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Study of metabolic disorders in rats under exposure to hypobaric hypoxia and development of correction approaches by simultaneous action on different elements of pathogenesis

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Abstract

Aim. To study the indicators of metabolic changes in the blood and brain structures of rats after exposure to hypobaric hypoxia and to determine possible pharmacological approaches to correction these changes.

Methods. Hypobaric hypoxia in rats was simulated for 30 minutes in a pressure chamber, simulating an ascent to 8500 m. 3 and 24 hours after hypoxia, the activity of alanine aminotransferase, aspartic aminotransferases, alkaline phosphatase, creatine phosphokinase, lactate dehydrogenase, the content of glucose, total protein, triglycerides, cholesterol, β-lipoproteins, iron and uric acid were determined in the blood serum. The level of malondialdehyde in the hippocampus and frontal cortex was examined. The studies of the effect of 2-chloroethoxy-aryl-dimethyl-aminophenylphosphorylacetohydrazide (CAPAH) (1 mg/kg) and Piracetam (100 mg/kg) after intraperitoneal injection 40 minutes before hypoxia and 1 hour after removing the rats from the pressure chamber were carried out. Statistical analysis was carried out using the GraphPad Prism software version 8.0.1, and the Student's t-test was used to test statistical significance.

Results. After 3 hours of hypobaric hypoxia, rats showed hyperenzymemia and dyslipidemia, the activity of almost all studied enzymes in the blood serum of rats was increased, the content of triglycerides was decreased, and the concentration of cholesterol was increased, the content of malondialdehyde in the hippocampus and frontal cortex was increased. In 24 hours after hypoxia, an increased level of creatine phosphokinase in the blood serum and malondialdehyde in the brain structures were noted. The use of 2-chloroethoxy-aryl-dimethyl-aminophenylphos phorylacetohydrazide prevented the development of hyperenzymemia, dyslipidemia and corrected the increased level of creatine phosphokinase after 24 hours; in both modes of administration, it reduced the serum level of malondialdehyde. Piracetam showed little effect only when administered prophylactically, preventing an increase in serum alkaline phosphatase activity and cholesterol levels.

Conclusion. The revealed efficacy of 2-chloroethoxy-aryl-dimethyl-aminophenylphosphorylacetohydrazide and its previously studied complex mechanism of action suggest that 2-chloroethoxy-aryl-dimethyl-aminophenylphosphor ylacetohydrazide is a potential drug for the prevention of hypoxic disorders and acceleration of adaptation to high-altitude hypoxia.

Keywords: hypobaric hypoxia, hyperenzymemia, dyslepidemia, malondialdehyde, complex mechanism of drug action, 2-chloroethoxy-aryl-dimethyl-aminophenylphosphorylacetohydrazide, (CAPAH).

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Background. Hypoxia is the major pathogenetic factor of many diseases and it affects all systems, both at the organismic and subcellular levels. The brain is mostly affected by hypoxia due to the high intensity of its metabolism [1, 2]. Changes caused by metabolic disorders, accumulation of lipid peroxidation {LPO} products caused by hypoxia of various origins, and intensities are becoming a ma-

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jor pathogenetic factor in the deterioration of cognitive-mnestic functions.

Hypobaric hypoxia {HH} represents the main syndrome of altitude sickness. Atmospheric air at all altitudes comprises oxygen by 21%; with an increase in the altitude, atmospheric pressure and partial pressure decreases, and the level of inhaled oxygen is significantly reduced. This leads to the development of a hypoxic state, causing several pathological reactions including biochemical, molecular, and genomic changes, most of which are focused on the brain [3]. Oxygen deficiency in the brain structures may lead to oxidative stress, triggering the processes of neurodegeneration with a subsequent disruption of the functions of neurotransmitter systems [4], primarily in the hippocampus and frontal cortex, which leads to impairment of memory and learning processes [5].

Since the most essential disorders in the pathogenesis of hypoxic damage are the changes in the energy processes, the integrity of membrane structures and enzyme systems, it is necessary to prevent and correct these disorders using pharmacological substances that may interfere specifically with the processes of increased resistance to hypoxic exposure, accelerate adaptation, and preventing brain dysfunctions [6].

