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ADAPTIVE REACTIONS INVESTIGATION UNDER EXPERIMENTAL HYPOXIA CONDITIONS WITH THE PRIOR TREATMENT OF THE 4-THIAZOLIDINONE DERIVATIVE

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ABSTRACT: The effects of 4-thiazolidinone-derivative – Les-589 as perspective anti-hypoxic agent under the experimental hypoxic hypoxia actions were studied. The values of peripheral blood and hematological indexes as well as parameters of cellular oxidative metabolism (level of lipoperoxidation products, activity superoxide dismutase and catalase, levels and ratio of lactic and pyruvic acids in the blood and liver tissue) of the experimental animals were investigated. The presented data confirm the anti-hypoxic properties of the Les-589 under study conditions and are the strong argument for further Les-589 study as perspective antihypoxant with preventative effect.

INTRODUCTION: Optimal flow of all reactions in the organism requires enough energy, which is supplied by the oxygen dependent metabolism. A lot of diseases can cause the phenomenon of hypoxia that considerably affects the energy balance of the body.

Hypoxia, as well as any other extreme factors, leads to formation of different adaptive reactions types: compensation, super-compensation and decompensation reactions [1-3].

Versatility of hypoxic syndrome needs permanent extension of the range of antihypoxic agents which must initiate the adequate anti-stress reactions.

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At the same time effectiveness of anti-hypoxic treatment depends on pathogenesis of the oxygen deficiency and degree of its manifestation [4, 5]. Defining the protective properties of the potential anti-hypoxic agents needs the most sensitive and available tests to assess adaptive functions of the body and predict the adaptive function type.

Blood system and hematopoietic cells, in particular, show high sensitivity to hypoxia, thus, assessment of the peripheral blood state is an adequate test for dynamic control of the post hypoxic adaptation quality. At the same time, little research is carried out to study the state and direction of adaptive-compensative reactions based on the defining of the peripheral blood state with the anti-hypoxic agents application.

One of the promising groups of 4-thiazolidinones are 4-thiazolidinone-3-carboxylic acids extensively explored as compounds with anti-inflammatory, anti-diabetic, antibacterial and antitumor activities [6,7].

A majority of the biologically active 4-thiazolidinone-derived compounds are 5-ylidene derivatives, probably because of the substituents at the C5 position of basic heterocycle which is crucial for the pharmacological effects [8-10]. On the other hand, ylidene fragment conjugation to the C4 carbonyl group makes compounds to be electrophilic and potentially reactive due to possible Michael addition of the nucleophilic protein residues to the exocyclic double bond. These Michael acceptors are one of the most efficient activators of Nrf2 through the modification of Keap1 [11] and new highly specific and potent inhibitors of the mitochondrial pyruvate carrier [12].

Thus, 5-ylidene-thiazolidinones can be treated as main building blocks for various derivatives series synthesis in so-called privileged substructure-based diversity oriented synthesis strategy [13], which has proven to be a fruitful tool to rapidly discover biologically active lead compounds.

Modification of the oxygen homeostasis/oxidative stress and/or ROS inhibition is considered to be possible modes of action of 4-thiazolidinone-3-carboxylic acids derivatives [14, 15]. Important in this context is the fact of combination of anti-oxidant properties with anti-inflammatory, immunomodulatory and antitumor pharmacological profiles [16], specifying the direction of novel 4-thiazolidinone-3-carboxylic acids study in the spirit of modern triad: stress-inflammation-cancer.

Information about new anti-hypoxic agents search among discussed compounds is scrimpy, although some anti-hypoxic agents with antioxidant properties are identified [17].

Thus, the aim of the presented manuscript was to define the types of the adaptive reactions as the response to the experimental hypoxic hypoxia actions with the prior treatment of the 5-ylidene-4-thiazolidinone-3-carboxylic acid derivative (Les-589).

For this purpose, the values of peripheral blood and hematological indexes were assessed, as well as some oxidative metabolism parameters.

