ONCOLOGY

Ca^{2+}-Induced Erythrocyte Hyperpolarization in Patients with Tumors of Different Localization

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In patients with lung cancer and malignant tumors of the head and neck, erythrocyte hyperpolarization associated with the opening of Ca^{2+}-activated potassium channels develops with lower rate and amplitude compared with that in healthy donors. Restoration of normal membrane potential is Ca^{2+}-ATPase-dependent. The rate of restoration is significantly higher in patients with tumors. Two groups with different hyperpolarization response and their dynamics during chemotherapy were identified in patients with lung cancer. Ca^{2+}-induced erythrocyte hyperpolarization and its dynamics during cytostatic therapy depend on tumor localization and histological type.

Key words: *Ca^{2+}-activated potassium channel; erythrocyte; lung cancer; head and neck tumors*

Neoplasia is accompanied by dramatic disturbances in red blood cells [1,2,11]. They involve considerable structural modifications of membrane phospholipides, increased microviscosity of the lipid bilayer, and cell shape modifications [6]. These modification cause changes in the activity of membrane-bound enzymes, specifically, increase Ca^{2+}-ATPase activity [8]. This enzyme plays an important role in the regulation of the intracellular Ca^{2+} concentration in erythrocytes, which affects Ca^{2+}-activated potassium ($K^+(Ca^{2+})$) channels responsible for the cell shape [15].

Study of the erythrocyte membrane $K^+(Ca^{2+})$-channels in patients with malignant tumors may enlarge our knowledge on the mechanisms of fast erythrocyte aging in oncology patients. In view of strong correlation existing between the membrane changes in erythrocytes and in the visceral cells, one can use erythrocytes as a natural model for investigation of general cellular characteristics, including permeability of biomembranes [13].

Most antitumor drugs produce toxic effect on erythrocytes and erythropoiesis [2,3]. It cannot be excluded that spherocytosis and a short erythrocytic life under these conditions [1,7,12] are due to improper functioning of Ca^{2+}-ATPase and $K^+(Ca^{2+})$-channels in the erythrocyte membrane.

Our aim was to study the parameters of Ca^{2+}-induced erythrocyte hyperpolarization response in patients with lung cancer (LC) and carcinomas of the head and neck (HNC) before and after chemotherapy.

MATERIALS AND METHODS

Male patients with LC of III-IV stages (n=14) and with HNC (n=9) were involved in the study. Diagnosis was based on the results of roentgenography, endo-

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scopy, and morphological investigation. Some patients (n=7) had differential forms of LC (planocellular, macrocellular, adenocarcinoma), others (n=7) had microcellular form. HNC patients had cancer of the larynx (n=2), mucinous carcinomas of the oral cavity (n=4), and carcinoma of the tongue (n=3).

Antitumor therapy for patients with LC was as follows: cyclophosphamide (750 mg/m^2 on the 1st and 8th days), adriamycin (25 mg/m^2, days 1 and 8), and metotrexate (20 mg/m^2, days 2 and 9). Another scheme was used for patients with HNC: platidiam (100 mg/m^2 on the 1st day), 5-fluorouracyl (1000 mg/m^2, days 1-5), and metotrexate (7 mg/m^2, days 1-5).

The control group included 16 healthy male volunteers of the similar age.

Peripheral blood was collected from the median cubital vein before eating. To prevent coagulation, heparin (25 U/ml) was added to blood. The erythrocyte preparation technique was described elsewhere [9]. Medium A (150 mM NaCl with 5 mM Na-phosphate buffer) was used for washing. Incubation was carried out in medium N (150 mM NaCl, 1 mM KCl, 1 mM $MgCl_2$, and 10 mM glucose). All solutions were prepared on deionized water.

The following chemicals were used: NaCl, KCl, NaH_2PO_4, Na_2HPO_4, $MgCl_2$, $CaCl_2$, and glucose (Reakhim); A23187, CICCP (carbonylcyanide-m-phenylhydrazine), Triton X-100 (Sigma). A23187 and CICCP were dissolved in alcohol. The concentration of alcohol in the incubation medium was < 0.5%, which did not affect activity of $K^+(Ca^{2+})$-channels.

