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Use of a New Infusion Preparation Containing a Complex Compound of a Polysaccharide and a Natural Metabolite of the Krebs Cycle in Experimental Toxic Hypoxia

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Abstract The aim of this work is to evaluate the efficacy of Rheoambrasol on the expression of hypoxia markers (HIF-1α, erythropoietin), the activity of lipid peroxidation and antioxidant protection during single-step and prolonged nitrite intoxication. Experiments were carried out on male Wistar rats (n=185) using sodium nitrite on two models, i.e. on the model of acute nitrite hypoxia (NH). Experimental model of toxic methemoglobinemia. The results showed that single and prolonged use of sodium nitrite leads to methemoglobinemia, increased levels of hypoxic markers HIF-1α and erythropoietin (EPO), as a protective response to oxygen deficiency, activated lipid peroxidation and reduced overall antioxidant status. The new drug "Rheoambrasol" has an antihypoxic effect, which is confirmed by a decrease in values of hypoxia markers: HIF-1α and EPO, both in toxic hypoxia and in methemoglobinemia. Also, the corrective effect of the drug "Rheoambrasol" on the activity of lipid peroxidation and the activity of antioxidant protection enzymes under conditions of single and prolonged nitrite intoxication was established.

Keywords Acute hypoxia, Methemoglobinemia, Polysaccharide complex, Natural metabolite, Hypoxia markers, Antioxidant status

1. Introduction

As is known, among the means of pharmacological correction of pathological conditions accompanying intoxication, the key place in the treatment are drugs of antihypoxic and antioxidant action, capable at the cell level to restore metabolism and its vitality.

The widespread distribution of xenobiotics in the modern world, which includes sodium nitrite, can lead to the development of hypoxia and formation of methemoglobin. Toxic hypoxia is accompanied by acute or chronic oxygen deficiency, impaired metabolism, cell functions, and can long have a significant impact on the formation and development of the underlying pathological process and increase the recovery time. At the same time hypoxia leads to energy deficit, activation of free radical processes in cells and decrease in reserves of mobilization of antioxidant protection [1,5,8].

Considering the above, for pharmacological correction of toxic hypoxia we used a new blood substitute "Rheoambrasol", which has antihypoxic, antioxidant and membranoprotective effects, containing a complex compound of polysaccharide and natural metabolite of Krebs cycle [6].

The aim of the work is to evaluate the effectiveness of “Rheoambrasol” on the severity of hypoxia markers (HIF-1α, erythropoietin), on the activity of lipid peroxidation, on antioxidant protection in experimental single-step and prolonged nitrite intoxication.

2. Main Body

2.1. Material and Methods of Study

The experiments were performed on 185 male rats weighing 190-220 g of the "Wistar" line in two models using sodium nitrite [3]. In the 1st series of experiments (on 100 rats) the model of acute nitrite hypoxia was reproduced by a single injection of 4% sodium nitrite solution under the back skin of rats at a dose of 90 mg/kg.

Series 2 - model of toxic methemoglobinemia was created by prolonged intoxication, daily administration of sodium nitrite for 30 days at a dose of 50 mg/kg.

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All animals in series 1 used in the experiment were divided into the following IV groups: I - intact group consisted of rats on the normal laboratory diet, II - control group - animals with nitrite hypoxia without treatment, 48 hours after administration of the toxicant; III - comparison group - rats with nitrite hypoxia after infusion of "Rheopolyglukin", IV - experimental group - rats with nitrite hypoxia after infusion of "Rheoambrasol".

injecting blood substitutes into the tail vein of rats at a dose of 5 ml/kg body weight for 5 days, 48 hours after sodium nitrite solution under administration in series 1, and 30 days after sodium nitrite solution under administration in series 2.

The content of methemoglobin (metHb) in the blood of experimental animals was investigated in group II after sodium nitrite solution under injection in 1.5 hours and 48 hours in series 1, in series 2 in 1.5 hours and after the last sodium nitrite solution under injection on day 30, and in groups III and IV after treatment, in 1 hour and 5 days after administration of the studied preparations [2].

