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THE STUDY OF THE EFFECTIVENESS OF ANTIHYPOXIC PROPERTIES OF CERTAIN COMPOUNDS OF VARIOUS ACTION MECHANISMS ON HEMIC HYPOXIA MODELS

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The article presents experimental data on the effectiveness of drugs that could stimulate the start of metabolic adaptation during the development of methemoglobinemia. The modeling of this type of hemic hypoxia led to the disruption of antioxidant protection, resulting in a rather significant decrease of superoxide dismutase and catalase (primary antioxidant enzymes) in the blood plasma of tested animals. We have established that anti-hypoxic activity was higher with the use of mexidol compared to the use of tocopherol. The differences in the indicators can perhaps be explained by the different mechanisms of action of the used antioxidants. The state of methemoglobinemia has also led to increased concentrations of medium-size molecules in the blood plasma. The use of mexidol and tocopherol did not show any significant effect on normalization.

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***Keywords*:** anti-hypoxic activity, hemic hypoxia, mexidol, tocopherol.

Introduction. Any type of hypoxia is accompanied by a decrease in oxidative metabolism and leads to disruption of the energy-producing system of the cell. As a result, a discrepancy between the energy demand and energy production of the cell is established, the process of synthesis of high-energy compounds, for example, ATP, is disrupted. ATP deficiency and the accompanying metabolic and structural changes become toxic factors that change the functions of membrane-bound proteins, the structure of lipid-protein interactions. ATP deficiency leads to the activation of anaerobic glucose breakdown, causes the accumulation of lactic acid and the development of acidosis. In conditions of O_2 deficiency, acidosis is aggravated by insufficient oxidation of fatty acids. The accumulation of under-oxidized active forms of fatty acids (acylcarnitine, acyl-CoA) inhibits adenine nucleotide translocase, thereby inhibiting the transport of ATP produced in mitochondria to the cytosol [1, 2]. This creates the prerequisites for the initiation of lipid peroxidation (LPO), which has a pronounced membrane damaging effect. The LPO activity causing modification of biological membranes is also stimulated by suppression of the activity of enzymes of the antioxidant system and, first of all, superoxide dismutase (SOD), catalase, which in turn is provoked by the formation of free radicals [3, 4]. Thus, a vicious circle is closed – a violation of energy

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metabolism leads to the activation of free radical oxidation, which damages the mitochondrial membranes, thereby increasing the energy deficit of the cell.

In recent years, it was found that under various pathological conditions, including hypoxia against the background of the formation of reactive oxygen species and the intensification of free radical oxidation processes, activation of LPO reactions in blood plasma, so-called molecules of middle mass (MM) appear in increased concentrations, covering the range with a molecular weight of 300–5000 *Da*. Molecules of this kind are degradation products of proteins and their complexes. An essential feature of MM is their clearly pronounced high biological activity and the ability to aggravate metabolic disorders that caused their formation. In fact, they acquire the role of "secondary toxins", influencing the vital activity of the cell [5]. Although a certain part of MM is a product of blood protein catabolism, it should not be ruled out that MM can be synthesized to bring homeostasis back to normal.

With the accumulation of information that the main indicators of hypoxia of any etiology, including endogenous hemic, are disorders of energy metabolism, it became clear that the antihypoxic defense of the body should consist in restoring the energy balance of the cell and the correction of energy metabolism can be achieved with the help of antihypoxants. Capable of influencing various links of energy exchange.

Currently, drugs that support the activity of the succinate oxidase link are finding practical use. One of these drugs is mexidol. The presence of succinate in the structure of mexidol is of fundamental importance for the manifestation of the pharmacological effects of the drug. In the presence of mexidol, the succinate oxidase oxidation pathway was activated, which under conditions of limiting NAD-dependent oxidation in the early stages of hypoxia allows the cytochrome portion of the respiratory chain to retain the ability of the cytochrome region to generate energy [6].

Some vitamins also exhibit pronounced antioxidant properties. So, vitamin E (α-tocopherol) has antioxidant properties. α-tocopherol has the ability to interrupt free radical oxidation chains due to the transfer of hydrogen from the phenolic ring to the peroxide radical, with the formation of a stable phenoxide radical, incapable of further free radical transformations [7, 8].

