Peculiarities of Ca^{2+}-Induced Erythrocyte Response in Patients with Lung Cancer Undergoing Antitumor Chemotherapy

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Characteristics of changes in the membrane potential induced by increased Ca^{2+} inward current in patients with lung cancer are presented. Both the amplitude and the rate of hyperpolarization induced by opening of Ca^{2+}-activated potassium channels are markedly decreased in erythrocytes from patients in comparison with healthy donors. Under conditions of moderate Ca^{2+} inward current, hyperpolarization is followed by restoration of the membrane potential. This process is more rapid in cancer patients. It is shown that the parameters of hyperpolarization in their dynamics during antitumor therapy depend on the type of lung cancer.

Key Words: *Ca^{2+}-activated potassium channel; erythrocytes; lung cancer*

Changes in the red blood can be noted even at the initial state of tumor process and become more pronounced during tumor growth [1,3,7]. Erythrocyte membranes in cancer patients are characterized by altered phospholipid composition and increased cholesterol content [7], which results in a rise of microviscosity of the lipid bilayer and changes in the cell shape [4]. This is accompanied by changes in activity of membrane enzymes, in particular, activation of Ca^{2+}-ATPase [5].

Ca^{2+}-ATPase is a key enzyme of the regulation of cell calcium concentration, which in turn considerably modulates the activity of Ca^{2+}-activated potassium channels in the erythrocyte membrane [9] and is responsible for the cell shape [10].

In light of this, the study of the functioning of Ca^{2+}-activated potassium channels of the erythrocyte plasma membrane in cancer patients can provide a new insight into the mechanisms of accelerated erythrocyte aging in the course of neoplasm process and in the pathogenesis of cancer. These problems are very important, since the majority of antitumor drugs disturb erythropoiesis and produce a damage to circulating erythrocytes [2]. It cannot be excluded that spherocytosis and reduced life-span of erythrocytes in cancer patients [1,8] are associated with disturbances in Ca^{2+}-activated potassium channels of erythrocyte membrane.

The aim of the present study was to evaluate kinetic characteristics of Ca^{2+}-activated potassium permeability of the erythrocyte plasma membrane in patients with lung cancer before and during antitumor chemotherapy according to the following scheme: cyclophosphane—adriamycin—methotrexate.

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MATERIALS AND METHODS

Twenty-one male patients with lung cancer were examined. In each patient lung cancer was verified

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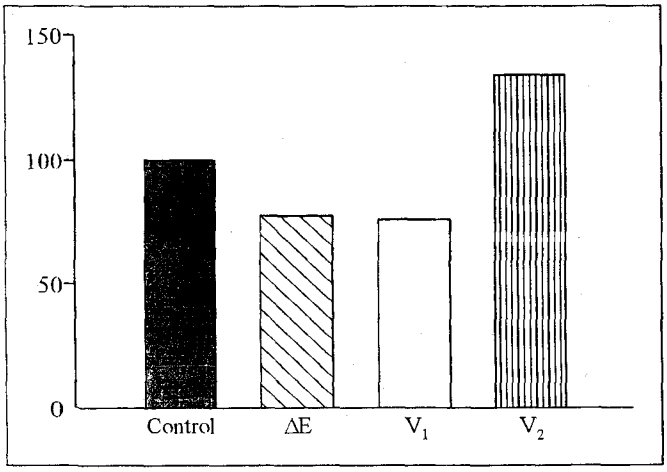

Fig. 1. Parameters of hyperpolarization response in erythrocytes from patients with lung carcinoma. Parameters of hyperpolarization in erythrocytes of health donors are taken as 100%.

