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INTENSITY OF LIPID PEROXIDATION IN PATIENTS WITH COLD INJURY

ABSTRACT

The authors report their study on the intensity of lipid peroxidation processes and the state of antioxidant protection in patients with cold trauma of varying severity. It was established that in the organism of patients with frostbite of extremities, the intensity of free radical oxidation and the level of antioxidant defense depended on the degree of frostbite.

Clinical and biochemical studies were conducted in 151 people, 81 of whom were patients with a cold trauma of varying severity, who entered the burn department of the Republican Hospital №2. The control group included 70 practically healthy people. The material of the study was fasting venous blood samples, drawn from the ulnar vein.

The parameters of the intensity of free radical lipid oxidation (malonicdialdehyde, diene conjugates) and antioxidant protection (low-molecular antioxidants, activity of superoxide dismutase, catalase, glutathione peroxidase, glutathionereductase) were determined by spectrophotometric methods. Clinical and biochemical parameters in blood serum were determined on the biochemical automatic analyzer CobasMiraPlus (Roche).

In the body of patients with frostbite of the extremities of the first and second groups, a significant increase in the parameters of lipid peroxidation - MDA and DC - is observed. In patients of the second group, the intensity of lipid peroxidation processes was higher than in patients of the first group.

The state of the body's antioxidant defense depends on the severity of the injury. In patients of the first group, the activity of SOD is increased by 8 times, CAT by 1.3 times, HP by 1.7 times, and GR activity decreased by 1.9 times in comparison with the control. The concentration of LMWA increases 2.1 times, and the content of ascorbic acid decreases by 1.2 times. In patients of the second group with respect to control, the activity of SOD increases 12.6 times, catalase 1.1 times, HP 1.5 times, and GH activity decreased 2.8 times. The content of LMAO increases 1.5 times, and the level of ascorbic acid in the blood decreases 2.4 times.

The shift in the prooxidant-antioxidant equilibrium toward the intensification of free radical reactions is also indicated by an increase in the coefficients: KSOD / CAT, KSOD / HP. The values of the coefficients (KSOD / CAT, KSOD / GP) depend on the severity of the cold trauma.

Keywords: lipid peroxidation, antioxidant protection, cold trauma, catatoxic and syntoxic adaptation programs.

Introduction

The cold in the Far North potentiates the development of not only adaptive rearrangements (cold adaptation), but the emergence of pathologies too (cold trauma). Under the cold trauma, most authors mean the effect of negative temperatures, leading to frostbite [5]. Cold trauma is one of the most common types of thermal trauma, which comprises almost 10% of all surgical diseases [20] and has a well-defined seasonal character. In Yakutia, where winter lasts 6-7 months in a year and the temperature drops to -60°C, these figures are much higher [14]. Despite some successes achieved in the treatment of cold trauma, some pathogenesis issues remain poorly understood. When using the traditional methods of treatment of cold trauma, 30-60% of the patients become severely disabled. This extremely high level of disability is a clear confirmation of the unresolved problem [1].

The few data, available in the literature, indicate that the effect of low temperatures on human and experimental animals is accompanied by the activation of free radical processes [9, 10]. Moderate activation of lipoperoxidation processes in response to the effect of an unfavorable factor is one of the variants of adaptation mechanisms and is aimed at increasing the permeability of the cell membrane and facilitating the work of membrane proteins. However, beyond certain limits, these shifts can become a pathogenetic factor by themselves, which are manifested by denaturation and inactivation of proteins, delipidization of membranes, disruption of cell division and growth [4]. Thus, the intensity of the processes of lipid peroxidation and the state of its main regulator - the antioxidant system - under the influence of cold on the human body as a stressful environmental factor, requires further study.

The objective of the study was to estimate the intensity of lipid peroxidation processes and the state antioxidant defense in the body of patients with cold trauma of varying severity.

Materials and methods

Clinical and biochemical studies were conducted on 151 people, 81 of whom were patients with a cold trauma of varying severity, who were admitted to the burn trauma department of the Republican Hospital No. 2 in Yakutsk. Among the injured were 74 men and 7 women. Patients were hospitalized in 1-5 days after receiving a cold injury, i.e. in the reactive period. Depending on the degree of frostbite, the patients were divided into two groups: the first group consisted of 35 patients with first and seconddegree frostbites, the second - 46 patients with the third and fourthdegree frostbites. The manifestation of frostbite in both groups was characterized by changes depending on the severity of the injury. The control group included 70 healthy people. The material of the study was fasting venous blood samples, drawn from the ulnar vein.

The study was approved by the local Committee on Biomedical Ethics at the Yakutsk Scientific Center of Complex Medical Problems (Yakutsk, Protocol 8, October 10, 2007). Patients and healthy subjects received informed consent for taking biological samples (venous blood) and participation in this study.