MATERIALS AND METHODS: The studies have been performed on the white rats (male, 180-200 g), within the general ethical principles of humane treatment to experimental animals [18]. Animals were divided into next groups: 1st - control, rats from the 2nd and the 3d group were given thiotriazoline (morpholin-4-ium (3-methyl-4H-[1, 2, 4]triazol-5-ylsulfanyl)-acetate) (2 mg/kg, ip, 2.5 % sol.), animals of the 4th and the 5th group were given Les-589 (100 mg/kg, ip, dissolved in Polysorbate 80). Animals of the 3d and the 5th groups in 45 min after treatment with thiotriazoline and Les-589 were exposed to the hypoxic hypoxia (identical to the height of 6000 meters above sea level) during 1 hour.

Les-589 (potassium salt of 3-[5-(3-phenylallylidene)rhodanin-3-yl]-propionic acid) was selected from department of Pharmaceutical, Organic and Bioorganic Chemistry *in home* library of 4-thiazolidinone derivatives, following our previous investigations [6, 10, 17] and primary data. Les-589 prolonged the survival time of the experimental animals under the hypoxia condition (identical to the height of 11000 meters above sea level) for the longer period (from (3.5±1.2) min. (control group) to (7.8±1.4) min.). The level of TBA-reactive products (TBARs) [19], activity of superoxide dismutase (SOD) [20] and catalase (CAT) [21] were estimated in blood and liver of the experimental animals for the study of the lipid peroxidation – anti-oxidation activity (LPO - AOA) system changes.

Hypoxia level was controlled by the levels and relation of lactic and pyruvic acids in blood and liver tissue [22, 23]. Blood parameters were defined 24 hours after hypoxia at the COULTER-T840 analyzer.

Based on the hematological parameters the following hematological indexes were calculated: adaptation index (I_A); granulocyte-agranulocyte index (GAI); lymphocyte-granulocyte index (LGI); relation of neutrophils and lymphocytes index (NLCI) [24, 25]. Statistical analysis of the obtained results was carried out (STATISTICA).

RESULTS AND DISCUSSION: The hypoxic exposition led to erythrocyte level decrease by 5,4% comparing to the control (**Table 1**).

TABLE 1: CHANGES IN ERYTHROCYTES AND THEIR PARAMETERS UNDER THE STUDIED CONDITIONS (M±m, n=10)

Parameters/ groups	Control	Hypoxia	Thiotriazoline	Thiotriazoline + Hypoxia	Les-589	Les-589+ hypoxia
Erythrocytes, $x10^{12}$/L	6.82±0.32	6.44±0.37	7.21±0.42	7.32±0.31●	7.42±0.21*	7.22±0.26●
Hemoglobin, g/L	129.8±10.4	131.4±11.2	132.0±10.9	134.3±11.4	137.3±11.2	132.0±10.9
Hematocrit, %	38.9±2.9	37.1±2.7	40.3±3.4	41.3±3.2	41.6±3.1	39.9±1.4

* – p < 0,05 to "control" group; ● – p < 0,05 to "hypoxia" group

At the same time, the tendency of erythrocyte and hemoglobin increase was observed in the experimental animals that were given thiotriazoline and Les-589, as well as groups with combination of hypoxia and tested compounds. The level of erythrocytes increased by the 5.7% and 9.1% in the 2nd and 4th group correspondingly comparing to the control group. Effects of tested compounds introduction showed the significant increase of erythrocyte level by 13.7% and 12.1% correspondingly (p < 0,05) in comparison with the groups only under the hypoxia (2nd and 4th groups). Analogical changes of hematocrit were observed. Thus, Les-589 and thiotriazoline, in case of their previous application before the hypoxia action, increased the oxygen capacity of blood following the increase of the erythrocyte and hemoglobin levels.

Under the influence of hypoxia the decrease of leucocytes by 32 % (p < 0,05) was detected (**Table 2**).

