

# **The influence of ozone on the brain oxidation-reduction enzyme activity in normal condition and postresuscitation period**

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## **Abstract**

The influence of the ozonated physiological saline (OPS) on the oxidative metabolism and lipid peroxidation system in brain tissue of the intact animals and in the reduction period after the clinical death has been studied. The OPS with the 135 µg/l ozone was infused to the intact rats at the background of the nembutal narcosis. The reperfusion period was studied at the 5 min clinical death model, the ozonised autoblood with the 80 µg/l ozone was intracarotid infused in the resuscitation period.

The influence of ozone both on the intact and hypoxic brain of rats in conditions of postresuscitation pathology was expressed in the neuron aerobic metabolism intensification and insignificant activation of peroxidation, causing the adaptive intensification of the cell antioxidant enzymatic system. In the period of reperfusion there is the pentosophosphate way activation, directed to provision of the synthetic processes in the brain at the postresuscitation period.

## **Introduction**

Now major attention is given to study of a bioenergy hypoxia as to a general purpose damaging part at many pathology conditions (1). The development of a histic hypoxia plays a major role in a pathogeny of reperfusion damages of organs. Excavation of a hypoxia promotes not only absence in the postreperfusion period of regeneration of a bloodflow and development of a phenomenon no-reflow at a microcirculation level, but also infringement of oxidation-reduction enzyme activity in cells of heart and, especially, brain.

As a result of the decrease of ability cells utilise  $O_2$  in oxidation-reduction synthetic reactions of high-energy phosphates in tissues the glycolytic responses with accumulation of lactate prevail. Besides owing to infringement of work of a respiratory chain (2),  $O_2$  acting to cells at reperfusion, in the greater degree enters in response of free - radical oxidation (4). In this case application of drugs with antihypoxical properties not only would allow to sustain more effective aerobic energy metabolism in cells, but also to reduce a pathological activation of peroxide oxidation of lipids at appointment.

Variability in the pathogenesis of the postischemic disorders explain the lack of unified methods capable to prevent the development of reperfusion complications. Studying the pharmacological effect of the typical medical drugs we got interested in ozone as a substance

capable to influence on the metabolic processes in different organs and in the whole organism through ozonolysis of organic substrates (3). Ozone metabolic effect on erythrocytes as the first target-cells are well-known at present time (14, 15, 16). As for the systemic effect of ozonated blood on healthy organs and tissues and on those with the pathology it remains unclear (5, 6, 7, 10, 11, 12, 17, 18, 19). So our task was to study the effect of the low concentrations of ozone dissolved in blood on the metabolism and functional state of the brain - the organ subjected to hypoxic changes.

### **Materials and methods**

The experiments were carried out on white non-linear rats with the weight of 200-250 g. On the rats was used in normality and on acute hypobaric hypoxia model were appeared an especial of variations in energy metabolism in cells of the brain, lipid peroxidation state and enzymes of antioxidant system, oxidoreductase enzymatic activity, ECoG and the behavior of the rats after parenteral treatment of ozonated physiological solution in the 135 µg/l ozone. Ischemia of the brain, followed by the postischemic period was studied on the model of 5-min clinical death resulted from acute haemorrhage. The choice of the model was stipulated by the fact, that acute haemorrhage is known to be one of the main causes inducing total ischemia of organs due to sharp decrease of pressure, cardiac arrest and respiratory failure. The animals on being anaesthetised with 35 mg/kg of Nembutal were done tracheotomy and carotid artery catheterization. The maximal amount of blood was let out through the catheter until the respiratory and cardiac failure was registered. To prevent coagulation the rats were injected heparin dose of 500 U/kg before the bloodletting. Conventional resuscitation measures were started in 5 minutes of the clinical death (the time counted off the last inhalation). In the course of resuscitation there were not used any preparations with the stimulating effect on cardio-vascular or nervous systems. During the postreanimation period the animals of the experimental group were intraarterially infused 1 ml of autoblood treated by ozone in the dose of 80 µg/l, ozone being generated by medical ozonator. The following data were registered: arterial pressure, respiratory rate, EKG, ECoG. Neurological deficiency was defined by the time of corneal and pain reflexes.

After the 60 minutes of postreanimation period the tissues of brain were placed into the liquid nitrogen and then taken for the lactate, pyruvate and adenilylic nucleotides analysis, products of lipid peroxidation and antioxidant enzyme activity - the superoxidedismutase and catalase. Histochemical methods were used to assess oxidoreductase enzymatic activity in the sensorimotor zone of the cerebral cortex considering the number and the character of the final product (diformasane) accumulation according to the 5-mark scale.

