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Correction of the degree of endogenic intoxication and lipoperoxidation processes by the infusion drug (rheoambrasol) with experimental hypoxia, induced by sodium nitrite

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Abstract---Rationale (relevance): Almost all known diseases, as a rule, are accompanied by a hypoxic state associated primarily with oxygen deficiency and impaired energy metabolism, which cause damage to the structure and functioning of organs and cells. Using a complex of a polysaccharide with a bioenergetic substrate, a blood-substituting infusion drug "Rheoambrasol" has been developed, which is able to protect cells from free radical oxidation and restore disturbed energy metabolism in cells during hypoxia. Purpose: to evaluate the effectiveness of correction of the hypoxia-inducible factor - HIF-1 α , endogenous intoxication, the intensity of lipid peroxidation processes in the liver during experimental nitrite hypoxia with the new blood-substituting infusion drug "Rheoambrasol". Material and methods. The setting of the experimental hypoxia model was carried out in outbred male rats weighing 185 ± 10.2 g by administering sodium nitrite at a dose of 90 mg / kg. In the blood plasma of experimental animals, the content of hypoxia-inducible factor (HIF-1 α), indicators of endogenous intoxication, the intensity of lipid peroxidation (LPO) and the activity of antioxidant enzymes systems (AOS) were determined in the liver. Results. The effect of the drug "Rheoambrasol" was clearly manifested and showed that the new blood substitute infusion drug reduces the content of the hypoxia-inducible factor (HIF-1 α), restores

the indices of endogenous intoxication, and the balance of the system of LPO / AOS during nitrite hypoxia more effectively than the drug "Rheopolyglukin".

Keywords---"Rheoambrasol", nitrite hypoxia, hypoxia-inducible factor (HIF-1 α), endogenous intoxication, lipid peroxidation (LPO), antioxidant system (AOS).

Introduction

Almost all known diseases, as a rule, are accompanied by a hypoxic state associated primarily with oxygen deficiency and impaired energy metabolism, which cause damage to the structure and functioning of organs and cells. It is known that the vicious circle that occurs during hypoxia, associated with a lack of oxygen and energy deficiency, stimulates free radical oxidation, which, in turn, damaging the membranes of mitochondria and lysosomes, aggravates the energy deficit. All this contributes to the penetration into the vascular bed of under-oxidized toxic products that can accumulate in cells [5,12]. The fundamental factor in the treatment of hypoxic conditions is the development and introduction into medical practice of means capable of correcting disorders of energy metabolism and their consequences and thereby increasing the resistance of cells, organs and the body as a whole to a lack of oxygen and other influences that disrupt energy production [1,5,7]. Using a complex of a polysaccharide and a bioenergetic substrate, a blood-substituting infusion drug «Rheoambrasol» has been developed, which is capable of protecting cells from free radical oxidation and restoring disturbed energy metabolism in cells during hypoxia [14].

Purpose

To evaluate the effectiveness of correction of the hypoxia-inducible factor – HIF-1 α , endogenous intoxication, the intensity of lipid peroxidation processes in the liver during experimental nitrite hypoxia with the new blood-substituting infusion drug «Rheoambrasol».

Material and Methods

The model of nitrite hypoxia (nitrite intoxication) was performed in 110 male outbred rats weighing 185 ± 10.1 g. The model of acute nitrite intoxication was reproduced by a single injection of 4% sodium nitrite solution in a dose of 90 mg/kg under the back skin of the rats [6]. All animals used in the experiment were divided into the following 4 groups: I - the intact group consisted of rats on the normal laboratory diet (n=10). Animals in which nitrite intoxication was induced were divided 48 hours after administration of the toxicant as follows: II – control group – animals with nitrite intoxication without treatment (n=10 rats); III - comparison group - rats with nitrite intoxication after «Rheopolyglukin» infusion (n=15); IV - experimental group - rats with nitrite intoxication after «Rheoambrasol» infusion (n=19). Thus, the efficacy of blood substituting infusion drugs in nitrite intoxication was studied in 54 rats.