The content of hypoxia-inducible factor (HIF-1α) was determined in the blood plasma of the experimental animals. The concentration of HIF-1α was determined by enzyme-linked immunosorbent assay (ELISA) using an enzyme immunoassay kit (Cloud-Clone corp., USA) according to the instructions enclosed with the kit.

EPO concentration was determined by enzyme immunoassay using "Erythropoietin ELISA-BEST" kit (Vector-Best, Russia).

Total antioxidant status (AOS) was also determined by enzyme immunoassay using an appropriate kit (Cayman, USA).

Immunoassay results were measured at 450 and 630 nm on a microplate photometer MR96 (Mindray, China). The results were expressed in ng/ml.

The intensity of lipid peroxidation (LPO) in erythrocyte hemolysates was determined by the level of malonic dialdehyde (MDA) according to the method of Titeeva G.R. (1996) [4]. Measurements were performed on a UNICO 2800 spectrophotometer (United products and instruments, Inc., USA).

Statistical processing of the obtained data was performed using "Excel" and "Biostat 4.03" programs. The criterion for statistical significance was $p<0.05$.

2.2. Results of the Study

The results of the study of methemoglobin showed that in the first series of experiments, the concentration of methemoglobin (metHb), under conditions of toxic hypoxia, already in 1.5 hours after the introduction of sodium nitrite increases by 56.6%. Then the concentration of metHb gradually decreases and by 48 hours we observe a partial recovery of the metHb concentration in the blood, but its value at the beginning of treatment is 13.2%. As seen in Figure 1, in the 1st series of experiments 48 hours after the first infusion of Rheoambrasol methemoglobin was restored to the baseline values, which was not observed after the use of Rheopolyglukin.

In the 2nd series of experiments, where sodium nitrite was administered for 30 days, the metHb concentration in the blood of experimental animals already increased in the first hours after the start of the experiment, and remains at this level for 30 days, was approximately 54.9±1.6 ($p<0.01$) of the level of total hemoglobin (Fig. 2). After application of the new blood substitute "Rheoambrasol" - % of methemoglobin in the blood decreased 1.6-fold ($p<0.05$), and by the end of treatment it recovered to the initial values ($p<0.05$).

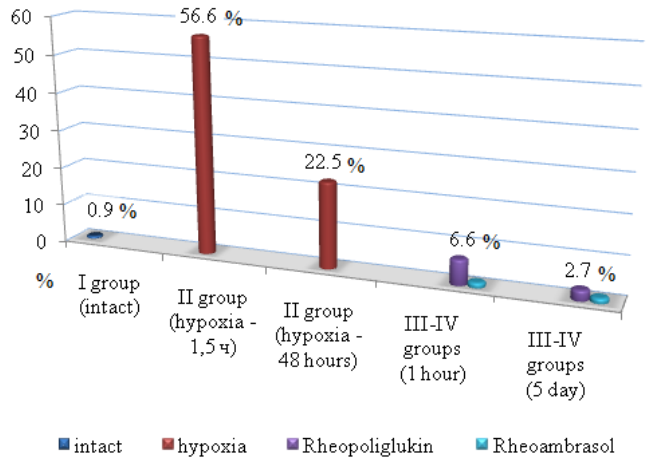

Figure 1. Changes in methemoglobin concentration during hypoxia (series 1: after 1.5 hours and after 48 hours) and after treatment with blood substitutes (on the 1st and 5th days)