Currently, anti-hypoxic drugs are used to treat the effects of hypoxic exposure, which increases the cell resistance during lack of oxygen, as well as antioxidants that may normalize the LPO processes, and also nootropic drugs that improve brain function [7]. Given the variety of mechanisms for developing hypoxic damage and various nonspecific effects, depending on the degree and consequences of metabolic disorders in various organs, it is necessary to use drugs that have a simultaneous effect on different links in the pathogenesis of these injuries.

Previously, it was revealed that the innovative drug 2-chloroethoxy-aryl-dimethyl-aminophenylphosphorylacetohydrazide {CAPAH}, which is at the final stage of preclinical trials, possesses a multitarget mechanism of action, which exhibits a protective effect in the hypoxia of various origins and prevents the degeneration of cortical neurons in rats subjected to the hypoxic action [8]. The ability of a wide ranged doses of CAPAH improves the processes of memory and learning, which could be impaired by hypoxic exposure, revealed in experiments on Mongolian gerbils, is the most important in the range of pharmacological action [9].

Considering the factors and to test the hypothesis about the efficiency of simultaneous pharmacological actions on different links of pathogenesis during hypoxia, it is necessary to analyze the feasibility of using CAPAH in the prevention and correction of the disorders caused by exposure to HH and to categorize it as a potential drug with a complex {multitarget} mechanism of action for the prevention and treatment of the altitude sickness and other pathological hypoxic conditions.

Materials and methods of research. The experiments were performed on 120 male Wistar rats weighing 180−210 g, which were divided into 12 groups of 10 animals each. Before the start of the experiments, all animals were kept under standard vivarium conditions with a natural light regime on a complete balanced diet {GOST R 50258-92}, in compliance with the International Recommendations of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes {1997}, and the rules of laboratory practice for conducting the preclinical studies in the Russian Federation, approved by the order of the Ministry of Health of the Russian Federation No. 708n dated 08/23/2010. All studies were approved by the local ethics committee of the Kazan State Medical University of the Ministry of Health of Russia {Protocol No. 10 of 12/19/2017}.

HH in rats was simulated for 30 min in a pressure chamber {the volume of a pressure chamber was 7.5 L, designed for one rat}, simulating an ascent to a height of 8500 m at a speed of 500 m/min [10].

The objects of the study were the potential drug CAPAH, which is at the final stage of preclinical trials, and the nootropic drug piracetam in ampoules {Organika, Russia}.

Two series of experiments were performed:

a) Study of biochemical parameters in blood serum 3 h after exposure to hypoxia with the prophylactic {40 min before placing the rat in a pressure chamber} administration of the drugs

b) Study of biochemical parameters in blood serum 24 h after the hypoxic exposure with the administration of drugs in the treatment mode, 1 hour after the animal was removed from the pressure chamber

Separate groups having the rats unexposed to HH {group of control animals; group of rats administered with CAPAH; and group of rats injected with piracetam}. Blood samples were collected from these groups of animals simultaneously.

Pharmacological agents were administered once intraperitoneally. CAPAH was injected at a dose of 1 mg/kg {1/1000 of $LD_{50,}$ which is most effective for hypoxic effects}, and piracetam was injected at a dose of 100 mg/kg, which is 1/100 of a half-lethal dose {the standard dose as a reference drug, used in the experiments}. The isotonic sodium chloride solution was administered in an equivalent volume in the rats exposed to HH {without correction with