Infusion therapy in groups III and IV was carried out by injecting blood substituting infusion drugs into the tail vein of rats at a dose of 5 ml/kg body weight for 5 days, 48 hours after sodium nitrite injection. The animals were slaughtered at experimental nitrite intoxication and 1 hour after infusion of blood substituting infusion drugs. The content of hypoxia-inducible factor (HIF-1 α) was determined in the blood plasma of the experimental animals [11]. The concentration of HIF-1 α was determined by enzyme-linked immunosorbent assay (ELISA) using a Cloud-Clone corp. (USA) kit, according to the instructions. Measurements were taken at a wavelength of 450 nm on a "MR96" microplate photometer (Mindray, China). The results of HIF-1 α obtained were measured in ng/ml.

During the experiment there were studied indices of endogenous intoxication: the level of medium-weight molecules (MWM) in plasma and erythrocytes, concentration of oligopeptides (OP) in plasma and erythrocytes (OPpl and OPer), sorption capacity of erythrocytes (SCE), calculated toxemia index (TI) in plasma and erythrocytes, and intoxication index (II) by standard formulas [9].

$$TI = MWM \times OP$$

where: MWM - medium-weight molecules; OP - oligopeptides in plasma and erythrocytes; II - intoxication index

$$II = TI_{pl} + TI_{er}$$

where: TI_{pl} - plasma toxemia index; - erythrocyte toxemia index; II - intoxication index.

To assess the intensity of LPO and AOS processes during nitrite intoxication and after infusion of blood substituting infusion drugs, liver was taken, chilled in a freezer, with metal forceps and then homogenized. The content of MDA, diene ketones, diene conjugates, glutathione reductase (GR), glutathione peroxidase (GPO), superoxide dismutase (SOD) and catalase activity was determined in the liver homogenate [10]. The content of lipid peroxidation products (MDA, diene ketones, diene conjugates) was determined according to the method of Titeeva G.R. et al. (1996) [16].

The activity of GPO and GR was determined spectrophotometrically at 340 nm. The activity of AOS enzymes was expressed in units (units) per gram of raw liver weight, and as specific activity [7]. Superoxide dismutase (SOD) activity was expressed in mmol/min/mg protein [3,4]. Purified SOD preparation (ICN Biomedicals, USA) was used as a standard [15]. The catalase activity of the samples under study was determined spectrophotometrically and expressed in mmol/min/g protein [10]. The measurements were performed on a UNICO2800 spectrophotometer (United products and instruments, Inc., USA). All measurements were performed on a spectrophotometer "UNICO2800" (United Products and Instruments, Inc., USA). Statistical processing of the obtained data was performed using "Excel" and "Biostat 4.03" programs. The criterion of statistical significance was $p < 0.05$.

Results and Discussion

As studies have shown, after sodium nitrite administration, animal death was observed within 48 hours and was 50.0% ($p<0.05$). Nitrite hypoxia led to a 6.4-fold increase in the concentration of hypoxia-inducible factor (HIF-1 α) in the blood ($p<0.05$) (Fig. 1).

