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THE ANTIOXIDANT ACTION OF CO2 - ONE OF THE UNIVERSAL MECHANISMS OF CARBOXYTHERAPY

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Abstract

Carboxytherapy (carbon dioxide treatment) is a promising area and an alternative method of treating many diseases. Carbon dioxide (CO2) is constantly present in the body, being the end product of cellular metabolism. It accumulates in tissues, diffuses into the blood, and is transferred to the lungs in three forms: dissolved in plasma, in bicarbonates, and as oxyhemoglobin in erythrocytes. CO2 is totally indispensable for the normal course of biochemical, humoral, and tissue processes in the body, as it is a pacemaker of these processes.

Materials and research methods. Scientific publications in foreign and domestic journals on relevant topics over the past 5 years, Internet resources.

Research results and their discussion. Analysis of the above changes in the body under the action of CO2 and states of biochemical markers (ADMA and d-ROM, ROS, NO, NOS) under oxidative stress indicate that CO2 has antioxidant properties, and carboxytherapy is a pathogenetic therapy for diseases which pathogenesis includes oxidative stress. The ROS-inhibitory effect of CO2 is universal for different cell types and, obviously, for different species of living organisms.

Conclusions. Thus, it has been established that CO2 is a universal inhibitor of the reactive oxygen species generation by various human and animal cells. These data allow explaining the physiological and pathophysiological (medical) effects of CO2 in the mechanism of carboxytherapy in a new way from the standpoint of the biochemical role of ROS.

Key words: *antioxidant action, carbon dioxide, carboxytherapy.*

Carboxytherapy (carbon dioxide treatment) is a promising area and an alternative method of treating many diseases [1, 2, 3, 4, 5]. Carbon dioxide (CO_2) is constantly present in the body, being the end product of cellular metabolism. It accumulates in tissues, diffuses into the blood, and is transferred to the lungs in three forms: dissolved in plasma, in bicarbonates, and as oxyhemoglobin in erythrocytes [6, 7]. The main amount of carbon dioxide - up to 90% is transferred by blood plasma in the chemically bound state as a part of bicarbonates. Transport of CO_2 by blood from the tissues to the lungs in these forms is as follows: the more CO_2 is formed, the greater amount of it is transported from the tissues to the lungs. Increased concentration of CO_2 in the body is observed at active physical loads, and also at deep controlled breath. Accumulated in the blood CO_2 is a physiological stimulator of respiration: causes the respiratory reflex, one of the main physiological effects of CO_2 in the body [8, 9]. Therefore, carbon dioxide can be considered a "signaling molecule" indicating the danger of hypoxia [7, 10]. On the content of CO_2 in the blood depends the durability of binding of oxygen to hemoglobin and O_2 ingress in tissues, as well as maintaining a stable acid-base balance (pH) in the body [10].

Thus, CO_2 is totally indispensable for the normal course of biochemical, humoral, and tissue processes in the body, as it is a pacemaker of these processes. The body needs CO_2 no less than oxygen, because, affecting the cerebral cortex, respiratory and vascular centers, it ensures the functioning of the parts of CNS, responsible for the tone of blood vessels and bronchi, heart function, metabolism, hormone secretion, electrolyte composition of blood and tissues, as well as the rate of biochemical reactions of the organism [11, 12, 13, 14, 15].

In physiological concentrations of CO_2 helps to reduce the intensity of oxidative processes: the mechanism of this action of CO_2 is associated with the blockade of hydrogen ions. Reactive species of oxygen (ROS) include superoxide anion radical, hydroxyl radical ('OH), and hydrogen peroxide [16, 17]. ROS are formed in all cells that use oxygen for respiration. At physiological hypercapnia, CO_2 inhibits the formation of ROS, thus protecting the cells of the body from the destructive effects of the latter [17]. At the same time, CO_2 acts as a mediator, launching a cascade of physiological and biochemical mechanisms of regulation of all body systems (respiratory, nervous, cardiovascular, excretory, hematopoietic, immune, etc.). All these systems of vital activity of the organism play an important role in maintaining its homeostasis [12, 13, 15]. Therefore, at low blood CO_2 and bicarbonate levels in the body disrupts the cascade of natural physiological and biochemical reactions essential to maintain homeostasis. To prevent adverse manifestations of hypoxia, ischemia, and hypocapnia, it is necessary to maintain the physiological concentration of CO_2 in the blood, which is provided by carboxytherapy [18]. This therapy can act on many parts of the pathological symptom complex, which is associated with the multifunctional involvement of CO_2 in metabolic and reflex processes of systemic self-regulation of an organism, and also carboxytherapy can eliminate local problems: pain, spasm, hypoxia, ischemia, etc. [4, 7, 14, 19].

