THE SUBCHRONIC TOXICITY OF DIFFERENT CONCENTRATIONS OF REACTIVE OXYGEN SPECIES AND BLOOD METABOLISM IN THE EXPERIMENT

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The study of the processes of free radical oxidation and energy metabolism of blood under the influence of different ozone concentrations during long use in the experiment have been conducted. Wistar rats were used in the work. Ozone were injected animals intraperitoneally daily for 30 days in the form of ozonated physiological solution with different doses of the drug in him – 0,6; 2,0 and 8 mcg. The intensity of lipid peroxidation, activity of superoxide dismutase, lactate dehydrogenase, the concentration of lactate and glucose was investigated in blood. General patterns of changes of blood biochemical parameters of rats show the toxic effects of ozone on the body with growth its concentration. The activity of superoxide dismutase increased when using only oxygenated physiological solution and O_3 at a concentration of 3000 mcg/l. The data obtained allow to conclude that optimal metabolic dose of ozone for the blood is 0,6 mcg.

Keywords: ozone, dose, lactate, glucose, lactate dehydrogenase, lipid peroxidation, blood

Ozone therapy is a new trend in modern medicine today [1–3]. The high redox potential of ozone is basis of its therapeutic action [4]. The use of oxidative therapies inextricably linked to the study of lipid peroxidation, as the change in the balance of pro – and antioxidant systems is one of the diagnostic criteria of severity of a pathological condition, describing the formation and progression of oxidative stress [2]. In addition, the use of ozone may be appropriate due to its multiple effects on metabolism [4]. Therefore, monitoring of free radical processes and the activity of oxidoreductase enzymes and their substrates in the blood allows you to monitor the effectiveness of ozone therapy, correcting the outcome of treatment [8]. However, the use of ozone is limited by the choice of doses. The use of this resource for the treatment and prevention of diseases is based on a wide range of therapeutic effects of different concentrations of ozone on the body. Oxygen radicals generated by the action of ozone, may have a positive effect and toxic effect on the cells and tissues of the body. To date, discussions have occurred, the subject of which is the question of "therapeutic" and "pernicious" effects of ozone on the body [10]. Given the known dose-dependent effect of ozone, it is necessary to assess the nature of the effects used in the clinic doses of ozone on the balance of pro – and antioxidant systems of the blood, the concentration of lactate and glucose in erythrocytes and plasma, as well as lactate dehydrogenase activity of erythrocytes. Taking into account this fact, this work is of practical and scientific interest. The aim of the study was to investigate the possible toxic effects of different concentrations of ozone on lipid peroxidation and energy metabolism in rat blood during prolonged use.

Materials and methods of research

Experiments were conducted on 50 white rats of Wistar line with weighing 160–180 g contained on a standard diet of vivarium. The animals were divided into 5 groups of 10 animals in each: group 1 – control (intact healthy rats); group 2 – animals were injected 1 ml oxygenated physiological solution daily intraperitoneally; 3, 4, 5 groups of animals were daily intraperitoneally injected ozone in the amount of 1 ml for 30 days in the form of ozonated physiological solution with different ozone doses in him – 0,6; 2,0 and 8 mcg at saturating concentrations of ozone in the ozone-oxygen mixture 3000; 10000; 40000 mcg/l, respectively. After month the animals were scored by decapitation under combined anesthesia (Zoletil (60 mg/kg) + Ksila (6 mg/kg)).

Ozone was obtained from the oxygen produced by the oxygen concentrator, using the medical device of ozone therapy "Medozons Systems" The ozone concentration in the physiological solution was determined with help of ozone analyzer in liquid media "IKOZH-5". In blood was investigated parameters of free radical oxidation (FRO), activity of superoxide dismutase and lactate dehydrogenase, the level of malonic dialdehyde, lactate and glucose.

The activity of the FRO processes were studied using the method of induced biochemiluminescence on the biochemiluminometer BCHL-06 (N. Novgorod). The following settings of biochemiluminogram were assessed:

tg 2α – parameter of the rate of decrease of free radical oxidation processes in the plasma shows the total antioxidant activity (TAA);

S – light sum of chemiluminescence for 30 sec – reflects the potential possibility of a biological object to free radical oxidation.

