blood. In ALL patients whose blood corpuscles were exposed to shear strain, ATP increased by a factor of 1.8 ($p < 0.05$) in comparison to the control group.

The surface potential of erythrocytes and platelets became more positive after mechanical stress. The charge of erythrocytes increased by 44% ($p < 0.05$), and the charge of platelets, by 40% ($p < 0.05$). In contrast, the potential of the lymphocyte surface became more negative, by 47% ($p < 0.05$) compared to the control (Fig. 1). In the blood of ALL patients exposed to mechanical stress the surface potential of blood cells became more positive in comparison to the control group. The surface potential of erythrocytes increased by 34% ($p < 0.05$); lymphocytes, by 27% ($p < 0.05$); and platelets, by 34% ($p < 0.05$) (Fig. 2).

The ATP level in blood exposed to mechanical deformation of corpuscles was higher than without mechanical stress. Our data point to ATP release to the intercellular space under mechanical stress, which is consistent with data reported by other authors [7, 15].

The modeled mechanically induced ATP release from blood cells affected their electric indices. We associate the change in the surface potential with the triggering and operation of the purinergic signaling pathway under mechanical stress [7]. It has been proven that erythrocyte membranes lack P receptors specific to ATP, while receptors for ADP and adenosine have been identified [16]. Thus, we infer that ATP metabolites might affect the change of electric properties of red blood cells owing to the entrance of calcium ions through an ionic pore [17], because purine receptors act as ion channels [9, 10]. Receptors of the P2X family located on lymphocyte surface have been described and the interaction of ATP molecules with these receptors causes Ca^{2+} channel opening [18].

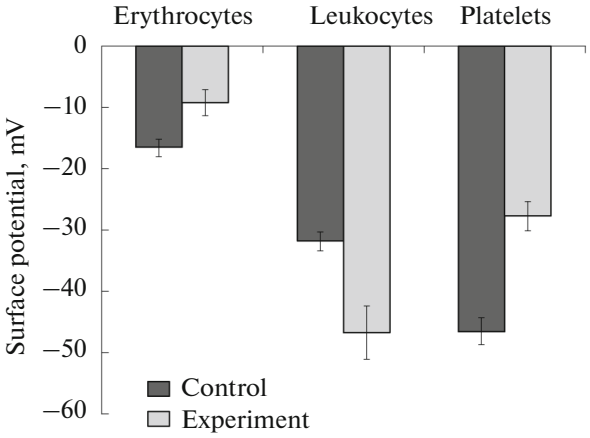

Fig. 1. The surface potential of blood corpuscles from healthy people. Experiment: under mechanical stress in vitro; control: intact blood. *Differences between the experimental and control groups are significant at $p < 0.05$, Student's t test.

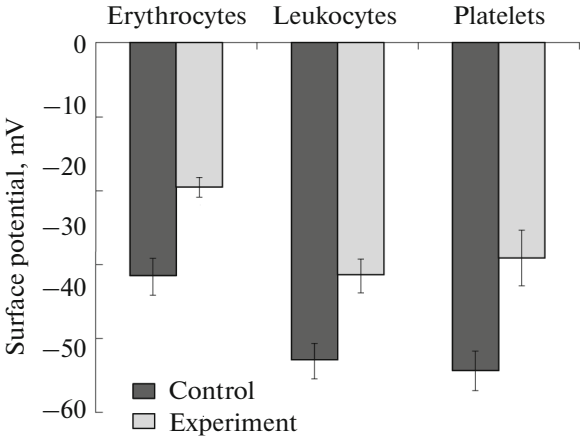

Fig. 2. The surface potential of blood corpuscles from patients with acute lymphoblastic leukemia. Experiment: under mechanical stress in vitro; control: intact blood. *Differences between the experimental and control groups are significant at $p < 0.05$, Student's t test.

This process may be related to the detected change in the lymphocyte surface potential. We presume that the increase in the surface charge of platelets is mediated by the interaction of ATP metabolites with P2Y receptors on the corpuscle surface [19].

In leukemia, the charges of erythrocytes and platelets increase, as in healthy people, whereas leukemic lymphocytes from ALL patients experience the opposite surface charge change in comparison to healthy cells. This phenomenon may be caused by elevated expression of the P2X7R receptor on the surface of leukemic cells [6], and the receptor may be activated by mechanical action on the cells. The increase in ATP concentration favors activation of the P2X7R purinergic receptor; its consequence is the instantaneous increase in the contents of intracellular Na^+ and Ca^{2+} in leukemic lymphocytes rather than Ca^{2+} alone [9]. With regard to this fact, we suppose that the higher positive charge on leukemic lymphocytes results from the opening of ion channels for both Na^+ and Ca^{2+}. The increase in platelet charge in ALL patients may be associated with activation of P2Y12 and P2Y1 by ATP molecules and also with direct action of metabolites of leukemic cells, causing depolarization of the platelet membrane [20, 21].

Thus, we demonstrated an increase in the ATP level in the intercellular space in response to mechanical stress in vitro. The ATP level in the blood of ALL patients is lower in both intact blood and at mechanical stress, which may be associated with altered erythrocyte functioning in the disease.

Our data bring us to the conclusion that mechanical stress may alter the electrical properties of the plasma membrane of blood corpuscles in healthy people and in patients with acute lymphoblastic leukemia. The detection of oppositely directed changes in the surface potential of lymphocytes in healthy people and in ALL patients is an essential result. In healthy people, the lymphocyte surface charge becomes more negative, while in ALL patients it becomes more positive. The revealed regularities may contribute to the study of intercellular interactions in the microvasculature. They can also be taken into consideration in the search for pharmacological regulatory targets to support the function of immunocompetent cells in disease.

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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, with later amendments. Informed consent was obtained from each participant of the study.

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