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Therapeutic hypercapnia. *Review*

Denis A. Tolstun, Khachik K. Muradian, Vladislav V. Bezrukov

D. F. Chebotarev Institute of Gerontology NAMS of Ukraine, Kyiv, Ukraine

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Correspondence: dtolstun1@gmail.com

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Abstract. In recent years, interest in hypercapnia and its practical applications has grown significantly. An analysis of literature data shows a wide range of systemic and local applications. Due to its powerful effect on blood circulation, vascular elasticity, activation of angiogenesis, and inhibition of pro-inflammatory factors, hypercapnia is already used in dermatology, phlebology, and therapy. Wide opportunities open up for practical use in neurology, given the powerful neuroprotective effect of carbon dioxide, which not only increases tolerance to ischemia, preventing the development of diseases but can also become a tool for the treatment of stroke and heart attack. The antitumor effect and the ability to reduce the level of metabolic processes also make hypercapnia an attractive geroprotector that will help in solving the issue of life extension.

Keywords: hypercapnia; hypoxia; cancer; diabetes; neuroprotection; longevity; carboxytherapy; ischemia; stroke

Abbreviations:

ATP — adenosine three phosphate
GRP — glucose-regulated protein
ETC — electron–transport chain
ERK — extracellular signal-regulated kinase
FAD — flavin adenine dinucleotide
HIF — hypoxia-inducible factor
HSP — heat shock protein
IFN — interferon
IL — interleukin
NAD — nicotinamide adenine dinucleotide
P_{CO_2} — partial pressure of CO_2
P_{O_2} — partial pressure of O_2
TNF — tumour necrosis factor
VEGF — endothelial vascular growth factor
V_{CO_2} — the rate of CO_2 production
V_{O_2} — the rate of O_2 consumption

The use of carbon dioxide in medicine has a long history. The first way of use can probably be considered the inhalation of gaseous fumes from volcanic solfataras (in Europe, for example, the Baia Cave in Naples), where carbon dioxide was used together with hydrogen sulfide and sulfur dioxide. In the 18th century, along with the study of the chemical properties of CO_2, at the suggestion of the Scottish physician Joseph Black and the French naturalist Antoine Lavoisier, high biological activity and the

involvement of carbon dioxide in metabolic processes were established. And already at the beginning of the 19th century, the German physician and chemist Friedrich Struve studied the therapeutic effect of CO_2 baths. He was the first who described the change like rheumatic diseases before and after the use of gas baths.

Since the beginning of the 20th century, the use of CO_2 as dry carbon dioxide baths and later subcutaneous injections — gas injections to improve skin blood supply and pain relief in case of various pain syndromes — have played a significant role in the medical arsenal. To date, the use of various therapeutic modifications for the use of CO_2 is expanding every year and is becoming not only an effective means of rehabilitation but also a real medicine.

Physiological mechanism of CO_2 action

CO_2 is a product of cellular respiration that requires the fastest removal from tissues. Therefore, in the body, there are many sensors and several systems that determine its concentration. An increase in CO2 levels, in particular, triggers the process of vasodilatation, increases blood circulation and respiratory intensity reduces muscle tone, which contributes to analgesic and anti-inflammatory effects [1, 2]. It is important to understand that these effects are seen in both exogenous and endogenous hypercapnia. But endogenous hypercapnia develops in pathological conditions accompanied by insufficiency of the external respiratory apparatus and a corresponding violation of gas exchange, which leads to negative consequences for the body due to the constant exposure to high levels of CO_2.

Carbon dioxide excites neurons in the respiratory centre of the medulla oblongata and chemoreceptors in the aortic arch, increasing external respiration, and at the same time increasing blood circulation due to the expansion of peripheral vessels, normalizing incl. venous return. That is, CO_2 has both central (through the nervous system) and peripheral effects (acting as a humoral factor). The humoral impact of CO_2 varies with its concentration. When breathing with a gas mixture with a CO_2 concentration of 5–7%, pulmonary ventilation increases by 6–8 times, and its concentration in the alveolar air increases by 1% [2, 3]. According to the data of most works, $PaCO_2$ in the alveolar air of 45–60 mm Hg is considered optimal for creating therapeutic hypercapnia (6–8% CO_2) and PaO_2 80–100 mm Hg (10,5–13% O_2).

