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Remaxol in the Correction of Lipid Peroxidation Processes of Biomembranes Induced by the Cold Exposure

ABSTRACT

In experimental conditions the possibility to correct free radical lipid oxidation of rats' organism membranes was studied with the introduction of the succinate containing drug called remaxol. The animals were divided into 4 groups and each of them had 30 rats: intact animals which were held in standard conditions of vivarium; the control group in which rats were exposed to cold during three hours daily; the experimental group in which before cooling animals had a daily intake of the remaxol in a dose of 50 mg/kg; the experimental group in which before cooling animals had a daily intake of the remaxol in a dose of 100 mg/kg. It was found out that in the blood of experimental animals a daily cold exposure during three hours contributes to the increase of lipid hydroperoxides level (by 25 – 41%), of diene conjugate (by 38 – 54%), and of malonic dialdehyde (by 27 – 74%) against the decrease of antioxidant system activity in the blood of intact animals.

The introduction of the succinate containing drug to rats in the conditions of cold exposure contributes to the reliable decrease in the blood of lipid hydroperoxides by 8-25%, of diene conjugates – by 10-29%, malonic dialdehyde – by14-38% in comparison with the rats of the control group. While analyzing the effect of the remaxol on the activity of the components of antioxidant system it was shown that the level of ceruloplasmin in the blood of animals was reliably higher by 10-50%, of vitamin E by 8-31%, of catalase by 7-28% in comparison with the same parameters of the rats of the control group.

During the comparative pharmacological analysis it was established that remaxol produced a direct antioxidant effect according to a dose during cold exposure on the organism of the experimental animals.

Keywords: remaxol, cold exposure, lipid peroxidation biological membranes, products of peroxidation (lipid hydroperoxides, diene conjugates, malonic dialdehyde), antioxidant system.

INTRODUCTION

Modern environmental conditions dramatically increased the level radicalopathy processes in the body [1, 8, 10]. Cold exposure stimulates the generation of reactive oxygen species that initiate the process of lipid peroxidation (LPO), due to the development of hypoxia, based on the increase in the rate of consumption of tissue oxygen necessary for energy supply,

in conditions of increased heat production [4, 7]. In these circumstances the use of the succinatecontaining antihypoxants is appropriate since the conversion of succinic acid in the body is associated with the production of energy necessary for life support, and under conditions of increasing load on any of the body systems, in particular during cold exposure, the maintenance of its work is provided mainly by the oxidation of succinic acid [3, 5, 7]. Given the above, the experimental evaluation of the effectiveness of polyionic infusion solution remaxol, comprising the salt of succinic acid, riboxinum, methionine, nicotinamide and excipients (magnesium chloride, potassium chloride, sodium chloride) in optimum concentrations developed by the scientific–technological pharmaceutical firm "Polysan" approbated on the clinical bases of the Department of anesthesiology and intensive care, St. Petersburg medical Academy of postgraduate education, for the correction of peroxidation processes induced by the effect of cold, is relevant and opens perspectives in the regulation of various stress factors.

The aim: to examine the effect of succinate containing preparationremaxol in doses of 50 mg/kg and 100 mg/kg on antioxidant state of warm-blooded organism in the conditions of cold exposure.

MATERIALS AND METHODS

To study the effect of remaxol in doses 50 mg/kg and 100 mg/kg on the organism of the experimental animals (white rats-males) cold model of the experiment was made [4]. In the experiment 4 groups of animals with the weight of 180g took part. There were 30 rats in each group: 1- intact group, the animals were in standard conditions of a vivarium; 2 – control group, the animals were exposed to the prolonged cooling in the climatic chamber "Fentron" (Germany) at the temperature -15°C during 3 hours daily within 21 days; 3- experimental group, before cooling the animals were made intraperitoneal introduction of remaxol in the dose of 50 mg/kg during 21 days; 4 – experimental group, before cooling intraperitoneal introduction of remaxol in the dose of 100 mg/kg was made to the animals during 21 days.

