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## The changes of ultrastructure and mechanical properties of lymphocytes' plasmalemma under the influence of chemotherapy

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### ABSTRACT

Atomic force microscopy has established the changes of mechanical properties of lymphocytes' membranes from patients with acute and chronic lymphoblastic leukaemia. The stiffness of cell membrane was increased under influence chemotherapy along with the ultrastructural organisation of the lymphocyte's surface was different between patients with acute and chronic leukaemia. In the blood of patients with acute leukaemia were circulated two types of lymphocytes with small and large spherical globules on the surface, respectively. The lymphocytes from patients with chronic lymphoblastic leukaemia carried on their surface the large structures of irregular shape. Significant differences in the structural properties of lymphocyte's surface in patients with acute and chronic lymphoblastic leukaemia point at differences cell response to the action of chemotherapy drugs.



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### INTRODUCTION

The main part of modern antitumor drugs aims to DNA damage (Belova *et al.*, 2014) and induction of active forms of oxygen initiating of the death of tumour cell (Katsuda *et al.*, 2010; Itoh *et al.*, 2011). However, cases of formation of cell resistance to chemotherapy are often which indicates the inclusion of cellular mechanisms that promote cell survival. According to the literature data, it may be the processes forming intracellular antioxidant, reaction blocking of apoptosis, the system of DNA reparation and ATP-dependent transporters pumping out the toxic substances out of cells (Volkova *et al.*,

2012). In the development of drug resistance, the cell membrane has an essential role. Biophysical properties of biomembrane (for example, stiffness and charge) predetermine the ability of the drug to achieve the intracellular molecular targets. In particular, according to a few studies, it was shown a decrease in the surface tension of membrane under the influence of drugs anthracycline drugs which are widely used in standard treatment regimens for various types of leukaemia (Bell *et al.*, 2013). The aim of current work was the investigation of features of ultrastructure and mechanical properties of lymphocytes in the patients after chemotherapy treatment.

### MATERIALS AND METHODS

In the study was used the peripheral blood from patients (men and women from 22 to 48 years old) with acute lymphoblastic leukaemia (ALL; n=25) and chronic lymphoblastic B-cell's leukaemia (B-CLL; n=25) in the primary diagnosis and after treatment (n=25 ALL; n=25 B-CLL). It was used the DNA-direct action chemotherapy drugs (doxorubicin and cisplatin) which patients gave according to the standard schemes prescribed by the doctor.

Blood sampling and diagnosis were performed by the Diagnostic Laboratory of the Belgorod Region Hospital of Sv. Ioasaf with the participation of specialised medical personnel. The study was approved by the local ethics committee of the Medical Institute of Belgorod State National Research University and informed consent of all subjects were obtained according to the recommendations of the Declaration of Helsinki (The International Response to Helsinki VI, The WMA's Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects, as adopted by the 52nd WMA General Assembly, Edinburg, October 2000).

The ultrastructure of lymphocytes' surface was studied by atomic force microscope (AFM) INTEGRA Vita (NT-MDT, Zelenograd, Ruccia, 2009) in the semi contact mode. It was used the probe NSG03 with a radius of curvature 10 nm for scanning. The blood preparation for AFM was carried out according to the methods described earlier (Sladkova, Skorkina, 2013). It was scanned 15 cells from each sample. On scans, the surface profile size 3.0X3.0µm were constructed and measure dimensions and calculated the number of globular protrusions by software Nova.

AFM studied the elastic properties of lymphocytes in the force spectroscopy mode (15 cells from each sample). The measuring of Young's module was performed on AFM according to the algorithm described in an earlier work (Skorkina *et al.*, 2012).

Variation statistics methods processed the results. The obtained data were within the normal distribution range and the significance of differences was evaluated using the Student *t*-test at  $p < 0.05$ .

## RESULTS

The globular protrusions of the different configuration and deepening on the lymphocytes' surface were identified (fig.1).

In the group patients with ALL in the primary diagnosis the relief of surface was smoothed (fig. 1a). In the group, ALL after treatment was identified two types of lymphocytes with a different configuration of plasmalemma (fig. 1 b,c). For the first type lymphocytes was typical by the presence of small globular protrusions and large invaginations of plasmalemma. For the second type lymphocytes in the relief of surface was found the globular protrusions with sphere form and rare invaginations.

