

Change of Erythrocytes Transport Function in Blood of Men with Prostate Adenocarcinoma (before and after Plastic Orchiectomy)

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Abstract

The changes of blood erythrocytes transport function in patients with prostate adenocarcinoma have been studied according to alterations in Na⁺/K⁺-ATP-ase activity and permeability of Na⁺ and K⁺ ions, before and after the plastic orchiectomy. The results of the study revealed that activity of Na⁺/K⁺-ATP-ase was increased compared with the data before the orchiectomy, but this parameter was reduced compared with control group. The increased amounts of the Na⁺ as well as K⁺ ions were also observed in the extracellular area compared with the same data before the orchiectomy. The amount of cholesterol, after plastic orchiectomy, was reduced compared with the data gained before orchiectomy, but the data were greater than those of the control group. It may be presumed that one of the reasons for change of Na⁺/K⁺-ATP-ase activity is variation of cholesterol content in erythrocyte membrane. Changes of the active transport system, in turn, affect the transport function of the erythrocyte membrane. In spite of surgical intervention, full recovery of patients does not take place after the plastic orchiectomy. Comparative normalization and approaching to the control group of the given indices in post-operation period (~6 months after surgery) indicate strengthening of the immune system and correspondingly—protective abilities of an organism, which was proved by the anamneses of patients.

Keywords

Prostate Tumors, Plastic Orchiectomy, Na⁺/K⁺-ATP-ase

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1. Introduction

Na^+K^+ -ATP-ase belongs to the transporter family of P-type ATP-ases [1]. It's known that the enzyme performs the signaling function in addition to the transport function [1] [2]. Na^+K^+ -ATP-ase interacts with neighboring membrane proteins and organized cytosolic cascades of signaling proteins to send messages to the intracellular organelles [3] and thus effects the expression of various genes, proliferation of cells, activation of some hormones and growth factors and phosphorylation of certain proteins [4].

Na^+K^+ -ATP-ase is exposed in highly specialized cells, such as erythrocytes [5]. Considering the fact that it is easy to extract and to investigate, erythrocyte membrane serves as a unique model for performing membrane investigations. So erythrocytes can be used as a model in the study of Na^+K^+ -ATP-ase activity research in biomembranes [6].

Plastic orchiectomy [7], as one of the most common treatments for prostate progressive cancer, implies regression of malignant tumor by means of androgen deprivation. It must be mentioned that application of this surgical method is palliative and does not mean a full recovery of patients [8]. If we take into account that in some cases the surgical treatment causes mutation of the cancer into a hormone-resistant form (which is characteristic with high aggression and metastases) [9], it becomes clear that in spite of some achievements in progressive cancer therapy, yet many problems remain to be solved.

Considering all the above mentioned, the main purposes of our investigation at this stage of the research were to study the changes of transport function of blood erythrocytes in patients with cancer of prostate (CaP) according to the alterations in Na^+K^+ -ATP-ase activity and permeability of Na^+ and K^+ ions, both before and after the orchiectomy, to make comparative analysis of pre- and post-operative results, and also to reveal the post-operative characteristics of disease according to gained results.

2. Materials and Methods

2.1. Materials

The erythrocytes of the patients with metastatic prostate cancer (before and after 6 months from castration) served as material for the studies.

Each study group, as well as control group, consisted of 15 patients of ages 60 - 75. Patients were not receiving any type of treatment during sample collection for this investigation.

The patients of the study groups were selected by the following criteria: Patients belonged to the same age group (60 - 75) and all patients belonged to the Caucasian race. Without therapy treatment, the analyses were done after the first reveal of the disease. Clinical stage of the disease of all patients has been diagnosed at the Al. Tsulukidze National Center of Urology (Georgia) by the rectal, histological and echographic investigations of prostate gland; Patients were of the similar anamnesis; Control group consisted of 15 practically healthy men of the same age group and same Caucasian race.

The Ethics Committee of Georgia approved the study and informed consent was obtained from each patient.