The level of malonic dialdehyde (MDA) in plasma and hemolysate of erythrocytes (1:10) was estimated by the method of M. Uchiyama and M. Mihara [7]. The concentration of glucose and lactate in plasma and erythrocytes was measured on the analyzer «SUPER GL ambulance» (Germany).

Lactate dehydrogenase activity in the direct reaction (LDHdr) was evaluated in the hemolysate of erythrocytes (1:40) using lactic acid as the substrate, in the reverse reaction (LDHrr) – using pyruvic acid [6]. The activity of superoxide dismutase (SOD) was determined in the hemolysate of erythrocytes (1:10) on the inhibition of the formation of the product of adrenaline autooxidation [5]. The protein concentration was determined by Lowry's method in the modification [9]. Statistical analysis of the results of studies was performed using Statistica 6.0.

Results of research and their discussion

During the studies the growth trend of free radical oxidation of blood plasma was noted when using oxygenated physiological solution and under the action of ozone at a concentration of 3000 mcg/l, the index *S* has increased by 7 and 12%, respectively, compared with healthy animals (Table. 1). The use of ozonized physiological solution with concentration of ozone 10000 and 40000 mcg/l was reduced lipid peroxidation in plasma of rats at 10 and 11%, respectively, compared to control animals. However in erythrocytes was a tendency to decrease free radical oxidation under the action of oxygenated physiological solution on 15%, at the concentration of 3000 mcg/l O_3 by 17%, the concentration of 10000 mcg/l O_3 – on 8% compared with healthy animals. When using an ozone concentration of 40000 mcg/l was marked the increase in lipid peroxidation in the erythrocytes of rats. Under the influence of oxygenated physiological solution and used ozone concentrations activation of total antioxidant activity of blood plasma was happened, the most marked when the ozone concentration was 3000 mcg/l: under the action of O_3 concentration of 3000 mcg/l TAA increased by 79%, in a concentration of 10000 mcg/l O_3 – 30%, at a concentration of 40000 mcg/l – 45%, when applying oxygenated physiological solution – on 59% compared with healthy rats. The increase of activity of antioxidant enzymes SOD under the action of ozone at a concentration of 3000 mcg/l at 34%, with a concentration of 10000 mcg/l is only 5%, when using oxygenated physiological solution by 57% and decrease of SOD activity at the concentration of ozone 40000 mcg/l at 45% compared with the control was revealed.

Table 1

Indicators of lipid peroxidation and antioxidant activity
in the blood of rats under the influence of reactive oxygen species

The experimental conditions	S in plasma, notional units	tg 2α, notional units	S in erythrocytes, notional units	SOD, notional units/mg of protein
Control rats	10,55 ± 0,90	0,44 ± 0,03	8,05 ± 0,73	242,02 ± 22,01
O_2	11,33 ± 1,02	0,70 ± 0,06*	6,86 ± 0,62	382,50 ± 32,21*
3000 mcg/l O_3	10,87 ± 1,02	0,79 ± 0,06*	6,70 ± 0,58	323,70 ± 28,20*
10000 mcg/l O_3	9,55 ± 0,86	0,57 ± 0,04*	7,44 ± 0,68	256,00 ± 23,21
40000 mcg/l O_3	9,34 ± 0,84	0,64 ± 0,06*	8,41 ± 0,72	132,70 ± 12,11*

N o t e : * – the differences are statistically significant compared to control rats ($p < 0,05$).

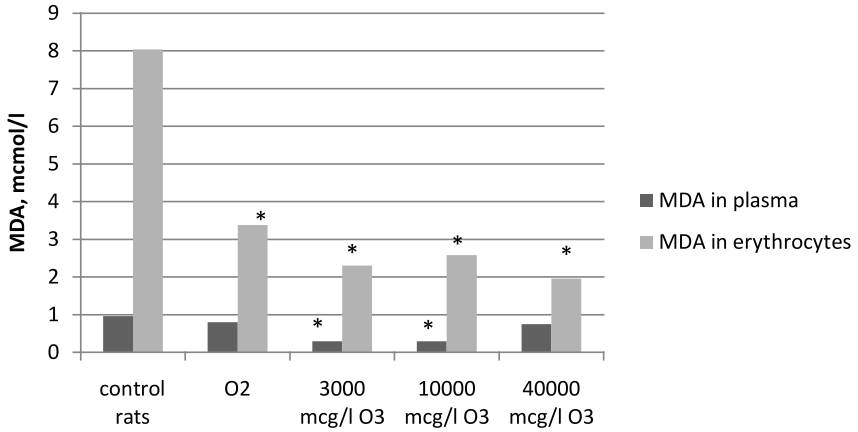

Fig. 1. The concentration of malonic dialdehyde (mcmol/l) under the influence of reactive oxygen species.
Note: * – the differences are statistically significant compared to control rats ($p < 0,05$)