The diameter and deepening invaginations of membrane the first type lymphocytes increased by 7 and 8 times ( $p < 0.05$ ) as compared with the same structures of lymphocytes in the patients with ALL at the primary diagnosis (table 1). The height of the globular protrusions on the second types of lymphocytes' surface increased by 6.8 times ( $p < 0.05$ ).

Invaginations on the second type of lymphocytes' surface were not found.

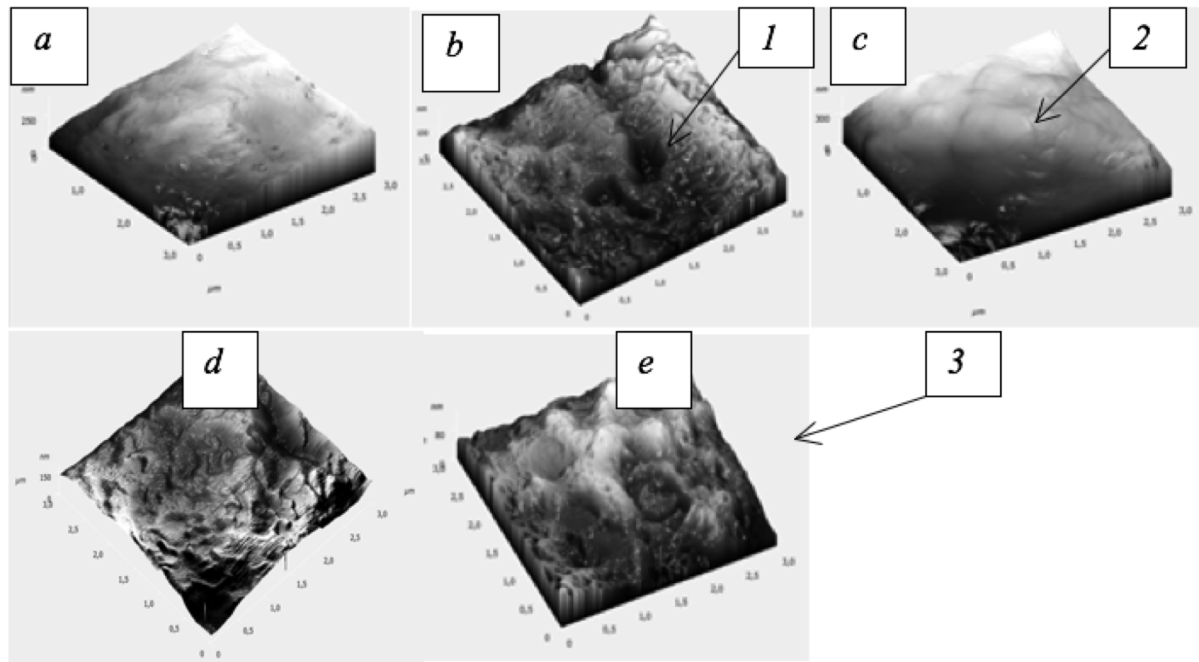
The elastic properties of the lymphocytes in both investigated group have not any significant differences. However, Young's module of the lymphocytes with ALL after treatment increased by 1.5 times ( $p < 0.05$ ; see table 1) as compared with lymphocytes from patients with ALL before treatment.

In the group patients with CLL, the ultrastructure of surface both at the primary diagnosis and after treatment was folded (see fig. 1 c, d). However, in the group patients with CLL after treatment, the total numbers of the morphological structures decreased and the height of globules and deepening of invaginations increased by 1.9 and 20.7 times ( $p < 0.05$ ) as compared with lymphocytes from patients with CLL before treatment. The stiffness of lymphocytes' surface in the patients with CLL after treatment increased by 5.2 times ( $p < 0.05$ ) as compared with cells before treatment.