Carboxytherapy for therapeutic purposes began to be used in the first half of the twentieth century. The first article on the medical use of CO_2 was published by Brandi et al. in 1932, where it is shown that CO_2 freely passes through cell membranes and shows a pronounced vasodilating effect [7]. It was then proved that the physiological function of endogenous antioxidant defense systems of the body in combination with exogenous antioxidant properties of CO_2 are necessary to maintain a balance between endothelial relaxing factors and endothelial narrowing

factors. This balance ensures normal vascular function [20, 21]. It is now established that the antioxidant action of CO_2 is associated with the active inhibition of ROS generation. The most powerful generators of ROS are phagocytes, their generation of ROS increases especially sharply with their excitation and phagocytosis. At present, the regulatory influence of CO_2 on the generation of ROS by cells of different tissues has been established. Currently, the study of this issue is of general biological and medical interest, as CO_2 is formed constantly in all cells of the body and it is important to know its modulating influence on ROS generation.

The solution to this issue may be the results of studies conducted on 75 healthy donors, 21 patients with bronchial asthma, and 186 outbred white mice [12, 22]. It was found that CO_2 inhibits ROS generation not only in blood phagocytes and alveolar macrophages but also in tissue cells of internal organs. This inhibitory effect of CO_2 is confirmed on seven types of phagocytic cells (blood phagocytes, alveolar macrophages, tissue phagocytes: macrophages of the liver, kidneys, brain, lungs, stomach) and on six types of tissue (total parenchymal and interstitial) cells of internal organs (liver, brain kidneys, skeletal muscle) [13]. The inhibitory effect of CO_2 on the generation of ROS by blood phagocytes was also confirmed clinically. Thus, in 22 donors: it was found that CO_2 at concentrations of 5.1% and 20% inhibits the generation of ROS by phagocytes, respectively, 3.15 and 5.13 times; $p < 0.001$) [23].

In addition, under the direct influence CO_2 can inhibit the generation of ROS by mitochondria of the liver. The formation of ROS in mitochondria can occur at ischemia, inflammation, hypoxia, as well as in borderline conditions ("physiological" hypoxia of newborns, "physiological" aging), accompanied by changes in the properties of mitochondrial membranes [15, 24]. Reduction of ROS generation under the influence of 8.2% CO_2 was found not only on blood phagocytes but also on alveolar macrophages in 21 patients with bronchial asthma. In bronchial asthma, carboxytherapy is more effective in those patients whose phagocytes have retained a high sensitivity to the inhibitory effect of CO_2 on ROS generation [16]. Given the analysis of these data, it can be assumed that the beneficial effect of CO_2 in bronchial asthma is associated with the inhibitory effect of CO_2 on ROS generation [12, 16]. Pre-inhalation by animals (42 mice) for 50 min of a gas mixture containing 8.2% CO_2 reduced the formation of ROS in the cells of the tissues of internal organs (liver, brain, lungs, myocardium, gastric pylorus, and skeletal muscle) 2.2-4.1 times.

Therefore, the ROS-inhibitory effect of CO_2 is universal for different cell types and, obviously, for different species of living organisms.

Another interesting feature, in terms of the antioxidant action of carboxytherapy, is that in the body CO_2 interacts with both active forms of O_2 and with active forms of nitrogen: in the presence of NO superoxide, peroxynitrite is formed, which reacts with CO_2 with the formation of nitrosoperoxycarbonate [25]. This compound is converted to nitrocarbonate, which is prone to further reactions. Thus, in the aquatic environment, the most probable reaction involving CO_2 is the hydrolysis of nitrocarbonate to form carbonate and nitrate [17, 25].

Thus, the antioxidant effect of CO_2 is also associated with the peroxynitrite conversion reaction resulting in the conversion of nitration reactions and oxidative damage. Whereas in a non-polar membrane environment, nitrocarbonate causes reactions, for example, leading to nitration of proteins and oxidative damage. However, when NO reacts with oxygen in the absence of superoxide, $N_2O_3.CO_2$ is formed, which in normal physiological conditions when interacting with CO_2 is hydrolyzed to nitric and carbonic acids [25, 26].

