Potential therapeutic effect of oxygen-ozone in controlling of COVID-19 disease

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Abstract

Atmospheric ozone is produced when nitrogen oxides react with volatile organic compounds. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) genome contains a unique N-terminal fragment in the Spike protein, which allows it to bind to air pollutants in the environment. 'Our approach in this review is to study ozone and its effect on the SARS-CoV-2 virus and patients with coronavirus disease 2019 (COVID-19). Article data were collected from PubMed, Scopus, and Google Scholar databases. Ozone therapy has antiviral properties, improves blood flow, facilitates the transfer of oxygen in hypoxemic tissues, and reduces blood coagulation phenomena in COVID-19 patients. Ozone has immunomodulatory effects by modulating cytokines (reduction of interleukin-1, interleukin-6, tumor necrosis factor-α, and interleukin-10), induction of interferon-γ, anti-inflammatory properties by modulating NOD-, LRR- and pyrin domain-containing protein 3, inhibition of cytokine storm (blocking nuclear factor-κB and stimulating nuclear factor erythroid 2-related factor 2 pathway), stimulates cellular/humoral immunity/phagocytic function and blocks angiotensin-converting enzyme 2. In direct oxygen-ozone injection, oxygen reacts with several biological molecules such as thiol groups in albumin to form ozonoids. Intravenous injection of ozonated saline significantly increases the length of time a person can remain hypoxic. The rectal ozone protocol is rectal ozone insufflation, resulting in clinical improvement in oxygen saturation and biochemical improvement (fibrinogen, D-dimer, urea, ferritin, LDH, interleukin-6, and C-reactive protein). In general, many studies have shown the positive effect of ozone therapy as a complementary therapy in the recovery of COVID-19 patients. All the findings indicate that systemic ozone therapy is nontoxic and has no side effects in these patients.

Key words: COVID-19; coronavirus; immunity; MERS; ozone; SARS; SARS-CoV-2

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) belongs to the group of beta-coronaviruses and has a diameter in the range of 50–200 nm, which is nearly 500 times larger than the size of an ozone molecule. The SARS-CoV-2 genome is surrounded by a coating of spike proteins. The main genetic material of this virus is a positive-strand RNA virus that is protected by a nucleocapsid (N), the virus genome encodes four structural proteins, all of which are required to produce the complete virus.[1] It is understood that interaction between angiotensin-converting enzyme 2 (ACE2) cell receptor and viral spike protein facilitates the coronavirus entry into respiratory epithelial cells, and ACE2 is extremely expressed in these cells. Along with viral-receptor interaction, the proteolytic cleavability of spike protein has been considered as the cause of disease strictness.[2,3]

The pathogenesis of the SARS-CoV-2 is still not well clear. Coronavirus disease 2019 (COVID-19) is characterized as a multi-systemic disease. Most patients suffer a self-limited course symptom; nevertheless, a few will experience serious or even fatal diseases.[4] In individuals, the special properties of these viruses are associated with viral pneumonia. The principle pathogenesis of COVID-19 involves; severe lung inflammation and immune deficiency, which are mutually associated with an excessive inflammatory immune response and enlarged production of cytokines (cytokine storm) against viral infection that cause damage to the host cells.[5,6]

Currently, ventilation oxygenation and fluid dealing stay the standard of maintenance for hospitalized COVID-19 patients. Numerous clinical trials and healing interventions presently purpose to recognize the most effective drug or combination against the disease.[7,8] Currently investigated approaches include antiviral (antivirals can be confirmed as harmless and active only in the context of randomized controlled clinical trial and anti-proinflammatory cytokines, anti-infectious and monoclonal antibodies, and passive immunotherapy (plasma therapy), especially in critical patients.[9]

Epidemiological data available in Tibet, China and high-altitude areas of Bolivia and Ecuador indicate that high-altitude inhabitants (2500 m above sea level) are less prone to severe side effects compared to low-lying areas in severe SARS-CoV-2 infection.[10] The two main findings of these studies include the risk of virus half-life due to the high

altitude of the environment and the second that hypoxia causes ACE2 to be down-regulated as the primary target for SARS-CoV-2 virus binding in the respiratory epithelium.[11,12] Article data were collected from PubMed, Scopus, and Google Scholar databases in 2020–2021 with terms of COVID-19, coronavirus, ozone, SARS-CoV-2, treatment and therapeutics. Therefore, this review aims to investigate the physiological and immunological mechanisms and beneficial possibilities of ozone on COVID-19 infection and review various clinical studies in patients with COVID-19.

