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Comparative Evaluation of Antioxidant Activity of Cytoflavin in Various Doses in the Conditions of Cold stress

ABSTRACT

The article reports results of study of the cytoflavin effect in doses of 50 mg / kg and 100 mg / kg on the antioxidant status of rats at chronic cold exposure.

It was established that the studied preparation prevented from stress-induced activity of lipid peroxidation in serum of the animals, exposed to cold. The effect is expressed in less formation of peroxidation products and in the prolonged conservation of a high level of antioxidant system components activity. During the comparative pharmacological analysis it was established that cytoflavin produced a direct antioxidant effect according to a dose during cold exposure on the organism of the experimental animals.

Keywords: antioxidants, cytoflavin, cold exposure, lipid peroxidation, experiment.

INTRODUCTION

Preservation of health and efficiency of people living and working in the conditions of the extreme north is one of the actual directions of medicine. In the zone of high latitudes, which occupy 1/3 of the territory of our country, a person meets with numerous unfavorable factors and temperature is the most important one among them [3, 6, 8, 9]. Prolonged exposure of a low temperature on the human organism may cause the development of such phenomena as syndrome of arctic hypoxia, syndrome of arctic tension, cold hypoxia, cold–associated symptoms [10]. Cold exposure induces the development of critical condition accompanied by exhaustion of energy and other reserves, reduction of tissue metabolism, forming proliferative and dystrophic disorders in all organs [5]. Stage development of consequences of cold exposure leads to polyorganic insufficiency, hypoxia, development of late cold hemolysis, collapse, disturbance of activity of coagulation system, function of the liver and the kidneys [2, 4].

It is known that in the base of any kind of hypoxia there is insufficiency of the main energy forming system of mitochondrial oxidative phosphorylation, conditioned by considerable decrease of oxygen delivery to the tissues or inhibition of oxidative enzymes [1, 11]. At present in the clinical practice compounds of succinic acid, having antioxidant and cytoprotective properties, are used as pharmacological active substances with a wide range of biological activity. Cytoflavin is a balanced complex consisting of two coenzymes vitamins – riboflavin – mononucleotide (vitamin B_2) and nicotinamide (vitamin PP).

Taking into consideration the above mentioned data **the aim** of the investigation is the study of cytoflavin effect in doses of 50 mg/kg and 100 mg/kg on the intensity of lipid peroxidation (LPO) and the condition of antioxidant system (AOS) in the conditions of cold exposure.

MATERIALS AND METHODS

To study the effect of cytoflavin 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 [1]. In the experiment 4 groups of animals with the weight of 200g 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 cytoflavin in the dose of 50 mg/kg during 21 days; 4 – experimental group, before cooling intraperitoneal introduction of cytoflavin 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 coloured 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 cytoflavin 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 cytoflavin in the dose of 50 mg/kg considerably less expressed decrease of this index was observed at the end of the second (by 18%) and the third weeks of the experiment (by 4%).

In the control group of animals a reliable accumulation of lipid hydroperoxides (LHP) in the blood by 27%, 40%, 24%, 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 cytoflavin in the dose 50 mg/kg before cold exposure some decrease of LHP in the serum by 14%, 10%, 5%, was observed on the on the 7th, 14th, and 21st days of the experiment correspondingly and these differences of index were not reliable. The increase of cytoflavin 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 12%-21%, the best result was received by the end of the second week of the experiment, on the 21st 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 cytoflavin in the dose of 100 mg/kg unlike experimental animals, receiving cytoflavin 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.

A reliable increase of malonic dialdehyde (MDA) content by 60-73% in the blood was observed during the experiment in the conditions of cold exposure on the experimental animals. In case of cytoflavin 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 cytoflavin in the dose of 100 mg/kg during all days of the experiment (the decrease of MDA content made up 32%-34% regarding the control group), the level of index, against the background of cytoflavin 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 32-34% 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 cytoflavin in the dose of 100 mg/kg before the cold exposure the content of ceruloplasmin in plasma inereases by 23%, 32% 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. Cytoflavin introduction in the dose of 50 mg/kg leads to small increase of ceruloplasmin content in plasma by 19%, 7% and 20% on the 7th, 14th and 21st days of the experiment correspondingly regarding the control. Thus, more pronounced effect of normalization of ceruloplasmin level is observed during cytoflavin introduction in the dose of 100 mg/kg.

