UDC: 612.273+615 **DOI: 10.18413/2500-235X-2017-3-1-49-72**

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N-ALKENYLIMIDAZOLE METAL COMPLEX DERIVATIVES AS EFFECTIVE AGENTS FOR THE HYPOXIC CONDITIONS

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Abstract. The relevance. The therapy of hypoxemic conditions by the substances, which are differing because of their key role in the vital processes of metabolism and also their high pharmacological activity, and which are known to be the regulators of redox systems, can be considered as one of the priority directions of the modern clinical and experimental pharmacology. Metal complexes of essential elements, which are the natural participants of the ligand formation, can be very interesting in this sense. **The research goal.** Experimental validation of the pharmacological correction of the hypoxic conditions of the N-alkenyl-, N-propargylimidazoles and 3-hydroxypyridine metal complex derivatives. **Test compounds and drugs.** In comparison with well-known antihypoxants, antioxidants and actoprotectors in animal studies investigated acute toxicity, cytotoxicity, antihypoxic and actoprotective activity, effect on Central nervous system, liver, kidneys, heart, system of blood microcirculation, biochemical and hematological parameters, redox potential, studied the main ways of mechanism of action and the possibility of combined use with drugs of different pharmacological groups of complexes of zinc, cobalt, iron, derivatives of N-alkenyl-, N-propargylimidazol and 3-hydroxypiridine. **The discussion of the results.** The researched drugs are moderately and low toxic, so it proves their safety. The antyhypoxic and actoprotective effect has been shown by the complexes of cobalt (CoALL) and iron (tetravim) and also by the derivatives of N-alkenylimidazole. Hemostimulating effect of CoAll has also been discovered. A possible mechanism of the antihypoxic effect of the test compounds could be attributed to the effect on coupling of oxidation and phosphorylation, maintenance of the mitochondria structure and functions in conditions of oxygen deficiency, elimination of the negative effect of hypoxia on carbohydrate metabolism, improvement of microcirculation and tissue oxygenation parameters and restore of the redox potential at hypoxia exposure. CoALL, probably, activates the key metabolism stages, which are responsible for energy supply by oxidizing organic substrates. The increasing of hematocrit, erythrocyte and hemoglobin levels can also be a protective factor of CoALL in condition of hypoxia. **Conclusions.** CoALL is promising for the further development as a hemostimulating drug, and CoALL and tetravim as antihypoxic and actoprotective drugs. Redox-regulating activity of metal complexes of the derivatives of N-alkenylimidazole offers the opportunities to construct new effective preparations of a wide spectrum of action on their basis.

Keywords: antihypoxic activity, metal complex compounds, N-alkenylimidazoles, redox potential.

Introduction.

The urbanization of the modern world, the industrial growth, the introduction of chemical technologies in our national economy and our life, the appearance of high-toxic compounds, the accumulation of the toxins, the generation of genetically modified foods and also some ecological catastrophes can affect the environment and cause real threats to all of us [1-8].

The oxygen, which used to be one of the greatest vital necessities, has to compete with some chemical emissions, so its specific consumption decreases, which leads to hypoxic conditions. Oxygen deficiency can cause a lot of cellular metabolism violations, and, consequently, an inevitable death of the cell, causing serious pathologies.

Oxygen insufficiency is one of the roots of the number of conditions: cardiovascular, oncological, pulmonary, endocrine and other diseases [9-21]. Hypoxic resistance increase has a great importance in military, marine, aviation and sport medicine, and also in the Emergency medicine [22-26].

The high-level capacity support during an acute hypoxia can be realized by antihypoxants. But their

diversity is very poor, their therapeutic effect is quite small, they can be useless for many types of hypoxia, and also they have a lot of side effects. That is why their application is limited.

Modern principles of the pharmacological therapy include the conversion to the economical oxygen consumption mode, using circulatory dynamics regulators, calcium channel blockers, membrane stabilizers, antioxidants and antihypoxants [13, 14, 27]. But despite on that, the main reason of the hypoxic damage, such as reductive index increase, still remains.

Unfortunately, complexes most of the modern drugs are presented by the different synthetic compounds of various compositions and are foreign for the human body. They are supposed to block certain biochemical processes and reach the therapeutic effect by the decrease of the organism's adaptive response to the damage. But the lack of the target selectivity can drain the organism's protective resource, up to its atrophy, so we can consider it as a great disadvantage. That is why, despite the rigorous selection of the new drugs, they all have a lot of side effects and also not very wide therapeutic effect, so it makes difficult to choose the right way of the therapy.

All the listed disadvantages of the modern drugs occur because of their incompatibility with some biological systems. Every pathological process depends on 3 biological flows (metabolic, energetical and informational flow) regulated by endogenous redox (Red-Ox) controllers. Hypoxia causes the Red-Ox system imbalance, and the protective resource induction and mobilization are going through the activation of specific proteins. So, as far as the amino acid chain specific areas form the signal transduction structural mark, we can use the modified signal molecules of those proteins as a drug. These substances (different Red-Ox active signal molecules) could play a role of safe and highly effective drug. They could be based on some native participants of ligand-forming process in biological systems, such as amino acids, vitamins, polyphenols or bio elements. Any insignificant change of Red-Ox potential balance can influence the whole body functions, and this ligand-forming process maintains this balance.

Based on the above mentioned, we have decided to choose the new way of the correction of the hypoxic conditions, based on the oxygen supply optimization and the simultaneous Red-Ox potential correction. The conception of the next-gen drug development could become a good alternative to replace the old-fashioned screening of the chemical substances [28].

Besides, the searching of the new chemical substances with wide therapeutic effect and with a lack of any side effects is an important task for experimental and clinical pharmacology.

So, the problem of finding and investigation of any effective and safe antihypoxants can be solved by the research, including the study of some chemical substances that play the key role in vital processes and which are known to be the Red-Ox systems regulators.

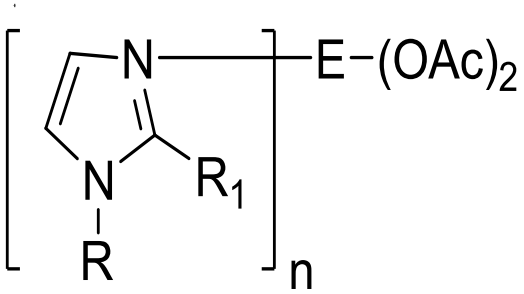
We have tested 18 complexes of zinc, iron, cobalt and copper, based on N-alkenyl-, N-propargylimidazoles and 3-hydroxypyridine as those perspective compounds. These compounds had been synthesized in the laboratory of heteroatomic compounds of A.E. Favorsky Irkutsk Institute of Chemistry SB RAS under the direction of the Academician of RAS B.A. Trofimov.

The research goal. Experimental validation of the pharmacological correction of the hypoxic conditions of the N-alkenyl-, N-propargylimidazoles and 3-hydroxypyridine metal complex derivatives.

Test compounds and drugs. Depending on the type of the ligand we can divide N-alkenyl-, N-propargylimidazoles and 3-hydroxypyridine derivatives into 7 groups (table 1).

The first group includes four vinylimidazole derivatives: acyzol, cobazol, tetravim and vim, containing zinc diacetate, cobalt dichloride, iron trichloride and iron diacetate, respectively. The second group is formed by two allenylimidazole derivatives: allim-1 and allim-2, which includes zinc diacetate. The third group consists of three isopropenylimidazole derivatives: pilim-1, pilim-2 and pilim-4, containing zinc diacetate, zinc diacetate and copper dichloride, respectively. The fourth group includes two allylimidazole derivatives: ALL and CoALL (containing zinc diacetate and cobalt dichloride, respectively); the fifth group contains complexes with zinc diacetate titled g-4, g-5, g-6, g-7 and g-8 immobilized on sulfo-AG. The sixth and seventh groups include derivatives of N-propargylimidazole and 3-hydroxypyridine, respectively.

N-alkenylimidazole derivatives have the general formula (fig. 1):



R – vinyl, allenyl, isopropyl or allyl; R_1 – hydrogen or methyl; E – Zn(II), Fe(III), Co(II) or Cu(II); Ac – chloride or acetyl; n – 1, 2, 4.

Figure 1. N-alkenylimidazole general formula

Table 1

N-alkenyl-, N-propargylimidazoles and 3-hydroxypyridine metal complex derivatives

№	Substance	Chemical name of the substances
I. N-vinylimidazole metal complex derivatives		
1	Acyzol	bis(N-vinylimidazole) zinc diacetate
2	Cobazol	(tetravinylimidazole) cobalt dichloride
3	Tetravim	(tetravinylimidazole) iron trichloride
4	Vim	bis(N-vinylimidazole) iron diacetate
II. N-allenylimidazole metal complex derivatives		
5	Allim-1	bis(N-allenylimidazole) zinc diacetate
6	Allim-2	(N-allenyl-2-methylimidazole) zinc diacetate
III. N-isopropenylimidazole metal complex derivatives		
7	Pilim-1	(N-isopropenylimidazole) zinc diacetate
8	Pilim-2	(N-isopropenyl-2-methylimidazole) zinc diacetate
9	Pilim-4	tetra(N-isopropenylimidazole) copper dichloride
IV. N-allylimidazole metal complex derivatives		
10	ALL	bis(N-allylimidazole) zinc diacetate
11	CoALL	bis (N-allylimidazole) cobalt dichloride
*V. Metal complex derivatives immobilized on sulfo-AG**		
12	g-4	Sulfo-AG* - bis(N-allenylimidazole) zinc diacetate
13	g-5	Sulfo-AG*-(N- isopropenylimidazole) zinc diacetate
14	g-6	Sulfo-AG*-(N-allenyl-2-methylimidazole) zinc diacetate
15	g-7	Sulfo-AG* - bis(N- propargylimidazole) zinc diacetate
16	g-8	Sulfo-AG* - bis(3-hydroxypyridine) zinc diacetate
VI. 3-hydroxypyridine metal complex derivatives		
17	BIS-3	bis(3-hydroxypyridine) zinc diacetate
VII. N-propargylimidazole metal complex derivatives		
18	BIS-N	bis(N-propargylimidazole) zinc diacetate

Notice. *Sulfo-AG – sulfated arabinogalactan

The modification of the imidazole cycle structure, deep research of its structure and the process of its coordination with metals are caused by the discovery of the specific pharmacological effect of complexes, depending on the metal nature, and the leading role of azole cycles in the pharmacy [29]. In light of the above, N-alkenylimidazole derivatives can be used as very perspective broad-spectrum drugs, particularly as antihypoxants.

Nowadays, the original domestic drug «Acyzole» based on the N-alkenylimidazole derivatives was developed. It is the most potent antidote for carbon monoxide and the other combustion products in the world [30]. Also it can be a prophylactic and a cure for neurotoxins poisoning [31]. The drug decreases the toxic damage on the CNS, which results in long-term memory processes improvement, spontaneous motor activity and emotional behavior normalization.

Acyzol decreases the intoxication severity during the carbon monoxide poisoning, and accelerates its elimination. It reduces the oxygen demand and increases the hypoxia resistance of the oxygen-sensitive organs (brain, myocardium, liver). Being a high effective antihypoxant, acyzole protects the organism, when the oxygen partial pressure is very low, and when hemoglobin oxygenation is lower, than it should be [32-34].

Besides, zinc, contained in acyzole, compensates its deficit in human body, helping to normalize metabolic processes, related to zinc-dependent enzymes. So, the drug could be used to treat any zinc deficiency conditions (Prasad disease, immunodeficiency, allergic dermatosis, prostate dysfunction etc.). Also, acyzole is established to be highly effective against psoriasis, one of the most common allergic dermatoses [35].

Nowadays, acyzole anti-inflammatory, reparative, detoxifying, immunomodulatory, bacteriostatic, hepatoprotective, adaptogenic, antioxidant, cardioprotective and other functions have been discovered [36, 37]. The drug helps to protect respiratory, cardio-vascular and urinary systems function [38].

Acyzole mechanism of action is known, and it is explained by its effect on hemoglobin subunit interaction. It comes together with Hill's constant decrease, and Haldane's effect removal. As a result,

CO affinity decreases, Douglas's constant is getting lower and carbon monoxide elimination accelerates [34, 39].