The intensity of lipid peroxidation was determined by the accumulation of malonicdialdehyde (MDA) [18] and diene conjugates (DC) [7] using spectrophotometric methods. The antioxidant defense system parameters of the organism were determined by the total content of low-molecular weight antioxidants (LMWA) [16] and the activity of superoxide dismutase (SOD) [22], catalase (CAT) [12], glutathione peroxidase (GPx) [7] and glutathione reductase (GR) [7]. Clinical and biochemical parameters: the activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), total protein, glucose, triacylglyceride (TAG) and urea in blood weretested on the automatic biochemical analyzer "COBAS MIRA Plus" (Roche).

Statistical processing of the data was carried out on a package of applied statistical programs SPSS Statistics 17. Standard methods of variational statistics

were used: calculation of average values, standard errors, 95% confidence interval. The reliability of the differences between the averages was estimated using the Student's t-test for independent samples. The data in the tables are presented in the form "M±m", where M is the mean, m is the mean error. Probability of the validity of the null hypothesis was assumed for p<0.05.

Results and discussion

According to our data, the rate of free radical reactions in the body caused by cold trauma depended on the degree of frostbite (Table 1). In patients of the first group, the MDA content was 1.8 times higher than the control, while in the second group the MDA level exceeded the control value by a factor of 2. The concentration of DC in the first group of patients was 1.9 times higher than control, and in the second group - 2.5 times.

Activation of lipid peroxidation in both groups of patients with cold trauma can be explained as an adaptive response of the organism to stress effects, since free radicals are a link in the development of urgent and long-term adaptation. At the stage of urgent adaptation, a deficit of macroerges arises in the cell, which leads to a «respiratory explosion» and excessive generation of reactive oxygen species, initiators of lipid peroxidation. Thus, the formation of a structural trace in the body under the influence of a stressing factor, including low temperatures, is accompanied by an acceleration of free-radical processes.

The development of inflammatory processes in the body is accompanied by a change in microcirculation, an increase in the permeability of blood vessels and the influx and activation of leukocytes in the affected tissues [17]. The chemotaxis factors of leukocytes are cytokines, leukotrienes, thromboxanes, prostaglandins, a substrate for the biosynthesis of which are polyunsaturated fatty acids with conjugated bonds. Therefore, the change in the ratio of the final and the initial products of lipid peroxidation towards the increase in the concentration in the blood of DC in patients with the third and fourth degree of frostbite is probably a consequence of the activation of membrane phospholipases and the release of polyunsaturated fatty acids, the oxidation of which increases the level of eicosanoids - mediators inflammation [11].

Peroxidation products have the ability to directly increase the ion permeability of the lipid bilayer by modifying proteins. The appearance of defects in the lipid layer of cell membranes and mitochondria is associated with the oxidation of thiol groups of membrane proteins, primarily Ca2+-ATPase [2, 3]. Inactivation of this enzyme leads to a delay in the evacuation of calcium ions from the cells and an increase in the intracellular concentration of calcium ions. Sodium ions enter the cell through the pores formed due to the difference in electrical potentials on the membranes, while potassium ions enter the mitochondria. As a result, the osmotic pressure within cells and mitochondria increases which leads to swelling. This further leads to inability of mitochondria to synthesize ATP, which induces energy hunger in the cell. A decrease in the stability of the lipid bilayer of the mitochondria can lead to an electrical breakdown of the membrane by its own membrane potential and electron leakage, which results in an increase in the concentration of superoxide radicals in the cell.

The increase in the products of lipid peroxidation reduces the rate of anabolic processes, which is manifested in slowing down the processes of tissue regeneration and wound healing. Our results are confirmed by the literature data [8, 19].

Accumulation of products of lipid peroxidation stimulates the antioxidant defense of the body. Assessment of the state of the enzymatic link of antioxidant protection in the blood of patients with cold trauma showed that the enzyme activity also depended on the degree of cold trauma (Table 2).

The activity of SOD - an enzyme that reduces and re-oxidizes the superoxide radical of oxygen to hydrogen peroxide, was increased in the first group of patients by a factor of 8.0, and in the second group – by a factor of 12.6.

The catalase activity in both groups of patients with frostbites was also increased. In the first group of patients catalase activity was 1.3 times higher than control, and in the second group – 1.1 times.