Against the background of cytoflavin in the dose of 100 mg/kg a reliable increase of vitamin E content by 16% and 24% is marked on the 7th and 14th days of the experiment correspondingly regarding the control group. Cytoflavin in the dose of 50 mg/kg affected considerably less the level of this index (by 2%, 9% and 1%) 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 cytoflavin 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 19-17%) 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 6-7%) against the background of cytoflavin introduction in the dose of 100 mg/kg. Cytoflavin 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 7%. 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 4% and by the 21st day of the experiment GL-6-PhDH activity during cytoflavin introduction in the dose of 50 mg/kg corresponds to the level of the control group.

Thus, cytoflavin in the dose of 100 mg/kg in comparison with cytoflavin 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. Cytoflavin in the dose of 100 mg/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 21-44% in comparison with the control group correspondingly. Effect of cytoflavin introduction in the dose of 50 mg/kg is less pronounced on the 7th and 14th days of the experiment, catalase activity increased by 4-5% correspondingly and on the 21st day of the experiment the level of enzyme activity corresponds to the control group.

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 cytoflavin 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 "Cytoflavin" which contains succinic acid is confirmed experimentally for the first time.
2. Intraperitoneal introduction of cytoflavin 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 (ceruloplasmin and vitamin E).
3. Statistically significant differences of changes of indices of LPO processes and components of AOS depending on the dose of cytoflavin and the duration of its application are determined (direct dose dependence – in case of application of cytoflavin greater dose, the antioxidant effect is more pronounced).
4. The results of the investigation give the grounds to recommend cytoflavin as an antioxidant as well as regulator of adaptation reactions of an organism at a low temperature.

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Table 1

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

Indices	Group / Periods of experiment	*Group1* intact n = 30	*Group 2* cold (control) n = 30	*Group 3* Cytoflavin in the dose of 50 mg/kg + cold n = 30	*Group 4* Cytoflavin in the dose of 100 mg/kg + cold n = 30
Dien conjugate (nmole/ml)	the 7th day	35,2± 4,3	48,7 ± 3,3* $P_{1,2}$< 0,05	36,1 ± 1,1** $P_{2,3}$ < 0,01	36,8 ± 1,0** $P_{2,4}$ < 0,01
	the 14th day	35,4 ± 3,0	49,2 ± 2,6* $P_{1,2}$< 0,01	40,4 ± 2,7 $P_{2,3}$ > 0,05	36,9 ± 1,1** $P_{2,4}$<0,01
	the 21th day	31,2 ± 2,6	48,1 ± 3,4* $P_{1,2}$ < 0,01	46,3 ± 3,4 $P_{2,3}$ > 0,05	36,6 ± 0,7** $P_{2,4}$ < 0,05
Hydroperoxides (nmole/ml)	the 7th day	26,0 ± 1,8	33,2 ± 1,1* $P_{1,2}$ < 0,01	28,8 ± 2,0 $P_{2,3}$ > 0,05	29,3 ± 1,1** $P_{2,4}$ < 0,05
	the 14th day	25,0 ± 2,7	35,2 ± 1,2* $P_{1,2}$< 0,01	32,0 ± 1,3 $P_{2,3}$ > 0,05	27,9 ± 1,1** $P_{2,4}$ < 0,01
	the 21th day	28,6 ± 1,5	35,6 ± 1,1* $P_{1,2}$ < 0,01	34,1± 1,6 $P_{2,3}$ > 0,05	28,4 ± 1,0** $P_{2,4}$ < 0,01
Malonic dialdehyde (nmole/ml)	the 7th day	3,8 ± 0,1	6,1 ± 0,2* $P_{1,2}$ < 0,001	4,3 ± 0,2** $P_{2,3}$ < 0,001	4,2 ± 0,1** $P_{2,4}$ < 0,001
	the 14th day	3,8 ± 0,2	6,6 ± 0,4* $P_{1,2}$ < 0,001	4,1 ± 0,1** $P_{2,3}$ < 0,001	4,4 ± 0,3** $P_{2,4}$ < 0,01
	the 21th day	4,4 ± 0,3	5,6 ± 0,4* $P_{1,2}$ < 0,05	4,7 ± 0,2 $P_{2,3}$ > 0,05	4,5 ± 0,2** $P_{2,4}$ < 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 cytoflavin in the dose of 50 and 100 mg/kg