Ozone

The ozone molecule, consisting of three oxygen atoms, is the strongest oxidant found in nature, which in gaseous form has a half-life of about 1 hour at room temperature and returns quickly to oxygen.[13] The Scripps Institute found that our bodies also produce ozone.[14] In ozone therapy (OT), 1–5% ozone in 95–90% oxygen is used as a gas mixture.[15] Ozone is the strong oxidant following fluorine and persulfate but has higher oxidizing power than oxygen. In OT, the oxygen/ozone gas mixture is produced using an ozone generator, in which due to the short half-life of ozone, the concentration of ozone is reduced by half in 40 minutes at 20°C and is reduced by half in 25 minutes at 30°C.[16]

Ozone has been widely studied in medicine and is presently used in various concentrations in various fields of medicine.[17] Ozone exhibits contradictory activity in contact with organic molecules, therefore triggering a strong antioxidant reaction.[18] Ozone exerts oxidative preconditioning that can reverse chronic oxidative stress conditions associated with stimulation of the production of radical free scavengers and cell wall protectors like glutathione peroxidase, catalase, and superoxide dismutase.[19] It also has the distinctive aptitude to inactivate biological contaminants, containing viruses, through the oxidation of double bonds. Ozone interrupts the wall integrity of bacterial cells, resulting in lysis and death, and thus effectively controls the growth of spores of various dermatophytes.[13] In minor autohemotherapies, inducing the oxidation of viral-free components extracted from blood samples containing 90 g/mL ozone may be practical, which could theoretically be considered as an inactive and immunogenic vaccine. Biochemically, when the blood is exposed to ozone for a few minutes, it immediately reacts with various molecules in biological fluids, including antioxidants, proteins, carbohydrates, and preferably unsaturated fatty acids, resulting in leads to the formation of α-hydroxy hydroperoxides, hydrogen peroxide (H_2O_2), ozonides, and 4-hydroxynonenal. They play a significant role in modulating inflammation, proliferation, growth, and cell death.[20] When blood is exposed to ozone, oxygen balances with extracellular fluid and intracellular fluid before it binds to hemoglobin. In contrast, ozone, which has 10 times more solubility than oxygen, provides a soluble and bimolecular composition in biological fluids and dissolves easily in water, reacts immediately with several biomolecules like ascorbic acid, urate, free cysteine, glutathione, and albumin thiol groups, and then disappears. The compounds produced in the reactions are "ozone messengers" and are accountable for their biological and therapeutic properties.[20]

Application of Ozone Therapy in Diseases

OT has proven, lasting, safe effects with minimal and avoidable side effects. Ozone is used in medicine to disinfect and treat diseases. Appliances of action of OT include reaction with polyunsaturated fatty acids and water, ozone creates H_2O_2 and at the same time, ozone forms a mixture of lipid ozonation products.[21]

Its mechanism of action is to inactivate bacteria, viruses, fungi, yeasts, and protozoa by stimulating oxygen metabolism and activating the immune system.[15] Newly, the potential effect of ozone on inactivation of the virus *in vivo* has been recognized, as well as the efficiency and safety of ozone administration on acquired immunodeficiency syndrome, B, and C hepatitis, Ebola, and influenza diseases, however, this method along with other managements can be acceptable and synergic.[22]

Ozone can attack proteins, spike lipids, and virus coatings, especially tryptophan, methionine, cysteine, arachidonic acid, linoleic acid, and oleic acid.[1] If the cytomegalovirus thiol groups are oxidized, it loses its pathogenicity. Regeneration of thiol groups with dithiothreitol restores 65% of the pathogenicity of the virus. human immunodeficiency virus for pathogenicity and the Ebola virus also need sulfhydryl groups to enter cells.[15] Cysteine is very susceptible to oxidizing disulfide (R-S-S-R) or extra residues, which disrupts the chemical activity of proteins by altering their three-dimensional structure. If the reduced thiols are oxidized, the enzymes may be inactivated, and ozone immediately oxidizes the thiol groups upon contact.[15] The ability of ozone to inactivate cysteine-containing proteins has been reported as an ozonide attack on cysteine-dependent papain protease enzyme. It is believed that ozone converts the active sulfhydryl group to sulfenate/sulfonic acid by oxidation and inactivates the enzyme. Also, the spike of coronaviruses is rich in tryptophan, which is after cysteine in terms of vulnerability to oxidation (**Figure 1**).[123]

