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Micromechanical properties of lymphoid cells in patients with acute lymphoblastic leucosis

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Abstract. The aim of this paper was to study the elastic and electrical properties of lymphoid cells in patients with acute lymphoblastic leucosis (ALL, $n = 15$). The mechanical properties of the membrane of lymphoid cells was recorded by atomic force microscopy (AFM). The electrical properties of the lymphoid cell membranes were detected by the Kelvin method. The elastic and electrical membrane properties of lymphoid cells were recorded by incubation with doxorubicin, one of chemotherapy drugs. We used different concentrations and incubation time. It was shown that in the acute phase of the disease and the stage of stable remission the lymphoid cell clones with a reduced stiffness and increased cell surface charge were found. In the experiments *in vitro* was demonstrated that the increased cell membrane rigidity may be one of the factors determining the tumor cell resistance to the chemotherapy. It was found that if to use the highest concentration of drug in the incubation medium (0.5–1.0 mg/ml) and its longest time, then the surviving cells had more elastic membrane (0.25–1.0 μPa) and the positive potential of the membrane surface (15–29 mV). These obtained data may have a significant prognostic importance for the evaluation of drug resistance of tumor cells.

Keywords: Elastic and electrical properties of cells, acute lymphoblastic leucosis, doxorubicin

1. Introduction

The development of acute forms of proliferative processes in leukocytes is accompanied by the accumulation in the bone marrow and peripheral blood cell subpopulations with altered molecular mechanisms of intracellular signal transmission [1–4]. It can be assumed that the result of the accumulation of abnormal cell forms is the development of drug resistance, and low patient survival [5]. In the development of drug resistance of tumor cells an essential role play cytogenetic and molecular abnormalities [6, 7]. However, this process can be affected by negative changes of micromechanical membrane properties. Coinciding, the mechanical and electrical properties of plasmatic membranes of cells may affect the delivery of the drug to the intracellular molecular targets. It was shown that under the influence of anthracycline drugs a reduction of the membrane surface tension occurs [8]. As a result of this the activity of vesicular transport and endocytosis intensity were altered [9]. In addition, it was shown that the vesicular drug transport depends on the biochemical composition and membrane fluidity. These properties of the tumor cell membranes, resistant to chemotherapy, were increased [10].

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Thus the aim of this study was to investigate the elastic and electrical properties of tumor lymphoid clones in patients with acute lymphoblastic leucosis at the acute stage and remission period of the disease.

2. Subjects and methods

2.1. Subjects

Blood samples for the cell micromechanical measurements (10 ml) were drawn via sterile venipuncture using heparin (5 IU/ml) as the anticoagulant and processed within four hours after collection; all preparations and measurements were carried out at room temperature ($20 \pm 1^{\circ}$C).

Two groups of patients have been formed to carry out the study, suffering from leukemia: 1) Patients with an acute form of the disease ($n = 20$), and 2) patients at the stage stable remission of the disease (after treatment with standard chemotherapeutic regimens, including the use of doxorubicin, $n = 20$). Diagnosis and treatment of acute leukemia were carried out according to cytochemical diagnostic algorithm adopted in clinical oncohematology [11] in Regional Clinical Hospital. St. Joasaph, Belgorod. Prior to entering this study, the patients had no chemotherapy.

The study was approved by the local ethic committee of Medical Institute of Belgorod State 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 52th WMA General Assembly, Edinburg, October, 2000). Twenty healthy volunteers participated in the study as a control.

2.2. Atomic force microscopy

Estimation of elastic properties of blasts forms of lymphocytes was performed using atomic force microscopy (AFM, Integra-Vita instrument configuration on the base of in-versed optical microscope Olympus IX-71, NT-MDT, Zelenograd, Russia, 2009). Samples were prepared by application native cell suspensions on the defatted glass substrate. Scanning of the cells was performed in the atomic force spectroscopy mode using the cantilever NSG03S. Thus from each sample were scanned at 20 cells. With the help of a special computer program «Nova» imposition of load at 25 plots of cell surface were performed. The obtained AFM curves were processed using "Ef3" software (NT-MDT, Russia). Processing of obtained scans was performed in software "Nova" (NT-MDT, Russia). Based on the data the index was calculated of rigidity of the membrane (Young's modulus, μPa).

