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Mechanisms of the Effects of Ozone

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ABSTRACT

The analysis of the literature and own data on the study of the mechanisms of the physiological effect of ozone on the erythrocyte link of the blood system was carried out. The effect of ozone on the oxygen-binding function of blood and its prooxidant-antioxidant balance is discussed. This effect of this gas under conditions of different partial pressure values of oxygen and carbon dioxide manifests itself in different ways. The effect of ozone is realized with the participation of gas transmitters, which realize their effect through intraerythrocyte mechanisms of formation of hemoglobin affinity for oxygen, which justifies its use as a means of improving the adaptive and antihypoxic capabilities of the body.

Keywords: Ozone; Blood; Oxygen; Gas Transmitters; Nitrogen Monoxide; Hydrogen Sulfide

Introduction

The use of ozone as an autohemotherapy based on taking blood from a patient, saturating it with ozone and then reintroducing it into the body is widespread, but the mechanisms of action of this gas under these conditions (*in vitro*) remain undisclosed [1]. In the body, the oxygen-hemoglobin dissociation curve (ODC) largely determines the diffusion of oxygen from the alveolar air into the blood, and then at the capillary level into the tissue. The shift of the oxyhemoglobin dissociation curve to the right is aimed at compensating for oxygen deficiency, and in conditions of oxidative stress, when oxygen utilization by tissues is disrupted, affects the activity of free radical oxidation processes [2]. There are isolated studies on the direct effect of O_3 on ODC [3].

Hypoxia and Hyperoxia

It seems important to evaluate the features of the effect of O_3 on blood oxygen transport function (OTF) under conditions of different values of pO_2 and pCO_2 [4,5]. We have shown that oxygenation enhances the effect of ozone on blood OTF. Sodium hydrosulfide and nitroglycerin increase this effect, especially the latter, namely, pO_2 and SO_2 increase by 25.95% ($p<0.05$) and 23.17% ($p<0.05$), respectively (the p50 real index increases by 24.54% ($p<0.05$)). The participation of gas transmitters in changes in blood OTF when exposed to ozone during oxygenation is noted. Hyperoxia under the action of ozone contributes to an increase in the content of NO_3-/NO_2- and H_2S, and the addition of nitroglycerin and sodium hydrosulfide increase these indicators. Pre-oxygenation under the action of O_3 leads to an increase in the content of NO_3-/NO_2- and H_2S in blood plasma by 24.73% ($p<0.05$) and 38.11% ($p<0.05$), respectively, compared with the group into which only ozone was injected. Nitroglycerin under oxygenation conditions contributes to an increase in the concentration of NO_3-/NO_2- by 97.9% ($p<0.05$) and H_2S by 59.99% ($p<0.05$). Erythrocytes due to the expression of catalytically active nitric monoxide synthase (NOS3 type 1), which is identical to that expressed in the endothelium, induces the synthesis of NO [6].

This substance is a strong mitochondrial regulator, reducing the oxygen affinity of cytochrome c oxidase, the terminal electron acceptor of the mitochondrial electron transport chain. Erythrocytes are involved in maintaining an oxygen-dependent balance between the formation and oxidation of H_2S due to their ability to generate this gas transmitter [7]. Pre-oxygenation does not significantly change the state of the prooxidant-antioxidant balance, and the addition of nitroglycerin and sodium hydrosulfide under these conditions contributes to an increase in diene conjugates, malondialdehyde, and catalase activity. Ozone can have both a pro- and antioxidant effect, depending

on the concentrations used. Thus, in blood samples exposed to O_3 at a dose of 160 mg/ml, an increase in malondialdehyde (MDA) is observed [8], and in patients with insomnia and coronary heart disease, ozone therapy promotes the growth of catalase. Under oxygenation conditions, when exposed to ozone and the addition of nitroglycerin, an increase in MDA and diene conjugates (DC) is observed, and sodium hydrosulfide does not have a similar effect.