Despite the widespread use of antioxidants and antihypoxants in medicine, until now there is no clear systematization of drugs and pathogenetic substantiation of the appropriateness of combinations of certain antioxidants and antihypoxants.

The aim of the study was to identify the severity of metabolic disorders in experimental animals under conditions of hemic hypoxia, to compare the dynamics of these parameters against the background of the introduction of antioxidants of various classes – mexidol and α-tocopherol.

Materials and Methods.

Laboratory Aimals. The studies were carried out on rabbits (*domestic rabbits, Oryctolagus cuniculus).* The animals were kept in standard vivarium conditions with free access to water and food, in a room with an air temperature of 22°C with a 12 *h* light / dark cycle. The studies were carried out in accordance with the bioethics rules approved by the National Bioethics Committee (Armenia).

Simulation of the Experiment. Modeled the average degree of methemoglobinemia (blood methemoglobin is 35–36%) by introducing $NaNO_2$ at a dose of 50 *mg*/*kg* subcutaneously [9].

To correct the disorders, mexidol was injected intraperitoneally at a concentration of 100 *mg*/*kg*, α-tocopherol – in the form of a 10% oil solution at a concentration of 200 *mg*/*kg* 30 *min* before the introduction of $NaNO_2$. Concentrations correspond to the dosages used in experiments [10].

For research, the animals were divided into 4 groups (n = 5 per group): I group – intact animals, II group – animals with the development of hemic hypoxia,III group – animals with hemic hypoxia developing against the background of the introduction of mexidol, IV group – animals with hemic hypoxia, developing against the background of the introduction of tocopherol.

Determination of Blood Biochemical Parameters. Blood was taken from the marginal vein 50 *min* after poisoning, when the concentration of methemoglobin (metHb) in the blood was at its maximum.

To identify the antioxidant status of the organism, the activities of SOD and catalase were determined. SOD activity was determined by the rate of adrenaline autooxidation [11]. Catalase activity was determined by the colorimetric method [12]. The intensity of lipid peroxidation was determined by the level of its molecular product: malondialdehyde (MDA). MDA was determined by the reaction with thiobarbituric acid spectrophotometrically at a wavelength of 535 *nm* [13]. The level of intoxication was assessed by determining the MM [14].

Processing of Results. Data are presented as arithmetic mean values with standard error in accordance with Microsoft Excel 2013. Standard error does not exceed 3% of absolute values (not shown in the figures). Statistical hypotheses were tested according to Student's *t*-test [15], the difference between the results of different series of experiments was taken as significant at $p<0.05$.

Results and Discussion. The reason for the development of hemic hypoxia can be the effect on the body of a number of chemical compounds (nitrates, nitrites, nitric oxide, some toxins of infectious origin, drugs), leading to the formation of metHb. Methemoglobinemia is expressed in a decrease in the oxygen-binding properties of hemoglobin. The process of metHb formation is reversible, but its reduction to normal hemoglobin occurs relatively slowly (over several hours), when the oxide form of iron (Fe^{3+}) again transforms into the ferrous form (Fe^{2+}). Clinical manifestations are observed when a significant amount of metHb accumulates in the blood – more than 30%.

We assessed the significance of violations of the prooxidant-antioxidant balance in the group of animals with simulated hemic hypoxia against the background of drug correction using antioxidants of various mechanisms of action.

The state of the enzyme link of antiradical protection of cells was studied according to the activity of SOD and catalase, LPO processes and their changes under the influence of α-tocopherol and mexidol, as shown by the experimental results, 50 *min* after the start of hypoxia there was a significant decrease in the activity of SOD (by 60%) and catalase (by 54%) in the blood plasma of animals as compared with those of the control group (Fig. 1). It should be noted that the activity of the enzyme link of the antioxidant blood system underwent phase changes: at the early stage of hypoxia (10 *min* after the start of hypoxia), there was some SOD activation, followed by suppression at the later stages of pathology (50 *min* after the start of hypoxia). In the group with mexidol correction, the activity of catalase and

SOD in the blood plasma of animals (III group) increased by 47% and 51%, respectively, with tocopherol (IV group) – by 30% and 34%, respectively, relative to the experimental group of animals without medical correction (Fig. 1).