It is important to know that the Verigo–Bohr effect occurs during hypercapnia. Without CO_2 in the blood, oxygen cannot be released from its bond with haemoglobin, which leads to oxygen starvation of cells even at a high concentration of O_2 in the blood. The higher the content of carbon dioxide in arterial blood, the easier it is for oxygen to be released from the bond with haemoglobin and pass it to tissues and organs, and vice versa — the lack of CO_2 in the blood promotes the bond of oxygen with haemoglobin [4]. At the same time, CO_2 plays one of the key roles in maintaining the acid-base balance of the blood due to the amphoteric properties of haemoglobin, which is capable of transforming into the state of oxyhemoglobin and carbohemoglobin.

An important effect of CO_2 is the change in energy metabolism. It has been shown that Vco_2 in mice in acute hypoxia and hypercapnia decreases by approximately 8% in response to each per cent increase in the CO_2 content in the respiratory mixture. In total, Vco_2 decreases by more than two times at high (8–10%) CO_2 concentrations in the respiratory mixture. At the same time, it is hypercapnia that has a greater metabolic inhibitory effect, since when hypercapnia is created, Vco_2 in an experiment on mice immediately decreases by almost two times, but such a decrease does not occur during hypoxia [5, 6].

Such inhibition of Vco_2 occurs when the concentration of CO_2 in the gas mixture is above 5%. The body compensates for the lower content of carbon dioxide by increasing blood circulation and respiration, resulting in an influx of oxidation substrates in the tissues and an outflow of CO_2 due to hyperventilation. The border of 5% is because the normal Pco_2 of the blood of mammals is 36–40 mm Hg, which corresponds to 5% of atmospheric 760 mm Hg, and the level of up to 5% of the body can still maintain with the help of hyperventilation, but over 5% hyperventilation only leads to an increase in the influx of exogenous CO_2, its concentration in the body increases and causes a decrease in the intensity of oxidative processes.

The inhibitory effect on metabolism is one of the fundamental differences between hypercapnia and hypoxia. While hypercapnia leads to a significant decrease in V_{O_2} and V_{CO_2}, hypoxia has practically no such effect. That is connected with the peculiarities of the process of obtaining cell energy. Spatially, it is divided into two microsystems (and, accordingly, two stages): the mitochondrial matrix, where the first stage takes place – the citric acid cycle, accompanied by three successive stages of pyruvate decarboxylation – and the inner mitochondrial membrane, on which the second stage occurs – the electron transport chain and "proton pump".

Hypercapnia inhibits the process of pyruvate oxidation to reduce NADH in the citric acid cycle, which occurs in mitochondrial plasma without the participation of O_2. The rate of this transformation depends on the level of hypercapnia – according to Le Chatelier's principle, the more $PaCO_2$, the slower the decarboxylation [7, 8].

Hypoxia, on the other hand, inhibits the level of metabolism at the second stage, when NADH is transferred to the ETC, on the inner membrane of mitochondria. There, NADH, together with $FADH_2$, triggers a flow of electrons, which, losing energy, pump protons into the intermembrane space. Upon reaching the cytochrome–C oxidase complex, the electrons completely lose energy and are utilized on the O_2 molecule. During hypoxia, there are not enough electrons for the "landing" of O_2 molecules, and the process is inhibited. But at the same time, the lack of oxygen leads to the accumulation of highly active NADH in the cell or a "plug" on the ETC, increasing the level of free radicals. Hypercapnia, on the other hand, gently limits the process of energy generation at its very beginning [9].

Evidence of such a decrease in the level of metabolism is not only a drop in V_{CO_2}, but also a decrease in body temperature, food intake, and blood glucose levels [6].

Permissive and therapeutic hypercapnia

Often, a side effect of modelling hypoxia, for example, using a rebreathing model or increasing the additional volume of dead space of the respiratory tract, is an increase in the partial pressure of CO_2 in the tissues. Until recently, practical medicine often treated hypercapnia negatively, using it only to test the reactivity of cerebral vessels due to a pronounced increase in volumetric and linear blood flow velocities, and a decrease in peripheral resistance in the arterial vessels of the brain [10].