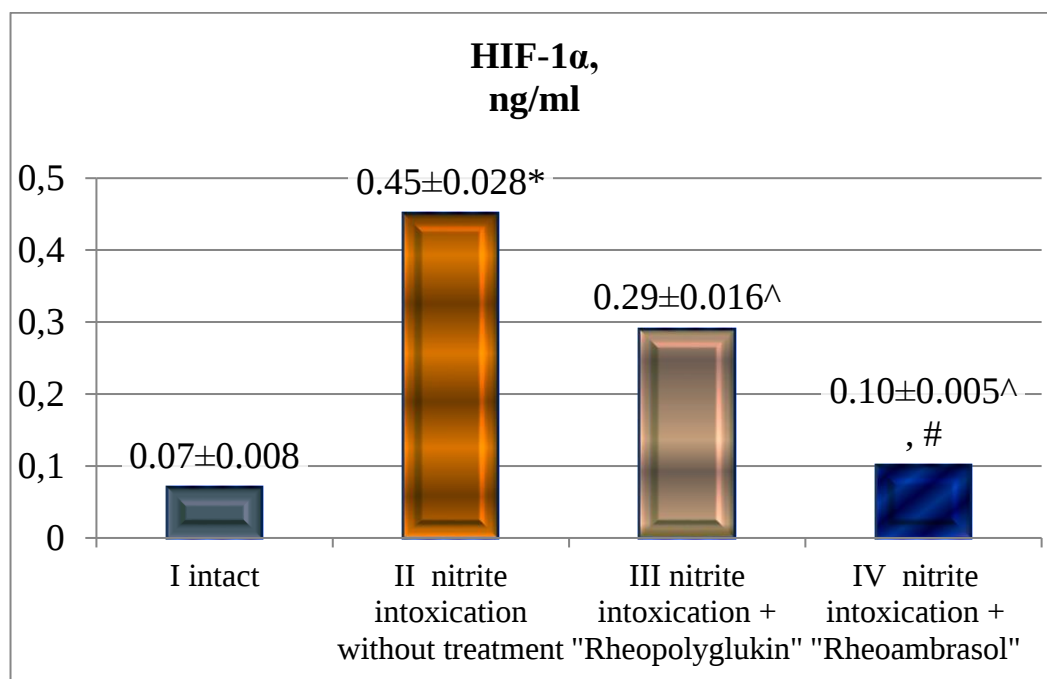


Figure 1: Changes in serum HIF-1 α content during nitrite hypoxia and after infusion of blood substituting infusion drugs in rats ($M \pm m$)

Under hypoxia there was an increase in endogenous intoxication: SCE increased 2.9 times, MWM in plasma and erythrocytes – 2.8 and 2.6 times ($p<0.05$), concentration of oligopeptides in plasma and erythrocytes – 2.3 and 2.5 times ($p<0.05$) (Table 1).

The toxemia index in plasma and erythrocytes in group II was higher than in group I by 6.2 and 6.4 times ($p<0.05$), respectively, as well as the intoxication index, which in group II was 6.3 times higher ($p<0.05$), relative to the indicators of intact animals, indicating the development of endogenous intoxication during nitrite intoxication. Interesting results were obtained when studying lipid peroxidation in the liver (concentration of MDA, diene conjugates and diene ketones) showed an increase in both intermediate and final LPO products (Table 2). In the study of LPO parameters during nitrite intoxication in the liver, the content of MDA increased 1.4-fold ($p<0.05$), diene ketones – 1.3-fold ($p<0.05$), diene conjugates – 1.2-fold ($p<0.05$). Hypoxia resulted in inhibition of the enzymatic system of antioxidant protection in the liver, whose activity decreased in the liver after 48 hours: catalase – 1.3 times ($p<0.05$), GR – 1.2 times ($p<0.05$), GPO – 1.1 times ($p<0.05$).

Table 1

State of endogenous intoxication parameters in the studied groups during nitrite hypoxia in rats and after infusion of blood-substituting infusion drugs (M±m)

Indicators	Intact, n=10	Nitrite hypoxia, n=10	Nitrite hypoxia + infusion of a blood-substituting infusion drug	
			Rheopolyglyukin, n=15	Rheoambrasol, n=19
	Group I	Group II	Group III	Group IV
In plasma				
MWM, conv. U.	9.9±0.5	27.7±1.5*	19.1±0.9^	12.5±0.6
Oligopeptides (OPpl), g/l	1.1±0.06	2.5±0.14*	1.6±0.07^	1.3±0.07
Toxemia index	11.5±1.3	71.1±7.5*	31.4±2.8*^	17.0±1.7
Intoxication index	23.4±2.5	147.6±13.4*	58.5±5.0*^	32.2±2.4
In erythrocytes				
MWM, conventional units	14.2±0.7	37.4±1.6*	23.5±1.2*^	16.0±0.6
Oligopeptides (OPer), g/l	0.8±0.04	2.0±0.09*	1.1±0.05^	0.9±0.03
Toxemia index	11.9±1.2	76.5±6.1*	27.1±2.3*^	15.3±1.2
K (MWMpl/MWMer)	0.7±0.005	0.7±0.03	0.8±0.01*^	0.8±0.04
SCE, %	20.2±1.0	58.6±2.5*	25.5±1.8*^	21.6±1.2

Thus, administration of sodium nitrite causes hypoxia, increased indices of endogenous intoxication, activation of LPO processes and depletion of antioxidant protection factors in the liver. Administration of the reference drug «Rheopolyglukin» in Group III resulted in 1.6-fold decrease of HIF-1 α concentration ($p<0.05$).