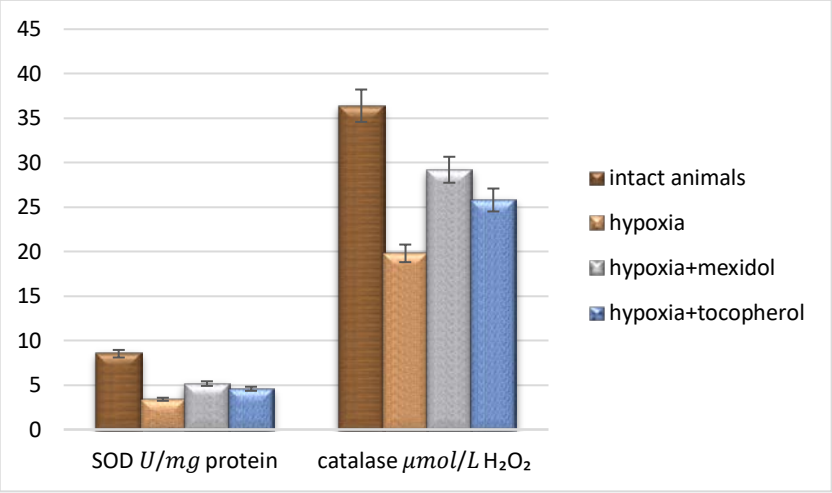

Fig. 1. Influence of mexidol and α-tocopherol on the antioxidant status of blood plasma of experimental animals in hemic hypoxia (differences are significant, n = 5, p <0.05).

It should be noted that synchronicity in changes in the activity of SOD and catalase is also noted by other authors [16, 17].

The presented data indicate, that under conditions of methemoglobinemia, inactivation of key enzymes of the antioxidant system occurs. The use of correction drugs leads to a partial restoration of enzyme activity, but not to the indices of a group of intact animals.

It was also found that in the blood plasma of rabbits there is a significant increase (by 3.5 times) in the MDA content: its concentration increased from 2.71 $\mu mol/L$ in the control to 9.54 $\mu mol/L$ in the group of animals subjected to hypoxia (Fig. 2). The MDA level in the III group (experimental group with the use of mexidol) was increased by 2.3 times, in the IV (experimental group with the use of tocopherol) – 2.9 times compared with the indicators of the control group (Fig. 2).

According to modern concepts, there is a relationship between the processes of LPO and accumulation in the blood of MM [18]. Under our conditions, the level of MM in the hypoxic period increased by 4.3 times (Fig. 3). Administration of mexidol and tocopherol did not seriously affect MM levels. The content of MM remained significantly higher than the blood plasma indices of intact animals both against the background of the introduction of mexidol and against the background of the introduction of tocopherol (3.8 times and 3.9 times, respectively).

Thus, 50 *min* after the development of acute hemic hypoxia, metabolic disorders appeared in the blood plasma of animals in the form of activation of LPO processes and insufficiency of the antioxidant system. The introduction of β-tocopherol and mexidol in the indicated doses caused a decrease in the intensity of lipid peroxidation, manifested by a decrease in the concentration of MDA. There

was also a tendency to restore the activity of antioxidant enzymes. The antihypoxic activity with the use of mexidol was more pronounced, than with the use of tocopherol. The differences in the mechanisms of action of the antioxidants used may explain the differences in the recorded values of the indicators.