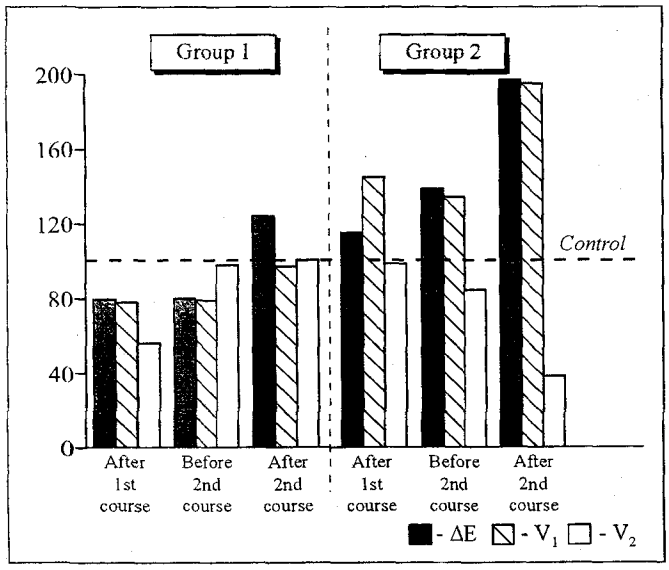

Fig. 2. Comparative characteristic of hyperpolarization response in patients with non-small-cell (group 1) and small-cells (group 2) lung carcinoma. Parameters of the control group are taken as 100%.

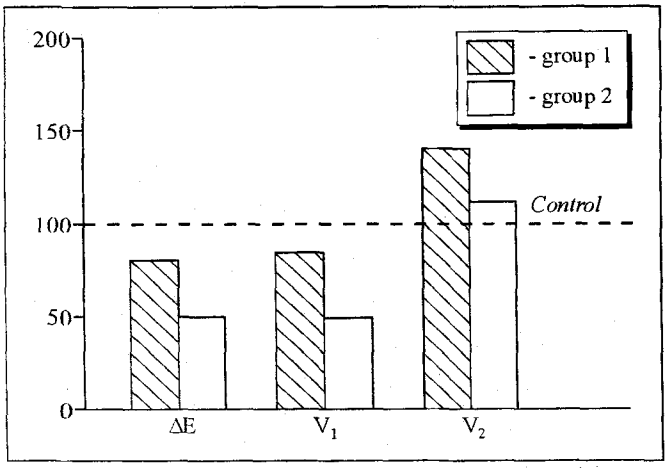

Fig. 3. Changes in parameters of hyperpolarization response in patients of two different groups during treatment. Baseline values (before treatment) taken as 100%.

by roentgenologic, endoscopic, and morphologic studies. Differentiated tumors (squamous or round-cell carcinoma or adenocarcinoma) were diagnosed in 11 patients, while 10 patients had small-cell carcinoma. The patients underwent chemotherapy according to the following scheme: 750 mg/m^2 cyclophosphane on days 1 and 8, 25 mg/m^2 adriamycin on days 1 and 8, and 20 mg/m^2 methotrexate on days 2 and 9. The 9-day cycles of chemotherapy were repeated at 2-week intervals.

Blood samples were obtained from the ulnar vein before and after each cycle of cytostatic treatment. Control group comprised 16 age-matched volunteers. Heparin (25 U/ml) was always used as anticoagulant. Blood was sampled, and erythrocyte suspension was prepared as described previously [6].

The following solutions and reagents were used:

1. medium A (for erythrocyte washing) contained 150 mM NaCl and 5 mM Na-phosphate buffer;

2. medium N (for erythrocyte incubation medium) contained 150 mM NaCl, 1 mM KCl, 1 mM $MgCl_2$, and 10 mM glucose.

All solutions were prepared using deionized water.

Reagents used were NaCl, KCl, NaH_2PO_4, Na_2HPO_4, $MgCl_2$, $CaCl_2$, and glucose (Reakhim), A23187, CCCP (carbonyl cyanide m-chlorophenylhydrazone), and Triton X-100 (Sigma). A23187 and CCCP were dissolved in ethanol; final concentration of the solvent in the incubation medium did not exceed 0.5%, so that ethanol had no effect on the activity of Ca^{2+}-activated potassium channels.

Previous studies showed that opening of Ca^{2+}-activated potassium channels induced by rise of cytosolic Ca^{2+} results in hyperpolarization of the erythrocyte membrane. This is a two-phase process: 1) an increase in cell calcium concentration induces potassium outward current, which is accompanied by a increase in the membrane potential; 2) a decrease in the cytosolic calcium concentration due to the action of Ca-pump closes the channels, and the membrane potential returns to normal.

Changes in the erythrocyte membrane potential were recorded as described elsewhere [11] with some modifications [6]. The ionophore A23187 was used to induce accumulation of calcium ions in erythrocytes. Changes in the membrane potential were assessed by pH shifts in the erythrocyte suspension in the presence of a protonophore. Proton distribution under these conditions depends on the erythrocyte plasma membrane potential.

