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Regulation by the Drug "Rheoambrasol" of the Processes of Lipid Peroxidation and the Activity of the Antioxidant System in Erythrocytes and in the Liver During Hemic Hypoxia

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Abstract Purpose of the study. To study the effect of a new infusion drug "Rheoambrasol" on lipid peroxidation and antioxidant system in erythrocytes and liver in acute hemic hypoxia. Materials and Methods. In the experiment on the model of hemic hypoxia induced by sodium nitrite in 100 male mongrel rats, the effect of "Rheoambrasol" on the indices of lipid peroxidation and AOS in erythrocytes and liver, and the general antioxidant status was studied. The results of the study showed that pharmacological therapy with "Rheoambrasol" suppresses the intensity of LPO, activates the activity of AOS enzymes in erythrocytes and liver, and increases the total antioxidant status.

Keywords Sodium nitrite, Lipid peroxidation (LPO), Antioxidant system (AOS), Erythrocytes, Liver, Blood substitute

1. Introduction

Recently, the danger to human health has been increasing due to the spread of chemical pollutants, such as oxygen-containing nitrogen compounds, which disrupt the oxygen transport function of blood and lead to the development of hypoxia. Regardless of the form of hypoxia, it is based on the disruption of the energy homeostasis of the cell, which leads to metabolic and structural changes, [5], activation of free-radical oxidation [1,2,8,10], damage to biological membranes of lipid bilayer and membrane proteins of the cell [2,4,6]. Nowadays, antioxidants capable of restoring the homeostasis of cellular and subcellular membranes and aimed at the correction of metabolic disorders are used to stop these changes in cells that occur under hypoxia [7,8,9]. The developed drug "Rheoambrasol" contains a biologically active complex of polysaccharide and bioenergetic substrate capable of correcting the work of oxygen transport systems to tissues and metabolic disorders in hypoxia.

Aim of the study

To study the effect of a new infusion preparation "Rheoambrasol" on lipid peroxidation and antioxidant system in erythrocytes and liver in acute hemic hypoxia.

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2. Materials and Methods

An experimental model of acute hemic hypoxia was set up in 100 male rats. The model was reproduced by a single injection of a 4% solution of sodium nitrite at a dose of 90 mg/kg under the skin of the back of rats. The choice of doses was determined by the preliminary titration of sodium nitrite, as well as data published in the works of the authors [5] (Igbaev R.K. 2006).

All animals used in the experiment were divided into the following 4 equal groups:

I – the intact group consisted of rats on normal laboratory diet (n=10).

Animals, in which hemic hypoxia was induced, 48 hours after toxicant administration were divided as follows:

II – control group - animals with hemic hypoxia without treatment (n=10 rats);

III – comparison group - rats with hemic hyproxia after treatment with "Rheopolyglukin" (n=15 rats);

IV – experimental group - rats with hemic hypoxia after treatment with "Rheoambrasol" (n=19).

Infusion therapy in groups III and IV was carried out by infusion of infusion drugs ("Rheoambrasol" and

"Rheopolyglukin") into the tail vein of rats at a dose of 5 ml/kg body weight for 5 days.

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

Immunoassay results were measured at wavelengths of 450 and 630 nm on a "MR96" microplate reader (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) was determined according to the method of Titeeva G.R. (1996) [11]. To assess the intensity of LPO and AOS processes in the liver during NI and after infusion of blood substitutes, the liver was taken, cooled in the 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 were determined in the liver homogenate. The content of lipid peroxidation products (MDA, diene ketones, diene conjugates) was determined according to the method of Stalnaya I.D. (1977) and Stalnaya I.D. and Garishvili T.G. (1977) using the TBK-AGAT kit ("Agat-Med", Russia). The activity of GPO and GR was determined spectrophotometrically at 340 nm. The activity of AOS enzymes was expressed in units (U) per gram of raw liver weight and in the form of specific activity. Superoxide dismutase (SOD) activity was expressed in mmol/min/mg protein [3]. The purified drug of SOD ("ICN Biomedicals", USA) was used as a standard. Catalase activity of the studied samples was determined spectrophotometrically and expressed in mmol/min/g protein [8]. The measurements were performed on a "UNICO 2800" spectrophotometer (USA).

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

3. Results of the Study

In I group studied of lipid peroxidation indices in erythrocytes allowed to reveal hyperlipoperoxidation in cell membranes under hemic hypoxia: MDA level increased 2.0 times ($p<0.05$), diene ketones - 1.9 times ($p<0.05$), diene conjugates - 2.1 times ($p<0.05$). Expressed suppression of AOS system enzymes activity in erythrocytes, which changed as follows: catalase activity decreased 1.6 times ($p<0.05$), GR decreased 1.4 times ($p<0.05$), GPO – 1.5 times ($p<0.05$), SOD – 2.4 times ($p<0.05$). The total antioxidant status in hemic hypoxia was decreased 1.7 times ($p<0.05$).