High concentrations of ozone can be dangerous to the lungs if inhaled directly for long periods. There are several ways to use ozone that are widely used. While controversial, direct intravenous injection of ozone gas is the simplest and most effective technique of ozonation and is widely used. Intravenous injection of ozone gas does not pose a similar risk because it is not composed of 80% nitrogen, and moreover ozone is highly solvable in blood and plasma. The solubility of ozone in water is 10–13 times higher than oxygen. Depending on temperature and pressure, about 50 mL of ozone gas dissolves in 100 mL of water, while only 4 mL of oxygen dissolves in 100 mL of water.[24] Some researchers prefer the usage of extracorporeally ozonated autologous blood transfusion with a volume of 100–200 mL, which is extensively recognized as a major autohemotherapy.[25] Different procedures for major auto-hemotherapy typically use only 100–200 mL of blood. The disadvantage of this method is that it needs blood sampling, heparinization, and ozonation of blood, followed by autologous blood transfusion. This procedure is problematic by the association of heparin with important side effects (allergic and thrombocytopenia) as well as the need for personal protective equipment against COVID-19.[26] In many organs, such as the pancreas, peritoneum, liver, mesenteric lymph

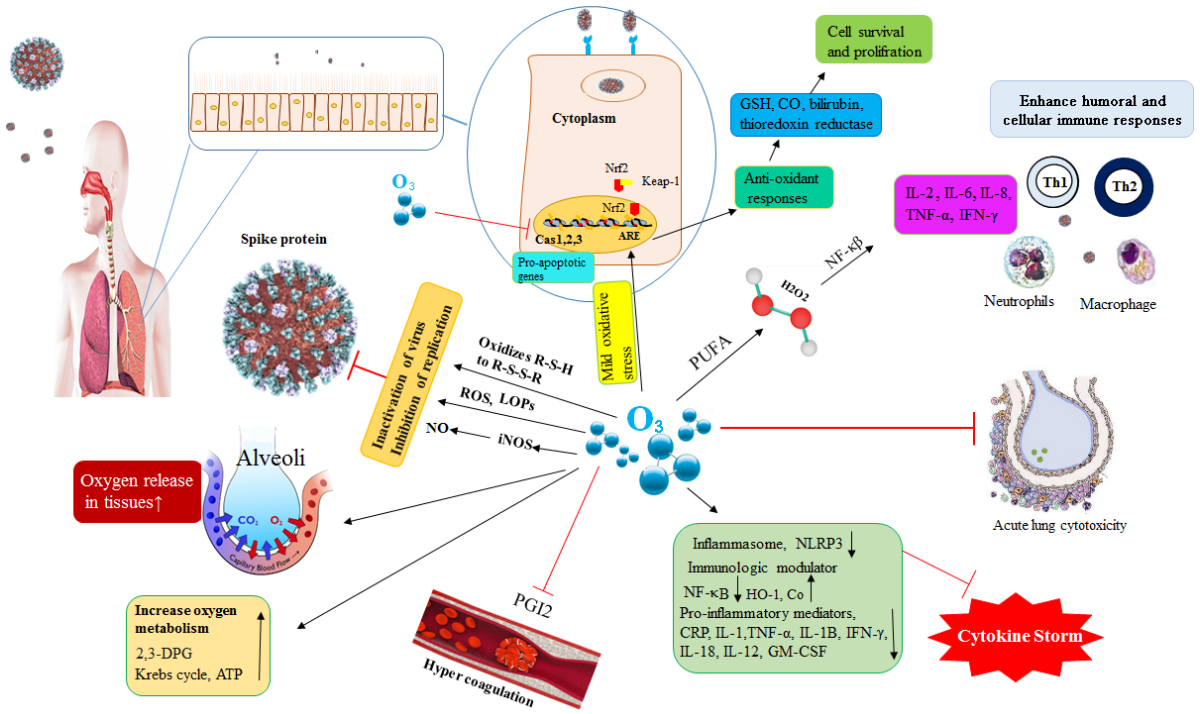

Figure 1: Proposed therapeutic effects of O_3 therapy for the treatment of COVID-19.
Note: COVID-19 serves as an ACE2 receptor to enter into human respiratory epithelial cells through its Spike proteins. The transcription factor Nrf2 bound to Keep-1 activated by alkenals. The released Nrf2 translocates into the nucleus and, after binding to Maf, docks on ARE and activates several genes leading to the synthesis of antioxidant proteins. O_3 can inhibit apoptosis and degradation of the cartilage matrix by inhibiting the activation of NF-κB resulting in cell survival. O3 has immunomodulatory effects by anti-inflammatory properties by modulating NLRP3, inhibition of cytokine storm (reduction of IL-1β, IL-6, TNF-α, IFN-γ, IL-18, IL-12, GM-CSF), O_3 can stimulate cellular and humoral immunity through secondary messengers (H2O2) and NFAT/AP-1 signaling pathway. O_3 is a multifunctional drug that can stimulate