The experiment was carried out according to the following scheme: 100 μl of packed erythrocytes were added to 1.9 ml medium N containing 50 μM $CaCl_2$; the samples were incubated at 37°C for 5 min and 20 μM CCCP was added to the mixture; after 2 min

2 μM A23187 was added. For determination of the intracellular pH after the incubation, Triton X-100 was added to the suspension to a final concentration of 0.2%.

The pH was recorded by means of an S904 electrode (Beckman) and a pH-121 pH meter (Russia).

The following parameters were recorded:

ΔE, membrane potential corresponding to maximum membrane hyperpolarization in response to addition of Ca-ionophore, mV;

V_1, the rate of alkalinization of the incubation medium reflecting the rate of membrane hyperpolarization, milliequivalent OH^-/(min×liter);

V_2, the rate of acidification of the incubation medium reflecting the rate of restoration of the membrane potential, milliequivalent OH^+/(min×liter).

Buffer capacity of the incubation medium, i.e., the amount of OH^- or H^+ necessary for shifting the pH by 1, were taken into account in the estimation of the rate of these processes.

The data were processed statistically using the Student's *t* test.

RESULTS

The amplitude and rate of hyperpolarization in the erythrocytes from patients with lung cancer are considerably decreased ($p<0.05$), while the rate of repolarization is increased ($p<0.05$, Fig. 1). These changes are most likely result from activation of Ca^{2+}-ATPase in erythrocytes from these patients [5].

Depending on the parameters of erythrocyte hyperpolarization response, all patients with lung cancer can be divided into 2 groups. Group 1 consisted of 10 patients in whom ΔE and V_1 were decreased by about 20%, while V_2 was elevated by 40% in comparison with the control group. Group 2 comprised patients in whom ΔE and V_1 constituted about 50% of the control, while V_2 differed little from the control (Fig. 2).

The dynamics of the parameters of Ca^{2+}-induced hyperpolarization during chemotherapy was also different in these groups. In group 1 patients, a decrease in the amplitude and rate of hyperpolarization was noted after the first and before the second course of treatment. After the second course of chemotherapy these parameters rose again. The rate of repolarization decreased after the first course of treatment and then returned to the initial value (Fig. 3).

In group 2 patients, the amplitude and rate of hyperpolarization increased after the first, before, and after the second course of treatment by 24, 40, and 90%, respectively, while V_2 considerably decreased (Fig. 3).

Histological examination shows that group 1 comprised primarily patients with nonsmall-cell lung carcinoma (8 of 10 patients). Group 2 primarily included patients with small-cell carcinoma (8 of 11 patients).

Thus, parameters of Ca^{2+}-induced hyperpolarization in erythrocytes and their dynamics in patients undergoing antitumor therapy strongly depend on morphological type of lung cancer.

REFERENCES

1. V. E. Gol'dberg, A. M. Dygai, and V. V. Novitskii, *Lung Cancer and the Blood* [in Russian], Tomsk (1992).
2. E. D. Gol'dberg and V. V. Novitskii, *Antitumor Anthracycline Antibiotics and the Blood* [in Russian], Tomsk (1986).
3. A. K. Zhuravlev and V. V. Murashko, *Byull. Eksp. Biol. Med.*, **98**, No. 11, 596-598 (1984).
4. A. P. Kashulina and I. P. Tereshchenko, *Pat. Fiziol.*, No. 5, 76-82 (1985).
5. S. N. Orlov, *Advances in Science and Technology* [in Russian], Moscow (1987).
6. S. N. Orlov, I. V. Petrova, N. I. Pokudin, *et al.*, *Biol. Membrany*, **9**, 885-903 (1992).
7. N. N. Slyusar', *Eksp. Onkol.*, **14**, No. 6, 56-62 (1992).
8. E. A. Stepovaya, E. D. Gol'dberg, and V. V. Novitskii, *Lab. Delo*, No. 6, 29-31 (1991).
9. V. L. Lew, *J. Physiol. (Lond.)*, **206**, 35-36 (1970).
10. V. L. Lew, *Biochim. Biophys. Acta*, **233**, 827-830 (1971).
11. R. I. Macey, T. S. Adorant, and T. W. Orme, *Ibid.*, **512**, 284-295 (1978).