Therefore, CO_2, preventing nitration reactions and oxidative damage has an antioxidant effect, which, for example, is confirmed by the normalization of the activity of superoxide dismutase (SOD). It is logical to assume that in the body hypercapnia (high CO_2 content in the blood) protects tissues from the destructive effects of toxic products of ischemia or hypoxia, which result in the formation of ROS.

In addition to the above mechanisms of antioxidant action of CO_2 in vivo one mechanism was proposed - stabilization with CO_2 of iron-transferrin complex, which also prevents the participation of iron ions in the initiation of free radical reactions [7]. Also, it has been proven that ROS such as H_2O_2, hydroxyl anion, and, mainly, superoxide found in the endothelium in ischemia and hypoxia, can be produced by modulation by mitochondria unrelated to nitric oxide synthetase, but with the participation of various oxidases: xanthine oxidase, COX, lipooxygenase, [27, 28, 29]. CO_2 blocking ROS, formed by the above mechanism, also contributes to the development of the antioxidant activity.

It is now known that increased levels of ROS are considered one of the main factors in the aging of organs, tissues, and the body as a whole [30, 31, 32]. On the other hand, physiological ROS production is required for signaling in normal cells because ROS serve as additional messengers involved in regulating the metabolism of mitogen-activated protein kinase, which acts as a pacemaker for cell growth, inflammation, apoptosis, and differentiation of cells. [5, 25], The pathophysiological link between these physiological and pathological conditions is the presence of severe oxidative stress.

In another study of the relationship between oxidative stress and CO_2, it was found that CO_2 plays a protective role in the process of removing free radicals and suppressing the expressed oxidative metabolism. Thus, it was found that asymmetric dimethylarginine (ADMA) inhibits the formation of NO and increases the level of free radicals in blood vessels, which leads to increased oxidative stress [33, 34, 35]. It is known that NO is a key vasodilator molecule that not only regulates vascular tone but also has antiplatelet, anti-inflammatory, and antioxidant properties. NO production is also closely linked to the activation of the endothelial nitric oxide synthetase (NOS) molecule, which is an indicator of vascular endothelial cell function: NOS promotes angiogenesis and suppresses myocardial fibrosis in cardiac dysfunction associated with high blood pressure [36].

As mentioned above, ADMA is an inhibitor of endogenous NO synthesis, and elevated concentrations of ADMA (a marker of oxidative stress) have been found in plasma in patients with classic cardiovascular diseases (CVD), as well as in the presence of other adverse risk factors: hypertension, diabetes mellitus, obesity [37, 38]. Therefore, ADMA is an informational natural factor for studying the relationship between the NO and CO_2 systems, especially under conditions of oxidative stress and inflammation, since ADMA is considered a mediator and marker of oxidative stress and inflammation. Not only ADMA can affect endothelial dysfunction but also cleaves electron transfer between NOS and L-arginine [39]. Whereas CO_2 inhalations enhance the activation of the NOS isoenzyme, and increasing this activity can lead to improved myocardial function[8]. The reliable prognostic value of CO_2 in the assessment of CVD risk was also confirmed by a systematic analysis of ADMA values in CVD patients. It is known that the decrease in ADMA activity is directly proportional to the severity of oxidative stress in CVD [33, 35, 37]. Other studies have shown that CO_2 can reduce the level of ADMA, and this also confirms that CO_2 is a natural physiological inhibitor of ROS generation [12].

Confirmation of the antioxidant action of CO_2 is also the result of the analysis of the content of serum d-ROM (an indicator of systemic oxidative stress) in myocardial infarction (MI). It is well known that oxidative stress is an important factor that negatively affects heart function [40]. Against the background of MI in systemic oxidative stress, the level of serum d-ROM is increased. It was found that the preliminary invasive administration of CO_2 leads to a significant decrease in the level of d-ROM [13]. Reduction of IM-induced content of d-ROM with pre-administration of CO_2 indicates that carboxytherapy can reduce both systemic and local oxidative stress, possibly by enhancing the compensatory effects of natural antioxidant systems, which in turn reduces oxidative and inflammatory processes in myocardium cells [22]. However, the exact mechanism of this action is not entirely clear.