The investigation was conducted simultaneously in all groups during 21 days, slaughter of the animals was made by means of decapitation on the 7 th, 14 th, 21 th days of the experiment. The intensity of LPO processes was estimated during examination of the content of lipid hydroperoxides, dien conjugates (according to methods, worked out by I.D. Stal'naja), malonic dialdehyde (according to colored reaction with thiobarbital acid) and the main components of AOS(ceruloplasmin – according to methods of V.G. Kolb, V.S. Kamyshnikov, vitamin E according to methods of R.Zh. Kiselevich, S.I. Skvarko, catalase and glucose-6-phosphate dehydrogenase

according to methods in modification of Ye.A. Borodin) in the rats' serum. Statistical processing of biochemical data was conducted by means of parametrical method with the use of "t" Student criterion.

RESULTS AND DISCUSSION

In the conditions of the prolonged cold exposure during the experiment a reliable increase of the content of dien conjugates (DC) in blood by 38-54% regarding intact animals was observed. Introduction of remaxol in the dose of 100 mg/kg in the conditions of cold model promotes a stable reliable decrease of DC content in the blood of the experimental animals during all days of the experiment on the average by 25% in comparison with the control. During the introduction of remaxol in the dose of 50 mg/kg considerably less expressed decrease of this index was observed at the end of the second (by 16%) and the third weeks of the experiment (by 10%).

In the control group of animals a reliable accumulation of lipid hydroperoxides (LHP) in the blood by 28%, 41%, 25%, on the 7^{th}, 14^{th}, and 21^{st} days of the experiments was observed correspondingly regarding intact rats. In the experimental group of animals receiving remaxol in the dose 50 mg/kg before cold exposure some decrease of LHP in the serum by 8%, 21%, 19%, was observed on the on the 7^{th}, 14^{th}, and 21^{st} days of the experiment correspondingly. The increase of remaxol dose to 100 mg/kg led to more pronounced reliable decrease of LHP in the serum during all days of the experiments in comparison with the control group average by 16%-25%, the best result was received by the end of the second week of the experiment, on the 21^{st} day of experiment the level of LHP content in the blood of experimental animals is comparable with analogous index in the intact group.

Thus, by the end of the experiment LHP content in the serum in the group of animals receiving remaxol in the dose of 100 mg/kg unlike experimental animals, receiving remaxol in the dose 50 mg/kg, became equal with the initial LHP content in the serum of the animals of the intact group. It shows the absolute stabilization of LPO processes.

Table 1

Content of LPO products in the rats' blood in the conditions of the prolonged cold stress against the background of applying remaxol in the dose of 50 and 100 mg/kg

Indices	Periods of experiment	*Group1* intact n = 30	*Group 2* cold (control) n = 30	*Group 3* Remaxol in the dose of 50 mg/kg + cold n = 30	*Group 4* Remaxol in the dose of 100 mg/kg + cold n = 30
Hydroperoxides(nmole/ml)	the 7th day	26,0 ± 1,8	33,2 ± 1,1* $P_{1,2} < 0,01$	30,6 ± 1,0 $P_{2,3} > 0,05$	27,9 ± 2,0 $P_{2,4} > 0,05$
	the 14th day	25,0 ± 2,7	35,2 ± 1,2* $P_{1,2} < 0,01$	27,9 ± 1,7** $P_{2,3} < 0,01$	26,4 ± 1,3** $P_{2,4} < 0,01$
	the 21th day	28,6 ± 1,5	35,6 ± 1,1* $P_{1,2} < 0,01$	29,0 ± 1,9** $P_{2,3} < 0,05$	28,6 ± 1,2** $P_{2,4} < 0,05$
Dien conjugate(nmole/ml)	the 7th day	35,2 ± 4,3	48,7 ± 3,3* $P_{1,2} < 0,05$	38,1 ± 2,8** $P_{2,3} < 0,05$	37,8 ± 1,9** $P_{2,4} < 0,05$
	the 14th day	35,4 ± 3,0	49,2 ± 2,6* $P_{1,2} < 0,01$	41,2 ± 1,2** $P_{2,3} < 0,05$	35,1 ± 1,4** $P_{2,4} < 0,01$
	the 21th day	31,2 ± 2,6	48,1 ± 3,4* $P_{1,2} < 0,01$	43,2 ± 2,3 $P_{2,3} > 0,05$	36,8 ± 1,1** $P_{2,4} < 0,05$
Malonic dialdehyde (nmole/ml)	the 7th day	3,8 ± 0,1	6,1 ± 0,2* $P_{1,2} < 0,001$	4,6 ± 0,3** $P_{2,3} < 0,05$	4,0 ± 0,1** $P_{2,4} < 0,05$
	the 14th day	3,8 ± 0,2	6,6 ± 0,4* $P_{1,2} < 0,001$	4,6 ± 0,2** $P_{2,3} < 0,05$	4,1 ± 0,2** $P_{2,4} < 0,01$
	the 21th day	4,4 ± 0,3	5,6 ± 0,4* $P_{1,2} < 0,05$	4,8 ± 0,1 $P_{2,3} > 0,05$	4,4 ± 0,3** $P_{2,4} < 0,05$