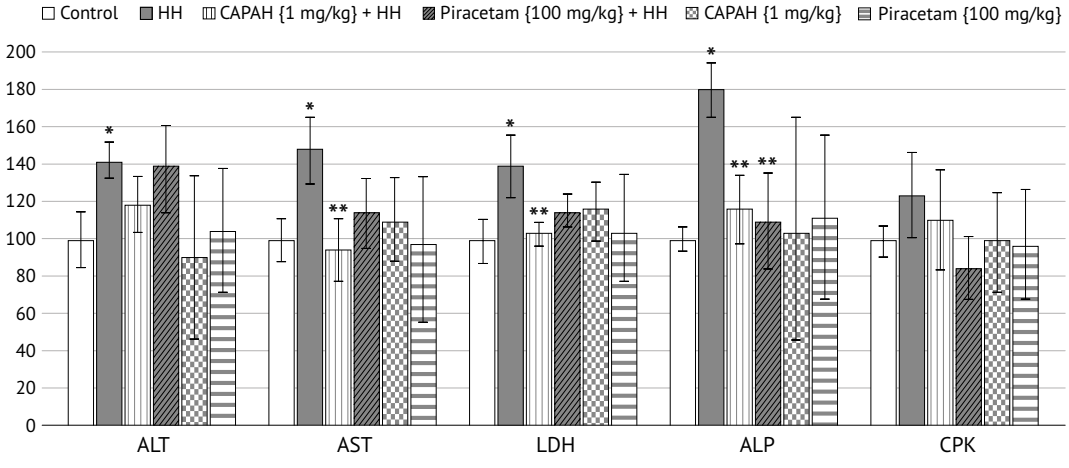

Fig. 1. The activity of enzymes in the blood serum of rats 3 h after the exposure to hypobaric hypoxia {HH} with the administration of 2-chloroethoxy-aryl-dimethyl-aminophenylphosphorylacetohydrazide {CAPAH; 1 mg/kg intraperitoneally} and piracetam {100 mg/kg intraperitoneally} for 40 min before placing the rat in the pressure chamber. The abscissa shows the names of enzymes; the ordinate indicates the enzyme activity {%} in relation to control values taken as 100%. The number of rats in each group was n = 10. ALT—alanine aminotransferase; AST—aspartate aminotransferase; LDH—lactate dehydrogenase; ALP—alkaline phosphatase; CPK—creatine phosphokinase; *difference is significance in relation to the control; **difference is significance in relation to the HH.

CAPAH or piracetam} and control animals {without HH}.

After 3 h {a} and 24 h {b}, blood was taken from the tail vein of the rats to determine the enzyme activity, after which the rats were decapitated using a guillotine {apparatus of the Open Science Scientific Production Association}, and the blood was taken to determine other biochemical parameters. In parallel, rapid isolation of the brain structures {hippocampus and frontal cortex} was performed to determine the level of malondialdehyde {MDA}.

Serum samples were prepared by settling the whole blood at room temperature for coagulation followed by centrifugation for ~10 min at 3000 rpm. The activity of alanine aminotransferase {ALT}, aspartate aminotransferase {AST}, alkaline phosphatase {ALP}, creatine phosphokinase {CPK}, lactate dehydrogenase {LDH}, γ-glutamyl transpeptidase, and the levels of glucose, total protein, triglycerides, cholesterol, β-lipoproteins, serum iron, and uric acid was measured using an analyzer Cobas Integra 400 Plus, Hoffman la Roche {Switzerland}, with the use of mono-tests of the same company following the instructions.

The accumulation of MDA as the main marker of LPO in the rat brain structures was determined by the reaction with Thio-barbituric acid according to the method [11]. The determination was performed on a BioMate 3S spectrophotometer {Thermo Scientific, USA} at a wavelength of 532 nm. The amount of MDA was calculated in μM/kg of wet tissue.

Statistical processing of the results was performed using the GraphPad prism 8.0.1 program with the calculation of the student's *t*-test.

Results. At the "altitude" of 8500 m, the animals showed signs of hypoxia development, namely, acrocyanosis, rapid breathing, decreased motor activity, increased amount of defecation, and development of "lateral position."

Acute hypoxia caused hyperenzymemia. Three hours after exposure to HH in the blood serum of the rats, the activity of enzymes ALT {HH 87.1 ± 6.9 U/L, control 61.7 ± 7.5 U/L at $p = 0.03$}, AST {HH 275.5 ± 29.3 U/L, control 185.5 ± 23.5 U/L at $p = 0.05$}, LDH {HH 3085.6 ± 326.7 U/L, control 2209.6 ± 228.6 U/L at $p = 0.02$}, ALP {HH 44.8 ± 3.5 U/L, control 24.7 ± 1.4 U/L at $p = 0.015$} increased.

Figure 1 presents a comparative assessment of enzyme activity after exposure to HH against the prophylactic administration of the drugs, and its results are presented as a percentage for clarity.

Prophylactic CAPAH prevented the development of HH-induced hyperenzymemia, and the activity of all enzymes studied did not differ from the control values. Against the administration of piracetam, a similar tendency was noted only in the case of ALP, while the activity of the other enzymes did not differ from those in HH rats.

