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STUDY OF THE ANTIHYPOXIC EFFICIENCY OF SUCCINATE UNDER THE CONDITIONS OF METHEMOGLOBINEMIA

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Modeling of methemoglobinemia (MetHb) led to a violation of antioxidant protection, which was manifested by a decrease in the activity of enzymes of the primary link of the antioxidant system – superoxide dismutase and catalase in blood plasma and brain homogenates of experimental animals. The state of MetHb also led to an increase in the concentration of malondialdehyde, an indicator of lipid peroxidation, as well as an indicator of oxidative modification of proteins. It has been established that the antihypoxic activity in the application of succinic acid significantly suppresses the biochemical changes caused by the hypoxic state.

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***Keywords*:** methemoglobinemia, antihypoxic activity, succinic acid.

Introduction. One of the most important consequences of a number of critical conditions is hypoxia, which is manifested by a disruption in the supply of oxygen to tissues and the development of multiple organ failures. Methemoglobinemia (MetHb) develops in some types of hypoxic conditions caused by toxic doses of nitro compounds leading, with a certain degree of its severity, to secondary tissue hypoxia.

MetHb depresses the respiratory function of the blood twice as much as a simple drop in oxygen levels. It has been shown that MetHb disrupts the normal course of metabolism, inhibiting the processes of tissue respiration and the activity of tissue respiration enzymes in cells due to a lack of oxygen and metabolic resources, developing oxidative stress. Studies have shown that MetHb in the range of 0.1–2.0% is protective, neutralizing cyanides, hydrogen sulfide, phenol, and other toxic substances, binding them into complexes with non-toxic compounds. Intoxication with a MetHb content in the blood below 10% may be asymptomatic, although a decrease in physical performance and the presence of functional changes in individual body systems are reported even with such a small concentration of MetHb. However, with an increase in the concentration of MetHb up to 20–40% (average degree of intoxication) patients usually develop headache, dizziness, weakness, shortness of breath. At higher concentrations (severe degree) loss of consciousness, development of severe hypoxia with depression of the respiratory center, generalized cyanosis are possible [1].

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MetHb is of particular interest due to the active use of sodium nitrite in recent years. It has been proven that nitrites are constantly present in the body endogenously, being synthesized in the human body, and synthesis occurs at any level of nitrates in the diet and drinking water. Some authors believe that the high risk of mass and sporadic poisoning with MetHb-formers is associated with intensive chemicalization of the national economy, insufficient efficiency of drinking water purification methods, an increased risk of contamination of drinking water, vegetables and various food products with nitrites and nitrates. At the same time, the authors note that cooking does not significantly reduce the concentration of these compounds in food products [2].

It is believed that exceeding the permissible concentrations of nitrites poses a serious threat to the body – nitrates and nitrites can cause carcinogenic processes.

The formation of toxic MetHb, accompanied by an increase in the level of MetHb in the blood, can provoke an increase in free radical oxidation products in the body [3, 4].

The foregoing makes it relevant to search for therapeutic methods that can cope with hypoxic damage. As a new approach, we observe the development of a class of pharmacological drugs – antihypoxic agents that can reduce or eliminate the manifestations of hypoxia by maintaining the minimum allowable energy metabolism in the cell, especially in the early post-hypoxic period. Antioxidants that directly affect the enzyme complexes of the respiratory tract of mitochondria have been identified. In order to prevent disorders of the respiratory chain, it is recommended to use NADH-oxide-dependent activators of the metabolism, especially activators of the second enzyme complex of the respiratory chain [5].

The purpose of the work is to identify the severity of metabolic disorders in MetHb in experimental animals, to study the effectiveness of succinic acid in order to correct the consequences of oxidative stress resulting from this type of hypoxia.

Materials and Methods.

Laboratory Animals. The studies were carried out on domestic rabbits (*Oryctolagus cuniculus*), weighing 2.2–2.5 *kg*. Animals were kept under standard vivarium conditions with free access to water and food in a room with an air temperature of 22℃ with a 12-hour light/dark cycle in accordance with the rules of bioethics approved by the National Bioethics Committee (Armenia).

Simulation of the Experiment. For experimental modeling of a mild degree of MetHb (methemoglobin in the blood around 20%) we have used sodium nitrite ($NaNO_2$) hypoxant, which is widespread in the modern human environment. Sodium nitrite is a derivative of nitric acid and is converted in the body to sodium nitrate, which oxidizes the ferrous iron of hemoglobin, resulting in the formation of methemoglobin, unable to bind oxygen. $NaNO_2$ was subcutaneously administered at a dose of 35 *mg*/*kg*.

