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ANTITOXIC ROLE OF TOCOPHEROL IN STABILIZATION OF BLOOD INDICES IN ACUTE HYPOXIA

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The character of deviations of blood clotting indices in acute hypoxia was studied, as this testifies to the development of hypercoagulation and thrombotic status. Maximum changes are recorded after the 3-hour period exposure, which indicates the deeper impact of the factor within the timeframe. Tocopherol use is considered effective, which as a powerful antioxidant and antihypoxant helps to stabilize blood cells membranes to create favorable conditions for biological oxidation processes and regulation of coagulation parameters and clotting time.

Keywords: acute hypoxia, blood clotting indices, tocopherol.

Introduction. The problem of organism and tissue hypoxia, in general, remains one of the most urgent problems in contemporary medicine. Hypoxia leads to insufficient energy supply to cells, because even in the presence of energy sources the oxidation processes do not occur for the release of energy [1–3]. Under these conditions the cellular activity is extremely hard. It is known that the hypoxia of the entire organism or individual organs and tissues may be due to various factors such as low oxygen content in the environment, respiratory and other pathologic conditions (shock, myocardial infarction, heart attack, asthma, craniocerebral trauma, diabetes) [3–5].

Hypoxia causes irreversible changes in vital organs. The central nervous and blood systems, cardiac, kidney and liver tissues are most sensitive to oxygen deficiency. Oxygen causes an euphoric condition, dizziness, weakening of muscle tension, loss of consciousness. Unfortunately, the hypoxic picture is often observed in large cities due to high levels of air pollution.

Pharmacological agents are used to reduce hypoxia, which increase oxygen supply to the body and improve oxygen absorption, reducing oxygen demand in organs and tissues [4].

Antioxidants and anti-hypoxants are widely used to provide broad-based activities while maintaining long-term effects. The latter are aimed at suppressing free radical oxidation of membrane lipids and thus hypoxic tissue injuries [1]. Antioxidants and anti-hypoxants contribute to the more “economical” consumption and absorption of oxygen in tissues and increase the body resistance to oxygen

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deficiency. The anti-hypoxic effect is associated with the presence of biologically active substances such as flavonoids, carotenoids, citric acid cycle components, which interact with vitamins and microelements (selenium, zinc, copper, magnesium, etc.), interfere with bioenergy processes raising the body resistance to oxygen deficiency [6–8].

Materials and Methods. This study focuses on the reveal of changes of some blood clotting indices and platelet count after the acute hypoxia, as well as the effectiveness of the use of tocopherol to correct the observed deviations.

The experiments were carried out on 8 rabbits of 2.2–2.5 *kg* weight, which were stored and fed in equal conditions. Blood samples were taken from rabbits by cardiopuncture method. Research has been done in two stages. In the first stage the clotting parameters were determined before hypoxia (norm), in an hour, after 3 hours and 10 days. In the second phase the effectiveness of protecting effect of tocopherol on hypoxia-related changes in the same diagram were studied.

Hypobaric hypoxia model was used for the experiments. During the experiment the velocity of compression and decompression in the hypobaric chamber was 15–20 *m*/*s*. The animal was "raised" at a height of 7000 *m* and stored for 30 *min*. Before the experiment, the animal was fixed on the experimental board by upper and lower limbs and kept for thirty minutes to adapt to the fixed state, followed by cardiopunction.

Tocopherol was purchased from the "SIGMA Pharmaceuticals" company. It was given to animals per os (350 μL/day) (according to dose described in the literature) for 10 days until the start of the study [5]. Blood indices were determined by the semi-automatic German HumaClot Junior device. Plastic vaccume test tubes with 3.2% sodium citrate were used for blood sampling.

Hemostat thrombin time (TT), thromboplastin (Hemostat thromboplastin (PT)), activated partial thromboplastin time (Hemostat APTT), fibrinogen (Hemostat fibrinogen) were determined. Trombine time is a test that can interfere with the transformation of fibrinogen into fibrin and the end of the thrombus occurrence. This test is used as a test for heparin therapy.