inflammatory cytokines (IL-1β, IL-6, IFN-γ, TNF-α), anti-inflammatory cytokines (IL-4, IL-10). Biological responses to Nrf2/ARE activation with mild OT oxidative stress increase the levels of direct antioxidants such as GSH, CO, and bilirubin through glutathione and thioredoxin reductase. O_3 can reduce biomarkers such as CRP, ESR, and uric acid, which have been shown to reduce CRP in patients with COVID-19. O_3 increases oxygenation to the blood and tissues prevents the formation of small thrombosis by secretion of some prostacyclins such as PGI2. O_3 increases the expression of HO-1 in endothelial cells, stimulates 2–3 diphosphoglycerates, so more oxygen to the delivered tissues in COVID-19 patients. O_3 or its mediators (ozonides [ROS, LOPs]) are capable of oxidizing cysteine and tryptophan residues on S-spike protein and preventing their binding to the ACE2. O_2-O_3 also affects NO and iNOS signaling pathways. NO, especially by inhibiting palmitoylation of S protein, reduces the viral replication of COVID-19, thus preventing the virus from binding to the ACE2 receptor. ACE2: Angiotensin-converting enzyme 2; AP-1: activating protein-1; ARE: antioxidant response element; CO: carbon monoxide; COVID-19: coronavirus disease 2019; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; GM-CSF: granulocyte-macrophage colony-stimulating factor; GSH: glutathione; H_2O_2: hydrogen peroxide; HO-1: heme oxygenase-1; IFN-γ: interferon-γ; IL: interleukin; iNOS: inducible nitric oxide synthase; Keep-1: Kelch like-ECH-associated protein 1; LOP: lipid ozonation product; Maf: musculoaponeurotic fibrosarcoma; NFAT: nuclear factor of activated T-cells; NF-κB: nuclear factor-κB; NLRP3: NOD-, LRR- and pyrin domain-containing protein 3; NO: nitric oxide; Nrf2: nuclear factor erythroid 2-related factor 2; O_2: oxygen; O_3: ozone; OT: ozone therapy; PGI2: prostaglandin I2; ROS: reactive oxygen species; TNF-α: tomur necrosis factor-α.

nodes, and cecum, OT has been shown to have decreasing properties on bacterial translocation. In an investigational sample of necrotizing pancreatitis, OT was found to be more operative in reducing oxidative stress, tissue damage, and bacterial translocation than hyperbaric oxygen therapy.[27] After using intraperitoneal ozone, Schulz et al.[28] observed a decline in polymicrobial peritonitis.