To correct the abnormalities, the animals were given succinic acid (SA) with drinking water half an hour before the injection of sodium nitrite. The dosage of 50 *mg*/*kg* was adjusted during the course of the experiment.

Animals have been divided into 5 groups (n = 3 per group): the first group – intact animals; the second group – animals with the development of MetHb after a single injection of $NaNO_2$; the third group – animals with the development of MetHb after daily administration of $NaNO_2$ for one week; the fourth group – with MetHb,

developing with the introduction of SA to animals of the second group; the fifth group – with methemoglobinemia, developing with the introduction of SA to animals of the third group.

Determination of Blood Biochemical Parameters. Biochemical parameters have been determined in blood plasma and brain homogenates.

Blood has been taken into heparinized vacuum vessels from the marginal vein. Animals have been taken out of the experiment by decapitation one hour after the last injection. The brain tissue has been homogenized in a Potter-Elvehjem glass homogenizer.

To identify the antioxidant status of the organism, the activities of superoxide dismutase (SOD) and catalase have been determined. SOD activity has been determined by the rate of adrenaline autooxidation [6]. Catalase activity has been determined by the colorimetric method [7]. The intensity of lipid peroxidation has been determined by the level of its molecular product – malondialdehyde (MDA). MDA has been determined by the reaction with thiobarbituric acid spectrophotometrically at a wavelength of 535 *nm* [8]. The intensity of oxidative degradation of proteins has been assessed by the level of their carbonyl derivatives recorded in the reaction with 2,4-dinitrophenylhydrazine. The optical density of the resulting dinitrophenylhydrazones has been recorded at a wavelength of 363 *nm* [9].

Processing of Results. Data are presented as arithmetic values with standard error according to Microsoft Excel 2013. Standard error does not exceed 3% of absolute values. Statistical hypotheses were tested using Student's *t*-test [10], the difference between the results of different series of experiments was considered significant at $p<0.05$.

Results and Discussion. In each specific case of hypoxia, the degree of normal provision of oxygen in tissues depends on how much the body's adaptation mechanisms can correct unfavorable factors that reduce the oxygen transport activity of the blood. Any hypoxic state, including MetHb, may be accompanied by an increase in the generation of reactive oxygen species, that is, by the development of oxidative stress.

In this study, the significance of disturbances in the prooxidant-antioxidant balance in the group of animals with simulated MetHb, as well as the possibility of correcting the resulting metabolic disorders has been evaluated. To this end, we used SA (succinate), which has antioxidant properties and, more importantly, the properties of a signaling molecule. Under the influence of extreme environmental factors, it can activate the most important biochemical mechanisms that ensure the adaptation of the organism [11].

In recent years, a secondary product of lipid peroxidation, MDA, has been considered as a trigger of oxidative stress, since an increase in MDA confirms the activation of the process of lipid peroxidation (LPO) leading to cell dysfunction [12]. The paper shows changes in the concentration of MDA during MetHb. According to the results of the experiments, with a single injection of $NaNO_2$ (group 2) in the blood plasma of rabbits, an increase in the concentration of MDA occurred (by about 34% in an hour) compared with the indices of intact animals (group 1). With daily administration of $NaNO_2$ for a week (group 3), the level of MDA in plasma increased by 43%, in brain homogenates by 39% compared with intact animals (Fig. 1).