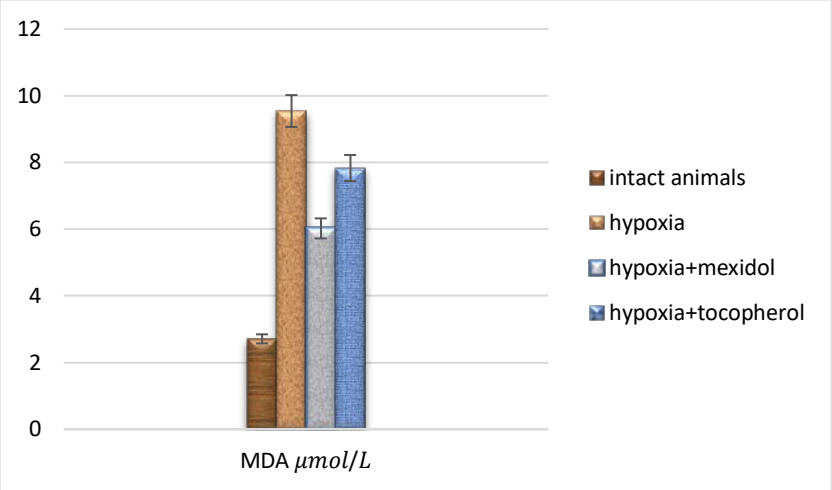

Fig. 2. Changes in the content of MDA in the blood plasma of animals against the background of the use of mexidol and α-tocopherol in hemic hypoxia (differences are significant, n = 5, p <0.05).

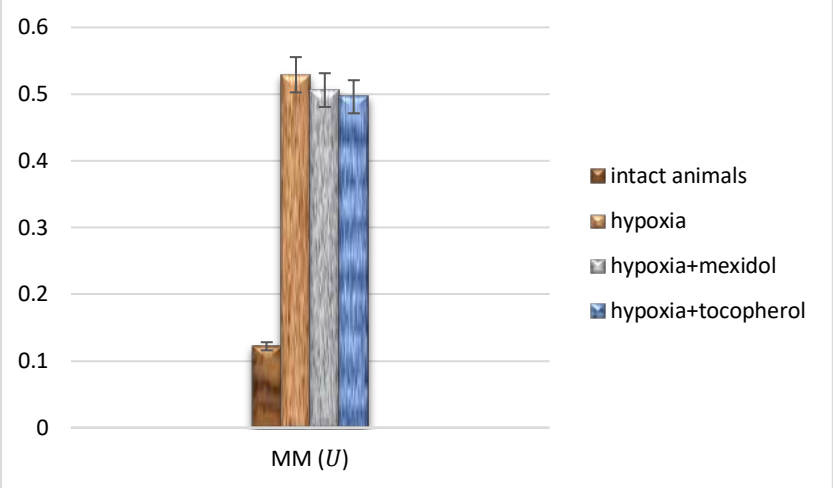

Fig. 3. Changes in the content of MM in the blood plasma of animals against the background of the use of mexidol and α-tocopherol in hemic hypoxia

Conclusion. As a result of the development of experimental hemic hypoxia, metabolic disorders appeared in the blood plasma of animals in the form of activation of LPO processes and insufficiency of the antioxidant system. The injection of α-tocopherol and mexidol in the indicated doses caused a decrease in the intensity of lipid peroxidation, manifested by a decrease in the concentration of MDA. A

tendency to restore the activity of antioxidant enzymes has also been observed. The antihypoxic activity with the use of mexidol was more pronounced than with the use of tocopherol. The differences in the mechanisms of action of the antioxidants used may explain the differences in the recorded values of the indicators. The obtained data suggest that the use of antioxidant protection agents can weaken the negative effects of chemical compounds causing intoxication and leading to the development of hypoxia.