Notes: * and **– differences, reliable regarding the intact group*and the control group of animals **

Table 2

The content of AOS components in the rats' blood in the conditions of the prolonged cold stress against the background of application of remaxol in the dose of 50 and 100 mg/kg

Indices	Periods of experiment	*Group1* intact n = 30	*Group 2* cold (control) n = 30	*Group 3* Remaxol in the dose of 50 mg/kg + cold n = 30	*Group 4* Remaxol in the dose of 100 mg/kg + cold n = 30
Ceruloplasmin (mkg/ml)	the 7th day	30,0 ± 1,9	20,5 ± 1,8* $P_{1,2}$ < 0,01	22,6 ± 4,6 $P_{2,3}$>0,05	26,6 ± 2,8 $P_{2,4}$ >0,05
	the 14th day	28,8 ± 1,4	19,1 ±1,2* $P_{1,2}$ < 0,01	28,7 ± 2,6** $P_{2,3}$< 0,05	27,7 ± 2,5** $P_{2,4}$< 0,05
	the 21th day	26,8 ± 1,4	20,3± 1,0* $P_{1,2}$ < 0,01	26,5 ± 1,1** $P_{2,3}$< 0,05	27,7 ± 2,1** $P_{2,4}$< 0,05
Vitamin E (mkg/ml)	the 7th day	48,7 ± 3,6	37,3 ± 1,5* $P_{1,2}$ < 0,05	40,2 ± 0,8 $P_{2,3}$>0,05	44,8 ± 1,7** $P_{2,4}$< 0,05
	the 14th day	47,5 ± 2,2	34,0 ± 1,6* $P_{1,2}$ < 0,01	40,9 ± 1,5** $P_{2,3}$< 0,05	44,7 ± 1,5** $P_{2,4}$< 0,05
	the 21th day	45,8 ± 2,0	38,0 ± 1,8* $P_{1,2}$ < 0,01	41,5 ± 1,3 $P_{2,3}$>0,05	44,8 ± 1,4** $P_{2,4}$< 0,05
GL-6-PhDH (mcmoleNADFH $л^{-1}c^{-1}$)	the 7th day	6,9 ± 0,2	5,6 ± 0,2* $P_{1,2}$ < 0,01	5,8 ± 0,2 $P_{2,3}$>0,05	5,8 ± 0,2 $P_{2,4}$> 0,05
	the 14th day	6,8 ± 0,2	5,9 ± 0,2* $P_{1,2}$ < 0,05	6,5 ± 0,1** $P_{2,3}$< 0,05	6,6 ± 0,2 $P_{2,4}$> 0,05
	the 21th day	6,7 ± 0,3	5,6 ± 0,2* $P_{1,2}$ < 0,05	6,5 ± 0,2** $P_{2,3}$< 0,05	6,4 ± 0,2** $P_{2,4}$< 0,05
Catalase (mcmole H_2O_2 $г^{-1}c^{-1}$)	the 7th day	93,0 ± 2,7	78,6 ± 5,1* $P_{1,2}$ < 0,05	83,8 ± 6,1 $P_{2,3}$>0,05	85,2 ± 6,4 $P_{2,4}$> 0,05
	the 14th day	95,2 ± 3,2	72,8 ± 5,9* $P_{1,2}$ < 0,05	84,4 ± 5,1 $P_{2,3}$>0,05	86,8 ± 4,8 $P_{2,4}$> 0,05
	the 21th day	97,0 ± 3,5	71,0 ± 4,2* $P_{1,2}$ < 0,05	81,0 ± 4,9 $P_{2,3}$>0,05	91,0 ± 5,0** $P_{2,4}$< 0,05

Notes: * and **– differences, reliable regarding the intact group*and the control group of animals **

A reliable increase of malonic dialdehyde (MDA) content by 27-74% in the blood was observed during the experiment in the conditions of cold exposure on the experimental animals. In case of remaxol introduction in different doses the the content of MDA in the blood was reliably lower, that in the control animals on the 7th and 14th days of the experiment. Antioxidant effect is more pronounced in remaxol in the dose of 100 mg/kg during all days of the experiment (the decrease of MDA content made up 21%-38% regarding the control group), the level of index, against the background of remaxol introduction in the of 100 mg/kg on the 21st day of the experiment is comparable with the level of index of the intact group.