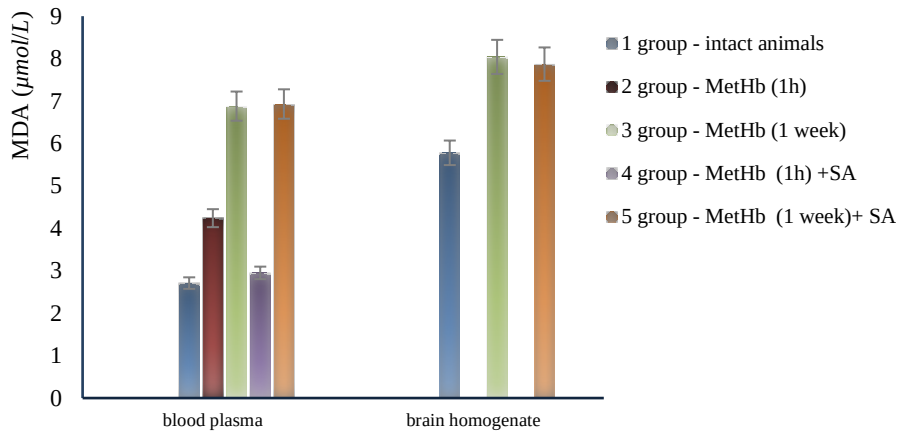

Fig. 1. Indicators of LPO of blood plasma and brain homogenates in normal conditions and in the experiment of the use of SA. ($p<0.05$ for difference between the data of the series and intact values; and $p<0.05$ for difference between the data of the series and the pathological state)

The graph also shows the MDA values in the presence of SA. As you can see, under the conditions of an hourly hypoxic exposure in the blood plasma of rabbits, a decrease in the concentration of MDA has been registered (in the 4th group – by 24%) compared with the indicators in the 2nd group, the value of the indicator approached the results of intact animals, which indicates a positive effect of amber acids. However, in the 5th group, both in blood plasma and in brain homogenates, a significant decrease in this indicator has not been observed. In this case, the use of SA had no effect on the concentration of MDA.

Taking into account that the development of hypoxia disrupts the balance between the formation of reactive oxygen species and the antioxidant protection of tissues, it was advisable to study the indicators characterizing the state of the antioxidant system. Enzymes of the antioxidant system, SOD and catalase, are powerful antioxidant factors that protect against superoxide anion and hydrogen peroxide, providing a favorable level of reactive oxygen species for cell activity. From the point of view of antioxidant protection, the manifestation of SOD activity without an indicator of catalase activity cannot be informative, since a balance between SOD activity and the activity of an enzyme that breaks down hydrogen peroxide is necessary for the normal functioning of the cell. And the level of antioxidant protection can only be determined by examining the activity of both enzymes.

The results of the study indicate that under conditions of MetHb, both in the blood plasma and in the brain tissue homogenates of rabbits, a similar dynamics of changes in the activity of these enzymes has been recorded.

At the initial stage (1 *h* after the administration of hypoxant), an increase in the activity of SOD and catalase has been recorded in the blood plasma of animals (by approximately 6% and 3.5%, respectively) (Fig. 2).

The increase in enzyme activity in the first hour of injury is probably associated with the launch of an adaptation mechanism. This is also evidenced by the fact that in the 3rd group (introduction of sodium nitrite during the week) a decrease in the activity of both enzymes has been registered. SOD activity in blood

plasma decreased by 10.8%, catalase activity by 12.2% (Fig. 1) in brain tissue homogenates, the decrease in SOD activity was 15%, catalase – 18% (Fig. 2).

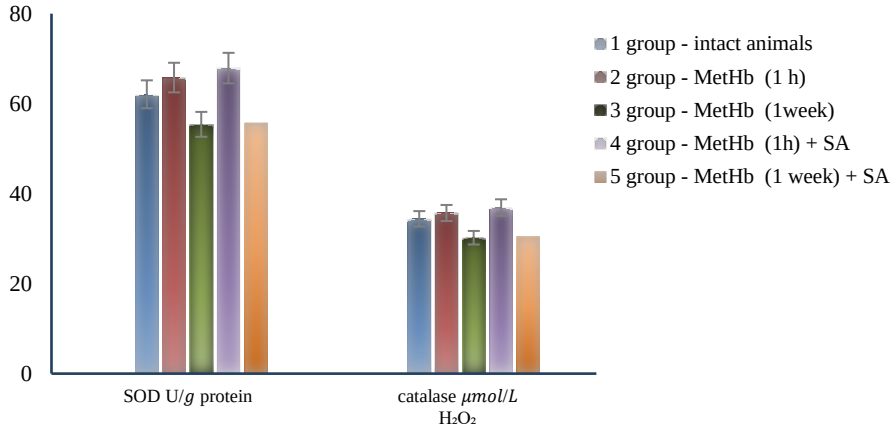

Fig. 2. Influence of SA on the antioxidant status of blood plasma of experimental animals in MethHb. ($p<0.05$ for difference between the data of the series and intact values; and $p<0.05$ for difference between the data of the series and the pathological state)

According to the data presented in the same graph, in the case of one-hour hypoxia in the presence of SA in the blood plasma, the levels of SOD and catalase activity are higher than in the 2nd group (by 3.2% and 3.4%, respectively). But in the 5th group, again, this dynamic does not persist, and in the group of animals with drug correction, the activity indicators of both enzymes remain low relative to the intact group of animals both in blood plasma and in homogenates (Fig. 3). The obtained results demonstrate the development of oxidative stress.