Acyzole increases hemoglobin affinity to the oxygen, oxygenated hemoglobin dissociation curve shifts to the left, increasing the vital organs oxygenation – brain, myocardium, liver. Also, acyzole, as universal energy exchange regulator, prevents the formation of highly reactive oxygen forms and balances Red-Ox processes in the cell [37]. Acyzole also has the membrane protective function, due to its antihypoxant and antioxidant effect [40].

Cobazol (cobalt complex), is passing the clinical trials at this moment. It is a potent broad spectrum hematopoiesis stimulator. Cobazol is known to have erythropoietic and leucopoetic activity, immunomodulatory and antibacterial functions. Its activity surpasses one of the iron-containing drugs – B_{12} vitamin and leucogen [41]. It also has the antibacterial activity, exceeding streptomycin and tetracycline one in 2–3 times [42]. Cobazole immunomodulatory functions, caused by the increasing of the number of lymphocytes and neutrophil phagocytic function, significantly expand its medical value [42].

The drug is very effective against different types of anemia: post-hemorrhagic, hypochromic, hemolytic, including the iron-containing drug resistant types and radiation-induced types. It prevents cytotoxic and post radial leucopenia, so it makes antineoplastic chemotherapy and radial therapy safer and more effective. Compared with ferroplex and ferrum-lek, cobasole causes faster increase of hemoglobin [43]. Cobazole effects make it indispensable for oncological diseases and emergency medicine.

3-hydroxypiridine derivatives are the perspective chemical compounds for the investigation during the different extreme factors influence [44].

Zinc, cobalt, iron and copper, contained in the studied complexes, are ecologically valuable and vital for human body, which confirms their essentiality.

Chemically pure nooglutyl and etomerzole, bemythil (metaprot, actoprotector, «Pharmproekt», Russia), bromantan (ladasten, psychostimulant with an actoprotective effect, «Lekko», Russia), mexidol («Pharmasoft», Russia) and hypoxen («Olifen corp.», Russia) were taken as comparison drugs.

Synergistic action of the N-alkenylimidazole derivatives was studied using commonly used in clinic drugs referred to different pharmacological classes: metaprot, mexidol, aktovegin, inosine, pikamilon, acetylsalicylic acid (ASA).

The investigated compounds and drugs were dissolved in water for injections or in Twin-80 emulsifier («V.A.G. Chemie», Germany), in concentration of 0,1–12%. They were once given intraperitoneally (i/p) or intragastric (i/g), 1 or 3 hours, respectively, before the selected indicators registration or the extreme factor effect. Metal complex compounds were given in different doses: from ineffective to toxic. The drugs were given in doses, which could be effective for different pathologies, according to the literature. The control group was treated with the equal volume of the solvent.

Research methods. In accordance with goals and objectives of the study, the experiments were conducted on 9877 white, outbread male mice 18–25 g, 192 white, outbred male rats 110–130 g and on 18 nonlinear male rabbits of the chinchilla breed 2.0–2.5 kg. The animals had been kept on a regular diet with an unlimited access to drinking water, according to the group accommodation rules (+18–20 ˚C, 40–70% relative humidity, native light).

The experiments were performed according to the following articles: The 11th article of The Helsinki declaration that had been accepted by the World Medical Association in 1964, The rules of the European Convention for the Protection of Vertebral Animals that are used for scientific research (ETS 123, Strasbourg, 1986) – GLP (decree 708n by the Ministry of Health of Russia, 23.08.2010), The manual of the conduction of preclinical trials of drugs (2012) [45].

The acute toxicity of the studied chemical compounds were studied on white male mice at a single i/p or i/g injection of aqueous solutions. The animal's death was registered every 24 hours. The supervision was being provided during 2 weeks. On the 7th and the 14th days the weight of survived animals was measured. On the 15th day these mice were euthanized with an overdose of ester. After all, the vital organs were weighted and all the pathomorphological changes were observed. The acute toxicity indexes (LD_{16}, LD_{50}, LD_{84}) were graphically calculated following the method of Miller and Tainter [46, 47] and estimated according to 4 grades of danger according to the National Standard 12.1.007-76 [48].

Cytotoxicity against MDCK cells (dog's kidney) and Vero cells (green monkey's embryonic kidney) was measured with MTT assay, characterizing cellular respiration intensity. The estimation of MTT-assay results was made by cytotoxic index (IC) calculation according to the following formula:

$$IC = \frac{(K - O) \times 100\%}{K},$$

K – optical density in the control wells; O – optical density in the studied wells.

Antihypoxic activity was studied in 4 models of acute hypoxia: hypobaric (AHBH), hypoxia with

hypercapnia (AHwHc), hemic (AHeH) and histotoxic (AHtH) [49, 50].

Mice physical performance in regular conditions was estimated according to the duration of the run on a 6-track treadmill which was set on a standard treadmill [50-53]. Besides that, it was estimated by measuring the duration of the swim in a swimming pool (+28º C) with a weight fixed to the beginning of tail which is equal to 7% of mouse's weight. Mice physical performance in case of AHBH was determined by measuring the duration of the run on a 2-track treadmill, put into an electrovacuum furnace. The altitude of the lift was 7500 m, the speed of the conveyer belt equaled 15–16 m/min, the speed of lifting – 50 m/sec [53]. Mice physical performance in case of AHwHc was studied according to the swimming trial in a can of transparent glass [53].

The functional status of the CNS of the animals was studied with 3 tests: conditioned protective reflex of avoidance (CPRA), anticonvulsant activity in case of subcutaneous introduction of corazol (125 mg/kg), and individual behavior in «open space» [54, 55].

To measure the functional status of animal liver on CLIMA MC-15 analyzer (RAL, Spain) the following biochemical indexes had been measured: total protein, albumins, globulins, total and conjugated bilirubin, cholesterol, triglycerides, aspartate aminotransferase (AST) and alanine aminotransferase (ALT). The blood was taken from neck blood vessels of the decapitated mice. Mice liver detox function was estimated by measuring the duration of their hexenal sleep after subcutaneous introduction (back derma) of 0.6% solution of hexenal (60 mg/kg) [56]. Liver carbohydrate metabolism was evaluated by the glucose blood level measurement after galactose loading test with the dosage 1 g/kg [57].

The kidney functional status was defined by the estimation of diuretic and nitrogen excretory functions, urine pH and density. The measurement was carried out with CLIMA MC-15 biochemichal analyzer (RAL, Spain).

Unanesthetized animal electrocardiographic indexes were registered with RM-6000 polygraph (Japan).

The mice oxidative exchange intensity was estimated according to their oxygen consumption index and their rectal temperature. Oxygen consumption was determined with S.D. Miropolky's closed-type apparatus during 9–12 min after preliminary 10 minute adaptation in the respiratory camera [50].

Glucose level in the blood serum of the decapitated mice was registered with CLIMA MC-15 biochemical analyzer (RAL, Spain).

Blood morphological indexes, hemoglobin and hematocrit level were determined with Exell 18-optioned automatic hematological apparatus (Drew, Netherlands). The blood was taken from neck blood vessels of the decapitated mice.

The options of microcirculatory dynamics and blood oxygenation in microcirculatory system were estimated using the laser doppler flowmetry (LDF) and tissue optical oximetry (TOO) at the same time. The measurements were provided with «LAKK-M» multifunctional laser diagnostic complex (Lazma, Russia). The capillary blood flow was measured on the external surface of the animal hind limbs.

For the Red-Ox potential calculation we used the fluorescent oxygen consumption index (FOCI) of the enzymes that take part in the respiratory chain and which is inversely proportional to the Red-Ox ratio:

$FOCI = A_{NADH}/A_{FAD}$

A_{NADH} – the amplitude of the fluorescent emission of the reduced nicotinamide adenine dinucleotide. A_{FAD} – the amplitude of the fluorescent emission of the oxygenated flavoproteins (FAD) [58]. The obtained dimensionless parameter reflects mitochondrial activity level and doesn't depend on apparatus factors or object dispersion properties.

The statistical treatment of the data was made with the computer program Microsoft Excel XP, based on Windows XP and STATISTICA 6.0. For the variational series of the sample, the average value (M), and its error (m) were calculated. The normality of the sample was tested with Shapiro-Wilk test. As all the samples had the distribution, which is close to the normal one, the significance of the differences between experimental groups was determined with one-dimensional dispersive analysis with the further treatment with Student's multiple comparisons method, using the Bonferron.

Discussion of the results.

Acute toxicity evaluation of N-alkenyl-, N-propargylimidazoles and 3-hydroxypyridine metal complex derivatives and analysis of the results, considering State Standard's requirements №12.1.007-76 [48] showed us, that tetravim is a low toxic compound (LD_{50} at intraperitoneal injection 1625 mg/kg), and pilim-4 is a high toxic compound (10 mg/kg accuses mice death). The rest of substances are moderately toxic (LD_{50} is in the range from 40 to 1250 mg/kg.) For CoALL and tetravim, which are the most effective for hypoxic conditions, LD_{50} were 500 mg/ kg (CoALL, i/g) and 2100 mg/kg (tetravim, i/g).

As we can see on fig. 2, tetravim concentration, inhibiting dehydrogenase metabolic activity (IC_{50}) is approximately 250 mg/kg for MDCK cell lines, and more than 2000 mg/kg for Vero.

Table 2

Effect of N-alkenyl-, N-propargylimidazoles, 3-hydroxypyridine metal complex derivatives and comparison drugs on the mice lifespan during acute hypoxia

Substance, drug	Hypoxic model	Dose, mg/kg, i/p								
		1	5	10	25	50	100	150	200	250
I. N-vinylimidazole metal complex derivatives										
Cobazol	AHBH	-	113±7	138±6*	154±12*	200±10*	238±8*	133±7*	-	-
Acyzol	AHBH	-	95±6	216±14*	276±18*	222±9*	162±12*	111±8	-	-
Tetravim	AHBH	114±9	154±5*	227±5*	182±5*	190±6*	187±9*	197±6*	207±6*	200±9*
Vim	AHBH	115±8	158±6*	153±7*	144±6*	150±8*	193±5*	175±6*	-	-
Cobazol	AHwHc	-	-	99±4	122±5*	139±6*	154±4*	152±4*	-	-
Acyzol	AHwHc	-	-	103±4	138±7*	199±4*	174±4*	180±2*	-	-
Tetravim	AHwHc	-	-	109±3	106±4	197±7*	175±6*	150±5*	145±3*	142±4*
Vim	AHwHc	-	-	111±5	114±7	159±7*	158±3*	154±5*	161±2*	-
Cobazol	AHeH	-	-	-	97±3	134±7*	157±5*	117±7	-	-
Acyzol	AHeH	-	-	-	114±12	161±11*	192±6*	166±10*	-	-
Tetravim	AHeH	-	-	-	113±4	123±5*	137±3*	131±9*	126±3*	126±5*
Vim	AHeH	-	-	-	98±11	94±10	105±6	-	-	-
Cobazol	AHtH	-	-	94±10	130±5*	140±7*	166±4*	244±4*	-	-
Acyzol	AHtH	-	-	-	98±9	109±10	-	-	-	-
Tetravim	AHtH	107±13	131±7*	135±6*	131±6*	136±8*	121±5*	129±8*	125±6*	129±8*
Vim	AHtH	-	-	-	97±9	99±7	92±8	-	-	-
II. N-allenylimidazole metal complex derivatives										
Allim-1	AHBH	-	112±15	179±6*	188±7*	159±6*	108±13	-	-	-
Allim-2	AHBH	-	96±10	143±5*	168±8*	191±7*	117±11	-	-	-
Allim-1	AHwHc	-	103±2	122±4*	123±2*	154±4*	-	-	-	-
Allim-2	AHwHc	-	101±9	118±5	121±5*	153±5*	-	-	-	-
Allim-1	AHeH	-	-	-	95±10	99±5	128±7*	-	-	-
Allim-2	AHeH	-	-	95±5	112±13	147±5*	98±9	-	-	-
Allim-1	AHtH	-	-	-	84±11	95±13	125±6*	-	-	-
Allim-2	AHtH	-	-	-	90±12	85±8	135±8*	-	-	-
III. N-isopropenylimidazole metal complex derivatives										
Pilim-1	AHBH	110±9	127±3*	184±6*	169±3*	135±5*	98±22	-	-	-
Pilim-2	AHBH	107±15	129±5*	181±6*	190±7*	175±4*	129±6*	108±16	-	-
Pilim-1	AHwHc	-	-	103±5	143±6*	204±8*	-	-	-	-
Pilim-2	AHwHc	-	98±6	98±4	120±5*	155±6*	-	-	-	-