## DISCUSSION

In the study, the ultrastructure and mechanical properties of lymphocytes in the patients with ALL and CLL before and after chemotherapy treatment have been investigated. It was established the significant differences between the features of the ultrastructure of plasmalemma in the patients with ALL before and after treatment. In the patients with ALL in the primary diagnosis for the relief of surfaced was characterised by smoothness but after treatment on the surface was identified two types of lymphocytes with a different configuration of plasmalemma. The presence in the peripheral blood of patients with ALL after treatment two type's lymphocytes with a different configuration of the surface may be related with forming two populations of heterogenous lymphocytes in the process of developing of leukaemia which respond differently to chemotherapy (Barret, 2007). Appearance lymphocytes with large sphere globules on the plasmalemma (the second types) may be an expression of toxic damage the blood cells of chemotherapy drugs (Charras, 2008) that it leads to the disruption of work the functional groups of the membrane proteins and proteins of cytoskeleton (Gough, 2009). The stiffness of lymphocytes' membranes in the patients with ALL after treatment increased that it points to the influence of chemotherapy drugs to the elastic properties of membrane and changes of microrheological properties of the cell (Tikhomorova *et al.*, 2016).

In the patients with B-CLL, the ultrastructure of plasmalemma was folded that pictures were pronounced after chemotherapy drugs. Reducing the



**Figure 1: Relief of lymphocytes' surface** *a* – patients with ALL in the primary diagnosis, *b* – patient with ALL after treatment (1 type), *c*– patient with ALL after treatment (2 type), *d* – patient with CLL in the primary diagnosis, *e* – patient with CLL after treatment; 1 – the large invaginations of plasma-lemma; 2 – the globular protrusion of spherical form; 3 –the clusters of globular protrusions.

**Table 1: Ultrastructure and mechanical properties of lymphocytes from patients with ALL and CLL**

Parameters		ALL (the primary diagnosis)	ALL (after treatment)		CLL (the primary diagnosis)	CLL (after treatment)
			Type 1	Type 2		
Globulus protrusions ( <i>M±m, n=25</i> )	height, nm	7.3±0.5	5.0 ±0.2	39.0±0.5*	17.6±0.9	52.0 ±0.8*
	number	3.0±0.1	12.0±0.7*	5.0 ±0.3	125.0 ±1.1	40.0 ±0.2*
Invaginations ( <i>M±m, n=25</i> )	diameter, nm	3.4±0.2	27.5±0.7*	3.0 ±0.8	149.4±12.9	14.0 ±0.9*
	deepening, nm	11.6±0.3	105.0±0.3*	12.0 ±0.5	8.0±0.9	174.4±0.2*
	number	5.0 ±0.5	3.0 ±0.3	1.0 ±0.3*	40.0 ±2.3	30.0 ±0.1*
Young's module, µPa ( <i>M±m, n=25</i> )		1.4±0.2	3.5±0.1*		1.8±0.01	11.2±0.5*

\*Statistically reliable differences in group patients at the primary diagnosis as compared with the group patients after treatment at  $p < 0.05$ ; ALL – acute lymphoblastic leukaemia, CLL – chronic lymphoblastic leukaemia.

elastic properties of the membrane while forming the large of globules collected in the irregular form may be related with degradation of the lipid bilayer of the membrane and damage the metabolism of glycerophospholipids in the cell of patients with CLL (Kazaryan, Galoyan, 2011).

In the whole, the stiffness of lymphocytes' membrane in the patients with B-CLL was increased as compared with lymphocytes' membrane in the patients with ALL at the primary diagnosis. For lymphocytes, after chemotherapy treatment in both groups, ALL and B-CLL has been established the increase of membrane's stiffness that it points at to

the similar influence of the chemotherapy drugs to the mechanical properties of lymphocytes' surface.

**CONCLUSION**

Thus, in the samples of blood from patients with ALL after chemotherapy treatment have been found in the lymphocytes with different ultrastructure of cell's surface. For the first cell's type was characterised by the small globules and large invaginations, but for the second types on the surface was presented the large globules with rare invaginations. Lymphocytes from patients with B-CLL after treatment was carried on the surface the large globules of irregular forms. It has been shown that

under the influence of chemotherapy drugs the stiffness of the cell's surface increased in both groups patients with ALL and B-CLL as compared with the groups at the primary diagnosis. At the same time, significant differences of the ultrastructure of plasmalemma between patients with ALL and B-CLL point at the different degree reaction of the cell's surface depend on the differentiation of lymphocytes and determinate conformational rearrangements under the influence of chemotherapy drugs. We can suppose that the response of lymphocytes to chemotherapy drugs may depend on the type of lymphoblastic leukaemia and the degree of maturity of the cell population.

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