However, in recent decades, attitudes towards hypercapnia have been changing, largely due to clinical practice and the SARS–CoV–2 pandemic. Already in the first wave of the pandemic, doctors confirmed the antioxidant, anti-inflammatory, and anti-cytokine effects of hypercapnia, as well as stimulation of the immune, cardiovascular and nervous systems as a preventive measure and treatment for COVID–19 [11].

The question came from a purely practical plane – should hypercapnia and hypercapnic acidosis be avoided, by all means, aggravating the condition of the lung tissue by excessive stretching and pressure during mechanical ventilation? Or one can allow the development of permissive hypercapnia in acute respiratory failure when the lungs are ventilated at low inspiratory volume and pressure. After all, this is how it is possible to minimize lung damage during mechanical ventilation by hypoventilation with an acceptable increase in $PaCO_2$ in the alveolar air [12–14]. With this strategy, reducing the tidal volume (Vt) and limiting the pressure of the inspiratory plateau leads to an increase in the CO_2 content in the body above the norm, up to the development of hypercapnic acidosis. The body, in an attempt to compensate for the change in blood pH, increases gas exchange, and the kidneys regulate bicarbonate levels in an attempt to make the blood more alkaline. Earlier in clinical practice, it was customary to fight acidosis by normalizing blood acidity [15, 16]. However, hypercapnic acidosis showed several protective effects: a decrease in the intensity of release of inflammatory mediators: tumour necrosis factor TNF–α, interleukin IL–1, nuclear factor NF–κB, and others. This is primarily due to acidosis that develops following hypercapnia since the use of sodium bicarbonate to normalize pH to the results of studies exacerbates damage to the lungs and myocardium [17].

Reducing the intensity of the inflammatory cascade in other organs and tissues. So, hypercapnic acidosis can have a protective effect on the myocardium due to a decrease in the degree of hypoxic/ischemic damage [18, 19]; on the brain, reducing ischemia-reperfusion injury and apoptosis of nerve cells [20]; liver, preventing the death of hepatocytes [20, 21]. In addition, hypercapnic acidosis

reduces the formation of free oxygen radicals and lipid peroxidation, which have a damaging effect on various organs and systems, and also reduces apoptosis in damaged tissues [22, 23].

Moreover, the protective properties of hypercapnia are best manifested when $PaCO_2$ in the tissues is at the level of 60–100 mm Hg, which corresponds to the content of CO_2 in the alveolar air from 45 to 60 mm Hg (5–8% CO_2 in the respiratory mixture). In contrast, severe hypercapnia ($PaCO_2$ 100–120 mm Hg) increases brain damage, which can be caused by increased cerebral oedema [24].

Some authors propose to distinguish between permissive and therapeutic hypercapnia. Permissive hypercapnia is achieved by a decrease in tidal volume during mechanical ventilation during lung injury, while therapeutic hypercapnia — a deliberate increase in arterial $PaCO_2$ —protects against in vivo reperfusion injury and injury caused by severe lung strain.

Clinical trials in adults with acute respiratory failure have shown improved survival and reduced incidence of organ failure in patients with low tidal volume and tolerable hypercapnia [25, 14]. Hypercapnia exerts a protective effect by modulating inflammation, lung mechanics and distensibility, and oedema, reducing tumour necrosis factor TNF–α and interleukin IL–6 in lavage and perfusate [26].

Of interest in this issue is the effect of CO_2 on the signalling pathways of mitogen-activated protein kinase (MAPK). They are crucial for the processes of development, oncogenesis, and inflammation, including the production of pro-inflammatory cytokines caused by reactive oxygen species, and in severe acute course of COVID–19.

At the moment, there are no drugs that can effectively prevent excessive inflammatory reactions in the endothelial cells of the lungs, heart, brain, and kidneys, which are considered the main causes of the severe course of this disease. However, the MARC of a person, i. e. extracellular signal-regulated kinases 1 and 2 (ERK1/2) are CO_2 sensors, and CO_2 is an effective anti-inflammatory compound that exerts its effect by inactivating ERK1/2 in cultured endothelial cells with increasing CO_2 concentration. That is, CO_2 acts as a potent inhibitor of cellular pro-inflammatory responses induced by H_2O_2 or the receptor–binding domain (RBD) of the SARS–CoV–2 spike protein. ERK1/2 is activated by the combined action of RBD and cytokines critical for the development of severe COVID–19, i. e. interferon-gamma (IFNγ) and tumour necrosis factor–α (TNFα) are more effectively inactivated by CO_2 than dexamethasone or acetylsalicylic acid in human bronchial epithelial cells [27].