Table 2

Table 2 (continueed)

Substance, drug	Hypoxic model	Dose, mg/kg, i/p								
		1	5	10	25	50	100	150	200	250
Pilim-1	AHeH	-	-	106±6	135±9*	169±10*	141±4*	-	-	-
Pilim-2	AHeH	-	-	-	99±6	109±5	-	-	-	-
Pilim-1	AHtH	-	-	-	84±8*	83±6*	-	-	-	-
Pilim-2	AHtH	-	-	-	73±4*	74±5*	-	-	-	-
IV. N-allylimidazole metal complex derivatives										
ALL	AHBH	-	114±14	159±5*	189±10*	228±5*	225±5*	-	-	-
CoALL	AHBH	-	118±17	133±7*	254±13*	281±15*	263±15*	-	-	-
ALL	AHwHc	107±7	108±6	127±4*	131±7*	183±10*	191±11*	-	-	-
CoALL	AHwHc	-	-	117±5*	118±2*	138±7*	136±8*	-	-	-
ALL	AHeH	-	-	94±4	94±9	109±4	108±6	-	-	-
CoALL	AHeH	-	105±6	151±8*	135±7*	155±5*	187±4*	-	-	-
ALL	AHtH	-	-	108±5	93±10	92±6	94±7	-	-	-
CoALL	AHtH	-	108±6	119±5*	139±6*	175±5*	180±5*	-	-	-
V. Metal complex derivatives immobilized on sulfo-AG										
g-4	AHBH	-	-	104±22	117±18	116±15	100±21	-	-	-
g-5	AHBH	-	-	116±16	104±27	106±20	106±22	-	-	-
g-6	AHBH	-	-	113±19	95±21	115±15	100±22			
g-7	AHBH	-	-	-	92±9	92±4	-	-	-	-
g-8	AHBH	-	-	-	100±9	98±9	-	-	-	-
g-4	AHwHc	-	-	97±4	100±3	112±2	102±8	-	-	-
g-5	AHwHc	-	-	99±4	98±6	102±5	116±9	-	-	-
g-6	AHwHc	-	-	93±2	103±6	101±8	108±9	-	-	-
g-7	AHwHc	-	-	-	93±8	108±12	-	-	-	-
g-8	AHwHc	-	-	-	81±6	105±12	-	-	-	-
g-4	AHeH	-	-	119±3*	109±11	103±4	103±10	-	-	-
g-5	AHeH	-	-	110±5	117±5	-	-	-	-	-
g-6	AHeH	-	-	115±4	111±3	-	-	-	-	-
g-7	AHeH	-	-	95±6	98±8	-	-	-	-	-
g-8	AHeH	-	-	95±8	96±5	-	-	-	-	-
g-4	AHtH	-	-	107±5	115±9	-	-	-	-	-
g-5	AHtH	-	-	107±8	116±7	-	-	-	-	-
g-6	AHtH	-	-	139±7*	108±5	-	-	-	-	-

Table 2 (continueed)

Substance, drug	Hypoxic model	Dose, mg/kg, i/p								
		1	5	10	25	50	100	150	200	250
g-7	AHtH	-	-	109±8	98±10	-	-	-	-	-
g-8	AHtH	-	-	106±9	109±7	-	-	-	-	-
VI. 3-hydroxypyridine metal complex derivatives										
BIS-3	AHBH	-	-	82±18	235±12*	267±13*	212±8*	-	-	-
BIS-3	AHwHc	-	-	115±4	127±3*	130±4*	185±4*	-	-	-
BIS-3	AHeH	-	-	-	108±4	113±6	-	-	-	-
BIS-3	AHtH	-	-	-	99±7	95±4	-	-	-	-
VII. N-propargylimidazole metal complex derivatives										
BIS-N	AHBH	177±9*	191±6*	262±5*	293±7*	417±10*	267±5*	-	-	-
BIS-N	AHwHc	-	93±4	131±6*	155±6*	193±2*	103±3	-	-	-
BIS-N	AHeH	-	-	119±4*	125±5*	135±3*	121±6*	-	-	-
BIS-N	AHtH	-	-	-	117±9	118±4	-	-	-	-
VIII. Comparison drugs										
Etomerzol	AHBH	-	-	-	98±10	108±11	157±9*	-	-	-
Mexidol	AHBH	-	-	-	94±11	90±16	91±19	-	-	-
Nooglutyl	AHBH	-	-	-	174±8*	134±8*	137±7*	-	-	-
Hypoxen	AHBH	-	-	-	110±5	244±9*	251±7*	267±8*	-	-
Etomerzol	AHwHc	-	-	-	96±8	96±9	132±3*	-	-	-
Mexidol	AHwHc	-	-	-	92±6	104±7	123±5*	-	-	-
Nooglutyl	AHwHc	-	-	-	119±4*	126±4*	138±4*	-	-	-
Hypoxen	AHwHc	-	-	-	-	97±5	126±4*	129±4*	-	-
Etomerzol	AHeH	-	-	-	125±7*	143±4*	106±8		-	-
Mexidol	AHeH	-	-	-	94±9	99±5	114±2*		-	-
Nooglutyl	AHeH	-	-	-	116±3*	130±5*	123±5*		-	-
Hypoxen	AHeH	-	-	-	-	-	112±2*	121±2*	-	-
Etomerzol	AHtH	-	-	-	114±4*	106±5	94±7	-	-	-
Mexidol	AHtH	-	-	-	106±9	106±7	108±9	-	-	-
Nooglutyl	AHtH	-	-	-	107±5	106±3	120±3*	-	-	-
Hypoxen	AHtH	-	-	-	104±4	143±3*	189±6*	119±3*	-	-

Notice.* – Significant differences compared with control animals at $p < 0.05$. Data are expressed as M ± m. M – arithmetic mean as % relative to the control group (100%); m – standard error of the mean as % relative to the arithmetic mean.

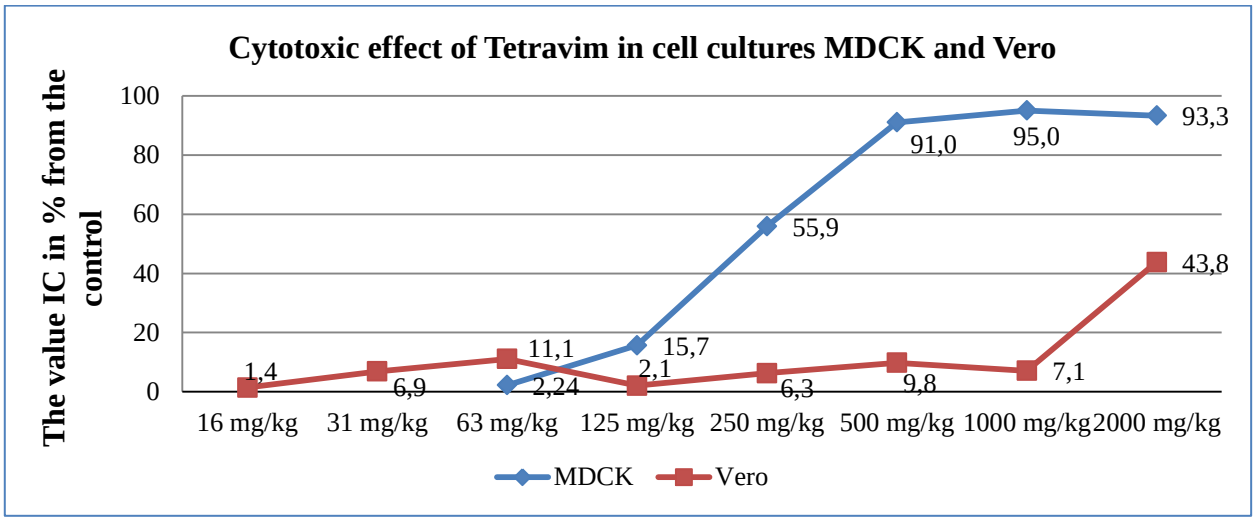


Figure 2. Cytotoxic effect of tetravim on MDCK and Vero cell cultures

Body mass' observation, data about autopsy and macroscopic study of internal organs of euthanized animals, which had survived after an injection of tetravim and CoALL, haven't revealed any statistic meaningful changes, comparing with the control group.

Antihypoxic activity of the researched N-alkenyl-, N-propargylimidazoles and 3-hydroxypyridine derivatives (i/p and i/g injections) was studied on 6130 mice. The results of the experiments show, that the researched metal complexes differently affect mice lifespan in acute hypoxic conditions. Antihypoxic effect severity and therapeutic effect range depends on the substance and a type of hypoxia.

On AHBH and AHwHc models acyzol, cobazol, tetravim, vim, allim-1, allim-2, pilim-1, pilim-2, ALL, CoALL, BIS-N, BIS-3 were effective in 1–250 mg/kg dose range i/p, increasing animal survival by 17–176%, but g-4, g-5, g-6, g-7 and g-8 substances were ineffective. In conditions of AHeH acyzol, cobazol, tetravim, allim-1, allim-2, pilim-1, CoALL, BIS-N, g-4 showed the protective effect in 10–250 mg/kg dose range, i/p. Mice lifespan was increased by 19–92%. Vim, pilim-2, ALL, g-5, g-6, g-7, g-8 and BIS-3 were ineffective. In conditions of AHtH cobazol, tetravim, allim-1, allim-2, CoALL, g-6 showed antihypoxic effect in 5–250 mg/kg dose range, i/p by 19–144%. Acyzol, vim, ALL, g-4, g-5, g-7, g-8, BIS-3 and BIS-N didn't have any protective effect, and pilim-1 and pilim-2 in 25 mg/kg and 50 mg/kg i/p doses demonstrated an oppressive effect.

Allim-1, allim-2, pilim-1 and pilim-2 antihypoxic results confirm the literature data [59-63].

Two of seventeen compounds showed the highest antihypoxic effect on 4 hypoxic models. So, the iron complex (tetravim) increased the mice lifespan by 21–127% in 5–250 mg/kg dose range on 4 acute hypoxic models, and the cobalt complex (CoALL) increased the lifespan by 17–181% in 10–150 mg/kg dose range, compared with the control group. The intensity and the effective dose range is higher, than the ones of the control drugs: etomerzole, mexidolum, nooglutyl and hypoxen (table 2)

Tetravim and CoALL antihypoxic effect was also shown on 4 acute hypoxia models, when they were injected i/g in a high dose range (50–1200 mg/kg and 25–300 mg/kg respectively). The mice lifespan was increased by 23–134% and 17–169%, respectively, compared with the control group.

In this way, the broad spectrum of tetravim and CoALL activity on hypoxic models of different origin in the different ways of administration lets us speak about the universality of their antihypoxic effect and recommend their further research as potential antihypoxants.

Effect of the coadministration of the N-alkenylimidazole metal complexes with different pharmacological classes drugs on the mice lifespan during acute hypoxia. Studying of the antihypoxic activity of the zinc (acyzol), cobalt (cobazol, CoALL) and iron (tetravim) complexes in combination with different pharmacological classes drugs can expand application field of the compounds and help to avoid potential side effects of acyzol and cobazol. It can also provide benefit in further investigation of tetravim and CoALL for their introduction into clinical practice.

We used acyzol, cobazol and CoALL at a dose of 25 mg/kg, and tetravim at a dose of 50 mg/kg. This doses correspond to the intermediate value among dosages which demonstrate antihypoxic activity and statistically significant increase mice lifespan after hypoxia of the different origin.