Thromboplastin (PT) is widely used as a test for an anticoagulation therapy with the activity of vitamin-dependent II, VII, IX, X factors, activation of C and S proteins. Activated Partial Thromboplastin Time (APTT) is a versatile test that is sensitive to the lack of all plasma factors, with the exception of factor VII. APTT is mainly used to determine the deficiency of factors XII, XI, X, IX, VIII, V, II, I and the pre-kallikrein.

Plasma fibrinogen is a useful test for diagnosis, controls of the course of the various diseases of bleeding. At present, the high level of fibrinogen is considered a risk factor for cardiovascular diseases.

The reliability of the recorded deviations in this research was determined by the Steward statistical method, at least at $p<0.05$.

The determination of the content of blood platelets in the blood of normal and Tocopherol consumed animals was carried out as well.

Results and Discussion. Rabbit blood protrombin time (PT) in norm was 15.8 ± 1.04 *s*, APTT was 33.0 ± 1.81 *s*, TT was 35.2 ± 1.48 *s*, fibrinogen content was 104 ± 11.47 *mg*/*dL*. After hypoxic exposure, deviations from indicators level were revealed (see Table). Thus, immediately after the hypoxia and, in particular, after

3 hours, PT deficiency was found to be approximately 13.9 ± 1.48 *s* ($p < 0.001$), being 12% less than the norm.

However, after 10 days, the increase in the index was 7%, compared with that by the 3rd. hours of exposure, remaining below the baseline (14.9±1.58 *s*) (Fig. 1). As for the trend of deviations of this indicator of tocopherol-received animals, there was a decrease in deviations on all time scales compared with those of tocopherol non-receive animals.

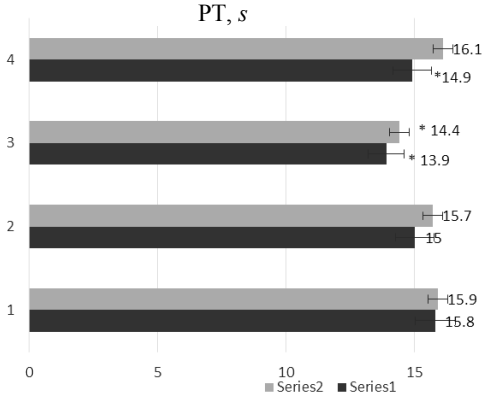

Fig. 1. Changes in protrombin time of natural and tocopherol received animals in acute hypoxia:
1 – norm;
2 – after one hour-exposure of the factor;
3 – after 3 hours of exposure;
4 – after 10 days of the exposure.
* – Specifies the degree of accuracy of the changes at least at $p < 0.05$.
Series 1 – tocopherol non-received animals.
Series 2 – tocopherol received animals.

The study of the deviations of APTT showed a gradual decrease in the effect within three hours, reaching 27.1 ± 1.58 *s* ($p < 0.04$), which is 12% lower than the norm. After 10 days, a slight increase in the index was observed (Fig. 2).

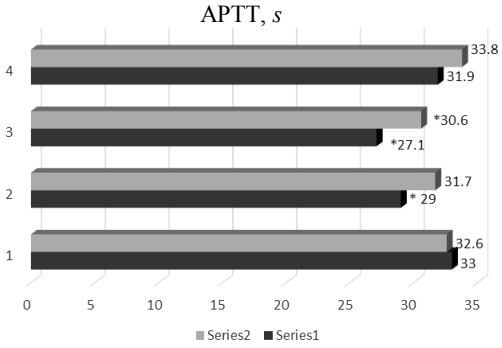

Fig. 2. Changes in activated partial thromboplastin time of natural and tocopherol received animals in acute hypoxia. Details see in Fig. 1.

Deviations of the indexes were also observed in the study of the thrombin time. A decrease was recorded by the first and, especially, by the 3rd hour, respectively reaching 31.7 ± 1.75 *s*, 35.2 ± 1.58 *s*) ($p < 0.05$). After 10 days, restoration of the indicator was observed (Fig. 3).