TABLE 2: CHANGES IN LEUCOGRAM UNDER THE STUDIED CONDITIONS (M±m, n=10)

Parameters/ groups	Control	Hypoxia	Thiotriazoline	Thiotriazoline + hypoxia	Les-589	Les-589+ hypoxia
Leucocytes, $x10^9$/L	11.5±1.1	7.8± 0.7*	15.1±1.2*	16.3±1.4*●	16.4±1.2*	16.8±1.3*●
Band neutrophils, %	2.9±0.1	3.1±0.,2	1.8±0.2*	2.5±0.1*	2.7±0.2*	2.3±0.2*
Segmented neutrophils, %	29.0±2.2	22.1±2.1*	28.3±1.9	26.0±1.6	25.0±1.4*	29.3±1.6●
Lymphocytes, %	63.7±4.1	73.0±4.2*	64.2±4.3	67.0±5.1	70.3±6.1	65.8±5.2
Monocytes, %	2.0±0.1	1.4±0.1*	4.5±0.4*	3.3±0.2●	2.3±0.1	4.1±0.3*●
Eosinophiles, %	0.9±0.1	0.3±0.02*	1.2±0.1*	2.2±0.2*●	0.15±0.01*	0.15±0.01*●

* – p < 0,05 to "control" group, ● – p < 0,05 to "hypoxia" group

Intense leukocytosis was observed in studied groups under the tested compounds treatment. The level of leukocytosis was almost the same in all groups. While, both thiotriazoline usage and hypoxia following the thiotriazoline treatment provided the increase of the percent of eosinophiles by 33% and 145% correspondingly, comparing to the control group. The trend of lymphocytes level increasing was observed, however its values were remained within the limits of maximum allowable upper limit. According to the literature, these findings indicate the mineralocorticoid production activation [24-26]. Modeling of hypoxic hypoxia following thiotriazoline usage led to the increase of the monocytes levels to 3.3%, comparing to 2.0% in the control group (remains within the norm limits), while the drug's introduction itself was accompanied with significant monocytosis (up to 4.5%). Effect of thiotriazoline introduction before the hypoxic exposition in comparison to the hypoxia modelling without protective agents was characterized in almost double increase of monocytes level (p < 0.05). Rats, which were treated with Les-589, had the following changes in leucogram: lymphocytosis – to 66% and 65% correspondingly, absence of eosinophiles, level of monocytes – within the norm limits, which certifies about the production of glucocorticoids at the same level as mineralocorticoids. In the group with hypoxia action under the above mentioned leukopenia, the increase of the lymphocytes level (by 17 %), decrease of segmented neutrophils (by

24 %), eosinophiles (by 62 %) and monocytes (by 43 %) compared to the control were observed. Based on the hematologic parameters the following relation indexes were calculated for estimation of the adaptive reactions types: adaptation index (I_A) (ratio of lymphocytes to segmented neutrophils); granulocyte-agranulocyte index (GAI) - ratio of the sum of band neutrophils, segmented neutrophils and eosinophils to the sum of monocytes and lymphocytes; lymphocyte-granulocyte index (LGI) – ratio of lymphocytes to sum of band segmented neutrophils, and eosinophils; index of the ratio of neutrophils and lymphocytes (NLCI) [24, 25]. According to the studied hypoxia conditions the relative increase of I_A (from 2.21±0.09 (control) to 3.37±0.12) and LGI, as well as decrease of GAI and NLCI were detected (**Table 3**).

TABLE 3: CHANGES IN HEMATOLOGICAL INDEXES UNDER THE STUDIED CONDITIONS (M±m, n=10)

Parameters/ groups	Control	Hypoxia	Thiotriazoline	Thiotriazoline + hypoxia	Les-589	Les-589+ hypoxia
I_A	2.21±0.14	3.37±0.31*	2.12±0.18	2.84±0.21*	2.95±0.23*	2.57±0.20*
LGI	2.04±0.14	2.56±0.20*	1.64±0.11*	2.65±0.21*	2.67±0.23*	2.33±0.20
GAI	0.51±0.03	0.34±0.01*	0.53±0.04	0.92±0.07*●	0.39±0.02*	0.64±0.04*●
NLCI	0.51±0.02	0.34±0.01*	0.59±0.05*	0.50±0.04●	0.40±0.02*	0.53±0.03●

* – $p < 0,05$ to "control" group, ● – $p < 0,05$ to "hypoxia" group..