Thus a reduction in the quantity of product of lipid peroxidation, malonic dialdehyde, was installed in plasma and erythrocytes under the influence of ozonized physiological solution. When using an ozone concentration of 3000 mcg/l, the concentration of MDA in plasma was reduced by 70%, in a concentration of 10000 mcg/l O_3 by 70%, at a concentration of 40000 mcg/l by 22%, when using oxygenated physiological solution by 17% compared with healthy rats. When use ozone concentration of 3000 mcg/l, the level of MDA in erythrocytes decreased by 72%, in a concentration of 10000 mcg/l O_3 – on 68%, in a concentration of 40000 mcg/l – on 76%, under the action of oxygenated physiological solution by 58% compared with healthy rats (Fig. 1).

Thus, in a wide range of ozone concentrations information about the dose-dependent nature [10] reaction of free radical oxidation for prolonged systemic use of ozone was confirmed. The regularity was confirmed not only on the basis of data of biochemiluminescence, but also study the quality of intermediate products of FRO, and SOD activity. In response to the introduction of O_3 the level of malonic dialdehyde decreases amid significant elevation of superoxide dismutase activity of erythrocytes. The total antioxidant capacity of plasma increases, probably due to the greater concentration of lipoproteins, ceruloplasmin, albumin, serotonin, insulin. Thus, the most pronounced changes in the system of pro- and antioxidant protection system of blood was noted when using ozone in a concentration of 3000 mcg/l.

The molecular mechanisms of action of various drugs and biologically active compounds depend on the peculiarities of the regulation of enzymes. There is no doubt that in this case LDH plays an important role in the regulation of energy metabolism of the cell [6]. In the direct reaction pyruvate is formed from lactate. Pyruvate can be used in the Krebs cycle under aerobic conditions. Return LDH reaction leads to the formation lactate from pyruvate and characterizes the severity of the anaerobic process in the cell. It is shown that under the action of ozone at a concentration of 3000 mcg/l LDH activity in the direct reaction increased by 66%, in a concentration of 10000 mcg/l O_3 – by 55%, at a concentration of 40000 mcg/l – by 78% compared with healthy animals (Fig. 2). The use of oxygenated physiological solution resulted in a decrease LDHdr by 22% compared with the control. The increased activity of the direct reaction of LDH is typical for aerobic conditions of the erythrocytes metabolism. Increased activity of LDH reduces of lactate and the accumulation of pyruvate. Therefore the activation intensity aerobic processes and the rate of metabolism in erythrocytes emanates under the O_3 influence. Lactate dehydrogenase activity in the reverse reaction was statistically significantly increased in ozone concentration of 3000 mcg/l to 58%, in a concentration of 10000 mcg/l O_3 – by 39%, at a concentration of 40000 mcg/l by 34% compared with control animals.