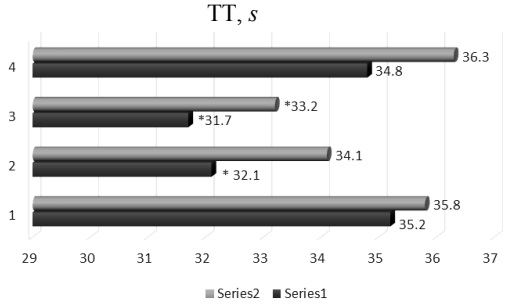

Fig. 3. Changes in trombin time of natural and tocopherol received animals in acute hypoxia. Details see in Fig. 1.

An hour after the hypoxia exposure, the level of fibrinogen was 156.0 ± 10.42 *mg*/*dL* (29.6 *s*), which reached its maximum change in 3 hours, amounting 158.0 ± 11.63 *mg*/*dL* (30.1 *s*); in norm was: 104.0 ± 11.47 *mg*/*dL* (22.2 *s*) ($p<0.00$). After 10 days, the decline of the index was recorded, remaining above the baseline. Accordingly, the time for the formation of platelets (PLT) has also decreased (see Table, Fig. 4). Due to the effect of tocopherol, all the deviations of indices were significantly mild in tocopherol-received animals compared with those of non-received ones.

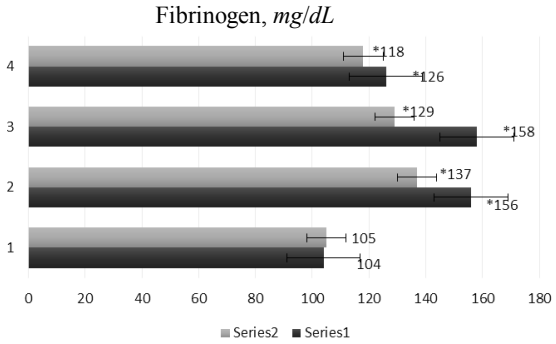

Fig. 4. Changes in fibrinogen content of natural and tocopherol received animals in acute hypoxia. Details see in Fig. 1.

Experimental data showed a large platelet reactivity against the hypoxia. An hour after the hypoxia exposure, the platelets count increased sharply and reached 499 ± 13.4 ($\times 10^9/L$) ($p < 0.000$), after 3 hours it reached 476 ± 13.68 ($\times 10^9/L$, $p < 0.000$), after 10 days – 406 ± 12.48 ($\times 10^9/L$), $p < 0.000$) (the initial level was 273 ± 11.47 ($\times 10^9/L$). Under the influence of tocopherol, the direction of changes was the same, but less expressed (see Table, Fig. 5).

Peripheral blood cells are an interesting object in acute hypoxia, as they differ not only in the functions they perform, but also in exchange processes, the degree of oxygen utilization, the occurrence of oxygen-reactive forms and the of resistance to them.

The analysis of the platelet count showed a significant increase in the rate and gave an indirect indication of increased thrombosis and a development of acute disseminated vascular coagulation syndrome during the organism reoxygenation [2, 3, 5, 9]. The structural and functional characteristics of platelet membranes play an important role in their functioning. Its role in hemostasis depends on the condition of the platelet shape.

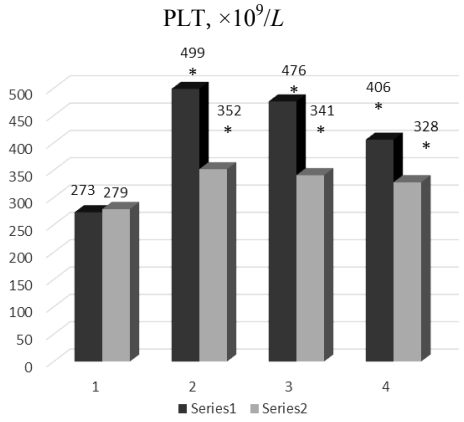

Fig. 5. Changes in the platelet count in natural and tocopherol received animals in acute hypoxia. Details see in Fig. 1.