The increase of I_A by 29 % (against the control) in the group which were treated with thiotriazoline before the hypoxic exposition, while the absence of significant changes in the case of the drug introduction itself was shown. The increase of I_A (on 33 %) was also detected in the group where Les-589 was used, as well as in animals previously treated with Les-589 (by 16 %). LGI values changed analogically to I_A, the first increased in all groups, but was the most expressed under the influence of hypoxia following the Les-589 application.

The changes of GAI and NLCI were opposite to I_A and LGI. As the result of the research, the relative decrease (comparing to control) of these indexes under Les-589 action and their renovation during combination of Les-589 and hypoxia almost to normal values were discovered. Thiotriazoline effect was characterized by the opposite direction of changes: increase of these indexes under thiotriazoline treatment and their relative decrease under the hypoxia conditions.

Based on the reactions of hematopoiesis, especially leukopoiesis, in complex with the state of immune organs, adrenals, the following types of adaptive reactions are differentiated: orientation, activation (quiet and raised), over-activation, deficient activation, stress [24, 25]. The complex of changes in leucogram, especially lymphocytosis, monocytosis, I_A increase, detected under hypoxia following the thiotriazoline indicated the development of the raised activation type of adaptive reaction, which belongs to the positive prognostic adaptive reactions.

Lymphocytosis, eosinopenia in 24 hours after Les-589 introduction and at the first 24 hours of post-hypoxic period following the compound usage, could indicate the increase of mineralocorticoids and glucocorticoids secretion. Together with the I_A increase it indicated the successful flow of the adaptive reactions at the optimal hormone status, which equally to the thiotriazoline effect, was realized via the reaction of the raised activation.

Worth noting is that based on the same parameters and hematological indexes the adaptive reaction to the hypoxic exposition was realized in form of the deficient type of adaptation. This was confirmed by leucopenia, decrease of the relative levels of monocytes, eosinophiles, significant increase of adaptation index.

Summarizing the presented results we can conclude the positive effect of the Les-589. The protective usage of which modified the adverse type of the adaptive reaction into the prognostically favorable anti-stress reaction. To confirm this finding, the study of the oxidative metabolism was the following step of our research. It is known that modification of oxygen dependent metabolism caused by hypoxia can form the basis for wide range of metabolic disorders accompanied by the formation of desadaptation states [4, 5], which requires timely identification.

Received data showed the activation of free radical peroxidation processes in blood and liver tissue of the experimental animals under hypoxic exposition. This is reflected in the increase of TBARs level together with the decrease of catalases and SOD activity (**fig. 1**).