The protective effects of so-called therapeutic hypercapnia currently remain experimental, but promising laboratory studies suggest the potential role of a possible effective agent in clinical practice both in mechanically ventilated individuals and in the prevention of severe conditions [16].

Neuroprotective effect

Stroke and ischemic brain damage are the second leading cause of disability and death in the world, accounting for 11.6%. And according to such an indicator as "disability-adjusted patient life years", equivalent to years of "healthy" life lost due to ill health, disability, or death, since 1990 humanity has lost a total of 143 million years of "healthy" life [28]. Therefore, the search for effective methods to increase the tolerance of the brain to ischemia is an urgent problem in modern medicine.

The use of hypoxic training to increase tolerance to cerebral ischemia has a proven efficacy [29, 30].

The effectiveness of the use of hypoxia has been proven both as a preventive and therapeutic agent. It has been established that hypoxic training for a month reduces the incidence of cystic infarction in the brain of rats associated with compression of the common carotid artery, and also positively affects the energy metabolism of the nervous tissue, increasing its resistance to ischemia [31, 32]. Interval high–altitude hypoxia has been successfully used to prevent the development of ischemic necrosis of the rat myocardium [33]. A significant protective effect is shown not only in the widespread laboratory model of rodents but also in humans, where the effectiveness of hypoxic training in the treatment of patients with neurocirculatory dystonia was shown [34, 35] in neurodegenerative lesions with cerebral circulation disorders, up to the effectiveness of hypoxic training in patients with cerebral palsy [36–39].

However, it has been shown that isolated hypercapnia also has a pronounced neuroprotective effect in ischemic–reperfusion brain injuries [24, 40]. The exact mechanisms by which hypercapnia increases tissue tolerance to ischemia have not been fully elucidated. It is believed that it includes the

processes of activation of mitochondrial metabolism of neurons and glia, activation of mitochondrial K^+ATP and adenosine receptors, inhibition of apoptosis, increased DNA repair, protective effects of HIF–1α and chaperones, as well as active synapto– and neurogenesis [41].

Therapeutic hypercapnia (arterial CO_2 tension ($PaCO_2$) 80–100 mm Hg) has also been shown to improve neurological deficits and attenuate histological lesions in cerebral ischemia-reperfusion. The use of hypercapnia significantly improves the ability of impaired sensorimotor and spatial memory in the model of focal cerebral ischemia, probably by attenuating the apoptosis of hippocampal neurons through the modulation of Bcl–2 and Bax proteins associated with apoptosis.

Nevertheless, the most popular is the study of the combined effects of hypoxia and hypercapnia as a means of increasing tolerance to ischemia. Numerous studies indicate that hypoxia in combination with hypercapnia has a much greater adaptogenic potential compared to hypoxia [42]. And the prospect of using hypoxia and hypercapnia is based not only on their pronounced effect on the vascular system.

The combination of hypercapnia and hypoxia increases the ischemic and hypoxic tolerance of the brain, more significantly compared to their isolated use [43, 44].

The neuroprotective effect of combined hypoxia and hypercapnia is realized through a variety of mechanisms. At the moment, we can confidently speak about the accumulation of hypoxia-inducible factor 1α (HIF 1α), which plays an important role in the cellular response to systemic hypoxia in all mammals. Along with an increase in HIF 1α, the amount of erythropoietin in the rat brain homogenate also increases [45]. Hypercapnia and hypoxia also increase the production of endothelial vascular growth factor (VEGF), heat shock protein HSP 70, and calcium-binding protein of astrocytic glia S 100B [46]. It has been shown that mitochondrial ATP–dependent potassium channels and A1 receptors for adenosine play an important role in the implementation of the neuroprotective effect of hypercapnia and hypoxia [47]. Also, the combined effect of hypoxia and hypercapnia maximizes the expression of the chaperone of the glucose-regulated protein (GRP 78) and the transcription factor NF–κB, which are key links in the adaptive branch in the stress of the endoplasmic reticulum of neurons in stroke. Another likely neuroprotective mechanism of action of hypercapnia is the inhibition of apoptosis in the peri-infarct area of cerebral ischemia [48].