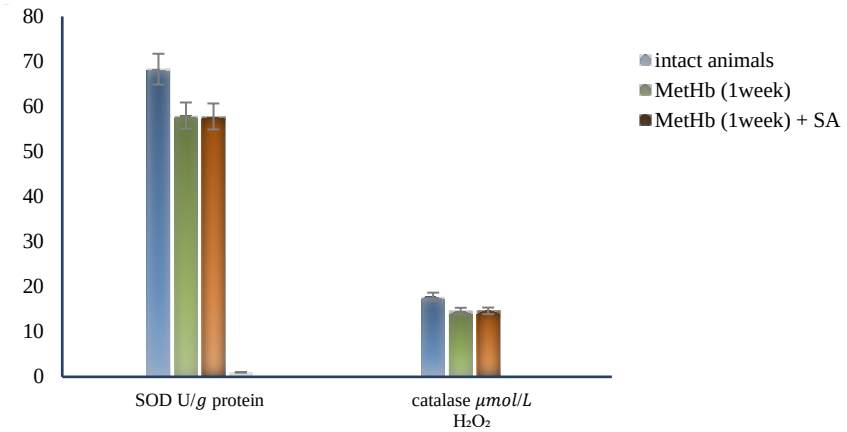

Fig. 3. Influence of SA on the antioxidant status of brain tissues of experimental animals in MetHb. ($p<0.05$ for difference between the data of the series and intact values; and $p<0.05$ for difference between the data of the series and the pathological state)

It is known that not only lipids but also proteins of cells and body fluids are attacked by reactive oxygen species. It should be noted that proteins may be more

sensitive to the harmful effects of free radicals: membrane lipids are to some extent protected from free radicals by endogenous fat-soluble antioxidants (vitamins E, A), while proteins, being hydrophilic, do not have such protection and are perhaps are more susceptible to oxidative modifications. Therefore, one of the early indicators of tissue damage is the process of oxidative degradation of proteins (generation of so-called “carbonyl groups” of proteins).

There is no single indicator of the degree of oxidative modification of proteins. For this purpose, under conditions of oxidative stress, various chemical or physicochemical changes in the protein molecule can be recorded, which will reflect the degree of its damage by reactive oxygen species. However, the determination of carbonyl groups of amino acid residues formed during protein oxidation has a number of practical advantages compared to other approaches and is considered as the most adequate and reliable indicator that quantitatively characterizes the severity of chemical changes in the protein structure depending on the intensity of oxidative free radical processes [13].

In order to identify the degree of protein oxidation under the conditions of our experimental model, we measured the level of carbonyl groups in blood plasma and brain tissue homogenates of rabbits. An increase in the number of carbonyl groups, detected at a wavelength of 363 *nm*, registers the concentration of ketone-dinitrophenylhydrozones, which characterizes later changes in proteins and indicates deep structural disturbances of these macromolecules.

The content of carbonyl groups of proteins in the blood plasma in both the 2nd and 3rd groups were significantly higher compared to those in the control group (by 32% and 46.8%, respectively). As a result of weekly intoxication, an increase in the content of protein carbonyl groups (by 45%) has also been noted in brain homogenates, which may indicate a serious change in the protein composition in the tissue (Fig. 4).