Ozone Therapy of COVID-19 Patients

Recently, China, Italy, Spain, and South America have reported that the use of the OT method is beneficial in patients with COVID-19-related acute respiratory failure.[29] The lipid structure of SARS-CoV-2 comprises fatty acids, including arachidonic acid, linoleic acid, palmitic acid, and oleic acid, and is rich in glutamic acid. In particular, arachidonic acid and linoleic acid have been shown to play a significant role in the infectivity mechanism of the coronavirus.[1] Also, the amino acids cysteine and tryptophan are abundant in the structure of coronavirus proteins, which are important in membrane fusion. The amino acid methionine also plays a significant role in stabilizing the protein structure and replicating the virus.[30] The glycoproteins in the spikes, which cause the virus to enter cells, are decorated with N-linked heterogeneous glycans and are the chief goal of antibodies.[1] Intravenous injection of ozonated saline significantly increases the length of time a person can remain hypoxic and delays the lowest point of the oxygen saturation curve.[31] Ozone is recommended due to its strong oxidizing power, which can inactivate and destroy SARS-CoV-2. This is justified not only by the fact that ozone is a potent oxidant but also by the fact that SARS-CoV-2 is a coated virus that is vulnerable to oxidant attack. Ozone can attack viruses at different points in their structure and oxidizing viral capsids and genetic materials prevent them from reproducing and multiplying, which by this mechanism damages the integrity of viruses. Although any viral structure can be attacked by ozone, structures with more double bonds or high electron density groups will be more vulnerable to ozone oxidation.[1] Another important feature of OT against COVID-19 infection is the ability of ozone to counteract the severe hypoxemia that the virus causes.[1,18] There are several

suggested mechanisms by which OT as an adjunct therapy for patients with COVID-19 may potentially improve outcomes.

Different Ozone Activity in COVID-19 Patients

Oxygen-ozone has a high solubility in plasma and produces secondary messengers such as H_2O_2, ozonoids, and alchenals (**Figure 1**). These are mainly competent, interacting with membrane proteins and cell receptors, especially immune cells. They enter the cells and interact with signal transduction proteins in the nucleus and mitochondrial surface. Ozone can inhibit the process of infection by oxidizing S proteins, ozone, and its reactive oxygen species can also attack the virus coat, and if they can penetrate the virus coat, they can attack its capsid, genome, and RNA, in which case the virus is not able to reproduce.[32]

The ability of ozone to efficiently inhibit cysteine-dependent proteins has recently been described as an ozone attack on cysteine-dependent papain. It is supposed that enzymes can be deactivated by oxidation of the active sulfhydryl group to sulfate or sulfonic acid. These mechanisms can openly reduce SARS-CoV2 infection and lead to faster recovery from pneumonia.[23,33] Also, coronavirus spike proteins are rich in tryptophan, which, like cysteine, is highly vulnerable to oxidation.[34] Also, an interesting conclusion about D-dimer expands the discussion of the role of oxygen-ozone therapy in thromboembolism or vascular and thrombotic disorders related to COVID-19, because D-dimer typically increases during COVID-19 and is greatly reduced in the existence of ozone. Oxygen-ozone also affects nitric oxide (NO) and inducible nitric oxide synthase signaling pathways. NO, especially by inhibiting palmitoylation of S protein, reduces the viral replication of SARS-CoV2, thus preventing the virus from binding to the ACE2 receptor (**Figure 1**). Ozone also increases the expression of inducible nitric oxide synthase, the major enzyme-producing NO in type 2 pneumocytes in rats.[35]

Immunomodulatory functions

The inflammatory response is characteristic of severe infection, and the modulation of cytokines is important to prevent disease exacerbation. Oxidative stress and innate immunity play vital roles in lung damage pathways that control the severity of acute lung cytotoxicity in viral infections such as severe acute respiratory syndrome.[16] During acute inflammatory procedures, to increase the response rate, nuclear factor-κB (NF-κB) increases the activity of mitochondrial NADPH oxidase, the major source of endogenous superoxide anion radical. There are strong relations amongst the coordinated activity of gene activation by both transcription factors, NF-κB and nuclear factor erythroid 2-related factor 2 (Nrf2), to resolve inflammatory procedures at the cellular and tissue levels. An imbalance among the NF-κB and Nrf2 pathways is also related to COVID-19 (**Figure 1**).[17]

An additional vital role that ozone plays in COVID-19 is its immunomodulatory properties. The main functional mechanism of oxygen-ozone therapy is active in the proteasome and cascade of inflammation, to control the inflammatory process associated with stimulation of nuclear factor Nrf2 and inhibition of nuclear factor NF-κB.[23] Ozone reacts with unsaturated fatty acids (polyunsaturated fatty acids) and aldehydes to produce hydroperoxides, especially H_2O_2, which spread rapidly through the cells of the immune system. It also regulates biological signal transduction, therefore, increasing immune responses, modulating interferon, and interleukins through NF-κB activation, hence increasing cytokine release (**Figure 1**). The human body can produce ozone to protect itself against infectious agents. This occurs with the participation of neutrophils and immune system antibodies that kill bacteria and viruses by producing ozone, using its oxidizing power.[1,36] It significantly reduces the concentration of NADH and contributes to the oxidation of cytochrome C, thereby stimulating oxygen metabolism, as well as showing anti-inflammatory function and cellular protection resulting from interaction with NF-κB and Nrf2.[37]