Life extension

Based on the very wide spectrum of action of carbon dioxide in the body, some researchers consider hypercapnia and hypoxia as a way to prolong life [9]. This is partly dictated by the active influence on the metabolic pathways of the body, as well as a noticeable antitumor effect.

At the organismic level, the positive effect of hypercapnia can be explained by the transition of regulatory and executive systems to a more "economical mode" of functioning, in which the energy generation pathways are reoriented from oxidative phosphorylation to evolutionarily older and safer glycolysis, which, in turn, stimulates the anabolic processes of the cell.

Thus, keeping mice in combined hypoxia and hypercapnia leads to a significant decrease in the intensity of oxidative processes and metabolism in general, which is expressed in a decrease in O_2 consumption and CO_2 production, as well as a decrease in food and water consumption; the resistance to various stressful influences and the life expectancy of the test animals (mice and fruit flies) are growing [49, 6].

From the point of view of the total effect of hypercapnia on the body and metabolic connections in it, the model of the naked mole rat (*Heterocephalus glaber*), which has become popular in gerontology, may be of interest. This small rodent lives in large underground colonies, where the level of CO_2 reaches 10%, and O_2 proportionally decreases [50, 51]. The naked mole rat demonstrates exceptional resistance to many forms of stress, hypoxia, reproductive ageing, cardiovascular diseases [52, 53], sarcopenia, diabetes [54, 55], and even the development of malignant neoplasms [56]. Since the life expectancy of naked mole rat is approximately an order of magnitude higher [57], and the metabolic rate and body temperature (33–34 °C) are significantly lower than in related species, for example, in mice [58, 59], its study can contribute to solving the problems of a chronic decrease in the level of metabolic processes and body temperature.

Based on a comparative analysis of the genomes of the naked mole rat and mouse, which revealed only minor differences in DNA, it was suggested that the difference in the processes of ageing and longevity is due to the peculiarities of their life and physiology [60]. Therefore, there is reason to believe that it is precisely such a hypoxic and hypercapnic environment that contributes to a decrease in the intensity of metabolic processes and the body temperature of a naked mole rat [61–63].

The ageing of the body is considered to be the gradual predominance of damaging factors over restorative ones with the accumulation of errors at all levels of biological organization. And it seems that one of the few adequate measures to counter such an accumulation of errors can be the impact on energy processes and temperature, as universal factors influencing the metabolic rate, directly related to ageing.

According to rough estimates, the metabolic rate of the naked mole rat is 4–6 times lower than that of mice [50, 58], and the temperature of the core of the body is lower by 3–4 °C. Thus, the sum of all these two factors gives an incredible difference in the lifespan of mice and naked mole rats [57, 59] against the background of a striking absence of typical signs of ageing — increased mortality and reduced reproductive activity [64].

Of course, it is impossible to attribute the fantastic differences of the naked mole rat only to the atmosphere in which it lives. But a whole group of animals of different taxa that exhibit extreme longevity, such as some species of whales, turtles, and the bivalve mollusc *Arctica islandica*, are united precisely by a combination of four partially interrelated, but practically independent factors: hypoxia, hypercapnia, hypothermia, and a low level of metabolism [65, 66].

And it is hypercapnia, as mentioned above, that can play a special role in interfering with the processes of energy and heat generation in the body, inhibiting the processes of pyruvate decarboxylation in the citric acid cycle. Whereas hypoxia only limits the flow of electrons in the electron transport chain, creating problems with the utilization of intermediate products and excessively active electrons in mitochondria.

Such assumptions are confirmed by experimental data, when, under conditions of hypercapnia, the level of gas exchange in mice decreases two times compared with hypoxia. And with chronic retention of mice in 7% hypercapnia and 12% hypoxia, they not only had a twofold decrease in the level of gas exchange, but also a decrease in body surface temperature, and voluntary caloric restriction of the diet occurred, which was accompanied by not only by a decrease in food intake by 30–40% but also by a decrease in the expression of hypothalamic neuropeptides, stimulating appetite [6].