Antioxidant enzymes SOD and CAT work together, promptly inactivate ROS, superoxide anion-radical and hydrogen peroxide, which are formed during the normal metabolism of cells, as well as during significant intensification of the processes of lipid peroxidation. However, these enzymes have little activity with respect to lipid peroxides formed during

Table 1

Concentration of MDA (µmol/L) and DC (µmol/L) in the blood of the control group and patients with cold trauma

Group	MDA	DC
Control	1.98±0.05	1.25±0.05
First	3.73±0.12**	2.40±0.10*
Second	4.05±0.26*	3.19±0.15**

Note: In Table 1-4 * p <0.05 compared with the control group, ** p <0.01 compared with the control group.

chain reactions of lipid peroxidation. The destruction of these products is carried out with the participation of the enzyme system of glutathione.

GPx is able to efficiently decompose hydroperoxide lipids and hydrogen peroxide. Its affinity to hydrogen peroxide is higher than that of catalase, therefore GPx effectively works at low peroxide concentrations [23, 24], however catalase plays a key role in protecting cells from oxidative stress caused by high concentrations of hydrogen peroxide.

Activity of GPx in the first group of patients was 1.7 times greater than control, in the second group - 1.5 times. GR activity decreased in blood of patients of the first group - in 1.9, and in the second group of x - in 2.8 times.

The concentration of the total content of LMWA in erythrocytes of patients with cold trauma also depended on the severity of the injury.

In the first group of patients, the LMWA content was 2.1 times higher than the control value, in the second group - 1.5 times. The concentration of one of the most important antioxidants - ascorbic acid in the blood of patients in the first and second groups was below the control value of 0.8 and 0.4 times, respectively (Table 3).

Probably, the decrease in the concentration of ascorbic acid, depending on the severity of the cold trauma, is related to the mechanism of action of this vitamin, since the utilization of ascorbic acid in the body has a close relationship with the exchange of catecholamines and steroid hormones of the adrenals. Thus, it has been established that under various stress effects on the human body (cooling, burn, blood loss, etc.), a sharp decrease in the concentration of ascorbic

Table 2

Activity of SOD, CAT, GPx and GR in the blood of patients with cold trauma of varying severity

Group	SOD, µmol/min*ml	CAT, µCat/L	GPx, IU/gHb	GR, µmolNADPH/min*gHb
Control	0.03± 0.01	0.60±0.01	0.04±0.01	0.86±0.03
First	0.24±0.02**	0.79±0.03*	0.07±0.01*	0.44±0.02*
Second	0.38±0.01**	0.69±0.02	0.06±0.01	0.31±0.01**

acid in the adrenal glands is observed. On the other hand, the introduction of vitamin C into the body increases resistance to various unfavorable environmental factors [25].

The data obtained by us testify that the state of antioxidant defense system of the organism of patients with cold trauma depends on the degree of frostbite of tissues. In both groups of patients there was an increase in the concentration of LMWA in the blood serum. Decrease in the content of LMWA in the second group of patients in comparison with the patients of the first group can probably be explained not only by its depletion during slaking of free radical and oxidative reactions, but also by a decrease in the activity of the GR -enzyme that reduces oxidized glutathione. The increase in SOD activity in the blood of patients of both groups is probably a consequence of an increase in the superoxide anion radical concentration. The change in catalase activity, depending on the degree of frostbite, is similar to the change in activity of GPx, probably because they have the same substrate.

Antioxidant enzymes form a single metabolic chain, in which the product of the first reaction is the substrate of the subsequent. In this regard, in order to sustain normal functioning of the whole enzyme antioxidant system, it is important to maintain certain ratios in the activity of individual enzymes in the chain. First of all, this concerns SOD-GPx and SOD-catalase, since unbalanced increase in the activity of SOD may lead to elevation of steady-state concentration of peroxides, which are toxic to the cell. Therefore, we carried out an analysis of the changes in the coefficients reflecting the state of the antioxidant system of the body, the KSOD/CAT, KSOD/GPx.

According to above data, in severe frostbite, the system of antioxidant defense of the organism is disturbed as a result of their imbalance (Table 4). The increase in the valuesof KSOD/CAT and KSOD/GPx in cold trauma of thirdand fourth degree is due to a sharp increase in SOD activity (6.5 times in the first and 10.4 in the second group) and comparatively a slight increase in the activity of GPx and catalase, enzymes that inactivate the product of the superoxide radical oxidation, which leads to an increase in the concentration of hydrogen peroxide and, as a result, the appearance of new forms of active oxygen (OH*, HClO) and intensification of lipid peroxidation.