Isoforms of NO are considered as dynamic sensors of cellular oxidative stress and regulators of redox-homeostasis. The mechanism of the positive effect of ozone therapy is manifested in an increase in the activity of antioxidant enzymes in blood serum [9]. In our experiment, the addition of nitroglycerin and sodium hydrosulfide under oxygenation conditions leads to an increase in catalase activity. Pre-deoxygenation weakens the effect of ozone on blood OTF, and nitroglycerin prevents the manifestation of this effect [10]. Deoxygenation reduces the effect of O_3 on pO_2 and SO_2 indicators in comparison with the group in which only ozonation was performed, while p50real decreases by 14.57% ($p<0.05$). In hypoxia, there is a deterioration in the processes of tissue transcapillary oxygen exchange, a decrease in the capabilities of the microcirculatory bed for oxygen delivery to tissues. The functional interrelations of various components of microhemodynamics regulation and blood OTF parameters during the hypoxic test were revealed [11]. Ozone significantly improves microcirculation by increasing the functional activity of capillaries, changing the physico-chemical characteristics of blood and, in particular, the oxygen-binding properties of blood [12]. As we can see from the experiments conducted, changes in blood OTF are noted under the influence of ozone under hypoxic conditions and with the addition of gas transmitter donors. The effect of ozone in hypoxic conditions leads to an increase in the content of NO_3-/NO_2- and H_2S, and the addition of nitroglycerin and sodium hydrosulfide increase these indicators [10]. NO ensures the body's adaptation to changes in pO_2 through its influence on the mechanisms of formation of the functional status of red blood cells. In addition, this gas transmitter is the main regulator of vascular tone and an angioprotector, which causes its effect on local microcirculation [11].

The complex relationship between O_3 and a group of NO synthase enzymes generating NO formation are described. O_3 can activate inducible nitric oxide synthase, which leads to an increase in the concentration of the latter, and also restores NO_2– at low pH values [13]. Another gas transmitter hydrogen sulfide is also involved in these mechanisms. Erythrocytes produce endogenous H_2S using 3-mercaptopyruvate as a substrate [14]. The production of H_2S in red blood cells depends on the level of NO in the blood serum [15]. Deoxygenation under the influence of ozone leads to a decrease in lipid peroxidation indicators (malondialdehyde, diene conjugates), as well as a decrease in retinol and α-tocopherol, including in the group with nitroglycerin. H_2S also performs antioxidant functions due to modifications of enzyme activity, including catalase [16].

Hypocapnia and Hypercapnia

Pretreatment with a hypocapnic gas mixture significantly increases the effect of ozone on blood OTF. Nitroglycerin under these conditions leads to an increase in the effect of this gas and is characterized by an increase in pO_2 by 17.81% ($p<0.05$), SO_2 by 9.83% ($p<0.05$), p50real increases by 3.23% ($p<0.05$), compared with the group that underwent pre-hypocapnia and ozone treatment [17]. It could be assumed from the nature of the change in pH and pCO_2 that due to the Borh effect, the ODC will shift to the left, however, the results we obtained indicate the opposite. A decrease in the concentration of hydrogen ions and the partial pressure of carbon dioxide (pCO_2) significantly reduces the affinity of blood to oxygen, which makes it difficult for oxygen to enter tissues at the level of capillaries of a large circle of blood circulation, and vice versa with an increase in these parameters (the Bohr effect). A number of studies have shown that hypocapnia contributes to an increase in the concentration of 2,3-diphosphoglycerate in erythrocytes [18].

In addition, it is known that hypocapnic conditions enhance the generation of ozone synthesis [19], the effects of which, in turn, are dose-dependent. It should be noted that the ability of peroxynitrite in the hypocapnic environment to increase the values of the hemoglobin to oxygen affinity (HOA) p50real and p50stand in comparison with the control group [20], which indicates a significant contribution of the nitrogen monoxide gas transmitter to the regulation of oxygen transport by blood. Probably, these facts lead to an increase in the effect of O_3 under given conditions. The addition of nitroglycerin under these conditions leads to an even more pronounced increase in the effect of ozone on blood OTF, accompanied by an increase in the content of NO_3-/NO_2- and H_2S. Sodium hydrosulfide does not have a similar effect on the oxygen-binding properties of blood, despite an increase in concentrations of NO_3-/NO_2- and H_2S. Nitroglycerin and sodium hydrosulfide under hypocapnia conditions promote the growth of NO_3-/NO_2- and H_2S [17]. The bioactivity of NO is preserved in erythrocytes due to its interaction with cystioles in hemoglobin with the formation of S-nitrosothiol, S-nitrosohemoglobin [21], the release of which occurs with a decrease in HOA. Under hypocapnic conditions, the addition of O3 probably leads to an increase in the content of NO derivatives, and the addition of nitroglycerin (a NO donor) enhances this effect. Ozone also contributes to an increase in the level of nitrogen monoxide, activating the mechanisms of its formation in the erythrocyte [22].