The pronounced decrease of concentration of ceruloplasmin by 24-34% in plasma was marked as a result of cold exposure on the organism of the laboratory animals. Against the background of the intraperitoneal introduction of remaxol in the dose of 100 mg/kg before the cold exposure the content of ceruloplasmin in plasma inereases by 30%, 45% on the 7th and 14th days of the experiment correspondingly regarding the control and it corresponds to the level of ceruloplasmin in the animals of the intact group by the end of the animals of the intact group by the end of the third week. Remaxol introduction in the dose of 50 mg/kg leads to small increase of ceruloplasmin content in plasma by 10%, 50% and 31% on the 7th, 14th and 21st days of the experiment correspondingly regarding the control.

Against the background of remaxol in the dose of 100 mg/kg a reliable increase of vitamin E content by 20% and 31% is marked on the 7th and 14th days of the experiment correspondingly regarding the control group. Remaxol in the dose of 50 mg/kg affected considerably less the level of this index (by 8%, 20% and 9%) on the 7th, 14th and 21 days of the experiment correspondingly, these differences regarding the control group were not authentic. Thus, there is a direct dependence of vitamin E content on the dose of preparation, but in the groups receiving remaxol in the dose of 100 mg/kg the effect is achieved and it allows to suppose stimulating effect of this dose of preparation on the intensified production of endogenous vitamin E, it may be a significant factor in prophylaxis of the cold stress.

Cold exposure causes (by 13-19%) the decrease of GL-6-PhDH activity in the blood of laboratory animals in comparison with the intact group. The partial normalization of enzyme activity is observed during all days of the experiment (by 4-14%) against the background of remaxol introduction in the dose of 100 mg/kg. Remaxol in the dose of 50 mg/kg leads to less pronounced effect partially normalizing GL-6-PhDH activity by the 7th day of the experiment to

4%. By the end of the second week the level of GL-6-PhDH activity is lower that the level of the control group index by 10% and by the 21st day of the experiment GL-6-PhDH activity during remaxol introduction in the dose of 50 mg/kg corresponds to the level of the intact group.

Thus, remaxol in the dose of 100 mg/kg in comparison with remaxol in the dose of 50 mg/kg gives more pronounced effect on normalization of GL-6-PhDH activity during its decrease against the background of cold exposure in the control group of animals.

A considerable decrease of catalase activity from (16%, to 27%) regarding group of the intact animals' takes place during the experiment in the conditions of cold exposure. Remaxol in the dose of 100mg/kg prevents from the decrease of catalase activity in the blood-partially after the first week of low temperature action (by 8%). In this case level of the intact group is not achieved. After the second week and by the end of the third week of cold exposure the enzyme activity increased by 19-28% in comparison with the control group correspondingly. Effect of remaxol introduction in the dose of 50 mg/kg is less pronounced on the 7th, 14th, and 21st days of the experiment, catalase activity increased by 7-16% correspondingly.

As a whole, the examination of LPO products content in the blood, content and activity of AOS components in the conditions of cold exposure and influence on these indices of remaxol in the dose of 50 and 100 mg/kg allows to establish antioxidant effect which is more pronounced in the dose of 100 mg/kg.

CONCLUSIONS

1. The possibility of the cold stress correction by means of introduction of preparation remaxol which contains succinic acid is confirmed experimentally for the first time.
2. Intraperitoneal introduction of remaxol to the laboratory animals (rats) decreases the intensity of LPO processes biomembranes induced by the prolonged cold exposure normalizing stationary level of peroxidation products against the background of a reliable increase of activity of the main AOS components.
3. Statistically significant differences of changes of indices of LPO processes and components of AOS depending on the dose of remaxol and the duration of its application are determined (direct dose dependence – in case of application of remaxol greater dose, the antioxidant effect is more pronounced).

4. The results of the investigation give the grounds to recommend remaxol as an antioxidant as well as regulator of adaptation reactions of an organism at a low temperature.

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