Acyzol, cobazol, CoALL and tetravim in combination with the different pharmacological classes drugs showed synergetic and antagonistic effects, which led to the increase and decrease of the N-alkenylimidazole derivatives antihypoxic activity. The effect of the compounds should be further investigated (table 3).

Table 3

Effect of the co-administration of the N-alkenylimidazole metal complexes with different pharmacological classes drugs on the mice lifespan during acute hypoxia

Substance, drug	Dose, mg/kg, i/p	Hypoxic model			
		AHBH	AHwHc	AHeH	AHtH
Metaprot	50	111±10	92±4	121±5*	108±13
Acyzol	50	222±9*	200±8*	160±7*	97±7
Acyzol+ metaprot	50+50	167±9*	167±9*	130±4*	102±14
Cobazol	50	200±10*	143±4*	130±7*	142±9*
Cobazol+metaprot	50+50	184±6*	140±7*	117±3*	140±4*
CoALL	25	254±9*	120±6*	135±5*	139±11*
CoALL+ metaprot	25+50	189±3*	131±4*	133±7*	154±8*
Tetravim	50	190±5*	195±6*	123±5*	135±9*
Tetravim+ metaprot	50+50	169±10*	175±7*	149±5*	151±9*
Mexidol	100	105±10	120±6*	119±4*	104±7
Acyzol	50	216±14*	195±2*	170±5*	108±5
Acyzol+mexidol	50+100	211±9*	190±4*	157±7*	100±5
Cobazol	50	193±13*	139±4*	135±6*	142±8*
Cobazol+ mexidol	50+100	202±13*	149±5*	128±5*	146±5*
CoALL	25	259±11*	116±4*	135±6*	139±7*
CoALL+mexidol	25+100	272±6*	123±5*	150±6*	143±7*
Tetravim	50	184±13*	199±4*	123±4*	147±8*
Tetravim+mexidol	50+100	240±6*	180±4*	140±4*	155±6*
Aktovegin	40	108±2	95±8	112±4	105±7
Acyzol	50	220±11*	198±5*	159±3*	97±8
Acyzol+aktovegin	50+40	166±10*	175±4*	131±6*	102±8
Cobazol	50	190±7*	140±5*	136±4*	137±4*
Cobazol+aktovegin	50+40	169±13*	144±5*	129±6*	145±3*
CoALL	25	253±10*	118±5*	133±10*	117±9*
CoALL+aktovegin	25+40	198±12*	119±2*	138±9*	119±10*
Tetravim	50	196±9*	194±6*	128±4*	139±8*
Tetravim+aktovegin	50+40	160±9*	191±5*	140±5*	141±7*
Inosin	200	91±16	98±7	93±4	85±10
Acyzol	50	199±13*	191±5*	160±3*	97±9
Acyzol+inosin	50+200	96±13	102±6	98±8	134±6*
Cobazol	50	182±8*	136±5*	141±4*	131±5*
Cobazol+inosin	50+200	110±10	96±7	111±9	176±2*
CoALL	25	245±15*	121±3*	133±11*	141±8*
CoALL+inosin	25+200	109±3	109±3	88±15	170±8*
Tetravim	50	190±9*	186±3*	133±4*	139±10*
Tetravim+inosin	50+200	98±14	101±8	109±7	147±8*
Pikamilon	20	110±12	101±7	109±7	130±8*
Acyzol	50	192±11*	197±6*	151±3*	108±9
Acyzol+ pikamilon	50+20	99±9	103±7	94±10	92±10
Cobazol	50	173±5*	141±4*	140±2*	133±5*
Cobazol+pikamilon	50+20	107±7	97±7	104±7	163±6*
CoALL	25	214±11*	122±2*	131±6*	141±7*
CoALL+pikamilon	25+20	92±14	109±3	98±10	156±11*
Tetravim	50	197±8*	173±3*	131±2*	141±12*
Tetravim+pikamilon	50+20	107±12	98±5	113±4	156±11*
ACK	125	105±18	95±8	80±6*	88±9*
Acyzol	50	198±10*	192±4*	163±4*	97±8
Acyzol+ASA	50+125	105±18	105±7	71±12*	97±8
Cobazol	50	188±8*	136±5*	142±4*	131±5*
Cobazol+ASA	50+125	110±10	96±7	87±9*	176±2*
CoALL	25	253±10*	121±4*	139±10*	141±8*
CoALL+ASA	25+125	106±14	109±3	102±14	188±7*
Tetravim	50	190±9*	186±5*	128±4*	139±10*
Tetravim+ASA	50+125	98±14	101±7	83±9*	105±11

Notice.* – Significant differences compared with control animals at $p < 0.05$. Data are expressed as M ± m.

M – arithmetic mean as % relative to the control group (100%); m – standard error of the mean as % relative to the arithmetic mean.

Metaprot in combination with tetravim showed summarized antihypoxic effect on AHeH model, while CoALL in combination with tetravim demonstrated slightly potentiated effect on AHtH model. Combination of mexidol, CoAll and tetravim had weak potentiating effect of the metal complexes protective properties on AHBH model and summarized effect on AHeH model. Other metaprot or mexidol combinations with N-alkenylimidazole derivatives were ineffective on the different acute hypoxia models. Actovegin in combination with acyzol, cobazol or CoALL was inefficient and didn't affect or reduced metal complexes antihypoxic activity. Use of the inosine combined with the N-alkenylimidazole derivatives led to potentiation of the antihupoxic activity on AHtH model and was ineffective on the other models. Picamilonum administration and its combination with cobazol, CoALL and tetravim potentiated antihypoxic activity of the N-alkenylimidazole derivatives on AHtH.

Use of ACA with cobazol or CoALL led to the potentiation of the antihypoxic activity of the cobalt complexes on AHtH. Other combinations on the other models didn't affect or reduced N-alkenylimidazole derivatives protective activity, likely due to the inhibition of the cellular respiration in the mitochondrias [64].

Table 4

Effect of the N-alkenylimidazole metal complex derivatives and officinal drugs on the mice physical performance in normal and complicated conditions of hypoxia

Substance, drug	Dose, mg/kg, i/p							
	1	2,5	5	10	25	50	100	200
Run in normal condition								
Acyzol	96±6	89±10	92±7	96±10	90±9	-	-	-
Cobazol	120±6*	161±8*	146±6*	122±5*	74±6*	-	-	-
CoALL	98±9	90±12	91±8	87±12	69±9*	-	-	-
Tetravim	96±12	90±8	91±10	90±6	88±10	84±5	-	-
Acyzol+cobazol	132±5*	153±8*	182±9*	123±4*	-	-	-	-
Metaprot	-	-	-	-	112±4	131±4*	135±2*	106±2
Bromantan	-	-	-	-	115±6	128±3*	126±3*	113±4
Etomerzol	-	-	-	-	-	104±7	-	-
Swimming in normal condition								
Acyzol	94±11	110±10	109±10	106±7	96±8	-	-	-
Cobazol	124±8*	175±9*	154±9*	112±7	79±10*	-	-	-
CoALL	109±11	99±9	101±10	93±8	65±10*	-	-	-
Tetravim	98±12	95±14	104±7	101±7	94±10	91±8	-	-
Acyzol+cobazol	136±6*	186±6*	158±8*	119±8	-	-	-	-
Metaprot	-	-	-	-	89±5	124±5*	101±6	108±6
Bromantan	-	-	-	-	111±4	129±7*	122±3*	97±4
Etomerzol	-	-	-	-	-	95±9	-	-
Run in AHBH condition								
Acyzol	-	-	-	-	149±5*	-	-	-
Cobazol	130±4*	143±4*	197±5*	128±5*	135±9*	-	-	-
CoALL	-	-	-	-	156±7*	-	-	-
Tetravim	-	-	-	-	-	153±6*	-	-
Acyzol+cobazol	124±5*	132±9*	146±7*	189±3*	-	-	-	-
Metaprot	-	-	-	-	-	98±10	127±8*	-
Bromantan	-	-	-	-	-	94±8	98±8	-
Etomerzol	-	-	-	-	-	94±8	-	-
Swimming in AHwHc condition								
Acyzol	-	-	-	-	128±7*	-	-	-
Cobazol	108±6	102±8	104±13	97±8	120±5*	-	-	-
CoALL	-	-	-	-	128±7*	-	-	-
Tetravim	-	-	-	-	-	133±8*	-	-
Acyzol+cobazol	116±4*	120±4*	98±7	108±9	-	-	-	-
Metaprot	-	-	-	-	90±8	111±6	93±8	-
Bromantan	-	-	-	-	109±9	99±5	-	-
Etomerzol	-	-	-	-	-	96±10	-	-

Notice.* – Significant differences compared to control animals at $p < 0.05$. Data are expressed as M ± m. M – arithmetic mean as % relative to the control group (100%); m – standard error of the mean as % relative to the arithmetic mean.

Pharmacological properties analysis of N-alkenylimidazole metal complex derivatives. The influence of CoALL and tetravim on physical activity in normal and hypoxic condition was studied in the experiments on laboratory animals (mice, rats, rabbits). The functions of CNS, liver, kidneys, oxidative and carbohydrate metabolisms, blood count indexes, blood microcirculation, oxygenation processes, Red-Ox potential were studied. The preference was given to these parameters because of their importance in maintaining homeostasis in conditions of acute hypoxia. The results were compared with the effect of acyzol and cobazol, which had already found their application in clinical practice. The study in comparison lets us estimate a full picture of the influence of the zinc, cobalt and ferric complexes on animal organism functions. It will also help us to extend their clinical application, to suppose any contraindications and side-effects, and to judge about the mechanism of antihypoxic action.

Physical performance in normal and complicated conditions. According to the table 4, in normal conditions physical performance of animals was decreased or not changed because of metal complexes used in active antihypotoxic doses.

In lower doses (1–10 mg/kg) acyzol, tetravim and CoALL showed no effect, but cobazol increased the duration of running by 20–61% and the duration of swimming by 24–75% ($p < 0.05$). The highest efficacy (up to 82%) was shown when cobazol and acyzol were used together. Indeed, combined injection of cobazol and acyzol in doses of 1; 2,5; 5; 10 mg/kg increased the duration of the run in studied mice by 32, 53, 82, 23% and the duration of swiming by 36, 86, 58, 19% ($p < 0.05$). In conditions of AHBH and AHwHc test drugs showed actoprotecting effect, that overperformed metaprot, bromantan and etomerzol. Further studies of actoprotecting effect of N-alkenylimidazol derivatives and their combinations is an interesting subject.

Central nervous system function. According to the results of CDRA-test, studied metal complexes did not show notable influence on CDRA of rats, but remarkably decreased the influence of AHBH on reflex lassitude and positive reaction generating to conditioned excitator, that proved the increasing resistance of the higher regions of animals CNS to acute hypoxia (fig. 3).