Çakır et al.[38] described that systemic markers of the inflammatory response (tumor necrosis factor-α (TNF-α) and interleukin (IL)-1β levels) decreased after ozone treatment. Biological responses to Nrf2/antioxidant response element activation with mild OT oxidative stress increase the levels of direct antioxidants such as glutathione, carbon monoxide, and bilirubin and stimulate glutathione regeneration through glutathione and thioredoxin reductase (**Figure 1**). It also increases the levels of enzymes that detoxify oxidants and electrophiles and increases the levels of phase II enzymes along with the inhibition of cytokine-mediated inflammation through the induction of leukotriene B4 reductase, decreasing iron overload and the following oxidative stress. Ozone can reduce biomarkers such as C-reactive protein (CRP), erythrocyte sedimentation rate, and uric acid, which have been shown to reduce CRP in patients with COVID-19 (**Figure 1**).[39]

Ozone shows strong anti-inflammatory properties by modulating NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammation, that plays a vital role in the onset and persistence of inflammation in various diseases.[40]

Ozone is known to have the ability to activate the immune system, while ozone is a weak inducer, lymphocytes, and monocytes reinfused during major autohemotherapy can migrate through the lymphatic system and activate other cells over time, stimulating the immune system. Ozone can stimulate cellular and humoral immunity through secondary messengers (H_2O_2) and the signaling pathway nuclear factor of activated T-cells and the activator protein 1 pathway. These ways are important transcription factors because they induce gene expression for the release of inflammatory cytokines (IL-2, IL-6, IL-8, TNF-α, and IFN-γ). Ozone can disable the virus by direct oxidation (ozone) or indirect (reactive oxygen species) and lipid oxidation products and can stimulate the cellular and humoral immune systems early in the COVID-19 infection stage.[17]

Ozone is a multifunctional drug that can stimulate inflammatory cytokines (IL-1β, IL-6, IFN-γ, TNF-α), anti-inflammatory cytokines (IL-4, IL-10), nitric oxide secretion or stem cells and can inhibit the NF-κB pathway, that can play the main role in stimulating hyper inflammation or cytokine storms.[41]

Physiological functions

Ozone has several beneficial properties that can be beneficial in the treatment of COVID-19. Systemic oxygen-ozone was used to evaluate adjuvant therapy in the initial control of disease development in patients with COVID-19 pneumonia. The data show that OT can improve the clinical condition of COVID-19 patients in a very short period, and increase many gas exchange parameters in people experiencing forced non-invasive ventilation in intensive care units (ICUs).[20]

Ozone can be through; activation of the pentose phosphate pathway, increasing the content of 2,3-diphosphoglyceric acid as well as increasing glucose-6-phosphate dehydrogenase and stimulating the metabolism of oxygen in red blood cells to provide sufficient energy and oxygen to the tissues. This may allow for a drastic change in functional activity that shifts tissues and organs from hypoxic to normoxic Also, it improves blood flow and capillary action, which has been reported to be beneficial for patients with ischemic vascular disease.[17,22] Therefore, ozone stimulates the glycolysis of red blood cells and increases the oxygen released to the tissues and stimulates the Krebs cycle, and thus increases ATP production (**Figure 1**).[13]

OT stabilizes hepatic metabolism and causes affinity, plasma levels of fibrinogen, and prothrombin to normal in infected patients, indicating a development in hepatic protein synthesis.[29] Ozone has an antiplatelet effect and increases the secretion of some prostacyclins such as prostaglandin I2, which is useful for patients with microthrombosis (**Figure 1**). All of these effects can help reduce the phenomenon of hypercoagulation in patients with COVID-19.[40] OT rapidly returns patients to normal respiratory physiology of the lungs, reducing inflammation and ischemia and thromboembolic effect, and ultimately leading to a complete recovery of oxygen saturation parameters.[32]