The results of the study of other biochemical parameters presented in Table 1 reveal that 3 h after the hypoxic exposure, dyslipidemia occurred in the blood serum of the rats; the triglyceride level decreased and the cholesterol concentration increased, which was accompanied by an increase in the cholesterol/triglyceride ratio. We did not es-

Table 1. Biochemical parameters of rat blood 3 h after the exposure to hypobaric hypoxia {HH}, and the effect on these parameters of 2-chloroethoxy-aryl-dimethyl-aminophenylp hosphorylacetohydrazide {CAPAH} and piracetam when administered 40 min before placing the animal in a pressure chamber.

Group of animals	Number in the group	Glucose, mM/L, M ± m	Total protein, g/L, M ± m	Triglycerides, mM/L, M ± m	Cholesterol, mM/L, M ± m	Cholesterol/ triglycerides, M ± m	β-Lipoproteins, mg/%, M ± m	Urea, mM/L, M ± m	Creatinine, mM/L, M ± m	Iron, mM/L, M ± m	Uric acid, mM/L, M ± m
Control	10	4.5±0.3	79.5±1.8	0.81±0.1	0.62±0.09	0.77±0.1	74.2±4.1	5.81±0.56	44.4±2.4	27.03±4.2	384.0±94.5
HH	10	4.6±0.3	81.7±2.5	0.53±0.06* p=0.04	0.84±0.05* p=0.03	1.66±0.3* p=0.01	68.3±5.1	5.00±0.46	45.3±1.7	27.7±3.8	433.2±10.7
CAPAH {1 mg/kg} + HH	10	4.4±0.3	80.0±2.2	0.58±0.04	0.60±0.05** p=0.03	1.01±0.03** p=0.05	70.2±6.0	3.60±0.29** p=0.02	37.0±1.5** p=0.015	22.4±2.1	350.3±101.2
Piracetam {100 mg/ kg} + HH	10	4.3±0.3	79.6±1.9	0.54±0.06	0.55±0.05** p=0.03	1.05±0.17	70.4±4.2	5.03±0.32	44.9±2.6	24.2±3.5	424.7±127.1
CAPAH {1 mg/kg}	10	4.7±0.4	76.4±2.1	0.76±0.2	0.68±0.05	0.89±0.1	73.2±4.1	6.10±0.62	48.0±0.51	28.7±4.1	350.7±80.2
Piracetam {100 mg/kg}	10	4.6±0.3	78.5±2.1	0.71±0.2	0.72±0.09	0.87±0.3	69.2±3.8	5.61±0.42	46.2±0.31	29.4±4.5	381±73.1

Note: *difference is significant relative to control; **difference is significant relative to HH.

tablish any changes in the other blood serum parameters {urea, creatinine, uric acid, protein, glucose, β-lipoproteins, iron, and bilirubin} after exposure to HH at that term of the follow-up.

Prophylactic administration of CAPAH prevented an increase in the cholesterol levels and changes in the serum cholesterol/triglyceride ratio. Additionally, when CAPAH was used in rats, a decrease in the levels of urea and creatinine was noted in comparison with the HH group. The use of piracetam also normalized the cholesterol level; however, the cholesterol/triglyceride ratios did not differ from those in the group of rats exposed to HH.

The results of the series, two of the experiments demonstrated that 24 h after the exposure to HH, only an increased level of CPK was registered in the blood of the rats, while the activity indices of other enzymes did not differ from the control ones (Table 2). The use of CAPAH after hypoxia normalized the activity of CPK, which was increased during hypoxic exposure.

As for the biochemical parameters of the blood, 24 h after HH, we did not reveal any differences between the experimental and control values, including in terms of triglycerides and cholesterol, the change in which was noted 3 h after exposure to hypoxia.

It should be noted that the administration of CAPAH and piracetam to rats without exposure to hypoxia caused no changes in the activity of enzymes and other biochemical parameters of blood serum (Tables 1 and 2).

Oxidative stress is a key link in the pathogenesis of HH and other types of hypoxia; it primarily affects the brain structures. Analysis of the level of the main marker of LPO process activation {MDA} in the hippocampus and frontal cortex of the rat brain 3 h after an exposure to the HH showed a significant increase in its level compared to control animals, by 1.9 times in the frontal cortex {51.4 ± 3.7 μmol/kg} at $p = 0.05$ relative to control rats {27.4 ± 2.1 μmol/kg} and by 1.7 times in the hippocampus {44.2 ± 2.8 μmol/kg} at $p = 0.01$ relative to control rats {25.8 ± 1.9 μmol/kg}. Prophylactic administration of CAPAH reduced the accumulation of MDA in both structures under stud by 1.4 times in the frontal cortex {39.4 ± 1.9 μmol/kg}, $p = 0.03$ relative to HH and by 1.5 times in the hippocampus {29.3 ± 2.6 μmol/kg}, $p = 0.02$ relative to HH. Prophylactic administration of piracetam {100 mg/kg} did not result in a significant decrease in the level of this indicator compared with the animals after exposure to HH.