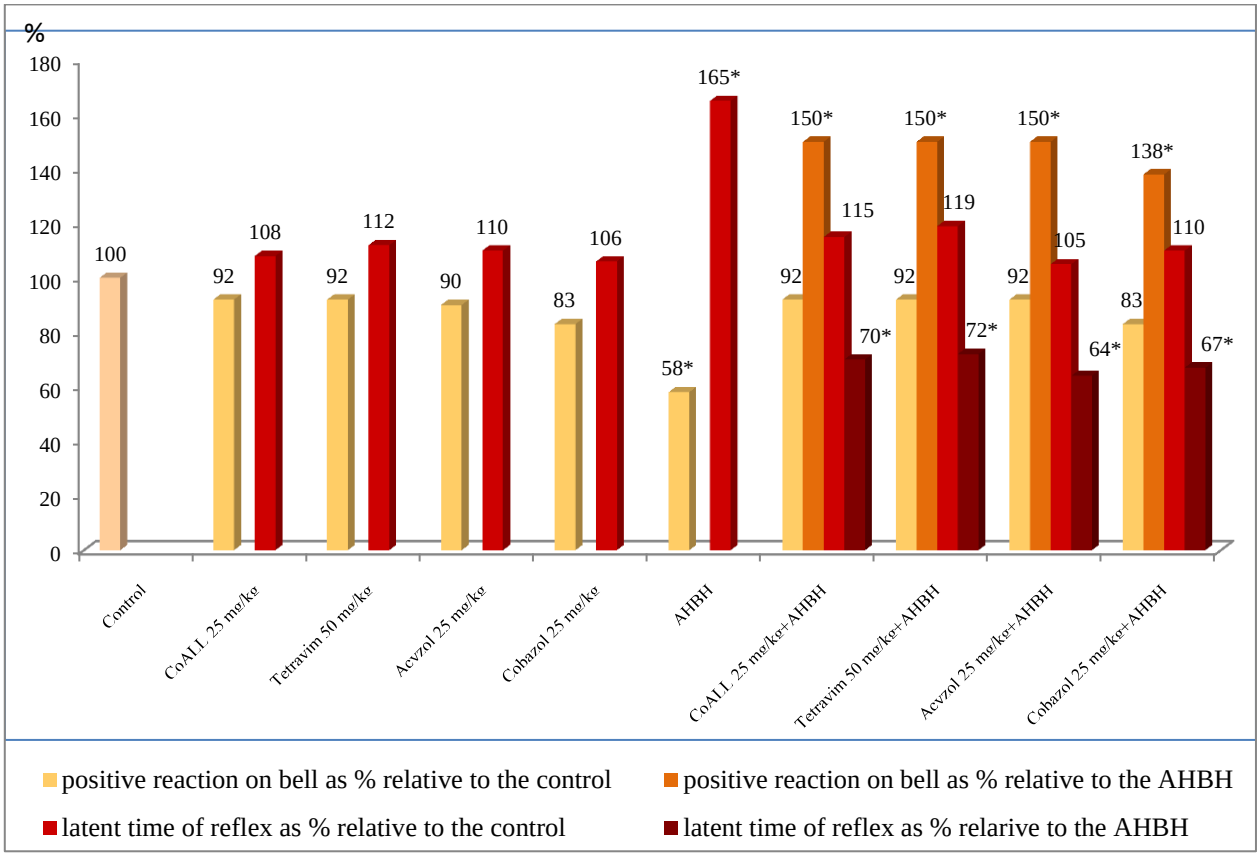


Figure 3. The effect of derivatives of N-alkenylimidazole on CPRA on rats
Notice.* – Significant differences compared to control animals at $p < 0.05$. Data are presented as % relative to the control group (100 %)

Noted, that with corazol injected, cobalt, ferric and zinc complexes reliably increased latent time of cramps and lifespan in studied mice compared to the control group. Considering that corazol increases brain demand in oxygen, the mechanism of anticonvulsant action of studied drugs could be established with their effect on cellular hypoxia and their capability to reduce neuronal oxygen consumption.

Conducted ethological experiment showed, that in 1 hour after intraperitoneal administration of metal complexes, tendency of decreasing activeness was noticed in all experimental animals compared to the control group. Indeed, after CoALL, acyzol, cobazol (25 mg/kg) and tetravim (50 mg/kg) administration the depression of patterns in behavioral structure was noted, such as: «sniffing» by 35, 35, 40 and 24%; «movement on the spot» by 44, 48, 43 and 33%, «burrow» by 70, 69, 68 and 40%, «moving» by 71, 64, 65 and 33% respectively. Volume of pattern «sitting» increased by 69, 83, 64 and 55% consequently. Patterns «grooming», «stand with support», «vertical stand», «defecation» were not observed (fig. 4).

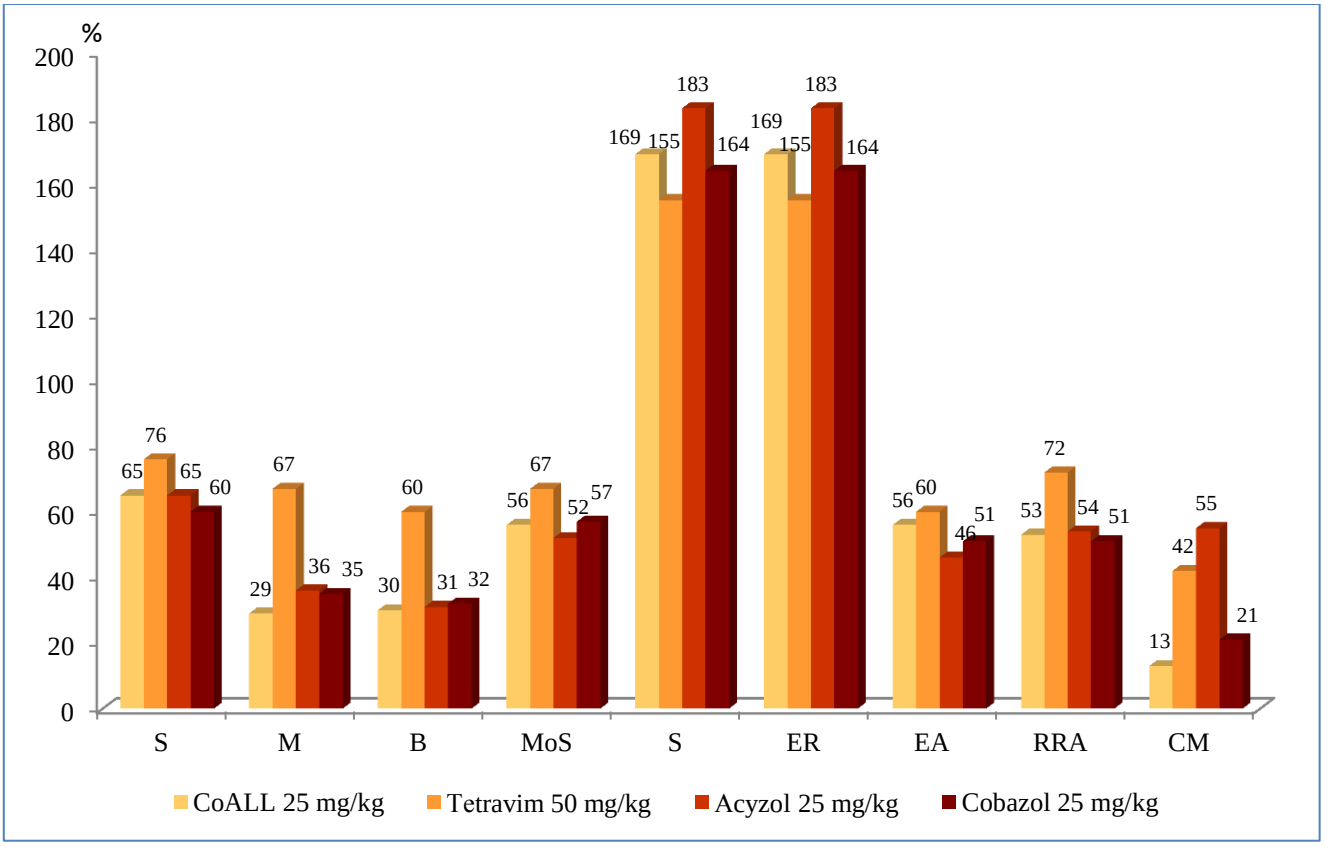


Figure 4. The effect of the N-alkenylimidazole derivatives on the volume and structure of behavior of rats in «open space»
Notice. S – sniffing, M – moving, B – burrow, MoS – movement on the spot, S – sit, ER – emotional reactivity, EA – emotional anxiety, RRA – reference research activity, CM – coefficient mobility.
Significant differences compared to the control animals at $p < 0.05$. Data are presented as % relative to the control group (100 %).

Metal complexes derivatives were changing integral characteristics of individual animal behavior. Indeed, in 1 hour after CoALL, acyzol, cobazol and tetravim administration ER increased by 69, 83, 64 and 55% respectively; EA, RRA and CM decreased by 44, 54, 49 and 40%, 47, 54, 49 and 28, 87, 45, 79 and 58% respectively, compared to the control values. ER increase is a result of depressing effect of the test drugs on emotional and motional spheres of the CNS.

Therefore, cobalt, iron and zinc complexes with N-alkenylimidazol intensify ability of the higher regions of animals CNS to resist acute hypoxia. Materials about depressing effect of the studied drugs on emotional and motional spheres of CNS received with trials could be considered as one of the possible mechanism of the antihypoxic action. Most possibly a few complementary mechanisms and not just one

mechanism form a basement of raising resistance of the CNS to hypoxia that was observed after metal complexes had been used, that seems to be an interesting subject to research.

Liver function. In experiments on rabbits, CoALL and tetravim administration one hour before AHBH restored the increased level of glucose in blood after galactose loading, that proves the gain in carbohydrate liver function (fig. 5).

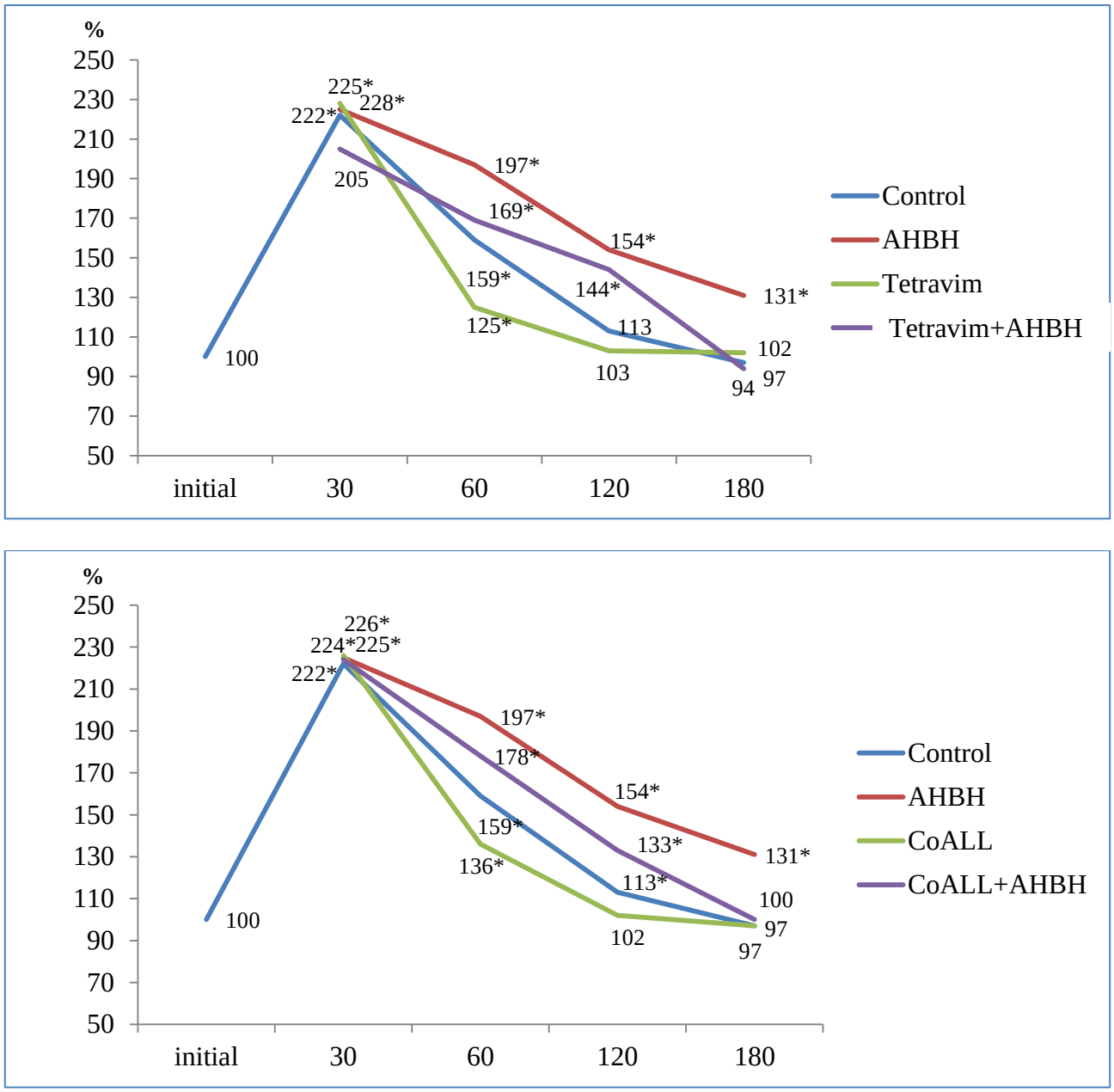


Figure 5. The effect of CoALL (25 mg/kg), tetravim (50 mg/kg), AHBH and their combination on blood sugar in rabbits after intravenous administration of galactose (1 mg/kg)
Notice. * – Significant differences compared to control animals at $p < 0.05$. Data are presented as % relative to the control group (100 %).