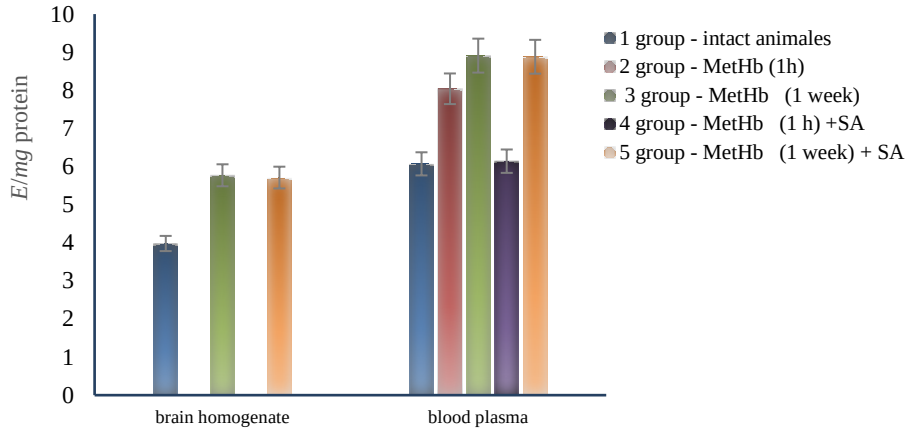

Fig. 4. Indicators of oxidative modification of blood plasma proteins and brain homogenates in normal conditions and in the experiment of the use of SA. ($p<0.05$ for difference between the data of the series and intact values; and $p<0.05$ for difference between the data of the series and the pathological state)

In case of one-hour hypoxia, the use of succinic acid brought the value of this indicator closer to the norm, but had little effect on the degree of protein degradation during weekly intoxication.

Perhaps it is under our conditions of relatively long hypoxia that the oxygen deficiency, which limits the rate of oxidation of the Krebs cycle substrates, reduces the value of succinate.

Conclusion. Thus, the development of MetHb suppresses antioxidant defense enzymes, enhances the processes of lipid peroxidation, and leads to the degradation of protein structures. A positive effect of SA on the correction of disorders has been revealed, although it has been shown that the effect of SA is short-term. At the initial stage the exposure to SA to some extent inhibits the activity of LPO and affects the processes of oxidative modification of proteins, which can possibly "even out" the negative effect of intoxication. But it should be noted that with a longer toxic effect, the SA does not affect the damage caused by the development of oxidative stress, which is probably due to the low permeability of the compound. The bioavailability of the compound may be facilitated when it is co-administered with other compounds. Higher doses of succinic acid should also be considered, taking into account the exclusion of an overdose of the drug.

The obtained data expand the understanding of the adaptive capabilities of the body in this type of hypoxic conditions.

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Ն. Կ. ՀԱՅՐԱՊԵՏՅԱՆ

ՍԱԹԱԹՎԻ ՀԱԿԱՀԻՊՈՔՍԻԿ ԱՐԴՅՈՒՆԱՎԵՏՈՒԹՅԱՆ ՈՒՍՈՒՄՆԱՍԻՐՈՒԹՅՈՒՆԸ ՄԵԹՀԵՄՈԳԼՈԲԻՆԵՄԻԱՅԻ ՊԱՅՄԱՆՆԵՐՈՒՄ

Մեթհեմոգլոբինեմիայի (MetHb) մոդելավորումը հանգեցրել է հակաօքսիդանտային պաշտպանության խանգարմանը, ինչն արտահայտվում է հակաօքսիդանտային համակարգի առաջնային օղակի ֆերմենտների՝ սուպերօքսիդդիսմուտազի և կատալազի ակտիվության նվազմամբ փորձարարական կենդանիների արյան պլազմում և գլխուղեղի հյուսվածքներում: MetHb արդյունքում գրանցվել է լիպիդային գերօքսիդացման ցուցանիշ մալոնային երկալդեհիդի կոնցենտրացիայի, սպիտակուցների օքսիդային փոխակերպման ցուցանիշի կոնցենտրացիայի աճ: Հաստատվել է, որ սաթաթթուն զգալիորեն ճնշում է հիպոքսիկ վիճակի հետևանքով առաջացած կենսաքիմիական փոփոխությունները:

Н. К. АЙРАПЕТЯН

ИССЛЕДОВАНИЕ АНТИГИПОКСИЧЕСКОЙ ЭФФЕКТИВНОСТИ ЯНТАРНОЙ КИСЛОТЫ В УСЛОВИЯХ МЕТГЕМОГЛОБИНЕМИИ

Моделирование метгемоглобинемии (MetHb) приводит к нарушению антиоксидантной защиты, что проявляется снижением активности ферментов первичного звена антиоксидантной системы – супероксиддисмутазы и каталазы в плазме крови и тканях головного мозга экспериментальных животных. Состояние MetHb приводит к увеличению концентрации малонового диальдегида – показателя перекисного окисления липидов, а также показателя окислительной модификации белков. Установлено, что янтарная кислота достоверно подавляет вызванные гипоксическим состоянием биохимические изменения.