The electrical properties of the tumor cell membrane were measured in the Kelvin probe mode. Cells suspensions for measuring of surface potential were prepared by way described in details elsewhere [12]. Measuring of surface potential was implemented by probe with conductive titanium coating series NSG03/TiN (Nanoworld, USA). The surface potential was also measured for 20 cells in each sample.

2.3. Protocols for in vitro lymphoid cells studies

Lymphoid cells were selected by centrifugation at 1500 rpm for 5 min with followed three times rewashed by RPMI-1640 and then resuspended in the same medium. Before the basic experiments, were evaluated on cell survival by staining Blue. For this purpose was combined 1 μl of the cell suspension mixed with 0.4% Trypan Blue in phosphate buffer (pH 7.2-7.3). Then counted number dead

cells using a light microscope Olimpus. For the subsequent experiments were used cell populations with survival not less than 98%.

Investigation of mechanical and electrical properties of doxorubicin resistance cells was performed *in vitro* study. Doxorubicin (adriamycin) is chemotherapeutic antibiotic that used like anti-neoplastic agents some solid tumors, leukemia and lymphoma [13]. In these experiments a sample of blood of 15 patients with ALL were included. In well cell culture plate was added in increasing concentrations of doxorubicin (0.001; 0.01; 0.1; 0.5; 1.0 mg/ml): to 10 μl cells suspension was added 1 μl doxorubicin. As a control 1 well without added doxorubicin was used. The cell incubation was performed into the CO_2 incubator at 37°C for one and 24 hours. After incubation, the cell survival was assessed. The doxorubicin resistance cells were washed three times by medium 199 with Hank's BSS. Then was studied elastic and electrical properties of the cell membrane surface by used various mode scanning of AFM according to scheme described above.

2.4. Statistics and data presentation

The results are presented as mean ± SEM. Comparisons were made using paired *t*-tests, and a two-tailed p value of $p < 0.05$ was considered to be significant; given the exploratory nature of these studies, p values are reported as determined.

3. Results

3.1. Biomechanical properties of tumor blood cells from patients with ALL before and after the treatment

In the blood samples of patients with ALL before the treatment were found circulated blast forms of lymphoid type (Fig. 1). It has been found that the Young's modulus of lymphoblasts was reduced by 49% ($p < 0.05$), whereas the cell surface charge – increased by 32% ($p < 0.05$) compared with normal lymphocytes (Table 1). After doxorubicin-based chemotherapy on the stage of ALL remission did not reveal blasts forms in the blood of patients (Fig. 2). In these patients after treatment index lymphocyte rigidity has remained reduced by 26% ($p < 0.05$), and the surface charge was increased by 27% ($p < 0.05$) compared with normal cells (Table 1).

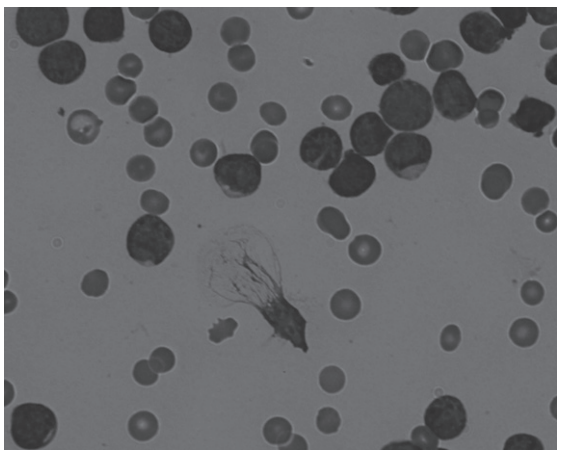

Fig. 1. The sample of blood from the patient with ALL (before the treatment).

Table 1
The value of the surface potential and Young's modules of lymphoid cells from patients with ALL (M ± m; $n = 50$)

Samples	Young's modules (μPa)	Surface potential (mV)
Normal lymphocytes (control)	3.5 ± 0.2	-37.3 ± 0.6
Lymphoblast (before the treatment)	1.77 ± 0.2[a]	-28.36 ± 0.1[a]
Lymphocytes (after treatment)	2.58 ± 0.1[a]	-29.43 ± 0.3[a]

[a]Statistically reliable differences in experiment as compared with the control at $p < 0.05$.