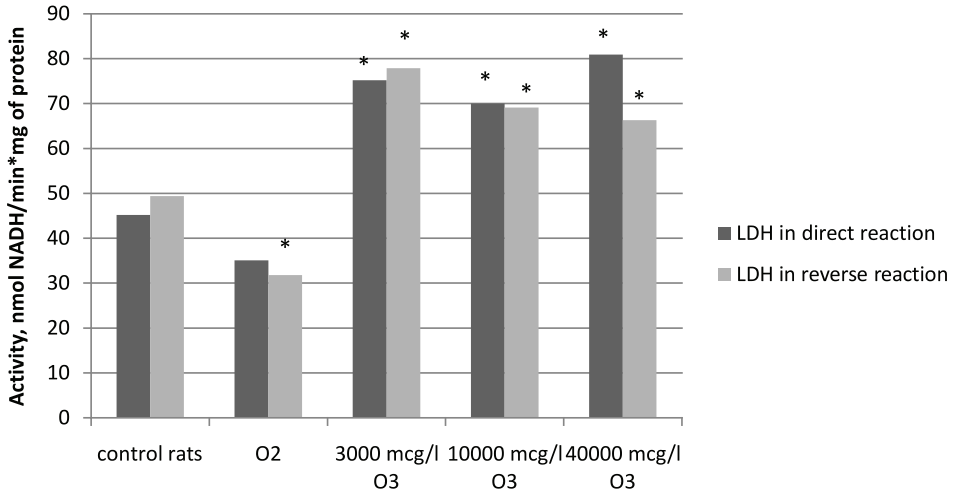

Fig. 2. The activity of lactate dehydrogenase (nmol NADH/min·mg of protein) under the influence of reactive oxygen species. Note: * – the differences are statistically significant compared to control rats ($p < 0,05$)

Table 2

The level of glucose and lactate in the blood of rats under the influence of reactive oxygen species

The experimental conditions	Erythrocytes		Plasma	
	glucose, mmol/l	lactate, mmol/l	glucose, mmol/l	lactate, mmol/l
Control rats	1,26 ± 0,11	1,21 ± 0,11	9,24 ± 0,09	1,95 ± 0,16
O_2	0,83 ± 0,07*	1,53 ± 0,14	7,90 ± 0,72*	2,65 ± 0,24*
3000 mcg/l O_3	0,69 ± 0,06*	0,79 ± 0,07*	10,41 ± 0,94	2,94 ± 0,14*
10000 mcg/l O_3	1,85 ± 0,16*	1,54 ± 0,14	17,62 ± 1,61*	2,72 ± 0,19*
40000 mcg/l O_3	3,21 ± 0,30*	3,11 ± 0,29*	17,32 ± 1,57*	2,47 ± 0,22*

N o t e : * – the differences are statistically significant compared to control rats ($p < 0,05$).

The lactate level in erythrocytes was significantly decreased only when using an ozone concentration of 3000 mcg/l by 35%, under the action of ozone concentration of 10000 mcg/l and 40000 mcg/l, and oxygenated physiological solution concentration of lactate increased by 27%, 156 and 26%, respectively, compared with healthy animals (Table 2). In plasma it was revealed statistically significant increase of lactate by the action of ozone at a concentration of 3000 mg/l by 50%, concentration of 10000 mcg/l by 39%, in a concentration of 40000 mcg/l by 27%, when using oxygenated physiological solution – by 36% compared with the control. The level of glucose in the plasma under the action of ozone at a concentration of 3000 mcg/l did not differ from the level of the substrate control animals, at a 10000 mcg/l O_3 concentration of glucose in plasma was increased by 90%, at a 40000 mcg/l by 87% compared with control animals. When using an ozone concentration of 3000 mcg/l glucose levels in erythrocytes decreased by 45%, under the influence oxygenated physiological solution by 34% compared with control animals. The application of ozone concentration of 10000 and 40000 mcg/l resulted in increased levels of glucose in erythrocytes by 47 and 54%, respectively, compared with healthy rats. Thus, the ozone at a concentration of 3000 mcg/l affects on carbohydrate metabolism in the form of increased glycolysis, activation of glucose utilization, reduce the level of glucose in the blood. This is achieved through stimulation of the pentose phosphate shunt and aerobic glycolysis. The ozone concentrations 10000 and 40000 mcg/l resulted in an increase in the level of glucose in erythrocytes by 47 and 54% compared with the control.

Conclusion

Summarizing, we can conclude that under the influence of ozone observed the processes of activation of glucose utilization, lactate, pyruvate, reactions of oxidative phosphorylation, increases resistance of erythrocyte membranes. It should be noted that the general regularities of changes of blood biochemical parameters in rats by intraperitoneal injection of ozone at different concentrations indicate the possibility of toxic effects of O_3 on the body along with the growth of its concentration. A dose-dependent effect of ozone on the metabolism of erythrocytes was revealed. The data obtained in the study of free radical oxidation and energy metabolism allows to draw the conclusion that the optimal metabolic ozone dose for blood is 0,6 mcg, in which there are positive changes in concentrations of glucose and lactate in the blood, the activity of lactate dehydrogenase in erythrocytes of animals, as well as the normalization of the balance of pro- and antioxidant systems in the plasma and erythrocytes.

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