Clinical Practice of Ozone in the COVID-19 Therapy

OT was discovered in the mid-1800s and has been used in medicine for nearly a century. This substance is used specifically in the pre-antibiotic period for infectious diseases. Ozone improves gas exchange, decreases inflammation, and modulates the antioxidant system, making it beneficial for the inflammatory phase or the cytokine phase, and in the hypoxemia or multifocal stage.[42]

Supplemental use of OT for patients with COVID-19 through auto-hemoinfusion can improve oxygenation of tissues, reduce lung inflammation and regulate the immune response, prevent cytokine storm, slow viral growth, regulate pulmonary microcurrents and prevent or reduce the rate of vascular hypertrophy and subsequent hyperemia, especially in the early stages, by counteracting endothelial damage, similar to what occurs in peripheral arterial injuries.[29]

Evaluating new therapeutic resources to combat COVID-19 is a priority in clinical trials due to the limited number of available options. Probiozovid is a progressive, interventional, randomized, and prospective, double-arm trial in which COVID-19-induced pneumonia patients participate.[43] In this study, 28 patients were included and were randomly divided into ozone autohemotherapy and control groups. This treatment contained systemic administration of oxygen-ozone twice a day for 7 days. All patients were treated with the best temporary treatment accessible. Mortality within 30 days was 3.3% for the ozone group and 10% for the control group. But he did not report any toxicity.[43]

One study found that OT increased the antioxidant action of Nrf2 and inhibited apoptosis through attenuating nucleotide-binding receptor domain-like oligomerization, which includes NLRP3-mediated inflammation. As a result, given that systemic oxygen therapy has all of these positive effects: controlling inflammation, stimulating the immune system, antiviral ability, protecting against ischemia-reperfusion injury, acting on the proteasome and inflammation. OT can be considered as a new method of immunoceutical therapy. They also showed that the protective effect of OT with its anti-inflammatory properties was achieved by modulating NLRP3 inflammation. Mixing ozone and oxygen at low concentrations can effectively improve organ ischemia-reperfusion, which is what happens in the lungs of patients with COVID-19 infection. Ischemia-reperfusion is the leading cause of lung dysfunction in many pathological diseases.[23,44]

Tanaka et al.[45] showed how influenza viruses are deactivated by low concentrations of ozone in the environment and on smooth surfaces. Further studies have shown that ozone can play a key role in fighting bacteria, viruses, and fungal diseases. Murray et al.[46] showed a reduction in viral infection after ozone exposure. This causes lipid peroxidation in the virus capsid, therefore disturbing its reproductive cycle and preventing essential interaction between the virus and the receptor. Study has shown how ozone can deactivate strains with or without virus coverage.[47] Some strains, such as herpes simplex virus type 1 and vesicular stomatitis virus decrease after receiving ozone. Significant showed in infectious particles in 15 minutes. VAC strains (Elstree strain) and H1N1 strain (Influenza A virus) showed a decrease in 40 and 30 minutes, respectively. These outcomes indicate significant changes in the morphology of diverse virus species.[18]

In one study,[48] the effect of rectal ozone on COVID-19 patients with severe pneumonia at the University Hospital of Santa Cristina in Madrid has been investigated. Four patients with severe bilateral pneumonia due to COVID-19 confirmed by reverse transcription polymerase chain reaction (+) for SARS-CoV2 were treated with rectal ozone. The intra-rectal ozone treatment procedure consists of five sessions (one session per day) in a volume of 100 mL and a concentration of 35 μg/mL. This method improved the oxygen saturation of patients. Inflammatory biomarkers such as fibrinogen, D-dimer, urea, ferritin, lactate dehydrogenase, IL-6, and CRP were also decreased. In conclusion, radiological symptoms of bilateral viral pneumonitis improved by 1 to 2 degrees based on Taylor's radiological scale. Therefore, it was concluded that rectal ozone is safe, effective, inexpensive and a simple option that can affect the SARS-CoV-2 virus and is offered as an adjunctive treatment option in the management of severe bilateral pneumonia in COVID-19 patients.[48]