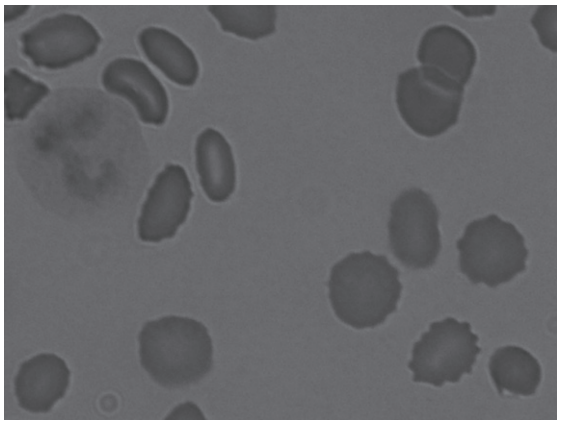

Fig. 2. The sample of blood from patient with ALL (after the treatment).

3.2. Micromechanical properties of tumor blood cells after incubation with doxorubicin

Upon incubation the cells with doxorubicin it was found that the number of viable lymphoblasts was significantly reduced. This reduction was dose-dependent manner (Fig. 3). By increasing the drug concentration in the incubation medium to about 0.5 mg/ml, the percentage of viable cells after one hour of incubation decreased almost two times, and at concentration up to 1.0 mg/ml – 4 times as compared with the control. It is interesting to note that after an hour of incubation at drug concentrations of 0.001 mg/ml 97% survived tumor lymphoblasts, whereas increasing the dose to 1.0 mg/ml viability retained only 24% of the cells (Fig. 3).

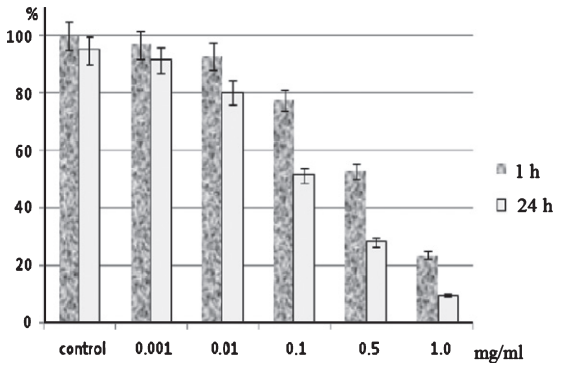

Fig. 3. Percent doxorubicin-resistant cell from patients with acute lymphoblastic leucosis.

Table 2
The value of the surface potential and Young's modules of the doxorubicin resistance cells (M ± m; $n = 50$)

Samples	Surface potential (mV)		Young's modules (μPa)	
	1 h	24 h	1 h	24 h
Control	–28.36 ± 0.12		1.78 ± 0.001	
0.001 mg/ml	–26.71 ± 0.34	–29.36 ± 0.38	1.84 ± 0.002	1.71 ± 0.001
0.01 mg/ml	–30.71 ± 0.25*	–35.82 ± 0.17*	2.24 ± 0.001*	1.38 ± 0.002*
0.1 mg/ml	–39.18 ± 0.71*	–51.27 ± 0.24*	3.47 ± 0.002*	1.21 ± 0.002*
0.5 mg/ml	–54.22 ± 1.13*	–11.23 ± 0.22*	5.14 ± 0.001*	0.90 ± 0.001*
1.0 mg/ml	15.76 ± 2.19*	29.18 ± 0.27*	0.58 ± 0.001*	0.39 ± 0.001*

*Statistically reliable differences in experiment as compared with the control at $p < 0.05$.

After 24 h incubation, the percent of doxorubicin resistance cells was decreased depending on the concentration of drug. So in the medium containing 0.001 mg/ml of doxorubicin have survived 91.5 ± 0.8% cell population, but with increasing the concentration of drug to 1.0 mg/ml the tumor cell survival was 9.6 ± 1.3% only. The survivability of malignant cells closely connected with their functional activity in this connection in current study was measured the charge surface of membrane in the doxorubicin resistance cells. According to the findings in studied samples in the concentration of doxorubicin 0.0001 mg/ml significant changes of charge membrane as compared with a control were found (Table 2). After hour incubation, with increasing in the medium of drug concentration to 0.01; 0.1 and 0.5 mg/ml in the resistance malignant cells were observed rose the surface potential of membrane respectively by 8%, 38% and 91% ($p < 0.05$) as compared with control. It is important to note, that with increasing drug concentration in the incubation medium to 1.0 mg/ml the resistance cells lost their negative charge.