It was previously shown that the rise of intracellular Ca^{2+} content results in two-phase hyperpolarization. Increased concentration of Ca^{2+} in the cytosole induces K^+ efflux due to opening of $K^+(Ca^{2+})$-channels, which increases transmembrane potential. When Ca^{2+} concentration in the erythrocyte is decreased as a result of Ca^{2+}-pump work, the channels close and the transmembrane potential returns to its initial level.

Membrane potential was recorded by a standard method [16] with some modifications [9]. In order to increase intracellular concentration of Ca^{2+} the calcium ionophore A23187 was used. Dynamic membrane potential changes were evaluated by the shifts in pH in the cell suspension in the presence of the protoionophore CICCP. Under these conditions the distribution of hydrogen ions reflects the membrane potential in red cells.

Experimental protocol was described elsewhere [10]. An S904 electrode (Beckman) and a pH-121 pH-meter (Russia) were used for pH-measurements.

The following parameters were analyzed:

ΔE, membrane potential corresponding to the maximum hyperpolarization during injection of A23187, mV;

V_1, alkalinity rate in the incubation medium, reflects the rate of membrane hyperpolarization (mEq. OH^-/liter×min);

V_2, acidosis rate in the incubation medium, reflects the rate of membrane potential restoration (mEq. H^+/liter×min).

The rate of the process was calculated taking into account the buffer capacity of the incubation medium, i.e., the number of OH^- or H^+ ions that are necessary for changing pH by one.

To verify the difference between the parameters of erythrocytic hyperpolarization in different groups of patients and in healthy donors, Hotteling's mean vector statistics was applied. In addition, we calculated the coefficients of correlation between the parameters. The groups of patients were separated according to their hyperpolarization response using the cluster analysis.

RESULTS

In LC and HNC patients, ΔE and V_1 decreased, and V_2 increased compared with control (p<0.001). All three parameters of erythrocytic hyperpolarization in HNC patients turned to be significantly lower compared with those of LC patients (p<0.001).

These findings indicate that activity of $K^+(Ca^{2+})$-channels in LC patients is reduced, which is even more pronounced in HNC patients. The activity of Ca^{2+}-ATPase, which is characterized by V_2, is higher in patients with LC than in HNC patients.

Among the factors that are responsible for such changes in $K^+(Ca^{2+})$-channel and Ca^{2+}-ATPase activities could be certain structural and functional membrane rearrangements in erythrocytes due to neoplastic processes [1,5,11] resulting in cell volume and shape transformations [7,12]. $K^+(Ca^{2+})$-channel opening lifetime depends on the intracellular Ca^{2+} concentration in red cells [14]. Thus, it can be concluded that $K^+(Ca^{2+})$-channels are modified by changes in Ca^{2+}-ATPase activity.

To estimate the relationship between the parameters of erythrocytic hypolarization, correlation coefficients were calculated.

Strong positive correlation between ΔE and V_1 (r=0.87) in healthy subjects and negative correlation between DE and V_2 (r=-0.66), and V_1 and V_2 (r=-0.61) were established. This implies that the amplitude and rate of hypolarization response are determined by the $K^+(Ca^{2+})$-channel opening. Negative correlation between V_1 and V_2, or ΔE and V_2 indicates that the mechanisms underlying these processes are interdependent but different by their origin. Indeed, we have demonstrated that the hypolarization phase is associated with $K^+(Ca^{2+})$-channel opening, while restoration to initial potential corresponds to Ca^{2+}-ATPase activation leading

TABLE 1. Parameters of Ca^{2+}-Dependent Erythrocytic Hyperpolarization Response in Patients with LC and HNC After Chemotherapy ($M \pm m$)