Indices	Group / Periods of experiment	*Group1* intact n = 30	*Group 2* cold (control) n = 30	*Group 3* Cytoflavin in the dose of 50 mg/kg + cold n = 30	*Group 4* Cytoflavin in the dose of 100 mg/kg + cold n = 30
Ceruloplasmin (mkg/ml)	the 7th day	30,0 ± 1,9	20,5 ± 1,8* $P_{1,2} < 0,01$	24,4 ± 1,8 $P_{2,3} > 0,05$	25,4 ± 0,9** $P_{2,4} < 0,05$
	the 14th day	28,8 ± 1,4	19,1 ± 1,2* $P_{1,2} < 0,01$	20,5 ± 1,5 $P_{2,3} > 0,05$	25,4 ± 1,4** $P_{2,4} < 0,05$
	the 21th day	26,8 ± 1,4	20,3 ± 1,0* $P_{1,2} < 0,01$	24,5 ± 2,0 $P_{2,3} > 0,05$	26,1 ± 1,7** $P_{2,4} < 0,05$
Vitamin E (mkg/ml)	the 7th day	48,7 ± 3,6	37,3 ± 1,5* $P_{1,2} < 0,05$	38,4 ± 1,4 $P_{2,3} > 0,05$	43,5 ± 1,7** $P_{2,4} < 0,05$
	the 14th day	47,5 ± 2,2	34,0 ± 1,6* $P_{1,2} < 0,01$	37,1 ± 1,0 $P_{2,3} > 0,05$	42,2 ± 1,0** $P_{2,4} < 0,01$
	the 21th day	45,8 ± 2,0	38,0 ± 1,8* $P_{1,2} < 0,05$	37,8 ± 2,9 $P_{2,3} > 0,05$	44,4± 1,8** $P_{2,4} < 0,05$
GL-6-PhDH (mcmoleNADFH $л^{-1}c^{-1}$)	the 7th day	6,9 ± 0,2	5,6 ± 0,2* $P_{1,2} < 0,01$	6,0 ± 0,2 $P_{2,3} > 0,05$	6,0 ± 0,3 $P_{2,4} > 0,05$
	the 14th day	6,8 ± 0,2	5,9 ± 0,2* $P_{1,2} < 0,05$	5,7 ± 0,3 $P_{2,3} > 0,05$	6,3 ± 0,1 $P_{2,4} > 0,05$
	the 21th day	6,7 ± 0,3	5,6 ± 0,2* $P_{1,2} < 0,05$	5,6 ± 0,3 $P_{2,3} > 0,05$	6,0 ± 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* $P_{1,2} < 0,05$	82,4 ± 3,1 $P_{2,3} > 0,05$	85,0 ± 3,8 $P_{2,4} > 0,05$
	the 14th day	95,2 ± 3,2	72,8 ± 5,9* $P_{1,2} < 0,05$	77,0 ± 3,8 $P_{2,3} > 0,05$	88,8 ± 5,0 $P_{2,4} > 0,05$
	the 21th day	97,0 ± 3,5	71,0 ± 4,2* $P_{1,2} < 0,05$	72,8 ± 3,2 $P_{2,3} > 0,05$	81,4 ± 4,2 $P_{2,4} > 0,05$

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

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