Metal complex derivatives of CoALL, cobazol, tetravim, acyzol, AHBH and their combined action didn't influence the concentration of total protein, albumins, globulins, cholesterol, triglycerides, direct and total bilirubin.

In mice that were receiving CoALL and cobazol in dosage 25 mg/kg, concentration of ALT decreased by 43 and 28% respectively. Concentration of AST increased by 138 and 59% respectively compared to the control mice (table 5).

Table 5

The effect of CoALL (25 mg/kg), tetravim (50 mg/kg), acyzol (25 mg/kg), cobazol (50 mg/kg), AHBH and combined on the activity of enzymes of blood serum of mice

Substance and type influence	Activity of enzyme, ED/l						AST/ALT	
	ALT			AST				
	abs.	%	*p*	abs.	%	*p*	abs.	%
Control	42.0±1.5	100	-	119.0±4.4	100	-	2.8	100
AHBH	49.3±1.8	117	< 0.001	157.1±6.7	132	< 0.001	3.2	14
CoALL	24.0±1.2	57	< 0.001	283.3±12.0	238	< 0.001	11.8	421
Tetravim	40.7±1.8	97	> 0.05	122.4±4.4	103	> 0.05	3.0	107
Acyzol	44.2±2.3	105	> 0.05	126.4±6.4	106	> 0.05	2.9	104
Cobazol	30.2±3.3	72	< 0.01	189.5±14.0	159	< 0.001	5.3	189
CoALL+AHBH	23.2±3.2	55	< 0.001	133.1±9.5	111	> 0.05	5.7	204
Tetravim+AHBH	10.1±3.3	24	< 0.001	42.0±4.1	35	< 0.001	4.2	150
Acyzol+AHBH	28.1±3.7	67	< 0.01	69.0±6.9	58	< 0.001	2.5	89
Cobazol+AHBH	31.5±3.8	75	> 0.05	129.7±12.9	109	> 0.05	4.1	146

In mice that were treated with tetravim and acyzol, concentrations of ALT and AST were equal to the control group. In case of cobalt compounds administration one hour before AHBH concentration of AST was equal to the control value and was decreasing when using acyzol and tetravim, the level of ALT was decreased.

High activity of AST when ALT is decreased could be a sign of suppression of peripheral metabolic reactions with multiple crossings and stimulation of central routs of catabolism close to CTA. AST/ALT ratio, determined after cobazol administration to mice were relatively significantly higher than control, that indicates an activation of central branches of catabolism, geared towards power supply by means of oxidizing organic substances, increase of substrate supply in CTA and more efficient energy metabolism.

Mice hexenal sleep duration after the treatment with acyzol, cobazol, CoALL (25 mg/kg) and tetravim (50 mg/kg) didn't change, so the liver detox function wasn't disrupted.

Therefore, the test compounds didn't affect liver protein, lipid metabolism, pigmentary and detox function. Carbohydrate metabolism improvement after tetravim' and CoALL administration, the elimination of hypoxia negative influence on carbohydrate metabolism and the change of AST and ALT enzymes after CoALL administration could be one of the supposed mechanisms of its action.

In researched doses, CoALL and tetravim didn't change kidneys nitrogen excretion and diuretic functions, and also urine physicochemical characteristics.

The results of the research of electrocardiography dynamic also didn't reveal any statistically significant changes, compared with the control group.

Analysis of the ferric and cobalt complexes of the N-alkenylimidazole derivatives antihypoxic activity mechanism. Possible antihypoxic mechanism of the metal complex compounds CoALL and tetravim could be attributed to a few reasons, the main of which are the following:

– cell Red-Ox potential regulation during the hypoxy;

– energy consumption optimization under the oxygen deficiency conditions;

– blood microcirculation improvement;

– oxygen supply optimization due to cobalt influence on the carbonic anhydrase;

– cobalt activation of the endogenous erythropoietin synthesis, gain of the erythropoeisis and HIF-1 stabilization;

Other reasons of the test compounds antihypoxic activity could not be excluded, which proves the need for the further investigation of the protective activity under the oxygen starvation conditions.

To clarify possible antihypoxic activity mechanism of the new N-alkenylimidazole derivatives CoALL and tetravim blood morphology and biochemistry, oxidative and carbohydrate metabolism, microcirculation intensity and Red-ox potential regulation were evaluated on the experimental animal models.

Oxidative metabolism. Oxidative processes intensity and their association with the phosphorylation could be estimated by the oxygen consumption and body temperature.

Oxygen consumption significantly decreased by 38 and 40% in an hour after administration of CoALL and tetravim at the dose of 25 mg/kg and 50 mg/kg respectively in comparison with initial level and restored initial values at 24 hours. Decoupler of the oxidative phosphorylation 2,4-dinitrophenol (2,4-DNF) administration at the

dose of 5 mg/kg increased mice oxygen consumption in a hour after administration on 38% ($p < 0.05$). Coadministration of 2,4-DNF (5 mg/kg) with CoALL (25 mg/kg) or tetravim (50 mg/kg) significantly decreased by 16 and 18% oxygen consumption in a 1 hour respectively in comparison with initial level and raised back to the initial values at 24 hours (fig. 6).

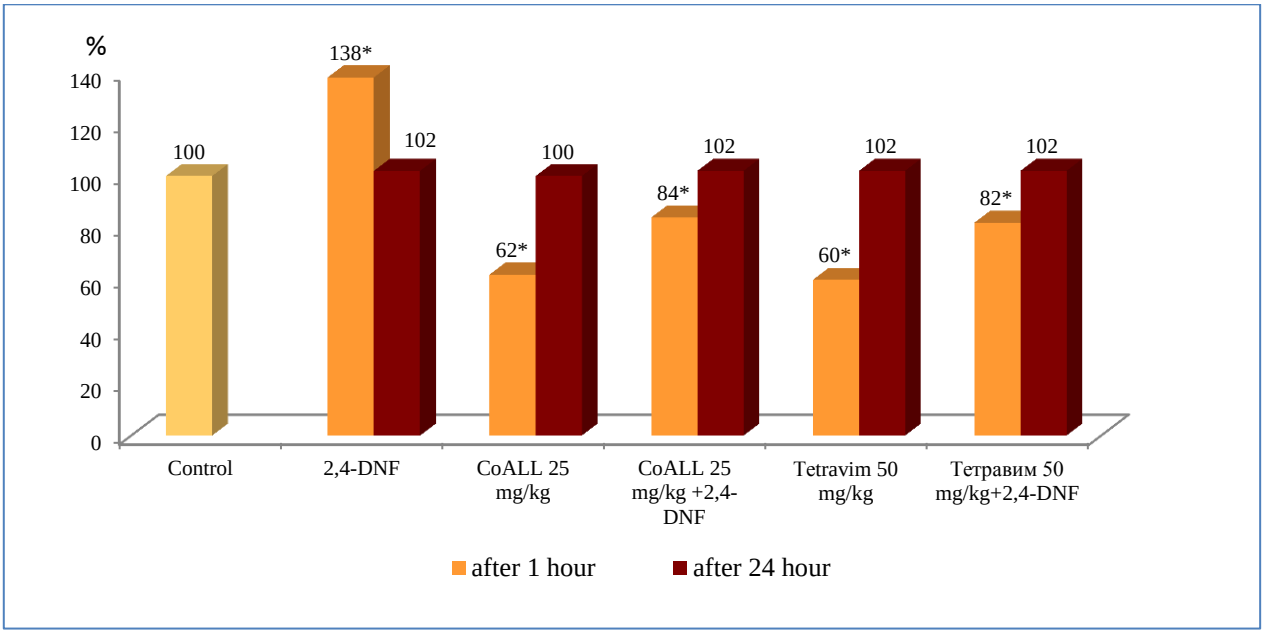


Figure 6. Effect of CoALL (25 mg/kg), tetravim (50 mg/kg), 2,4-DNP (5 mg/kg) and combinations thereof on mice oxygen consumption (n = 10)
Notice. * – Significant difference ($p < 0.05$). Data are referred to the control value, which was taken as 100%.

So, CoALL and tetravim decreased oxygen consumption and reduced 2,4-DNF adverse influence on it.

Rectal temperature of the test animals decreased by 10 and 7% ($p < 0.05$) an hour since the CoALL and tetravim administration, respectively, in comparison with the control values. In an hour after 2,4-DNF administration (5 mg/kg) temperature rose up to 39,6°C, which was 7% above the control values, and only 24 hours after administration temperature came back to the initial level (fig. 7).

Coadministration of CoALL, tetravim and 2,4-DNF at the same doses did not affect rectal temperature in the both time points.

N-alkenylimidazole derivatives effect on the oxidative metabolism could be attributed to their ability to transform aerobic respiration into anaerobic, which decreases oxygen demand. Gas exchange restore in 24 hours since compound administration could happen because of its excretion in this period of time and/or formation of the metabolites which doesn't affect oxygen consumption. The more economy energy uptake could be explained by the compound interference into oxidative phosphorylation coupling mechanism in mitochondria, which decreases decoupling of this processes.

Oxygen consumption and rectal temperature restore analysis after 20 min of simulation of the presence at a height of 7500 m showed decreased oxygen consumption after hypoxia in control animals for 60 min, which indicates that mitochondrial respiratory function is disturbed (table 6).

Animals from the test group did not demonstrate decrease in the oxygen consumption after hypoxia, which could be the result of the CoALL and tetravim beneficial effect on mitochondria structure and function under the oxygen deficiency conditions. the height reduces body temperature up to 3,0□C in control group. Body temperature of mice treated with CoALL and tetravim did not decrease after the simulation. Influence on oxidation and phosphorylation coupling and mitochondria structure and function maintenance during the hypoxia may play the main role in the antihypoxic mechanism of the test compounds.

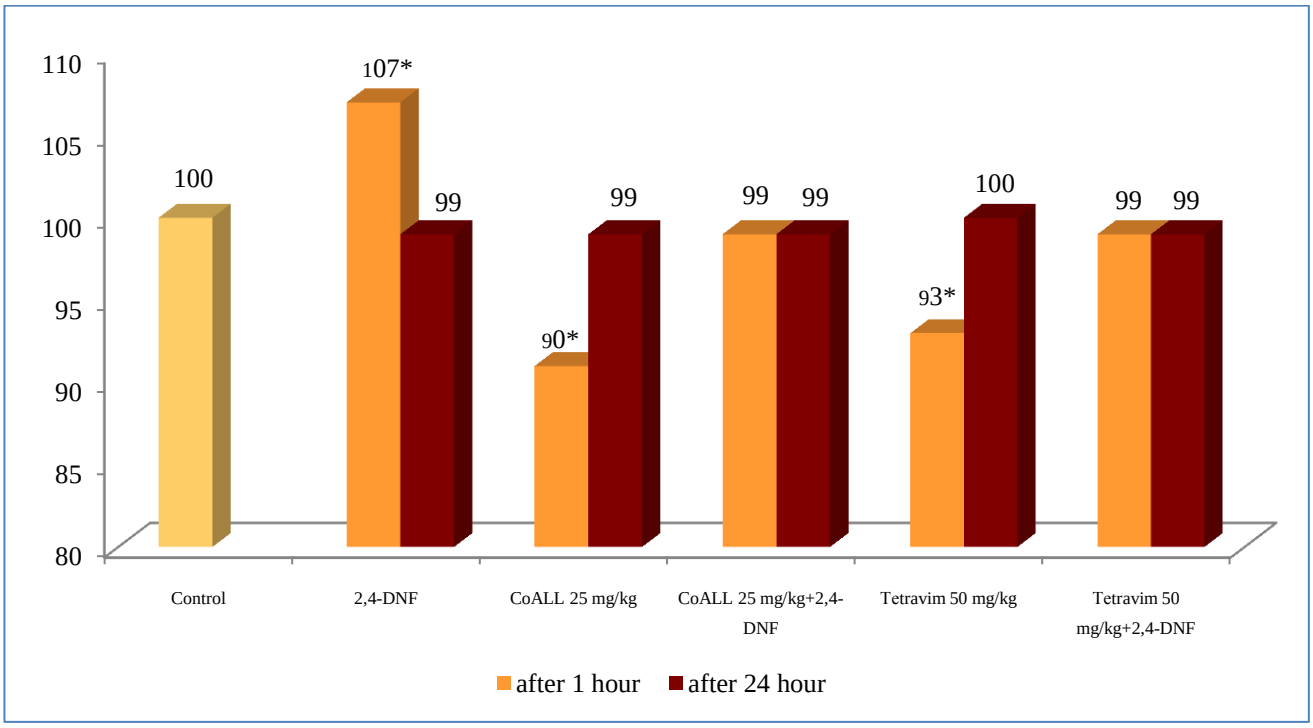


Figure 7. Effect of CoALL (25 mg/kg), tetravim (50 mg/kg), 2,4-DNP (5 mg/kg) and combinations thereof on mice rectal temperature (n = 10)
Notice. * – Significant difference ($p < 0.05$). Data are referred to the control value, which was taken as 100%