### **Results**

The introduction anaesthetized rats ozone physiological solution with concentration of the ozone 135 µg/l rendered influence on activity some dehydrogenase. Through 60 minutes of the observable period the decrease of activity PDG was marked at increase of activity NADH DG and  $\beta$ -obDG, that testified to possible increase of inclusion in aerobic metabolism not characteristic for a brain substrates of oxidation – free fatty acid.

Therefore, probably, both the activity PDG is reduced and the recycling pyruvate utilisation in a cycle Krebs, despite of sufficient activity aerobic ways of glucose transformation in brain is braked. The experiments have shown, that at model operation of a hypobaric hypoxia the ozonated physiological solution has the expressed antihypoxic properties.

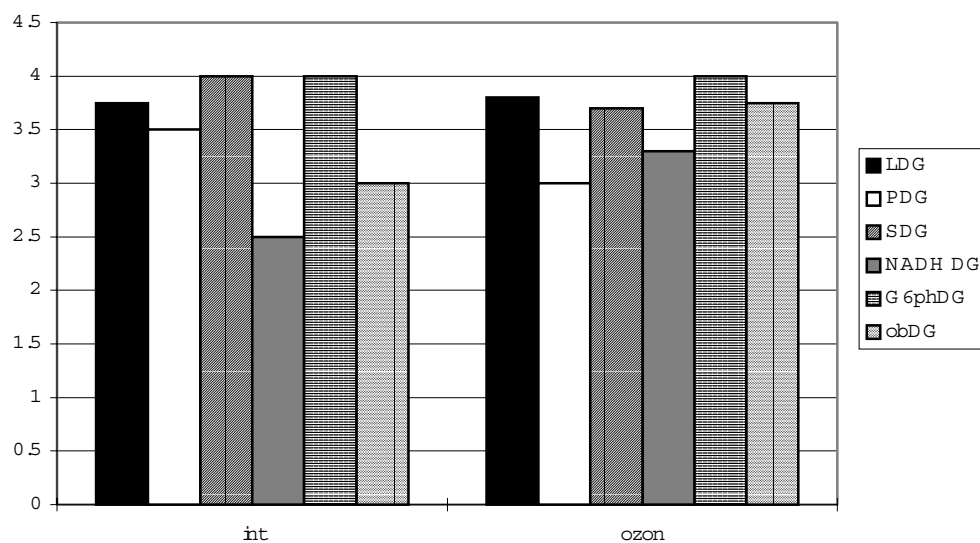


Fig.1. Oxidoreductase enzymatic activity in the sensoriomotor zone of cerebral cortex of the intact rat

The rising of a resistance of a brain to a hypoxia at the rats descended for account of an activation of aerobic energy processes at preventive application, to what the augmentation of activity NADH DG and  $\beta$ -obDG, increase of an interrelation ATP/ADP testified, and also adaptable intensifying of activity of ferment system of antioxidant protection of cells, about what it is possible to judge on accumulation in a tissue of a brain of Schiff bases through 60 mines after introduction of solution on 18 % and rising of activity SOD on 64 %. On a background of narcosis the introduction of an ozonized solution enlarged activity of narcosis on the data ECoG, that, probably, is connected to an activation opioid system.

After the introduction of an ozonated blood the early postreanimation period experimental animals compared with the control ones were found to restore the functional indices of their cardiac and brain activity more quickly and in a better volume (Fig.2).

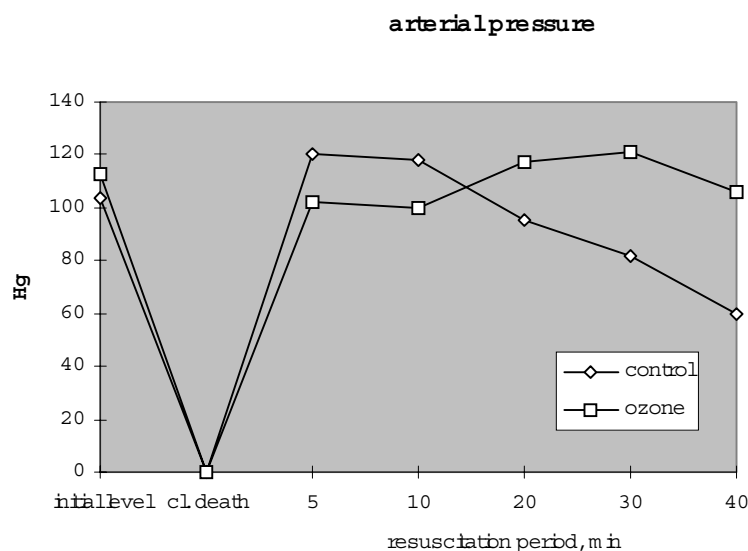


Fig. 2. Influence of blood ozonation on the arterial pressure respiratory rate

Blood ozonation helps to shorten the time necessary to restore independent respiration and corneal reflex, but does not influence on the pain reflex (Fig. 3). ECoG data reveal the initial bioelectric brain activity to be restored completely. During 30 mines the prevalence of low-frequency fluctuations was observed, and to 40 mines there were high-frequency waves, the increase of waves of a  $\theta$ -range was especially characteristic. However to 60 mines in frequency a spectrum ECoG of a brain waves of a  $\delta$ -range nevertheless dominated.