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REFERENCES

1. Prijanti A.R., Iswanti F.C., et al. Hypoxia Increased Malondialdehyde from Membrane Damages is Highly Correlated to HIF-1α but not to Renin Expression in Rat Kidney. *IOP Conf. Ser. Earth Environ. Sci*. **217** (2019), 012062. https://doi.org/10.1088/1755-1315/217/1/012062
2. Souza e Silva L.F., Brito M.D., et al. Mitochondrial Dysfunction and Changes in High-Energy Compounds in Different Cellular Models Associated to Hypoxia: Implication to Schizophrenia. *Sci. Rep*. **9** (2019), Article number: 18049. https://doi.org/10.1038/s41598-019-53605-4
3. Ayala A., Munoz M.F., Argüelles S. Lipid Peroxidation: Production, Metabolism and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. *Oxid. Med. Cell. Long*. (2014), Article ID: 360438. https://doi.org/10.1155/2014/360438
4. Rauchová M. Vokurková, Koudelová J. Hypoxia-Induced Lipid Peroxidation in the Brain during Postnatal Ontogenesis. *Physiol. Res*. **61** (2012), 89–101. https://doi.org/10.33549/physiolres.932374
5. Nikolskaya V.A., Danilchenko Yu.D., Memetova Z.N. Biochemical Aspect of Consideration of the Role of Average Mass Molecules in the Body. *Bulletin of the Crimean Federal University: Biology, Chemistry* **26** (2013), 139–145 (in Russian).
6. Tretter L., Patocs A., Chinopoulos C. Succinate, an Intermediate in Metabolism, Signal Transduction, ROS, Hypoxia, and Tumorigenesis. *Biochim. Biophys. Acta* (BBA) – *Bioenergetics* **1857** (2016), 1086–1101. https://doi.org/10.1016/j.bbabio.2016.03.012
7. Bel'skaya L.V., Kozenok V.K., Massard G. Endogenous Intoxication and Saliva Lipid Peroxidation in Patients with Lung Cancer. *Diagnostics* **6** (2016), 39. https://doi.org/10.3390/diagnostics6040039
8. Rudolphi-Skorska E., Filek M., Zembala M. α-Tocopherol/Gallic Acid Cooperation in the Protec–tion of Galactolipids Against Ozone-induced Oxidation. *J. Membr. Biology* **249** (2016), 87–95. https://doi.org/10.1007/s00232-015-9851-4
9. Karkischenko N.N., Karkishchenko V.N., et al. *Biomedical (Preclinical) Study of the Antihypoxic Activity of Drugs*. Moscow (2017), 98 p.
10. Volchegorskii I., Miroshnichenko I., et al. Effects of Derivatives of 3-Hydroxypyridine and Succinic Acid on Stereotypical Behavior and Catalepsy in Mice. *Neurosci. Behav. Physiol*. **48** (2018), 947–953. https://doi.org/10.1007/s11055-018-0654-9
11. Sirota V. A Novel Approach to Study the Reaction of Adrenaline Autooxidation: A Possibility for Polarographic Determination of Superoxide Dismutase Activity and Antioxidant Properties of Various Preparations. *Biochem. Suppl. Ser. B Biomed. Chem.* **5** (2011), Article number: 253. https://doi.org/10.1134/S1990750811030139

12. Boriskin P., Deviatkin A., et al. Relationship of Catalase Activity Distribution in Serum and Tissues of Small Experimental Animals. *IOP Conf. Ser. Earth Environ. Sci.* **403** (2019), 012113. https://doi.org/10.1088/1755-1315/403/1/012113
13. Fogarasi E., Croitoru M.D., et al. Malondialdehyde Levels can be Measured in Serum and Saliva by Using a Fast HPLC Method with Visible Detection. *Revista Română de Medicină de Laborator*. **24** (2016), 319-326. https://doi.org/10.1515/rrlm-2016-0029
14. Shitov A.Yu. Molecules of Middle Mass as an Indicator of Divers' "Hyperbaric Intoxication". *Almanac of Clinical Medicine* **28** (2013), 48–52. https://doi.org/10.18786/2072-0505-2013-28-48-52
15. Kobzar A.I. *Applied Mathematical Statistics*. Moscow, Fizmatlit (2006), 816 p. (in Russian).
16. Muñiz P., Coma M.J., Terán J. Oxidative Stress and Vascular Damage in Hypoxia Processes Malondialdehyde (MDA) as Biomarker for Oxidative Damage. *Electron. J. Biomed.* **2** (2014), 50–53.
17. Parrilla-Taylor D.P., Zenteno-Savín T. Antioxidant Enzyme Activities in Pacific White Shrimp (*Litopenaeus vannamei*) in Response to Environmental Hypoxia and Reoxygenation. *Aquaculture* **318** (2011), 379–383. https://doi.org/10.1016/j.aquaculture.2011.05.015
18. Górnicka M., Ciecierska A., et al. α-Tocopherol Protects the Heart, Muscles, and Testes from Lipid Peroxidation in Growing Male Rats Subjected to Physical Efforts. *Oxid. Med. and Cell. Long.* (2019), Article ID: 8431057. https://doi.org/10.1155/2019/8431057