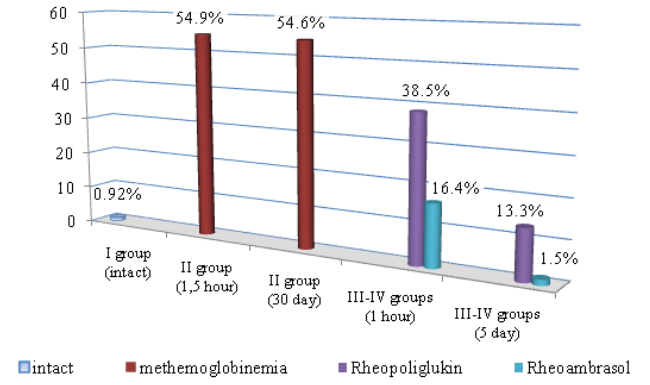

Figure 2. Changes in methemoglobin concentration in the model of toxic methemoglobinemia (series 2: after 1.5 hours and at day 30) and after treatment with blood substitutes (at day 1 and day 5)

The studies show that hypoxia markers (HIF-1α and EPO) reacted distinctly. When studying one of the most objective indicators of hypoxia development - hypoxia-inducible factor (HIF-1α) in blood in series 1, it was shown that it increased 6.4-fold ($p1<0.05$) (Table 1). The study of hematopoiesis-inducing properties of peripheral blood serum revealed a 4.1-fold ($p<0.001$) increase in erythropoietin (EPO) concentration in series 1 of experiments.

In series 2, study of the concentration of hypoxia index (HIF-1α) showed that on day 30, its level increased 3.0-fold ($p<0.001$) compared with the initial state (Table 2). In series 2, on the 30th day, in experimental toxic methemoglobinemia, the concentration of EPO increased 1.9-fold ($p<0.001$).

The use of blood substitutes in series 1 and 2 led to a decrease in hypoxia markers. Thus, in series 1 after using Rheoambrasol, HIF-1α concentration decreased by 3.8 times ($p<0.0001$), which was lower by 65.5% ($p<0.05$), compared with the result obtained after using Rheopolyglukin. Concentration of EPO after application of Rheoambrasol decreased to the initial level ($p<0.05$), which was not observed after application of the drug of comparison group "Rheopolyglukin".

In series 2 after using the new blood substitute “Rheoambrasol”, as shown in Table 2, the concentration of HIF-1α was 2.6 times lower ($p<0.0001$), which was 27.3% ($p<0.05$), compared with the result obtained after using the drug "Rheopolyglukin". After using “Rheoambrasol”, the concentration of EPO also decreased and was 16.0% ($p<0.05$) lower compared to the comparison group, where Rheopolyglukin was used.

In series 1, interpretation of lipid peroxidation indices by the final product of peroxidation MDA showed that during toxic hypoxia its content increased in plasma by 2.0 times ($p<0.05$). After administration of “Rheoambrasol”, MDA concentration was close to the initial values. Thus, MDA content in blood significantly decreased by 1.9 times ($p<0.05$). Hyperlipoperoxidation may be due to imbalance in AOS system. For this purpose, the total antioxidant status in blood plasma was studied. Total antioxidant status in toxic hypoxia decreased 1.7-fold ($p<0.05$) (Table 1). After using “Rheoambrasol”, the AOS values increased and were 19.2% higher compared with Rheopolyglukin.

The use of the drug "Rheoambrasol " leads to washout of toxic metabolites of impaired metabolism. Intravenous infusion of "Rheoambrasol" restores the parameters of lipid peroxidation (LPO) and activates AOS enzymes in the blood.

In the 2nd series of experiments, methemoglobinemia also activated LPO processes and decreased total antioxidant status. As can be seen from Table 2, MDA in plasma at 30 days after injection of sodium nitrite solution – increased 2.2-fold ($p<0.05$), and the total antioxidant status decreased 2.0-fold ($p<0.001$). When comparing the results obtained in series 1 and 2 of experiments, we can see that in series 2, prolonged sodium nitrite intoxication contributed to more pronounced changes in LPO and AOS. After application of the preparation "Rheoambrasol" in the 2nd series of experiments, the plasma level of MDA was restored to initial values and was 41,2% ($p<0,05$) higher in comparison with the comparison group (III group), which was not observed after application of the preparation "Rheopolyglukin". Total antioxidant status also recovered to baseline values after the use of “Rheoambrasol” and was 22.3% higher ($p<0.05$) compared with the result after the use of “Rheopolyglukin”.