Reflection of inflammatory-destructive processes in the body is an increase in the blood of patients with activity of enzymes: ALT, AST, GGP and ALP. According to our data, in patients with cold trauma the level of enzymes (IU) in the blood in the first and second groups corresponded to: ALT 31.17±1.69 IU and 77.20±2.05 IU, AST 47.43±2, 29 IU and 109.00±4.48IU, GGT 34.29±1.17IU and 64.09±2.49IU, ALP 150.55±6.43IU and 154.38±6.14IU, respectively. In the control group, the activity values of the enzymes ALT, AST, GGT and ALP were 21.11±0.50; 27.90±0.39; 24.12±1.35 and 116.20±5.45IU, respectively. The increase in the level of these enzymes in the blood of patients was in direct proportion to the severity of the injury.

Table 3

The concentration of ascorbic acid (mg / dL) and the total content of low molecular weight antioxidants (mgEq / ml * erythrocytes) in the blood of patients with cold trauma of varying severity

Group	LWMA	Ascorbic acid
Control	0,044±0,001	0,57±0,02
First	0,094±0,004**	0,48±0,01*
Second	0,066±0,003*	0,24±0,01**

Note: * p <0.05 compared with the control group, ** p <0.01 compared with the control group.

Table 4

Values of KSOD/CAT and KSOD/GPx

Coefficients (K)	Group		
	Control	First	Second
KSOD/CAT	0.06±0.002	0.30±0.017*	0.55±0.02*
KSOD/GPx	0.97±0.035	3.57±0.14*	6.21±0.24*

The concentration of MDA and total cholesterol in the blood in patients with frostbite of 3-4 degrees were in a directly proportional relationship. In the first group, a 8% decrease in MDA compared with the second group was associated with a 13.6% decrease in cholesterol (5.07±0.27 mmol/L); in the second group, the increase in MDA concentration was combined with high cholesterol (6.08±0.24 mmol/L). The cholesterol level in the control group was 5.85±0.30 mmol/L. This is probably due to the ability of lipid peroxidation products to inhibit the activity of the key enzyme of cholesterol catabolism - 7-ά-hydroxylase, which leads to the sustenance of its stably high level with the intensification of processes of free radical oxidation in the body [21]. The data obtained by us on the increase in cholesterol in the blood of patients with cold trauma confirm this position.

We found a relative decrease in the total protein in comparison with the control value (73.57±1.69 g/L): in the first group the concentration of the total protein was 59.08±1.05 g/L, and in the second group - 59.53±2.97 g/L. Probably, the decrease in blood plasma proteins is associated with the activation of catabolism, as evidenced by the tendency to increase the urea concentration depending on the degree of severity of the cold trauma. The concentration of urea in the first group was 3.72±0.10 mmol/L, in the second - 4.15±0.12mmol/L, in the control group was 3.59±0.06 mmol/L.

The level of TAG in the blood of patients also depended on the degree of frostbite, in the first group of patients its concentration was 1.14±0.03 mmol/L, in the second - 1.28±0.01mmol/L, in the control group - 0.81±0.02 mmol/L.

According to V.N. Morozov, A.A. Khadartsev [13], depending on the depth and area of the lesion, the organism chooses the adaptation mechanism: synthoxic or katatoxic, which will determine the course of the disease in the future. The area of tissue damage in the ourpatients was insignificant and amounted to approximately 1 to 3% of the entire surface of the body. Therefore, katatoxic adaptation programs developed in patients of both groups [6,15].

Conclusion

The development of cold trauma is associated with the intensification of lipid peroxidation. In the organism of patients with frostbite of the extremities of the first and second groups, a significant increase in the parameters of lipid peroxidation – MDA and DC – is observed. In patients of the second group, the intensity of lipid peroxidation processes was higher than in patients of the first group.

Accumulation of products of lipid peroxidation stimulates the antioxidant defense of the body. The state of the body's antioxidant defense depends on the severity of the injury. In patients of the first group, the activity of SOD increased 8-fold, CAT in 1.3-fold, GPx in 1.7-fold, and GR activity decreased 1.9-fold compared with the control. The concentration of LMWA increases 2.1 times, and the content of ascorbic acid decreases by 1.2 times. In patients of the second group with respect to control, the activity of SOD increases 12.6 times, catalase in 1.1, GPx in 1.5, and the activity of GR decreased 2.8 times. The content of LMWA is increased 1.5 times, and the level of ascorbic acid in the blood is reduced 2.4 times.

The shift in the prooxidant-antioxidant equilibrium toward the intensification of free radical reactions is also indicated by an increase in the coefficients: KSOD/CAT, KSOD/GPx. The valuesof these coefficients depend on the severity of the resulting cold injury.

Cold trauma is characterized by the development of inflammatory-destructive processes, manifested in the increase in the activity of enzymes in the blood: ALT, AST, γ-GT, ALP. In addition, patients with cold trauma experienced an increase in cholesterol, and its concentration in the blood increases in direct proportion to the degree of tissue damage and the level of inflammation.

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