It is important that in this case, behavioural reactions and motor activity do not change. A hypercapnic–hypoxic atmosphere is a model of a physiologically balanced voluntary calorie restriction, which is supported by a reduced expression of hypothalamic neuropeptides that stimulate appetite. Such an effect can be proposed as a non–drug remedy for the prevention and treatment of overweight and related diseases, incl. diabetes mellitus.

The available literature lacks information on the effect of hypoxia and especially hypercapnia on diabetes mellitus and carbohydrate metabolism. Smirnov et al. showed that hypercapnic–hypoxic training (2 hours a day) reduces blood glucose levels and promotes the treatment of type 1 diabetes mellitus and concomitant polyneuropathy in children [67].

Anti-tumour effect

Cancer remains one of the leading causes of death in developed countries. And although every year the methods of its treatment are improved, most of them have very serious side effects. Therefore, the main task of oncologists is not only treatment but also the development of an effective, at the same time non–toxic, preventive, and therapeutic strategy for combating oncology.

And as it is not paradoxical, but almost blind rodent *Heterocephalus glaber* can become one of the guides of mankind in the way to fight against cancer. He not only showed an example of truly successful biological ageing but also showed amazing resistance to the development of oncology [56]. It is possible that the harsh living conditions of the naked mole rat, namely severe hypercapnia and hypoxia, are related to such resistance, for example, through the *Wnt signalling pathway*, which regulates embryogenesis, and cell differentiation, and the development of malignant tumours. A large–scale

transcriptomic study of *Wnt pathway* interaction/integration/maintenance of genomic responses induced by hypercapnia in mammals (mice and humans) as well as invertebrates (*Caenorhabditis elegans* and *Drosophila melanogaster*) showed that hypercapnia activates genes that inhibit *Wnt signalling* in the lungs and skeletal muscles of mice in vivo and several cell lines of various tissue origins, and Wnt pathway homologues sensitive to hypercapnia have also been observed in human, fly, and nematode bronchial cell lines. Such data suggest an evolutionarily conserved role for high CO_2 levels in the regulation of *Wnt pathway* genes and their influence on organismal fate [68].

After all, life originated, and most of the evolution took place in atmospheres with an extremely low content of O_2 and a high content of CO_2, while in the modern atmosphere, on the contrary, the content of O_2 is more than 500 times higher than the content of CO_2 [69–71]. To survive, successful species seem to have had to modify old and/or invent new adaptive and defensive systems. However, taking into account that the main elements of life support (nucleotides, amino acids, etc.) are evolutionarily invariant or very conservative, there is reason to believe that the main life support and longevity systems remained essentially hypoxic/hypercapnic. And a return to hypoxia or hypercapnia makes it possible to "turn off" the evolutionarily recently acquired ones, leaving only highly conservative fundamental life support systems.

Indeed, experimental studies and clinical observations have shown that hypercapnia can have an anti-inflammatory effect and even help cure some diseases.

In addition to the suppression of generalized inflammation, the successful use of hypercapnia for the proliferation of cancer cells has been shown. The effect is achieved both on isolated cells, for example, SGC–7901 gastric cancer cells, by suppressing their invasion and migration, as well as inducing apoptosis when CO_2 heated to 42°C is injected into the abdominal cavity (pneumoperitoneum) by the laparoscopic method [72]. A similar effect was observed on human gastric cancer AGS inoculated into nude mice. After a 4–hour treatment of the tumour with CO_2, expression of the heat shock protein HSP 70 decreased in cancer cells, tumour growth was inhibited in nude mice, and immunostimulatory effects appeared [73]. The effective cytotoxic effect on AGS of gastric cancer cells through Bax–induced mitochondrial apoptotic signalling also indicates that local hypercapnia can be a potential therapy for peritoneal carcinomatosis of gastric cancer [74]. However, this technique should be treated with caution, as in another in vitro model of CO_2 pneumoperitoneum, investigators observed the proliferation of cervical cancer cells after a short period of inhibition. Presumably, the mechanism of which is associated with the inhibition of phosphorylation of the PI3K/Akt signalling pathway [75].