Analysis of the results showed that in series 1 and 2 of experiments sodium nitrite causes methemoglobinemia, especially in the first hours after the administration of sodium nitrite in series 1, and in series 2 experiments methemoglobinemia holds at the same level for 30 days. During the development of hypoxia, HIF-1α accumulates in the body, which is a protective reaction in response to oxygen deficiency [7]. This is explained by the fact that HIF-1α protein causes gene expression, provides mobilization of adaptation processes in the cell and increases erythropoiesis.

Table 1. Changes in values of hypoxia, lipid peroxidation, and total antioxidant status during experimental toxic hypoxia and after blood substitute infusion in rats (M±m)

Indicators	Intact	Toxic hypoxia	After 5 day treatment with blood substitutes	
			Rheopolyglukin	Rheoambrasol
	I group	II group	III group	IV group
HIF-1α, ng/ml	0.07±0.008	0.38±0.034	0.29±0.016	0.10±0.005
EPO, mIU/ ml	8.4±0.13	34.4±0.2	12.1±0.1	8.5±0.1
MDA in plasma, nmol/ml	3.2±0.17	7.1±0.34	5.3±0.21	3.4±0.15
TAS, U	1.41±0.12	0.82±0.09	1.14±0.11	1.36±0.12
Note: * - reliability ($p<0.05$) in comparison with group I; ^ - same ($p<0.05$) in comparison with group II; # - same ($p<0.05$) in comparison with group III.				

Table 2. Changes in hypoxia factor HIF-1α, lipid peroxidation index (LPO), and total antioxidant status in methemoglobinemia after blood substitute infusion (M±m)

Indicators	Intact	Toxic hypoxia	After 5 day treatment with blood substitutes	
			Rheopolyglukin	Rheoambrasol
	I group	II group	III group	IV group
HIF-1α, ng/ml	0.07±0.008	0.21±0.010	0.11±0.006	0.08±0.12
EPO, mIU/ ml	8.4±0.13	16.3±0.19	10.6±0.12	8.9±0.10
MDA in plasma, nmol/ml	3.1±0.06	6.7±0.1	5.1±0.06	3.0±0.1
TAS, U	1.39±0.11	0.70±0.07	1.12±0.09	1.37±0.12
Note: * - reliability ($p<0.05$) in comparison with group I; ^ - same ($p<0.05$) in comparison with group II; # - same ($p<0.05$) in comparison with group III.				

According to the experimental data, the value of these indices was higher in series 1 with toxic hypoxia. In series 1 and 2 of the experiment, the LPO processes are activated and the activity of AOS enzymes decreases, the indices of which were more pronounced in series 2 with methemoglobinemia.

Pharmacological correction with the new drug "Rheoambrasol" was unequivocally higher both in the 1st and in the 2nd series of experiments compared to the effect of administration of the drug of comparison group - "Rheopolyglukin". The effectiveness of the drug "Rheoambrasol" is associated with the presence in its composition polysaccharide complex with natural metabolite that contribute to the removal of toxic substances from the body and prevent the formation of free radicals, restore damaged cells in hypoxia. "Rheoambrasol" has high rheological activity, restores microcirculation, improving blood supply to tissues, eliminating hypoxia and stabilizing cell metabolism.

3. Conclusions

1. With single and prolonged use of sodium nitrite methemoglobinemia develops, indices of hypoxic markers HIF-1α and EPO increase as a protective reaction to oxygen deficiency, lipid peroxidation processes are activated and general antioxidant status decreases.
2. The antihypoxic effect of “Rheoambrasol” is confirmed by a decrease in the indices of hypoxia markers HIF-1α and EPO in both toxic hypoxia and methemoglobinemia.
3. Corrective effect of “Rheoambrasol” on the activity of lipid peroxidation and the activity of antioxidant protection enzymes under conditions of single and prolonged nitrite intoxication was established.

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