After using «Rheopolyglukin» we could observe a decrease of endogenous intoxication indices: SCE was 2.3 times lower ($p<0.05$), SM content in plasma and erythrocytes was 1.5 and 1.6 times lower ($p<0.05$), and oligopeptides - 1,6 and 1,8 times lower ($p<0.05$) respectively, than in group II. Plasma and erythrocyte toxemia index decreased - almost 2.3-fold and 2.8-fold ($p<0.05$), respectively, intoxication index - also 2.5-fold ($p<0.05$).

Table 2.

Changes in indicators of lipid peroxidation, the antioxidant system of the liver during nitrite hypoxia and after infusion of blood-substituting infusion drugs (M ± m)

Indicators	Intact, n=10	Nitrite hypoxia, n=10	Nitrite hypoxia + infusion of a blood-substituting infusion drugdrug	
			Rheopolyglyukin, n=15	Rheoambrasol, n=19
	Group I	Group II	Group III	Group IV
LPO				

Indicators	Intact, n=10	Nitrite hypoxia, n=10	Nitrite hypoxia + infusion of a blood-substituting infusion drugdrug	
			Rheopolyglyukin, n=15	Rheoambrasol, n=19
	Group I	Group II	Group III	Group IV
MDA, mmol/100g	3.4±0.14	4.7±0.17*	4.0±0.14*^	3.5±0.19^#
Dket, nmol/mg	0.89±0.04	1.16±0.05*	1.10±0.07	0.84 ± 0.07^#
Dcon, nmol/mg	0.90 ±0.05	1.1±0.05*	1.0±0.09*^	0.90±0.06^#
AOS				
Catalase, 104 units/h*kg	1095.4±42.9	912.5±20.4*	935.2±26.6*	1058.5±30.2^#
SOD, 103 units/h*kg	178.4±5.5	148.6±4.6*	161.4 ± 5.1*	181.2±4.9^#
GR, nmol/mg	27.4±1.8	21.0±0.9*	23.1 ± 0.9*	28.2±0.4^#
GPO, mmol/kg	6.58±0.28	5.9±0.13*	6.12±0.10*	6.49±0.11^#
LPO/AOS	0.004±0.0003	0.007±0.0003	0.005±0.0004	0.004±0.0003

After «Rheopolyglukin» application LPO parameters in the liver decreased: MDA content - 1.2 times ($p < 0.05$), diene conjugates - 1.1 times ($p < 0.05$) and there was a tendency for insignificant decrease of diene ketones. Activity of such enzymes of antioxidant protection in the liver as catalase, GR – 1.1 times and statistically insignificant increase of GPO and SOD.

After «Rheoambrasol» administration in group IV, HIF-1 α concentration was 4.5 times lower ($p < 0.001$), which was 65.5% lower ($p < 0.05$) in comparison with the result obtained after «Rheopolyglukin» administration in group III (Table 2). The use of blood-substituting infusion drug «Rheoambrasol» leads to a decrease in the content of toxic metabolites of impaired metabolism, as evidenced by the values of endogenous intoxication. Thus, SCE after «Rheoambrasol» administration was 2.7 times lower ($p < 0.05$) than in group II, which was 15.3% lower ($p < 0.05$) than after «Rheopolyglukin» administration in group III. After administration of «Rheoambrasol», the content of medium molecules in plasma and erythrocytes in group IV was 2.2 times ($p < 0.05$) and 2.3 times ($p < 0.05$) lower than in group II, respectively, and oligopeptides in plasma and blood were 1.9 and 2.2 times lower ($p < 0.05$) respectively.