Thus, based on the above, we can conclude that CO_2 paired with O_2 maintains the viability of our body. Violation of this balance leads to increased oxidative stress, the development of pathological conditions with the formation of ROS, which are the main trigger of aging, disrupting the subtle and complex biochemical intracellular processes of the body. Whereas CO_2 in physiological concentrations eliminates hypoxia, ischemia, reduces the rate of oxidative reactions, and thus exhibits antioxidant properties, contributing to the effect of "anti-aging". Therefore, prolonged hypocapnia and hypoxia can serve as a trigger for pathological processes in the body, in particular the suppression of natural antioxidant systems [41]. The accumulation of underoxidized metabolic products (ROS, etc.) in the tissues causes a painful reaction. Many studies have shown that the elimination of hypoxia with carboxytherapy, reduces the formation of these pathological products and promotes their removal from the body, thereby reducing pain [42].

Another aspect of the antioxidant effect of carboxytherapy application may be diabetes mellitus (DM) [41]. Currently, the cyclic process of LPO in the body is considered a universal pathological mechanism, which explains the main biochemical pathways of the toxic effects of hyperglycemia on the body. A significant breakthrough in understanding the pathological mechanisms of damage to β-cells of the pancreas in diabetes was the study of the biochemical significance of NO and cytokines that induce the expression of NO synthetase [44]. The latter, in turn, in β-cells promotes the formation of NO from L-arginine, which leads to the subsequent formation of free radical compounds and as a consequence to the destruction of β-cells [45]. Thus, in diabetes not only the formation of free radicals is activated, but also the activation of free radical oxidation processes in β-cells of the pancreas and other organs and tissues [41]

Therefore, to prevent or reduce the processes of free radical oxidation that cause damage in diabetes, it is justified not only to use drugs that can correct the development of hyperglycemia but also carboxytherapy that complements the treatment of diabetes, as one of the mechanisms of carboxytherapy in this pathology is its antioxidant action [41].

Thus, in the study of the intensity of the processes of LPO and the antioxidant activity of CO_2 by the method of induced biochemiluminescence it has been revealed that after "dry" carbon dioxide baths the antioxidant system is activated, as evidenced by a 21.2% decrease in plasma free radicals, malonic dialdehyde - by 11.9%, 6.5% increased activity of erythrocyte catalase, and there was a downward trend in diene conjugates noted [46].

Therefore, due to the antioxidant properties and participation in the maintenance of acid-base balance, carbon dioxide in natural (physiological) concentrations:

dilates narrowed small arteries and capillaries, while the reduction of carbon dioxide in the blood leads to their spasm and the opening of arteriovenous shunts, which impairs blood circulation in the tissues;

normalizes venous tone, as at insufficient tone of veins venous stagnation of blood develops, the outflow of blood from tissues is broken, their hypostasis amplifies, trophism and oxygenation worsens, toxic products of vital activity, including ROS, accumulate in tissues;

vasodilating and antioxidant action of CO2 on vessels leads to expansion of coronary vessels, bradycardia, decrease in BP, improvement of trophism of tissues, in particular, a myocardium;

Under the action of CO2 at the same time the need of heart muscle for O2 is reduced by 18-22% and increases myocardial tolerance to exercise;

Blood CO2 level reduction strengthens the binding of oxygen to hemoglobin that complicates the ingress of O2 in tissues, while Russian scientist BF Verigo and Dane K. Bohr have independently proved that without CO2 oxygen cannot be released from binding to hemoglobin, which leads to oxygen starvation of cells at high concentrations of O2 in the blood;

due to the direct and reflex action of CO2 on the vasomotor center of the medulla oblongata and the center of n. vagus redistribution of blood in an organism occurs, peripheral vessels dilate, the anaerobic energy exchange is mobilized and trophic processes improve;

Analysis of the above changes in the body under the action of CO2 and states of biochemical markers (ADMA and d-ROM, ROS, NO, NOS) under oxidative stress indicate that CO2 has antioxidant properties, and carboxytherapy is a pathogenetic therapy for diseases which pathogenesis includes oxidative stress.

Thus, it has been established that CO2 is a universal inhibitor of the reactive oxygen species generation by various human and animal cells. These data allow explaining the physiological and pathophysiological (medical) effects of CO2 in the mechanism of carboxytherapy in a new way from the standpoint of the biochemical role of ROS.

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