The blood samples were obtained before the surgery and after 6 months from castration at the National Centre of Urology (Georgia).

2.2. Methods

Gaining of the erythrocyte membranes was performed by the method of Dodge [10]. Erythrocyte suspension was lysed using 20 mmol/l of phosphate buffer of pH 7.4 and centrifuged. Then, the procedure was repeated using the same solution of the concentration 10 mmol/l and 5 mmol/l in order to wash off the haemoglobin from the erythrocyte ghosts.

Protein concentration was detected by the method of Lowry [11].

Na^+ , K^+ -ATP-ase activity determination method was based on the spectrophotometric determination (635 nm) of free orthophosphate (Pi) released due to the Na^+ , K^+ -ATP-ase catalyzed hydrolysis of ATP. Pi is usually transformed to a phosphomolybdate complex in an acid medium followed by a reduction with ascorbic acid followed by complexation with basic dyes that yield colored complexes. Specific activity of the enzyme was expressed as μmol of Pi released per min per mg of protein [12].

Na^+ and K^+ ions were studied by ionometric method [13].

The concentration of cholesterol was determined by using Liebermann-Burchard reagent spectrophotometri-

cally at 635 nm [14].

Experimental data were processed by means of standard variation statistics MINITAB (Basic statistic) $P < 0.05$ was taken as statistically significant.

3. Results and Discussion

Investigations have revealed the changes in Na^+/K^+ -ATP-ase activity in blood erythrocytes of CaP patients after the plastic orchietomy. In particular, the index increased compared to the data of pre-operation period [15], but it remained lower than the control variant (Table 1). At the same time, increase of concentration of Na^+ and K^+ ions in extracellular area was observed. Though, both—the enzyme activity and ion concentration remained lower compared to similar indices of the control group (Table 1).

It is established that there is a two-side relationship between Na^+/K^+ -ATP-ase activity and concentration of Na^+ and K^+ ions. In particular, Na^+/K^+ -ATP-ase is the main enzyme regulating the concentration gradient of Na^+ and K^+ ions in intra- and extra-cellular areas; But mentioned ions, in turn, play the role of enzyme's regulators [1] [16].

Thus, decrease of Na^+/K^+ -ATP-ase activity in membrane of erythrocytes, compared to the control group, which was revealed in CaP cases both, before and after orchietomy, may be somehow connected with the decrease of K^+ ion concentration in extracellular area. It is known that increased concentrations of K^+ ions suppress the inhibitory effect of ouabain, causing the rise of enzyme's activity [13]. Taking into account the above mentioned, it may be supposed that one of the reasons for decreased Na^+/K^+ -ATP-ase activity (compared to the control group) in case of CaP, both before and after orchietomy, may be the reduction of K^+ ion concentration in the surrounding medium (which was expressed more clearly before the orchietomy). Presumably accumulation of K^+ ions in the extracellular area is insufficient for reducing the inhibitory effect of ouabain. As a result Na^+/K^+ -ATP-ase inhibition takes place in case of the mentioned pathology (this was expressed more clearly before the orchietomy) (Table 1) [15].

As for changes of Na^+ ion concentration in the extracellular area, we suppose that while suppressing Na^+/K^+ -ATP-ase activity, ouabain causes transport of insignificant amount of Na^+ ions into the extracellular area, which was revealed as a decrease of Na^+ ion concentration (compared to the control group) both before and after orchietomy (Table 1). This result was especially evident in pre-orchietomy data. Our supposition is based on data demonstrating the linear relation between changes of Na^+ ion concentration in the extracellular area and alterations in enzyme's transport function [17], and also is supported by our previous results [15] (Table 1).