Local action and subcutaneous administration

In addition to the total effect on the body, carbon dioxide has a pronounced local effect, allowing it to be used as a local remedy. Moreover, in cosmetology and dermatology, hypercapnia has been used for a long time and very successfully. In particular, it actively affects tissues, triggering a cascade of reactions to reduce inflammation, increase blood microcirculation and lymphatic drainage, as well as angiogenesis.

The list of diseases that can be treated with carboxytherapy is expanding every year. It includes, for example, chronic venous and veno–lymphatic insufficiency, ulcers of various origins (keloid, atrophic, venous ulcers), rheumatism, psoriasis, arterial pathologies (acrocyanosis, Raynaud's disease, Buerger's disease, diabetic ulcers, acrocyanosis, atherosclerotic ulcers), localized fatty deposits, alopecia areata, and aesthetic skin diseases.

Such a wide range of actions can be conditionally divided into physiological and biochemical ones. Physiologically, CO_2 promotes vasodilation using a mechanism similar to that of adenosine. An increase in interstitial CO_2 tension was indeed considered a potential signal, responsible for the metabolic control of coronary blood flow, which leads not only to increased myocardial activity but is also used to autoregulate local blood flow, adjusting local perfusion to metabolic and tissue oxygen demand.

A consequence of the activation of blood flow during carboxytherapy is a significant positive effect on improving the movement of the lymph. Using lymphoscintigraphy, Manzo and co-authors showed an improvement in local parameters of blood circulation and tissue perfusion in patients with

severe lymphocytosis [76]. Positive changes in blood flow and lymphatic drainage also resulted in active lipolysis, so this type of therapy can be effectively used to treat localized obesity and cellulite [77, 78].

Long–term regulation of local blood flow is carried out by the expansion of the microcirculatory bed with the help of angiogenesis. Moreover, with local hypercapnia, false angiogenesis is first activated. It is a result of an increase in tissue blood flow and recanalization of dormant and fading capillaries [79, 80]. With true angiogenesis, CO_2 can promote the release of endothelial and fibroblast growth factors that can stimulate angiogenesis [81, 1]. After the application of hypercapnia, an acceleration of wound healing by 25% was shown compared with the control [82, 6].

At the biochemical level, one of the main properties of hypercapnia, which reduces tissue inflammation and systemic inflammation, is the suppression of the production of tumour necrosis factor (TNF–α), pro-inflammatory interleukins IL–1β, IL–6, IL–8, as well as an increase in the production of anti-inflammatory interleukin IL–10. Decreased nuclear transcription factor (NF–κB) in hypercapnia, which controls expression of immune response genes and apoptosis, and activated by the already mentioned cytokines TNF–α and IL–1, also reduces local inflammation in tissues and stress [83–86].

Thus, carbon dioxide acts not only on the mechanisms of direct feedback, affecting the movement of blood or the elasticity of blood vessels, it helps to restore the function of local microcirculation and tissue perfusion in case of damage. It also reduces tissue oxygen consumption (Bohr effect) and affects tissue blood flow in the long term, leading to the creation of new vessels and tissue vascularization, increasing the elasticity of red blood cells, reducing both generalized and local inflammation in the body, inhibiting the production of pro-inflammatory factors and cytokines.

Concluding remarks

Despite its negative reputation in biology and medicine, carbon dioxide is a very powerful humoral factor in the body, which acts as a pacemaker for a huge number of processes. Thus, it intensifies blood circulation, both at the body level and in the area of its administration, while being a powerful vasodilator, it reduces the basal tone of arterioles, recanalizes dormant capillaries, and triggers the process of neoangiogenesis. Reducing the permeability of ion channels, increases the resistance of the cell membrane, has an inhibitory effect on pro-inflammatory factors, and stimulates anti-inflammatory ones. Due to the pronounced antioxidant action, it increases the body's resistance to adverse environmental factors and accelerates wound healing. It has a powerful neuroprotective effect, helping to prevent ischemic lesions and even recover from them. Carbon dioxide can reduce the rate of metabolic processes, exhibit pronounced anti-cancer properties, and even be used as a geroprotector.

Of course, for a clearer understanding of when and how to use this substance known to all of us since childhood, more than one hundred experimental and clinical studies have yet to be carried out.

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