In experimental animals it takes less time to restore spontaneous respiration and to normalise arterial pressure without the hypofunctional stage on the 30th minute of the postischemic period. The use of ozone was not found to produce any effect on the cardiac contraction rate.

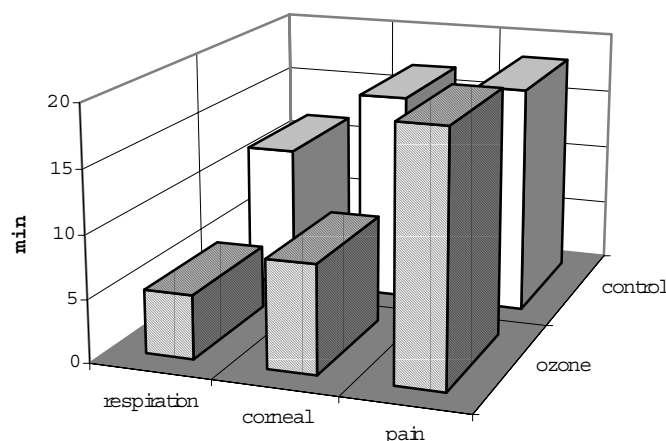


Fig. 3. Revivification of independent respiration, corneal and pain reflexes in postischemic period

Biochemical tests data show the increase of ATP level in the brain. The amount of ATP in the brain of experimental animals was higher than that in intact rats (Table 1). Lactate and pyruvate content was diminished compared with the control group but still higher than that in intact animals. At the same time lactate/pyruvate ratio in the experimental group was lower compared with the cardiac tissue of intact rats and brain tissue of control animals.

Table 1. The concentration of ATP (mmol/g w.w.), lactate and pyruvate (mmol/g w.w.) in brain tissues in postischemic period

|         | ATP            | Lactate        | Pyruvate       | Lactate/Pyruvate |
|---------|----------------|----------------|----------------|------------------|
| intact  | 1,431 ± 0,109  | 0,528 ± 0,031  | 0,228 ± 0,021  | 2,414 ± 0,191    |
| control | 1,571 ± 0,016  | 1,122 ± 0,082* | 0,388 ± 0,045* | 3,287 ± 0,268*   |
| ozone   | 1,782 ± 0,070* | 0,866 ± 0,076* | 0,311 ± 0,048* | 2,780 ± 0,162    |

• \* -  $P < 0,05$  as compared to intact group

Histochemical analysis of oxidoreductase enzymatic activity in the sensorimotor zone of cerebral cortex revealed the decrease in the activity of succinate dehydrogenase (SDG) and NADH- dehydrogenase with the increase in the activity of lactate dehydrogenase (LDG), favouring the lactate acidosis decrease (Fig. 4).. At the background of the pyruvate

dehydrogenase (PDG) activity normalisation, the  $\beta$ -obDG activity increase is observed, compared with the controls. The glucose-6-phosphate dehydrogenase (G-6phDG) activity increase is also noted.

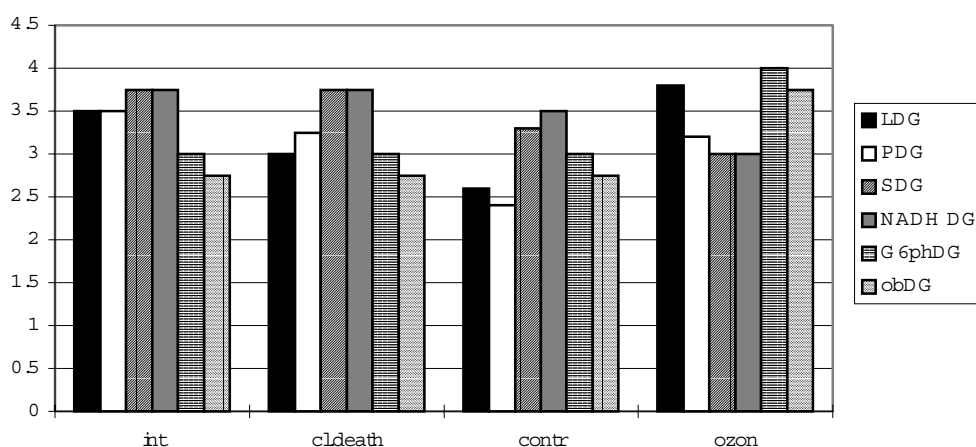


Fig.4. Oxidoreductase enzymatic activity in the sensorimotor zone of cerebral cortex in postischemic period

The study of processes lipid oxidation of a head brain in the given series is especially urgent, as the additional application of oxidizing methods of therapy alongside with restoration oxygen metabolism of the organism is capable to cause excessive activation a LPO at failure antioxidant protection of cells in reperfusion conditions.