Discussion. HH is an essential sign of a rarefied environment, which may result from a decrease in the barometric pressure when climbing

Table 2. Activity of rat blood enzymes 24 h after exposure to hypobaric hypoxia {HH}, and the effect on these parameters of 2-chloroethoxy-aryl-dimethyl-aminophenylphosphorylacetohydrazide {CAPAH} and piracetam when administered 1 hour after removing the rat from the pressure chamber

Group of animals	Alanine aminotransferase, U/L, M ± m	Aspartate aminotransferase, U/L, M ± m	Lactate dehydrogenase, U/L, M ± m	Creatine phosphokinase, U/L, M ± m	γ- Glutamyltranspeptidase, U/L, M ± m
Control	67.37±7.90	107.37±17.91	2336.3±240.5	794.5±72.7	1.65±0.36
HH	77.21±13.69	134.78±23.59	2639.8±182.4	1248±109.2* p=0.01	1.46±0.44
HH + CAPAH {1 mg/kg}	64.86±6.85	132.75±18.1	2131.9±286.6	867.6±100.4** p=0.03	2.92±0.71
HH + piracetam {100 mg/kg}	66.75±7.35	128.75±19.1	2224.7±344.4	1195.8±119.4*	2.74±0.91
CAPAH {1 mg/kg}	72.35±10.07	119.48±19.49	2578.0±470.1	706.2±150.9	2.57±0.27
Piracetam {100 mg/kg}	69.47±8.90	127.35±27.91	2435.3±280.5	786.5±79.7	1.85±0.39

Note: *difference is significant relative to control; **difference is significant relative to HH.

to an altitude, a decrease in the partial pressure, and oxygen levels in the inhaled air, which leads to various nonspecific effects in relation to the various organs. First and foremost, the brain is affected. Studies have shown that HH, which occurs at an altitude > 2500 m, leads to a decreased number of nerve cells in the frontal cortex and hippocampus and dissolution of chromatin in neurons of the frontal cortex [12]. Developing oxidative stress and concomitant metabolic disorders lead to impaired memory and cognitive functions [6, 12].

Literature data indicate that the most significant changes in metabolism occur in the first hours of the recovery period after the end of hypoxic exposure [13, 14]; therefore, at stage 1 of research, we studied changes in some biochemical parameters 3 h after exposure to hypoxia.

At this time, pronounced hyperenzymemia was noted, manifested by an increase in the activity of ALT, AST, LDH, and ALP, which is associated with an increase in the passive permeability of biological membranes, their disorganization, releasing enzymes into inter-tissue liquid and blood, which causes metabolic disorders and secondary hypoxic tissue alteration [15].

Damage to the integrity of cell membranes and the release of enzymes into the blood was accompanied by an increase in cholesterol, a decrease in triglyceride levels, and an increase in cholesterol/triglyceride ratio. The activation of lipolysis during hypoxia occurs because of an increase in lipase activity and developed acidosis [3], which is accompanied by the accumulation of excess ketone bodies and higher fatty acids. The latter may have an uncoupling effect on the processes of oxidation and phosphorylation, thereby aggravating hypoxia [16].

The mechanisms causing damage to biological membranes of cells and the disintegration of various biosystems under hypoxia first include the activation of free radical oxidation of lipids [6, 17]. The brain is the most vulnerable and sensitive to the intensification of this process [5, 18], and we also registered a significant increase in the MDA level, the main marker of LPO, in the hippocampus and frontal cortex of rats after exposure to HH.

Thus, the analysis of the results obtained at stage 1 showed that, 3 h after 30 min of exposure to HH, numerous biochemical changes develop regarding the pathogenesis, which indicates the need for a comprehensive drug approach for their prevention and correction.

At stage 2 of the study, 24 h after exposure to HH, many biochemical parameters normalized in the rat blood serum; however, an increased level of CPK was noted, whose activity, unlike other enzymes, did not change at an early stage. The question arises that why the activity of CPK in the blood serum increased only after 24 h, while the high level of other enzymes, caused by the release into the blood due to the damage to cell membranes during HH, had already normalized by this time.