In response to the action of ozone in erythrocytes, the formation of NO and hydrogen sulfide gas transmitters changes, which directly affects the modification of hemoglobin properties, and indirectly through hemoglobin-independent mechanisms, changes in the structural organization of the erythrocyte membrane. Carbon dioxide molecules can also be involved in this process, namely, reducing the partial pressure of this gas. Hypercapnia enhances the effect of ozone

on blood OTF and is characterized by a shift of ODS to the right, however, under these conditions, nitroglycerin and sodium hydrosulfide do not change the parameters of the blood gas transport function, and hypercapnia enhances the effect of O_3 [23]. An increase in the concentration of hydrogen ions and pCO_2 significantly reduces the affinity of blood for oxygen, which changes its flow into tissues at the level of capillaries of a large circle of blood circulation. In our studies, a decrease in pH and an increase in pCO_2, arising through the implementation of the mechanisms of the Borh effect, lead to an increased effect of ozone on blood OTF indicators, manifested in a more pronounced shift of HOA to the right.

The mechanisms of intraerythrocyte regulation of HOA are realized at various levels: a change in the structural organization of erythrocytes, modeling the effect of allosteric effectors on the hemoglobin molecule. In response to the action of ozone in erythrocytes, the formation of NO and H_2S gas transmitters changes, which directly affects both the modification of hemoglobin properties and indirectly – through hemoglobin-independent mechanisms – a change in the structural organization of the erythrocyte membrane. As you can see, a carbon dioxide molecule is also involved in this process, namely, when its partial pressure increases. In some cases, gas transmitters (NO and H_2S) can have a synergistic effect, but in experiments with hypercapnia this was not observed – an increase in hydrogen sulfide content was noted in the absence of a change in the activity of the L-arginine-NO system, which does not lead to an increase in NO concentration, and preliminary hypercapnia with the addition of ozone leads to an increase in hydrogen sulfide levels [23],

This may be due to an increase in the production of 3-mercaptopyruvate sulfurtransferase under these conditions, which promotes the synthesis of H_2S in erythrocytes [14]. Under conditions of preliminary hypercapnia and the introduction of ozone, an increase in the level of hydrogen sulfide by 59.21% ($p<0.05$) was noted without changing the content of NO metabolites in comparison with the group into which only ozone was injected. It is noteworthy that the most pronounced increase in nitrate/nitrite is observed in the group with nitroglycerin, and the highest level of H_2S is in the group with sodium hydrosulfide. However, there are no changes in the nitrate/nitrite content in this group, which may be due to the participation of erythrocyte carbonic anhydrase in regulating their distribution between plasma and erythrocytes under these conditions [24]. When nitroglycerin and sodium hydrosulfide are added, an increase in NO and H_2S is observed, which is due to their mutually conjugate effect on each other's synthesis [25]. Preliminary hypercapnia does not eliminate the effect of ozone on free radical oxidation processes, and gas transmitter donors also do not change the studied parameters [23]. A significant increase in MDA, DC in groups with O_3, indicates the activation of lipid peroxidation processes, which reflects a certain risk when using ozone as a therapeutic agent. Consequently, hypercapnia does not eliminate the activating effect of this gas on lipid peroxidation processes, and nitroglycerin and sodium hydrosulfide, moreover, aggravate it.

The tension of the antioxidant defense mechanisms, judging by the increased concentration of retinol and α-tocopherol, is obviously associated with their release from the erythrocyte membrane and is a consequence of oxidative damage caused by ozone [26]. At the same time, there is an increase in catalase activity in hemolysates with the addition of O_3. This gas, being an oxygen donor, affects the enzymatic component of the antioxidant system of erythrocytes, which provides protection from reactive oxygen species [27], when neutralized, hydrogen peroxide is formed, which leads to an increase in catalase activity. Our studies demonstrate the effect of ozone on blood OTF at the level of the erythrocyte unit of the blood system, which is realized by modifying the intraerythrocyte system of regulation of HOA, which allows us to meet the needs of aerobic metabolism at the regional and systemic levels.

The modification of HOA is achieved as a result of rearrangements in the membrane organization of erythrocytes (Band-3), changes in their metabolism, ionic composition, the action of a number of modulators (2,3-Bisphosphoglyceric acid, glutathione, etc.), as well as the effects of Borh and Haldane. According to the data we had obtained, which were described in detail above, the adaptive changes in the functional properties of hemoglobin under the action of O_3 and under conditions of different values of pO_2 and pCO_2 involve a system of gas transmitters (NO, H_2S), providing local tissue oxygen needs. Thus, the effect of ozone has a significant effect on the systemic mechanisms of the blood responsible for the formation of oxygen homeostasis of the body.

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