In a comparative analysis after treatment, SM values in plasma and erythrocytes were 34.6% and 31.9% ($p < 0.05$) lower after treatment with «Rheoambrasol», respectively, and oligopeptides were 18.8% and 18.2% ($p < 0.05$) lower, compared with «Rheopolyglukin». The plasma and erythrocyte toxemia index in group IV was 4.2 and 5.0 times ($p < 0.05$) lower than in nitrite intoxication in group II, which was 45.9% and 43.5% lower, respectively ($p < 0.05$), than after infusion of «Rheopolyglukin». Intravenous infusion of «Rheoambrasol» restores the indices of lipid peroxidation (LPO) and activates the enzymes of the AOS system in the liver. In the liver, after the application of «Rheoambrasol», the MDA content decreased, recovering to the initial values, which was not observed after the infusion of «Rheopolyglukin». After the application of «Rheoambrasol», MDA was 50.0% ($p < 0.05$) lower, diene ketones - by 23.6% ($p < 0.05$), diene conjugates - 10.0%

($p < 0.05$), compared with «Rheopolyglukin». The activity of AOS enzymes was also restored to the initial level and was higher after the infusion of «Rheoambrasol»: catalase activity was higher by 13.1%, GR - by 22.1%, GP - by 6.0% and SOD - by 19.7% ($p < 0.05$). The use of a blood-substituting infusion drug «Rheoambrasol» containing a polysaccharide with a bioenergetic substrate during intoxication with sodium nitrite led to a decrease in mortality to 5.0% ($p < 0.05$), which after the use of «Rheopolyglukin» reached 25.0% ($p < 0.05$).

Discussion

Thus, the infusion of «Rheoambrasol» had an antihypoxic effect, as evidenced by a decrease in the level of hypoxia-inducible factor in the blood - by 4.5 times ($p < 0.001$), by a detoxification effect - by a decrease in endogenous intoxication indicators: so the SCE indicator decreased by 2.7 times ($p < 0.05$). The use of «Rheoambrasol» during hypoxia reduces the intensity of LPO processes, and restores the activity of AOS enzymes in the liver. The effect of the blood-substituting infusion drugs «Rheoambrasol» was clearly manifested, it was more effective in comparison with «Rheopolyglukin».

This is due to the fact that the anti-hypoxic action of «Rheoambrasol» is based on the bioenergetic mechanisms of the metabolite succinate, which is oxidized through the succinate oxidase pathway of the respiratory chain in the mitochondria. This ensures that during hypoxia, when the NADH-dependent oxidation pathway is restricted, the activity and capacity for oxidative phosphorylation in the second and third conjugation sites is provided, which contributes to the maintenance of higher macroergic levels. Thus, the mechanism of the antihypoxic action of «Rheoambrasol» is associated with its specific effect on energy metabolism with an increase in the degree of cell energetization [13].

Succinate is the most potent of all oxidation substrates, capable of reducing oxidized NAD^+ of the initial site of the respiratory chain through electron transfer reverse reactions. A polysaccharide of plant origin facilitates succinate penetration into the cell and its subsequent oxidation in the respiratory chain, which enhances the antihypoxic properties of «Rheoambrasol». Biologically active composition including polysaccharide and bioenergetic substrate has good antihypoxic, antioxidant, detoxifying, membranoprotective effect that allows to recommend it at various hypoxic toxic conditions.

Conclusions

1. New blood-substituting infusion drug «Rheoambrasol» has an antihypoxant effect, reducing the level of hypoxia-inducible factor HIF-1 α by 4.5 times ($p < 0.05$).
2. «Rheoambrasol» has a detoxifying effect, as evidenced by the reduction of endogenous intoxication: so the SCE index decreased by 2.7 times.
3. «Rheoambrasol» reduces the intensity of LPO processes and restores the activity of AOS enzymes in the liver.

Conflict of Interest

J.D. Khujahmedov confirmed the absence of a conflict of interest to be disclosed
 L.I. Shevchenko confirmed that there is no conflict of interest to be disclosed
 Kh.Ya. Karimov confirmed the absence of a conflict of interest to be disclosed

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