Changes in blood coagulation rates in acute hypoxia

Test	PT, *s*	APTT, *s*	TT, *s*	Fibrinogen, *mg/dL*, *s*
Norm (1)	15.8±1.04	33.0±1.81	35.2±1.58	104.0±19.47, 22.2 *s*
Norm (2)	15.9±0. 84	32.6±1.46	35.8±1.41	105.0±11.65, 22.0 *s*
After 1 hour exposure (1)	15.0±1.04	29.0±0.81*	32.1±1.02*	156.0±11.7*, 29.2*s*
After 1 hour exposure (2)	15.7±1.86	31.7±1.02	34.1±1.47	137.0±12.78*, 26.8 *s*
After 3 hour exposure (1)	13.9±1.48*	27.1±1.58*	31.7±1.75*	158.0±11.63*, 30.1 *s*
After 3 hour exposure (2)	14.4±1.60*	30.6±1.41*	33.2±0.48*	129.0±10.89*, 24.9 *s*
After 10 days (1)	14.9±1.58*	31.9±1.08	34.8±0.47	126.0±11.53*, 26.3 *s*
After 10 days (2)	16.1±1.41	33.8±1.04	36.3±1.11	118.0±10.60*, 24.8 *s*

(1) – natural (non-tocopherol) animals; (2) – tocopherol received animals.
* – specifies the degree of accuracy of the changes at least at $p < 0.05$.

According to literary data, the platelets that have a disk shape (discolored), round, smooth or wavy surface, belong to calm forms and constitute 75% of the total population of the platelets. Cells with external characteristics of functional activity are characterized by the presence of some formations on the surface membranes, which play a crucial role in the interaction of platelets, endothelium and other molecules, ensuring the ability of platelets to adhere and aggregate [1–3, 10].

In this study the deviations of above-mentioned indexes testify to the development of hypercoagulation and thrombotic status. Maximum changes were recorded after the 3 hours period, which indicates the deeper impact of the factor within the timeframe. Among the compensatory reactions to mitigate the effects of hypoxia, the organs of the cardiovascular and respiratory systems, as well as biochemical processes occurring in tissue cells of organ-systems lacking oxygen are mainly involved. Compensative rehabilitation reactions during hypoxia take place against the background of long-term conditions or illness, so they are subject to constant changes and deviations from the norm [3–5].

The use of tocopherol in acute hypoxia conditions has essentially mitigated the deviations of coagulation indicators. It is known from many sources of literature that tocopherol is a strong immunomodulator that promotes the strengthening of immune-defending systems [6, 7].

Tocopherol is placed in a manner that prevents oxygen from contacting with unsaturated membrane lipids, which protects biomembranes from peroxide damage. Membrane-stabilizing effect of tocopherol is manifested by its ability to protect SH-groups from oxidation of membrane proteins [7, 8].

Tocopherol, as an anti-hypoxant, stabilizes the mitochondrial membrane and conservates oxygen by the cells. Due to that, mitochondria increase an oxidation phosphorylation and formation of ATP and createinfosphate. Tocopherol also controls heme synthesis, thereby enhancing hemopoiesis, hemoglobin and mioglobin synthesis [6, 7, 11].

On the other hand, tocopherol possesses the ability to suppress the activity of the phospholipase A2 of lysosomes that destroys the membrane phospholipids. The damage of lysosome membrane leads to the removal of proteolytic enzymes to the cytosol and cell degradation [1, 12].

The effect of tocopherol is explained by its ability to stabilize the mitochondrial membrane and save the oxygen absorption by cells. Due to film-stabilizing effect of tocopherol the combination of oxidative phosphorylation in mitochondria increases the formation of ATP and creatinphosphate.

In conclusion, tocopherol use is considered effective, because it is a powerful antioxidant and antihypoxant, wich helps to stabilize blood cells membranes to create favorable conditions for biological oxidation processes and regulation of coagulation parameters and clotting time.

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