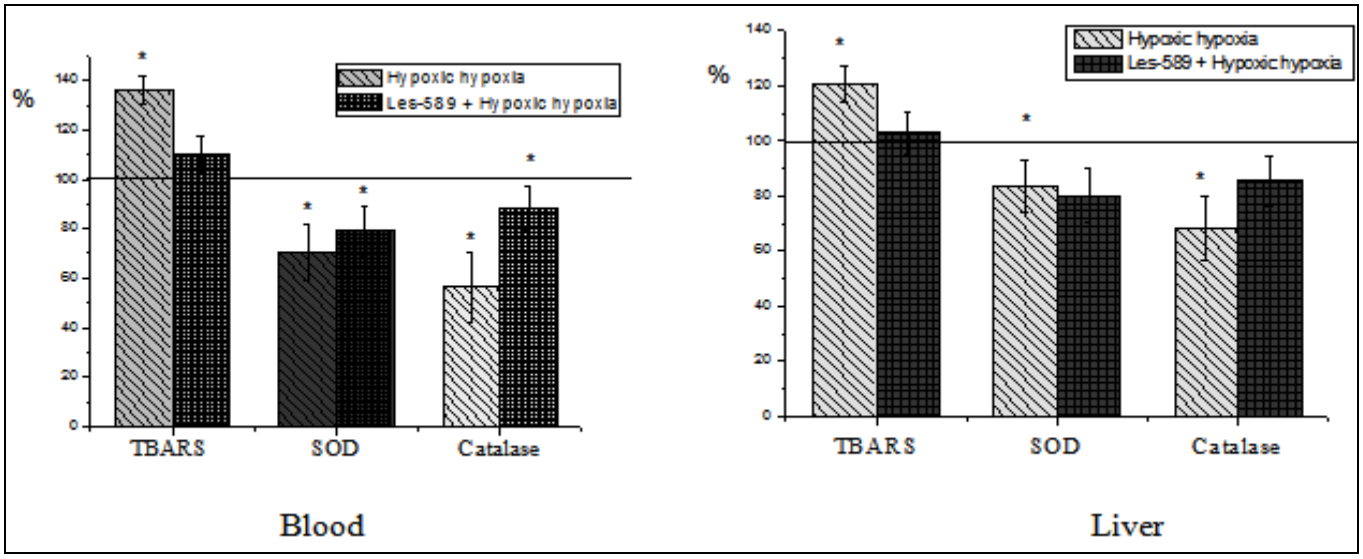

FIGURE 1: CHANGES OF TBARS AND ANTIOXIDANT ENZYMES ACTIVITIES LEVELS IN THE BLOOD AND LIVER OF EXPERIMENTAL ANIMALS

Analysis of the literature and our data certified that the coefficient of ratio of AOD (antioxidant defense) parameters to the lipid peroxidation (LPO) activity (K=AOA/LPO) [5] is the most informative for estimation of pro/antioxidant system.

Our findings showed that the value of such coefficient (K = 100% in the normal condition) under the hypoxia exposition was drastically decreased both in the blood and liver of the experimental rats (**fig. 2**).

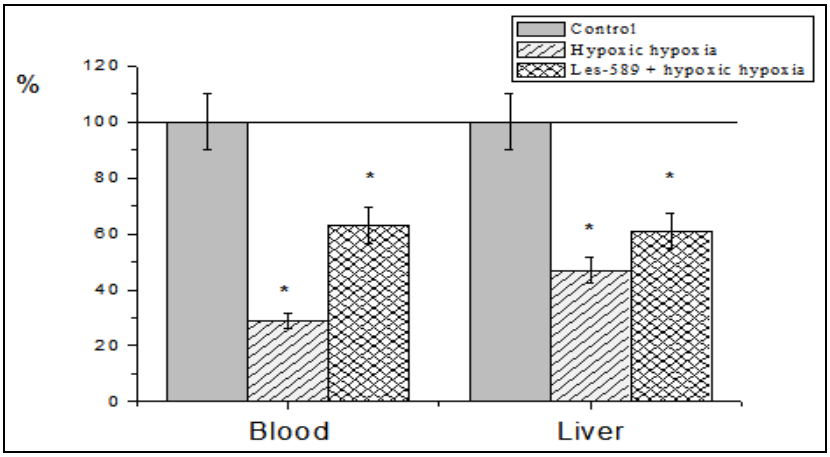

FIGURE 2: CHANGES OF COEFFICIENT (K = AOA/LPO) IN THE BLOOD AND LIVER OF EXPERIMENTAL ANIMALS

The coefficient value decreased by 70% in blood and in the liver tissue by 53 % ($p < 0.05$) correspondingly.

Application of Les-589 in the corresponding group led to the increase of the coefficient values in the blood in 2,1 times ($p < 0.01$), in the liver - in 1.3 times ($p < 0.05$). This was mainly provided by the increase of the catalase activity and suppression of LPO. This significant increase of the coefficient's values could indicate the restoring of the balance in the system of pro- and anti-oxidant processes and favorable type of the adaptive reaction. The hypoxic exposition caused the relative increase of the lactate level and increase in lactate/pyruvate ratio both in blood and liver tissue (**fig. 3**).