In a control series in postreanimation period through 60 minutes of restoration of activity of the organism the high level of the contents of products a LPO - quantity TK is marked the index oxidation of double communications - on 41 % is increased by 129 %, Schiff bases - on 45 %, at constant activity SOD. The use of the OPS in the period of reperfusion increases the catalase activity and due to this fact doesn't cause the LPO response intensification in the brain tissue, stimulated by reoxygenation at the blood flow renewal. The SOD activity in that period was decreasing because of that link of the antioxidant protection exhaustion or the enzyme inactivation in that period of time.

## Discussion

Ozonated solution induces an increase of metabolism processes rate in cells of the brain and also some activation of lipid peroxidation and enzyme system of antioxidant protection. In the sequent hypoxic stress was registered the antihypoxic properties of ozonated physiological solution.

The use of ozonated blood in 60 minutes of postischemic period leads to activation of oxidative metabolism in brain and heart cells. Small doses of ozone on being infused into blood cause the increase in the activity of a number of glycolytic enzymes in the erythrocytes (15), similar effect is observed in lung cells on ozone inhalations.

In our experiments we noticed specific changes in the indices of carbohydrate metabolism. In the control group the brain cortex revealed prevailing anaerobic processes of glucose utilisation, lactate accumulation with decreased oxidoreductase activity. The experimental

group was found to have the increase in LDG, PDG activity with the decrease in lactate amount. It might be explained by the intensity of aerobic ways of glucose transformation. Ozone effect, or to be more precise, the effect of blood ozonolysis products on the oxidative processes that take place in the brain cells makes it possible to restore the functional activity of the organs responsible for the viability of the whole organism in less time.

Thus, the received tendency of changes in metabolism and function of the examined organs makes it possible to recommend ozone as a preventive or corrective method in hypoxia pathologies in post ischemic period.

## **Conclusions**

The influence of the ozonated physiological saline on the oxidative metabolism and lipid peroxidation system in brain tissue of the intact animals and in the reduction period after the clinical death has been studied.

The pyruvate level increase at the lactate amount preservation is observed in 60 min after the OPS infusion in the intact group which testify to the glycolysis activation in the brain tissue along the aerobic way. At the same time, the slight decrease of the pyruvate dehydrogenase activity is observed, but the  $\beta$ -oxybutyrate dehydrogenase activity.

It is possible that the glucose metabolism at that period was primarily directed to provision of the synthetic processes in the brain. The increase of the NADH-dehydrogenase, the first enzyme of the aerobic oxidative process intensification at the OPS infusion to the intact rats.

Besides, the single infusion of the OPS to the intact animals causes the adaptive increase of the SOD activity in the brain tissue at the LPO slight intensification. The ozonated autoblood infusion in the reperfusion period causes the lactate accumulation decrease in the brain and the pyruvate content increase. The lactate dehydrogenase activity in this period is increased, favouring the lactate acidosis decrease. At the background of the PDG activity normalisation, the  $\beta$ -obDG activity increase is observed. The glucose-6-phosphate dehydrogenase activity increase is also noted. The use of the OPS in the period of reperfusion increases the catalase activity and due to this fact doesn't cause the LPO response intensification in the brain tissue, stimulated by reoxygenation at the blood flow renewal (8). The SOD activity in that period was decreasing because of that link of the antioxidant protection exhaustion or the enzyme inactivation in that period of time. Ozone effect, or to be more precise, the effect of blood ozonolysis products on the oxidative processes that take place in the brain cells makes it possible to restore the functional activity of the organs responsible for the viability of the whole organism in less time.

The received tendency of changes in metabolism and function of the examined organs makes it possible to recommend ozone as a preventive or corrective method in hypoxia pathologies in post ischemic period (13).

Thus, the influence of ozone both on the intact and hypoxic brain of rats in conditions of postresuscitation pathology was expressed in the neuron aerobic metabolism intensification and insignificant activation of peroxidation, causing the adaptive intensification of the cell antioxidant enzymatic system. In the period of reperfusion there is the pentosephosphate way activation, directed to provision of the synthetic processes in the brain at the postresuscitation period.

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