After 24 h incubation the number of doxorubicin-resistant cells was decreased depending on the concentration of the drug. For example, in a medium containing 0.5 mg/ml of doxorubicin, survival rate was 92% of the whole cell population, while increasing the drug concentration up to 1.0 mg/ml led to 10% of tumor cell survival only. When the concentration of doxorubicin in the medium was 0.001 mg/ml, significant alteration in the membrane surface potential was not detected (Table 2). Whereas the rise in drug concentration from 0.01 to 0.5 mg/ml has led to the gain of potential increase from 8 to 91% was found ($p < 0.05$).

In a population of doxorubicin-resistant lymphoblasts observed changes not only electrical but also the elastic properties of the membrane. So after one hour incubation with the drug at concentrations of 0.01, 0.1 and 0.5 mg/ml of cell membrane rigidity increased by 26%, 95% and 189% ($p < 0.05$), respectively, compared with the control. However, when the maximum concentration of drug at this series of experiments was used, equal to 1.0 mg/ml, in the remaining populations of the doxorubicin-resistant cells cell membrane rigidity was reduced by 67% ($p < 0.05$; Table 2). At the same time after 24 hours of incubation with the drug in the samples remained resistant to doxorubicin cell forms. Their rigidity was significantly lower than control values.

4. Conclusion

The results of study showed that at acute leukemia, the cell membrane rigidity of lymphoblasts is reduced, and their surface potential is increased. Such alterations of the membrane properties can provide a high degree of deformability of lymphoblasts and their migration activity in microcirculation.

In *in vitro* experiments detected a close relationship between the properties of the cell membrane of tumor cells and their resistance to chemotherapy. So for example at low concentrations of doxorubicin in the medium (0.001 mg/ml), a large percentage (up to 95%) surviving cells kept high membrane rigidity and its negative charge. These data are consistent with opinion that doxorubicin enters the cell by diffusion [14, 15]. It has been shown that penetration of drug through membrane by diffusion led to death of leukemic clones which partially differentiated in cell cycle (in our study the number of these cells doesn't exceed 4–6%). However, with increasing drug concentrations and incubation time to 24 hours, the cell membrane becomes softer and a cell surface charge increased gradually, reaching a positive value at the highest doxorubicin concentrations in the medium (1.0 mg/ml). These effects likely are associated with a high affinity of doxorubicin to membrane lipids [16, 17]. In model experiments on the cell lines it has shown that doxorubicin decreased the surface tension difference between the lipid layers in the membrane, but it does not change the surface potential of the cells [8]. However, according to our data, obtained on the living cell systems, there was an increase of the surface potential of lymphoblasts. At high drug concentrations were detected the cell populations which still exhibited resistance to chemotherapy. They had a positively charged cell surface and a quite soft membrane. The mechanism of cell survival is likely related to the change of the membrane mechanical properties and membrane transporters operation under the influence of high doses of doxorubicin. There is evidence that the drug accumulates in the lysosomes, which are involved in the formation of drug-resistant tumor cells [18]. In its turn, the formation of lysosomes related to the difference of the surface tension forces between the bilayers and the formation of membrane bends [9]. Since doxorubicin has high affinity for membrane lipids, it can change the fluidity of plasma membranes [19]. In the turn, this may alter the membrane transporter functions [20, 21]. It is known that multi-drug resistant cells of patients with acute forms of leukemia are associated with the expression and mutation in the cell membrane adenosine triphosphate cassette transporters [22, 23]. Under these conditions their functional activity was changed after using anthracyclines in chemotherapy regimens [24]. There is evidence that resistance of tumor cells correlates with the presence in the membrane transport pump, which can remove the drug from the cell [25].

Taken together these data showed that the electrical and mechanical properties of the surface of tumor cells may have importance in the prediction of drug resistance and can serve as a functional state of objective markers of lymphoid cells.

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