This effect is apparently because CPK serves as an enzyme of energy metabolism, and under hypoxic exposure, its blood level increases compensatory to provide cells with energy under conditions of oxygen deficiency [14, 19]; however, this action develops 3 h after the hypoxia. Therefore, at the early stage of the recovery period, we did not see these changes. It should also be emphasized that 24 h after exposure to HH in rats, there was no recovery of MDA level in the studied structures of the brain; the level of this marker of LPO process

remained increased in the hippocampus and frontal cortex than the rats of the control group.

The relatively rapid recovery of most metabolic parameters does not serve as evidence of the recovery of the whole organism and its functioning, which is confirmed by the development of neurological deficits in the early and late periods of the recovery period [13, 14]. For this reason, to increase resistance to the hypoxic effects and accelerate adaptation processes, targeted use of the drugs is required.

The ability of CAPAH to prevent the development of hyperenzymemia, when used for prophylactic purposes, may result from the previously proven membrane-stabilizing effect of this substance [20], which significantly weakens the effect of hypoxia. The same mechanism, apparently, contributes to the efficiency of CAPAH when administered for treating the effects of HH, and a decrease in the increased blood level of CPK leads to an adequate energy supply to the body in HH.

The prophylactic administration of CAPAH also prevented an increase in the blood cholesterol levels and normalized the cholesterol/triglyceride index, that is, it reduced the severity of dyslipidemia disorders that was developed after exposure to HH. Additionally, in animals that were administered with the CAPAH before an exposure to the hypoxia, a decrease in the urea and creatinine levels was recorded in the blood compared to hypoxic animals. This factor may indicate that under the influence of the drug during the hypoxia, the intensity of energy consumption decrease by suppressing urea formation, which could protect the cells during hypoxia and ischemia [14, 21].

The ability of CAPAH to reduce the level of the main marker of LPO {MDA} in the frontal cortex and hippocampus is of particular importance. As mentioned above, the accumulation of LPO products in the hippocampus and frontal cortex deteriorates memory and cognitive functions, and their constantly high level results in the development of neurodegenerative processes [22]. Possible mechanisms for decreasing the MDA level upon administration of CAPAH can be the presence of its own antioxidant properties [9] and membrane stabilizing action [9, 20]. Additionally, the previously revealed the ability of CAPAH to interact with the glycine regions of NMDA receptors cannot be ruled out, thereby modulating the release of extracellular glutamate and the accumulation of calcium ions, which trigger the peroxidation process in the hypoxia [8].

It should be noted that the comparison drug piracetam was noticeably inferior in the efficiency to CAPAH in this model, as it reduced hyperenzymemia to a lesser extent, did not eliminate hypoxia-induced dyslipidemia, and did not decrease the MDA level in the hippocampus and the frontal cortex.

Our use of the drug piracetam, widely used in clinical practice as a reference drug, is due to its anti-hypoxic properties, the ability to correct impaired metabolic processes, and to improve the memory and learning processes [23].

The ability of CAPAH to reduce the HH-induced intensity of LPO processes in the brain structures with simultaneous correction of metabolic disorders in the blood serum of rats enables to categorize it as a potential drug with a complex mechanism of action for preventing hypoxic disorders and accelerating adaptation to high-altitude hypoxia.

Summing up the results of this study, we could conclude that for HH, it is necessary to use drugs with a complex mechanism of action, capable of exerting simultaneously an effect on different links of the pathogenesis of hypoxia.

CONCLUSION

The revealed efficacy of CAPH and its previously studied complex mechanism of action enable us to consider this pharmaceutical product as a potential drug for preventing hypoxic disorders and accelerating adaptation to high-altitude hypoxia.

Author contributions. I.I.S. created the study concept and design, and wrote the text; A.Z.B. performed the general analysis of the data obtained and the literature review; E.V.Sh. performed the experiments, processed the results obtained; N.A.T. analyzed the data obtained in terms of biochemical research; D.O.N. presented the results obtained in the tables and figures, compiled a list of references; E.V.B. conducted biochemical experiments; A.G.O. processed and analyzed the results obtained in terms of the study of lipid peroxidation.

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Conflict of interest. The authors declare no conflict of interest.

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