Table 1. Dynamics of changes in the state of LPO and AOS in erythrocytes under hemic hypoxia and after treatment with infusion drugs in rats (M±m)

Indicators	Intact,	Hemic hypoxia	After 5 days of treatment with infusion drugs	
			Rheopolyglukin	Rheoambrasol
	I group (n=10)	II group (n=10)	III group (n=15)	IV group (n=19)
Malon-dialdehyde, nM /mgHb	0,51±0,03	1,13±0,07*	0,84±0,05*^	0,54±0,05^
Diene ketones, U	0,17±0,016	0,33±0,04*	0,28±0,03	0,20±0,02
Diene conjugates, U	1,2±0,06	2,4±0,16*	2,0±0,10^	1,4±0,2^
Catalase, m/mgHb/min	45,7±2,3	28,4±1,5*	34,8±2,2,	44,4±3,5^
SOD, U/ mg Hb	2,6±0,14	1,1±0,08	1,8±0,09	2,4±0,13
GR, мкМ NADPH2/min x Hb	2,3±0,6	1,6±0,08	1,9±0,10,	2,2±0,09
GPO, U/mg Hb	0,3±0,02	0,20±0,04	0,24±0,01	0,29±0,02
AOS, U	1,41±0,12	0,82±0,09	1,14±0,11	1,36±0,12

Note: * - reliability ($p<0.05$) when comparing with the initial state; ^ - the same ($p<0.05$) when comparing with the indicators of the group with hemic hypoxia; # - the same ($p<0.05$) when comparing the results with the group after treatment with blood substitute "Rheopolyglukin".

Table 2. Dynamics of changes in the state of LPO and AOS in the liver during hemic hypoxia in rats and after treatment with infusion drugs (M±m)

Indicators	Intact,	Hemic hypoxia	After 5 days of treatment with infusion drugs	
			Rheo-polyglukin	Rheo-ambrasol
	I group (n=10)	II group (n=10)	III group (n=15)	IV group (n=19)
Malon- dialdehyde, µmol/100g	3,4±0,14	4,7±0,17*	4,0±0,14*^	3,5±0,19^#
Diene ketones, nmol/mg	0,89±0,04	1,16±0,05*	1,10±0,07	0,84±0,07^#
Diene conjugates, nmol/mg	0,90±0,05	1,1±0,05*	1,0±0,09*^	0,90±0,06^#
Catalase, 10^4 U/h•kg	1095,4±42,9	912,5±20,4*	935,2±26,6*	1058,5±30,2^#
SOD, 10^3 U/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^#

Note: * - reliability ($p<0.05$) when comparing with the initial state; ^ - the same ($p<0.05$) when comparing with the indicators of the group with hemic hypoxia; # - the same ($p<0.05$) when comparing the results with the group after treatment with blood substitute "Rheopolyglukin".

In IV group "Rheoambrasol" pharmacotherapy in hemic hypoxia fully restores the shifts of prooxidant-antioxidant equilibrium in erythrocytes. LPO indicators were lower than after "Rheopolyglukin" infusion, so MDA by 35.8% ($p<0.05$), diene ketones – by 28.6% ($p<0.05$) and diene conjugates - by 30.0% ($p<0.05$), respectively.

There was an activation of AOS enzymes in erythrocytes under hemic hypoxia, the values of which were higher compared to the effect of "Rheopolyglukin" administration: catalase activity was higher by 27.1% ($p<0.05$), SOD – by 33.3% ($p<0.05$), GR - by 15.8% ($p<0.05$) and GPO – by 20.8% ($p<0.05$) (Table 1).

In the liver, the shifts of prooxidant-antioxidant equilibria under hemic hypoxia (group II) were less pronounced than in erythrocytes. It was found that MDA content increased 1.4-fold ($p<0.05$), diene ketones – 1.3-fold ($p<0.05$), diene conjugates – 1.2-fold ($p<0.05$) (Table 2). The activity of catalase decreased – 1.3-fold ($p<0.05$), GR – 1.2-fold ($p<0.05$), GPO – 1.1-fold ($p<0.05$), SOD – 1.2-fold ($p<0.05$).

Experimental therapy with "Rheoambrasol" in hemic hypoxia (group IV) restores lipid peroxidation (LPO) indices and activates enzymes of the AOS system in the liver. After "Rheoambrasol" application, MDA was lower by 50.0% (0.05%), diene ketones by 23.6% (0.05%), and diene conjugates by 10.0% ($p<0.05$), compared to "Rheopolyglukin" (group III). AOS enzyme activities were also restored to baseline and were higher after «Rheoambrasol» infusion: catalase activity was higher by 13.1%, GR by 22.1%, GPO by 6.0% and SOD by 19.7%. The total antioxidant status also after "Rheoambrasol" infusion in hemic hypoxia was restored to the baseline level, compared to the result after "Rheopolyglukin" infusion and was 22.3% higher ($p<0.05$).

4. Discussion

In this way, the main expression of the pro-oxidant -antioxidant balance in blood hypoxia is manifested in erythrocytes. Pharmacological therapy with the medicinal drug "Rheoambrasol" regulates the process of free-radical oxidation of lipids and increases the activity of enzymes AOS, calcium and erythrocytes, and restores general antioxidant status and blood hypoxia.

This can be explained by the biologically active composition of the drug, including a complex of polysaccharide and bioenergetic substrate, which has the ability to support energy production processes under hypoxia. "Rheoambrasol" has a good antioxidant effect and regulates redox processes in hemic hypoxia.

5. Conclusions

1. «Rheoambrasol» pharmacotherapy suppresses the intensity of LPO in erythrocytes and liver during hemic hypoxia.
2. Infusion of the drug «Rheoambrasol» markedly increases the activity of enzymes of the AOS system in erythrocytes and liver and increases the total antioxidant status in blood under hemic hypoxia.

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