Table 6

The effect of CoALL (25 mg/kg) and tetravim (50 mg/kg) on the oxygen consumption of mice in the early after hypoxic period (n = 10)

Chemical compound	Basic data	Since 60 min after leading chemical compounds	Since 5 min after sinking with the «height»	Since 60 min after sinking with the «height»
	Oxygen consumption, ml/мin/100 g of body weight			
	M ± m	M ± m	M ± m	M ± m
Control	5.0 ± 0.2	5.0 ± 0.2	2.5 ± 0.2*	2.8 ± 0.3*
CoALL	5.0 ± 0.2	3.1 ± 0.3*	5.1 ± 0.4	5.2 ± 0.3
Tetravim	5.0 ± 0.2	3.0 ± 0.3*	4.9 ± 0.3	5.0 ± 0.4
Rectal temperature, ºC				
	M ± m	M ± m	M ± m	M ± m
Control	37.0 ± 0.2	36.9 ± 0.2	34.0 ± 0.5*	34.3 ± 0.5*
CoALL	37.0 ± 0.4	33.5 ± 0.1*	33.7 ± 0.6*	33.9 ± 0.4*
Tetravim	37.0 ± 0.1	34.5 ± 0.3*	34.8 ± 0.3*	35.1 ± 0.5*

Notice.* – Significant differences compared to control animals at $p < 0.05$. Data are shown as M ± m.

The data showed, that simulation of the presence at

Glucose blood level. Glucose content decreased by 25% with comparison to control level in the AHBH conditions caused by the simulation of the presence of the animal at a height of 7500 m for 20 min. Glucose blood level of mice treated with the CoALL (25 mg/kg) and tetravim (50 mg/kg) did not change. Glucose blood level of the animals treated with the metal complexes compounds an hour before hypoxia performance was relatively similar to the control group values. Increase in glucose content was 28 and 40% in comparison with the animals, which were not treated with CoALL and tetravim, respectively, before AHBH exposure. Thus, test compounds did not affect glucose blood level, but prevented hypoglycemia, caused by AHBH.

Complete blood count. Single dosing of the CoALL (25 mg/kg) and tetravim (50 mg/kg) had different influence on hemogramm parameters. Hematocrit, hemoglobin and formed elements levels in mice blood increased significantly in an hour after CoALL administration. The amount of the leukocytes, lymphocytes, monocytes, granulocytes, thrombocytes and erythrocytes increased by 97, 88, 90, 180, 29, and 30% respectively. Hematocrit and hemoglobin level significantly increased by 27 and 28%, respectively, in comparison to the control values. Tetravim administration did not affect morphological parameters, hemoglobin level or hematocrit (table 7).

Table 7

The effect of CoALL (25 mg/kg), tetravim (50 mg/kg), AHBH and combined on the hemogram

Index, unit	Control	AHBH		CoALL		AHBH+CoALL		Tetravim		AHBH+tetravim	
		abs.	%	abs.	%	abs.	%	abs.	%	abs.	%
Leucocytes, $x10^9$ g/l	5.8±0.3	5.9±1.1	102	11.4±1.1	197*	7.9±0.7	136*	6.5±0.2	112	6.5±0.2	152*
Lymphocytes, $x10^9$ g/l	4.3±0.5	4.4±0.3	102	8.1±0.8	188*	5.0±0.3	116	4.9±0.5	114	4.9±0.5	116
Monocytes, $x10^9$ g/l	1.0±0.1	0.9±0.1	90	1.9±0.2	190*	1.7±0.4	170*	1.1±0.1	110	1.1±0.1	210*
Granulocytes, $x10^9$ g/l	0.5±0.1	0.6±0.1	120	1.4±0.1	280*	1.2±0.3	240*	0.5±0.1	100	0.5±0.1	340*
Erythrocytes, $x10^{12}$ g/l	5.6±0.5	7.1±0.3	127*	7.3±0.3	130*	5.9±0.2	105	6.1±0.3	109	6.1±0.3	100
Hemoglobin, g/l	131.0±3.9	152.0±1.2	116*	166.0±5.8	127*	135.0±7.4	103	125.0±6.7	96	125.0±6.7	110
Hematocrit, %	30.2±2.5	34.8±0.2	115*	38.8±1.6	128*	32.9±1.2	109	28.8±3.0	95	28.8±3.0	99
Thrombocytes, $x10^9$ g/l	375.0±28.0	338.0±19.0	90	485.0±19.0	129*	405.0±25.0	108	338.0±23.0	90	338.0±23.0	108

Notice.* – Significant differences compared to the control animals at $p < 0.05$. The data are presented as M ± m.

AHBH, induced by the presence for 20 min in vacuum chamber, simulating conditions at a height of 7500 m, significantly increased erythrocyte and hemoglobin levels, and hematocrit on 27, 16 and 15%, respectively compared to the control animals. Neither thrombocyte nor leukocyte parameters had any significant differences.

Mice treated with CoALL one hour before the hypoxia exposure had the same erythrocyte and hemoglobin levels, and hematocrit as at the beginning of the experiment. This results could be attributed to the erythrocyte level regulation, based on the feedback principle. Hypoxia activates erythropoietin synthesis, which in turns affect bone marrow and stimulate erythropoiesis. Increase of the amount of the erythrocytes enhance oxygen supply and reduces the degree of the hypoxia, which leads to inhibition of the erythropoietin synthesis [65]. CoALL and AHBH together increased the levels of the leukocytes, monocytes and granulocytes on 36, 70 and 140% respectively in comparison to the control levels. The lymphocytes counts did not differ from the initial values.

It is known, that cobazol has stimulating effect on hemopoiesis [29]. Specific character of this effect is its targeting on increase of erythropoiesis rate and the amount of the leukocytes and thrombocytes. Immunomodulating effect of the cobazol is confirmed by the persistent increase of the lymphocytes amount and neutrophil phagocyte activity.

Experiments showed, that cobazol effect was implemented through the hystotoxic hypoxia, caused in mesangial cells of the renal glomeruli, which resulted in the raise of the cAMP and cGMP levels and activation of the lysosomal enzymes of the erythropoietic fraction, which in turns increase the erythropoietin blood level [43]. Since CoALL and cobazol are analogous, it could be proposed that they share the hemostimulating mechanism.

So, in our experiments ferric complex compound tetravim did not affect blood cells level, hemoglobin concentration and hematocrit, while the cobalt complex CoALL increased all the mice hemogramm parameters. The increase of the erythrocyte and hemoglobin levels, and hematocrit caused by the CoALL administration could be the key point of its antihypoxic activity.

Microcirculation system. Microcirculatory bloodstream is the key point of the connection of the local and integral structural-functional homeostasis regulation systems, which provide energy substrate and oxygen supply at the appropriate level to the cell metabolic rate. By means of blood stream inner processes of the cell Red-Ox status control combined with homeostasis control through the changes in general and local bloodflow [65]. That is why microcirculation disorder, independently of the origin, leads to the cell hypoxia and disturbance in redox processes.

It is known that the vascular tone control mechanism is based on reactive oxygen species [66]. At the same time, hypoxic conditions that affect tissue energetics usually result in hemodynamic disorders in the microcirculatory bloodstream [67].

Vasoactive and toxic metabolites release during the hypoxia affects bloodstream and leads to microcirculation disorders progression. It is known, that antihypoxic activity of the different drugs targets the microcirculation improvement (mexidol, actovegin et

al.). That is why it is important to investigate the effect of the new compounds with the significant antihypoxic activity on the microcirculation.

To evaluate the metal complexes compounds CoALL and tetravim influence on the microcirculation state, we tested their effect on microhaemodynamics and tissue oxygenation.

Microhaemodynamics. We estimated statistical parameters of the perfusion value, and this allows to calculate tissue perfusion level (TP) in perfusion units (p.u.), the mean square deviation, characterizing the temporal variability of the erythrocyte flow (σ or flax, p.u.), significant for assessing the microcirculation state and maintenance of its regulation mechanisms, as well as the ratio between variability of perfusion (flax) and average perfusion (TP), expressed in the variation coefficient (Kv) – the index of vasomotor activity of the microvessels.

TP decreased approximately 4-fold after AHBH exposure, possibly, due to bloodstream redistribution (more efficient in the vital organs and less efficient in skin capillaries) and increases of the erythrocyte and thrombocytes aggregation activity and whole blood and plasma viscosity. Flax level decreased by 26%, which resulted in reduction of the oxygen return. Kv, characterizing vasomotor activity of the microvessels, increased by the 112%, which could be explained by the catecholamine and other vasoactive compounds production during acute hypoxia.

TP was 18,4±1,9 p.u. an hour after the CoALL administration, that was 38% higher than control level. Flax level increased by 65%, Kv decreased by 25% compared to the control values. CoALL administration an hour before the AHBH exposure decreased negative influence of the acute hypoxia on microcirculation. TP was similar to control values, and Kv and flax level were higher than in control group by 46 и 86% respectively.

Tetravim affected microcirculation less than CoALL in the same conditions. TP increased significantly by 28% one hour after the compound administration at the dose of 50 mg/kg. At the same time Kv decreased by 40%, and flax level was relatively similar to the control values. Animals treated with tetravim one hour before the AHBH exposure demonstrated flax and Kv increase by 65 and 56% respectively, and TP was similar to the control values (table 8).

Table 8

Effect of CoALL (25 mg/kg), tetravim (50 mg/kg), AHBH and their combination on microhaemodynamics (n=10)

Substance and type influence	TP		σ		Kv	
	abs.	%	abs.	%	abs.	%
Control	13.3±1.4	100	3.7±0.5	100	37.8±9.2	100
AHBH	3.1±0.8	23*	2.3±0.6	74*	80.2±7.9	212*
CoALL	18.4±1.9	138*	6.1±0.5	165*	28.3±6.0	75*
CoALL+AHBH	15.3±2.3	115	6.9±1.2	186*	55.1±5.2	146*
Tetravim	17.0±1.2	128*	3.6±0.5	97	22.7±3.2	60*
Tetravim+AHBH	13.7±1.0	103	6.1±0.9	165*	59.0±5.2	156*

Notice. * – Significant differences compared to control animals at $p < 0.05$

In general, the increase of the perfusion level after CoALL and tetravim administration indicates the ability of the compound to improve microcirculation.

Tissue oxygenation. Optical tissue oxymetry showed that CoALL administration increased SO_2 by 23%. Oxygen utilization enhanced by the compound because of parameter U increased by 2,2-fold. Red-Ox reaction level can be evaluated by the 40% decrease of Sm and 17% decrease of the oxygen concentration in the mixed blood of the microcirculatory bloodstream (table 9).