Subjects	Before treatment			After treatment		
	ΔE, mV	V_1, mEq OH^-/lxmin	V_2, mEq H^+/lxmin	ΔE, mV	V_1, mEq OH^-/lxmin	V_2, mEq H^+/lxmin
Healthy donors (n=16)	38.36±1.83	1.61±0.06	0.25±0.064			
Patients with LC						
total (n=14)	24.25±1.76	1.02±0.09	0.38±0.046			
cluster 1 (n=7)	18.44±0.76	0.75±0.07	0.27±0.077	20.69±2.93	1.088±0.16	0.24±0.073
luster 2 (n=7)	30.05±1.30	1.30±0.08	0.501±0.057	24.43±4.97	1.057±0.26	0.23±0.09
Patients with HNC (n=9)	21.23±2.78	1.097±0.14	0.34±0.066	24.36±4.46	1.067±0.22	0.04±0

to a decrease in intracellular Ca^{2+}, and, therefore, to $K^+(Ca^{2+})$-channel closure [9].

In patients with LC, significant positive correlation between all the parameters was found. The correlation coefficients for ΔE and V_1, ΔE and V_2, and V_1 and V_2 were: 0.91, 0.87, and 0.86, respectively.

Only the correlation between V_1 and V_2 proved to be significant in the group of HNC patients (r=0.95).

These findings indicate that the same ion transport systems are involved in the Ca^{2+}-dependent erythrocytic hyperpolarization in patients with HNC and in healthy subjects, but the interaction between the systems is changed in the course of the disease. These changes can be attributed to structural and functional membrane modifications associated with neoplasia.

It was shown by cluster analysis that the groups of patients are not uniform in the respect of hyperpolarization parameters of erythrocytes. Thus, LC patients can be divided into two clusters. The first cluster consists of patients (n=7) with low ΔE and V_1, and V_2 near to control. The second cluster includes patients in which all the test parameters are higher than the mean level obtained for patients with LC. However, the amplitude and rate of the hyperpolarization response were below the control, but V_2 increased 2-fold compared with control (p<0.001).

Thus, the activity of $K^+(Ca^{2+})$-channels was decreased in erythrocytes of cluster 1 patients, while the activity of Ca^{2+}-ATPase was near the control value. The activity of $K^+(Ca^{2+})$-channels in cluster 2 was lower than in healthy donors, but higher than in cluster 1. Ca^{2+}-ATPase activity in patients of cluster 2 was significantly higher than that of the cluster 1 patients, and in healthy donors.

Hystological investigation showed that patients from cluster 1 had predominantly microcellular form of the LC (6 of 7). Cluster 2 consisted of patients with microcellular form (3 of 7) and with other forms of LC.

Cytostatic preparations substantially aggravate anemia in patients with neoplasmy. This is due to their toxic effect on erythropoiesis and erythrocytes in the peripheral blood [2,3]. Dynamic shifts in parameters of erythrocytic hyperpolarization during chemotherapy were in different patients (Table 1). The first course of chemotherapy increased ΔE and V_1 and reduced V_2 in LC patients from cluster 1. This suggests that activity of $K^+(Ca^{2+})$-channels increased and that of Ca^{2+}-ATPase decreased.

The first course of chemotherapy reduced all hyperpolarization parameters in erythrocytes in cluster 2 patients, i.e., inhibited both $K^+(Ca^{2+})$-channels and Ca^{2+}-ATPase.

Our attempt to assess the effect of the second course of chemotherapy in the different groups of patients with the LC failed. ΔE and V_1 showed a tendency toward an increase, while V_2 toward a decrease, reflecting further rise of $K^+(Ca^{2+})$-channel activity and fall of Ca^{2+}-ATPase activity.

In patients with HNC, ΔE increased while V_1 and V_2 decreased (Table 1). After treatment, $K^+(Ca^{2+})$-channel activity rose and Ca^{2+}-ATPase activity dropped.

One of the mechanisms leading to shifts in the erythrocytic hyperpolarization response in patients during the treatment with cytostatic drugs may be their influence on the plasma membrane. Thus, adriamycine enhances Ca^{a2+}-dependent potassium efflux from erythrocytes via activation of Ca^{2+} entrance into the cell [4].

It can be concluded that erythrocytic $K^+(Ca^{2+})$-channel and Ca^{2+}-ATPase activity and its changing in the course of cytostatic treatment depend on localization and hystological type of the tumor.

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