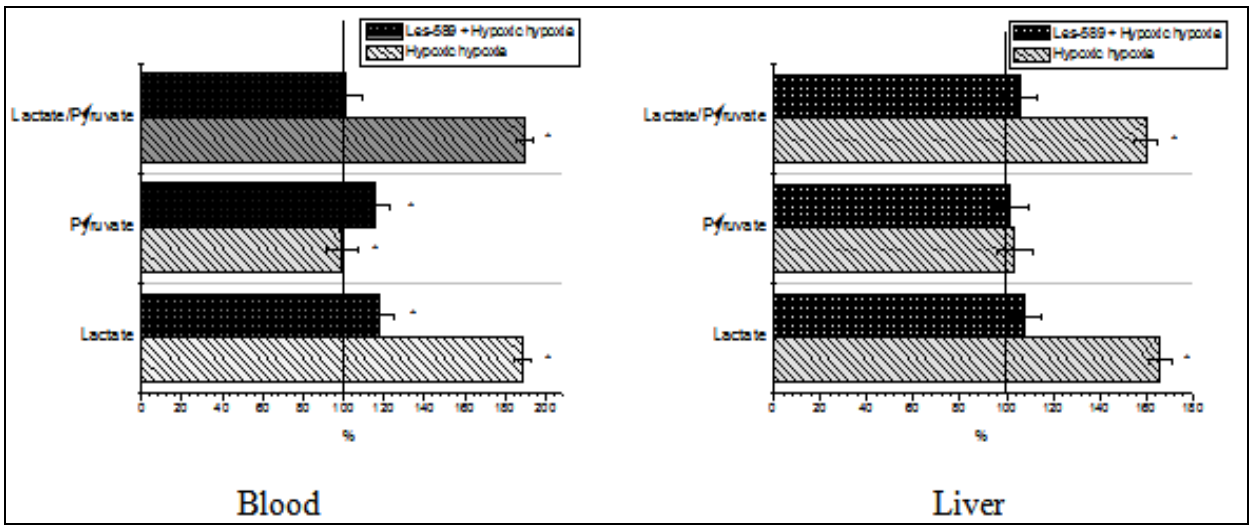

FIGURE 3: CHANGES OF LACTATE AND PYRUVATE LEVELS IN THE BLOOD AND LIVER OF EXPERIMENTAL ANIMALS

The preventative introduction of Les-589 led to decrease of the lactic acid level in blood by 70 % ($p < 0.05$) in comparison to the group without correction. While the pyruvic acid level in the blood increased slightly. The lactate/pyruvate ratio which was used as balance index of aerobic and anaerobic processes, decreased in the blood by 88 % ($p < 0.05$). The same character of changes was observed in the liver tissue under compound treatment.

However, the value of these changes was lower (decrease by 53 %, almost reaching the normal value). This data, in generally, showed the decrease of the post-hypoxic effects expression level and clearly confirmed the anti-hypoxic properties of the Les-589.

Also, the mentioned indexes (K = AOA/LPO and K = lactate/pyruvate) had been considered as the most prominent diagnostic parameters. Presented data demonstrated the necessity of the complex assessment of the states of pro- and anti-oxidant processes, aerobic and anaerobic reactions for defining the efficiency and quality of the adaptive reaction. Moreover, it grounded the validity of the hematological parameters and indexes for the control of adaptive reaction under hypoxic syndrome and its correction.

In general, the anti-hypoxic properties of Les-589 were confirmed and presented data are the strong argument for its further investigation as potential antihypoxic agent with preventative effect.

CONCLUSION: The decrease of the degree of post-hypoxic effects manifestation and the resumption of the pro- and antioxidant system balance under Les-589 treatment were shown. In accordance to the hematological and biochemical parameters analysis the protective usage of Les-589 before hypoxic hypoxia action converts adverse type of adaptive reaction (inferior adaptation type) in prognostically favorable type (increased activation).

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