Table 9

Effect of CoALL (25 mg/kg), tetravim (50 mg/kg), AHBH and their combination on tissue oxygenation (n=10)

Substance and type influence	SO_2		M		Sm		U	
	abs.	%	abs.	%	abs.	%	abs.	%
Control	69.0±4.7	100	60.6±4.2	100	4.8±0.58	100	1.9±0.39	100
AHBH	72.3±3.8	105	57.5±5.2	95	3.9±0.23	81*	0.9±0.21	47*
CoALL	84.6±4.1	123*	50.3±4.8	83*	2.9±0.60	60*	4.2±0.81	221*
CoALL+AHBH	75.1±4.3	109	52.0±2.3	86*	4.0±0.43	83*	3.7±0.62	195*
Tetravim	75.0±7.1	109	58.0±2.5	96	4.7±0.30	98	2.0±0.70	105
Tetravim+AHBH	65.8±5.3	95	62.0±3.1	102	5.0±0.90	104	2.0±0.45	105

Notice. * – Significant differences compared to control animals at $p < 0.05$

Considering the fact that microcirculation is variable and adapt to the specific physiological demands of the tissue, the most meaningful characteristic is oxygen exchange parameter (EOE). Control animals had integral EOE value 25.5±1.2 a.u. After CoALL administration its value increased by 85% compared to the control value (fig. 8).

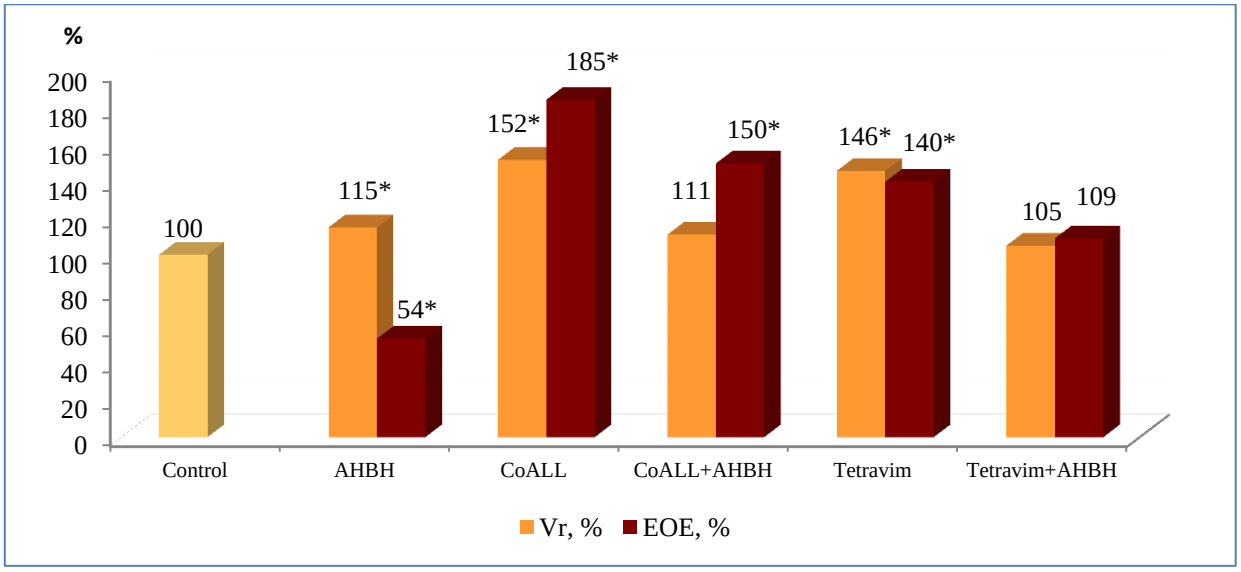


Figure 8. Effect of CoALL (25 mg/kg), tetravim (50 mg/kg), AHBH and their combination on erythrocyte concentration (Vr) and EOE (n =10)
Notice. * – Significant difference ($p < 0.05$). Data are referred to the control value, which was taken as 100%.

Control animals had 6.2±0.3% of the erythrocytes in the test skin region. One hour after the CoALL administration erythrocyte content increased by 52% resulting in binding more oxygen. These data are consistent with the haemogramm data, which showed the increase of the erythrocyte amount in mice blood after the CoALL administration.

After the perfusion restriction transcapillary exchange parameters in the skin of the test animals consistently decreased in AHBH model (Sm, U and EOE), because epidermal cells demonstrate enhance resistance to oxygen deficiency. Sm decreased by 19%, U by 53 and EOE by 46%. Vr increased by 15%.

CoALL administration one hour before AHBH performance kept oxygen saturation at the control level. Perfusion oxygen parameter and oxygen concentration in mixed blood were lower than control values by 17 and 14%. U and EOE exceeded the control values by 95 and 50% respectively.

Oxygen saturation in arterial and mixed blood, and specific consumption of the oxygen by the tissues were not affected by the tetravim administration. EOE value increased by 1,4-fold compared to the control values. As for the CoALL administration, the amount of the erythrocytes increased by 46% in the test skin region that more likely confirmed the Tetravim ability to mobilize the erythrocytes from the blood pool.

So, multifunctional laser diagnostic complex «LAKK-M» for the full-scale study of the microcirculation tissue state allows to study microhaemodynamic parameters and oxygen transport under the influence of the biologically active compounds. All of the above changes in the microcirculation system caused by the N-alkenylimidazole derivatives enhanced tissue tolerance for the hypoxia. At the same time microhaemodynamics and EOE in tissues improved and erythrocyte content in microvascular blood flow increased. These data are useful for clinical science and partially explain antihypoxic mechanism of the test compounds.

Red-Ox potential. The microcirculatory bloodstream closely associated with the redox systems of the cell, and provided the essential level of the oxygen and metabolites supply for the tissues [66]. Great changes of the Red-Ox potential always lead to the cell death activation. It is important to supply the cell with oxygen to maintain the redox balance, so it is obvious that the hypoxic states lead to a drastic change in the Red-Ox potential.

In our studies, we used the emission of the respiratory chain enzymes NADH and FAD to determine the Red-Ox potential of the cell. In the recent years, physicochemical characteristics of pyridine nucleotides and FAD have been intensively studied in connection with the possibility of their use as intracellular markers of the activity of the cell

energy systems. It is known that the NAD and FAD fluorescence spectra characterize the energy «status» of the cell. Pyridine nucleotides fluoresce only in the reduced state (460–490 nm) and lose this ability after oxidation, while FAD, on the contrary, fluoresce only in the oxidized form (520–530 nm) and lose this property upon the transition to the reduced form.

Transition from the rest state to the active energy metabolism is accompanied by the increase in the oxidized pyridine nucleotides (NAD^+, $NADP^+$), FAD and cytochromes concentration and corresponding decrease in the concentration of their reduced forms. The actively metabolizing tissues are characterized by spectra with approximately equal intensities of the emission of the reduced pyridine nucleotides and oxidized FAD. At the same time the rate of the respiratory chain electron transfer is as high as possible.

The study of the CoALL and tetravim effect on the Red-Ox potential under AHBH conditions could be useful for investigating the possible mechanism of their antihypoxic effect.

The FOCI and Red-Ox potential did not change 1 hour after the administration of CoALL and tetravim to white outbred rats (table 10).

Table 10

The effect of tetravim (50 mg/kg), CoALL (25 mg/kg), AHBH and their combination on FOCI and Red-Ox potential (n=8)

Substance and type influence	A_{NADH}	$A_{flavins}$	FOCI	%	Red-Ox potential	%
Control	350±13.5	230±8.8	1.53±0.073	100	0.66±0.032	100
AHBH	389±14.3	202±6.6	1.95±0.126	127*	0.53±0.034	80*
CoALL	382±12.6	250±14.1	1.55±0.115	101	0.66±0.045	100
CoALL+AHBH	370±8.6	256±16.9	1.46±0.081	96	0.69±0.042	105
Tetravim	518±14.6	361±13.8	1.45±0.054	94	0.70±0.034	106
Tetravim+AHBH	353±10.6	250±15.6	1.43±0.168	93	0.63±0.059	95

Notice.* – Significant differences compared to control animals at $p < 0.05$. The data are presented as M ± m, where M is arithmetical mean and m is standard error of the mean.

FOCI significantly increased by 27% Under AHBH conditions as a result of the increase in the fluorescence emission of reduced NADH and the decrease in the fluorescence emission of oxidized FAD. The Red-Ox potential decreased by 20% compared to the control values. The test substances administration 1 hour prior to AHBH exposure reduced the Red-Ox potential shift caused by the oxygen deficiency.

Hypoxia, regardless of its etiology and symptoms, causes a cascade of damage to the multidimensional system, which leads to the disruption of the metabolic processes and Red-Ox systems imbalance. NADH concentration increase during the oxygen deficiency can lead to the switching of aerobic glycolysis to anaerobic. The decrease in the energy potential of cells, observed in this case, results in the fact that they not only lose the ability to perform their network functional duties in the organism, but they are not even able to provide their own vital activity, which inevitably leads to their death.

The conducted studies indicate that the metal complex derivatives of N-alkenylimidazole tetravim and CoALL restore the Red-Ox potential shift induced by AHBH and may be useful as regulators of the Red-Ox state.

Conclusions

1. The strategic task of creating a new generation pathogenetic therapy drugs is the development of the drugs-regulators of the Red-Ox systems aimed at activation of the protective resource of the organism. Complexes of essential bioelements with corresponding ligands can claim the role of highly effective safe drugs.

2. The tested metal complex derivatives of N-alkenyl-, N-propargylimidazoles and 3-hydroxypyridine: acyzol, cobazol, vim, allym-1, allym-2, pilim-1, pilim-2, ALL, CoALL, BIS-N, BIS-3, g-4, g-5, g-6, g-7 and g-8 belong to the class of moderately toxic compounds, which proves their safety. The iron complex of the vinylimidazole tetravim refers to low-toxic substances (LD_{50} for intraperitoneal administration is 1625 mg/kg, for intragastric administration 2100 mg/kg), and the copper complex of propenylimidazole, pilim-4, corresponds to the class of highly toxic compounds.

3. Twelve of the 17 investigated metal complex derivatives of N-alkenyl-, N-propargylimidazoles and 3-hydroxypyridine had an antihypoxic effect on models of the acute hypoxia of different origin. The degree of the antihypoxic effect and the range of therapeutic doses depends on the model of hypoxia and the compound.

4. The antihypoxic effect of the cobalt complex named CoALL and iron complex named tetravim exceed the antihypoxic effect of the other compounds studied, as well as known antihypoxants and/or antioxidants: etomerzole, mexidol, nooglutyl and

hypoxen, in terms of the degree of the antihypoxic effect and the range of the effective doses in 4 types of hypoxia.

5. The combined administration of CoALL, tetravim, acyzol and cobazol with drugs of different pharmacological classes results in synergy and antagonistic effects.

6. CoALL and tetravim act as actoprotectors, increased resistance of the higher divisions of the animal CNS to the acute hypoxia, caused a depressing effect on the emotional and motor parts of the central nervous system, improved the carbohydrate function of the liver and eliminated the negative effect of hypoxia on carbohydrate metabolism; had no influence on protein and lipid metabolism, did not affect the function of the kidneys and heart under the conditions of AHBH or AHmHc.

7. A possible mechanism of the antihypoxic effect of the test compounds could be attributed to the effect on coupling of oxidation and phosphorylation, maintenance of the mitochondria structure and functions in conditions of oxygen deficiency, elimination of the negative effect of hypoxia on carbohydrate metabolism, improvement of microcirculation and tissue oxygenation parameters and restore of the redox potential at hypoxia exposure. CoALL, probably, activates the key metabolism stages, which are responsible for energy supply by oxidizing organic substrates. The increase in the erythrocyte and hemoglobin levels and hematocrit could also be a factor of antihypoxic action of CoALL.

8. Metal complexes CoALL and tetravim are promising for further development as drugs with antihypoxic, actoprotective and hemopoiesis stimulating activity. Red-Ox-regulating activity of metal complexes of the derivatives of N-alkenylimidazole offers the opportunities to construct new effective preparations of a wide spectrum of action on their basis.

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