It is known that in addition to the ion pumps, transport of substances to intra- and extra cellular areas is provided by passive and secondary active transport processes. Moreover, the conjugated functioning of the primary and secondary active transport through the membrane is also possible [1]. Taking into account the above mentioned supposition, it becomes clear that changes in Na^+ and K^+ ion transport abilities of erythrocyte membrane (which in case of CaP was revealed by the reduced amount of the ions in the extracellular area, both before and after plastic orchietomy) must be determined not only by the inhibition of Na^+/K^+ -ATP-ase activity, but the role of passive and secondary active transport must also be taken into account. Possibly, in case of CaP, the total transport system functioning is generally deregulated, which is reflected on the transport function of erythrocytes.

According to above mentioned, we suppose that decrease of Na^+/K^+ -ATP-ase activity and changes of Na^+ and K^+ ion concentration in case of CaP, both before and after orchietomy, reflects the general changes of a cancer-damaged organism and impaired reaction of its controlling systems against the cancer pathology.

Table 1. Alterations of Na^+ , K^+ -ATP-ase activity, ions permeability and cholesterol amount in red blood cells.

Object (tumor tissue)	Na^+ , K^+ -ATP-ase activity $\mu\text{M}/\text{min}$ (mg/protein)	$1/p\text{Na}^{+-}$	$1/p\text{K}^{+}$	Cholesterol (mg/%)
Control group	0.546 ± 0.2	0.66 ± 0.01	0.45 ± 0.002	160 ± 0.05
CaP (before castration) [15]	0.28 ± 0.1	0.48 ± 0.02	0.38 ± 0.001	572 ± 0.01
CaP (after castration)	0.335 ± 0.1	0.53 ± 0.01	0.40 ± 0.001	383 ± 0.04

$n = 15$ (the amount of the patients in each group); $P \leq 0.05$.

Comparative normalization of above mentioned indices (compared to pre-operation data) after the 6 month from orchiectomy may indicate to a partial recovery of the general condition of an organism and its homeostasis [6].

Next step of investigations comprised the study of cholesterol content in membrane of erythrocytes both, before and after orchiectomy.

It is well known that there is a direct relationship between the cholesterol concentration, Na^+/K^+ -ATP-ase activity and changes of Na^+ and K^+ ion transport function in erythrocyte membranes [18]. Moreover, cholesterol is responsible for membrane fluidity. Rise of the cholesterol content in membrane until it reaches the determined level is due to the increase of micro viscosity [18]. Enzyme Na^+/K^+ -ATP-ase, in turn, responds to alteration of membrane viscosity by a change in its activity [19] [20].

Decrease of cholesterol content in erythrocyte membrane after the plastic orchiectomy, compared to pre-operation data, was revealed by experimental results. But the index was higher compared to the results of the control group (Table 1). As the amount of cholesterol in erythrocyte membrane after orchiectomy was mean value between norm (control group) and pathology (CaP), activity of Na^+/K^+ -ATP-ase correspondingly would be intermediate, which was proved by our investigations as well (Table 1).

Thus, according to our studies and literary data it may be presumed that one of the reasons for Na^+/K^+ -ATP-ase activity change is alteration of cholesterol content in erythrocyte membranes. Changes of the active transport system, from its side, affect the transport function of the erythrocyte membrane.

4. Conclusions

It has been revealed that after plastic orchiectomy Na^+/K^+ -ATP-ase activity as well as the amount of Na^+ and K^+ ions in the extracellular area is increased compared with the results gained before the orchiectomy.

It has been revealed that reduced cholesterol content in erythrocyte membrane after plastic orchiectomy, compared with the same data before orchiectomy, should lead to increased activity of Na^+/K^+ -ATP-ase.

After orchiectomy the systemic effect of cancer on erythrocyte membranes of CaP patients is revealed by the number of alterations such as changes in cholesterol content, alterations of lipid peroxidation intensity [21] and morphological changes of erythrocytes [22]. All these alterations affect the transport function of erythrocyte membrane.

In spite of surgical intervention, full recovery of patients does not take place after the orchiectomy, because of the residual phenomena.

Comparative normalization and approaching of the studied indices gained in post-operation period (6 months after operation) to the control group data indicate strengthening of immune system and correspondingly—protective abilities of an organism, which was proved by the anamneses of patients.

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