Ն. Կ. ՀԱՅՐԱՊԵՏՅԱՆ

ՆԵՐԳՈՐԾՈՒԹՅԱՆ ՏԱՐԲԵՐ ՄԵԽԱՆԻԶՄՆԵՐ ՈՒՆԵՑՈՂ ՄԻԱՑՈՒԹՅՈՒՆՆԵՐԻ ՀԱԿԱՀԻՊՈՔՍԻԿ ՀԱՏԿՈՒԹՅՈՒՆՆԵՐԻ ԱՐԴՅՈՒՆԱՎԵՏՈՒԹՅԱՆ ՈՒՍՈՒՄՆԱՍԻՐՈՒԹՅՈՒՆԸ ՀԵՄԻԿ ՀԻՊՈՔՍԻԱՅԻ ՄՈԴԵԼՆԵՐՈՒՄ

Հոդվածում ներկայացվում են դեղամիջոցների արդյունավետության փորձարարական տվյալներ, որոնք կարող են խթանել նյութափոխանակության հարմարվողականության սկիզբը մեթեմոգլոբինեմիայի զարգացման պայմաններում: Հեմային հիպոքսիայի այս տիպի մոդելավորումը հանգեցնում է հակաօքսիդանտային պաշտպանության խանգարման, ինչն արտահայտվում է հակաօքսիդանտային համակարգի առաջնային օղակի ֆերմենտների՝ սուպերօքսիդդիսմուտազի և կատալազի ակտիվության կտրուկ նվազմամբ փորձարարական կենդանիների արյան պլազմում: Հաստատվել է, որ մեքսիդոլի կիրառման դեպքում հակահիպոքսիկ ակտիվությունն ավելի արտահայտված է, քան տոկոֆերոլի կիրառման դեպքում: Ցուցանիշների գրանցված արժեքների տարբերությունը կարելի է, թերևս, բացատրել կիրառվող հակաօքսիդանտների ազդման մեխանիզմների տարբերությամբ: Մեթեմոգլոբինեմիայի վիճակը հանգեցրել է նաև արյան պլազմում միջին զանգվածի մոլեկուլների կոնցենտրացիայի աճի: Հատկանշական է, որ ինչպես մեքսիդոլը, այնպես էլ տոկոֆերոլը նշանակալի դրական ազդեցություն չեն դրսևորել այս ցուցանիշի արժեքի վրա:

Н. К. АЙРАПЕТЯН

ИЗУЧЕНИЕ ЭФФЕКТИВНОСТИ ПРОТИВОГИПОКСИЧЕСКИХ СВОЙСТВ НЕКОТОРЫХ СОЕДИНЕНИЙ РАЗЛИЧНОГО МЕХАНИЗМА ДЕЙСТВИЯ НА МОДЕЛЯХ ГЕМИЧЕСКОЙ ГИПОКСИИ

В статье представлены экспериментальные данные об эффективности средств, которые могли бы стимулировать запуск метаболической адаптации при развитии метгемоглобинемии. Моделирование этого типа гемической гипоксии приводило к нарушению антиоксидантной защиты, что проявлялось довольно резким снижением активности ферментов первичного звена антиоксидантной системы – супероксиддисмутазы и каталазы в плазме крови экспериментальных животных. Установлено, что антигипоксическая активность при применении мексидола была более выраженной, чем при применении токоферола. Возможно, отличия в регистрируемых значениях показателей можно объяснить различием механизмов действия примененных антиоксидантов. Состояние метгемоглобинемии приводило также к увеличению концентрации молекул средней массы в плазме крови. Показательно, что как мексидол, так и токоферол не оказали значимого положительного влияния на нормализацию этого параметра.