In a study by Fernández-Cuadros et al.,[17] ozone was shown to be antiviral, to modulate the immune system, to stimulate cel-

lular and humoral immunity, and to facilitate oxygen transport in hypoxemic tissues, so it could be used in the treatment of SARS-CoV-2. In Italy, people are treated with ozone via autohemotherapy to manage patients with COVID-19 pneumonia. Improvement of general clinical condition, normalization of temperature, reduction of CRP, and improvement of oxygen saturation were observed.[17]

A report from a clinical case in China presented that a 49-year-old patient with severe pneumonia kept in the ICU showed evidence of clinical improvement from the outset after five sessions of ozone by autohemotherapy. The effect of OT on tissue oxygenation lasted nearly 9 hours and the patient was effectively transferred to the internal medicine ward. In addition to the reduction of IL-6 and SARS-CoV-2 negative polymerase chain reaction resulting from nasal swabs, significant reductions in D-dimer, fibrinogen, and CRP were observed.[48] In Spain, Hernández et al.[49] published the report of a 49-year-old patient with severe pneumonia. The patient needed to use a mechanical ventilator and be admitted to the ICU, and was prescribed ozone (autohemotherapy) as his last hope for treatment. The patient showed rapid recovery, to the point that after two sessions he did not need to be admitted to the ICU and after five sessions the need for oxygen was significantly reduced. Hernández et al.[49] emphasized that the clinical improvement was due to the modulation of the immune system, oxygen delivery, and the antioxidant role of ozone through autohemotherapy in this patient.

OT has been used successfully as an adjunct therapy for patients with COVID-19 in China, Spain, Italy, and South America.[50] One suggested mechanism is to improve blood/tissue oxygenation to prevent multi-organ system failure due to lack of oxygen.[26] In three patients with COVID-19-induced pneumonia who had respiratory failure, a rapid improvement in hypoxia was observed with a decrease in inflammatory markers and D-dimer, which was observed after 1–4 sessions of oxygen-ozone treatment. In these three patients, invasive mechanical ventilation was not essential. After treatment with oxygen-ozone, all patients were discharged home in 3–4 days. Oxygen-ozone therapy appears to be an effective treatment for COVID-19 patients with severe respiratory failure. Many controlled clinical trials are needed to evaluate the efficacy and safety of oxygen-ozone therapy compared to the typical supportive case in patients with COVID-19 in terms of the necessity for invasive ventilation and the length of stay in the hospital.[40]

Conclusion

Atmospheric ozone is an air pollutant produced during the reaction between nitrogen oxides and volatile organic compounds. The virus genome contains a distinctive N-terminal fragment in the Spike protein, which allows the coronavirus to bind to air pollutants in the environment, although the effect of the virus spike on the transmission of the infectious agent has not yet been proven. Some studies support the stimulant effect of ozone on increasing virus replication. Atmospheric oxygen may gradually degrade the thiol groups in the virus and also increase the rate of degradation at higher temperatures. Many viruses require regenerated thiol groups to enter the host cell, which is rich in the amino acid cysteine. These amino acids are found in the spike, membrane proteins, and coatings of the SARS-CoV-2 virus. By oxidizing the reduced thiol groups of the amino acids cysteine and tryptophan, ozone can prevent the virus from entering the host body and exert its lethal effect. OT against the SARA-CoV-2 virus has antiviral properties, improves blood flow, facilitates the transfer of oxygen in hypoxemic tissues, and reduces blood coagulation phenomena in patients with COVID-19. Ozone has immunomodulatory effects by modulating cytokines (reduction of IL-1, IL-6, TNF-α, and IL-10) that prevent disease exacerbation, induction of INF-γ has anti-inflammatory properties by modulating NLRP3, inhibition of cytokine storm (ozone blocks the NF-κB pathway and stimulates the Nrf2 pathway), stimulates cellular and humoral immunity, stimulates phagocytic function, and blocks the receptor of the virus, ACE2.

There are various methods for injecting ozone gas, containing penetration into the external ear canal, vagina, rectum, and bladder, as well as direct injection of ozone gas into muscles, joint spaces, and discs to relieve back pain. In direct oxygen/ozone injection, oxygen is equilibrated with extracellular fluid and intra-erythrocytic before binding to hemoglobin, and ozone, which is more soluble than oxygen, reacts with several biological molecules such as thiol groups in albumin to form ozonoids. Intravenous injection of ozonated saline significantly increases the length of time a person can remain hypoxic. Many physicians have used intravenous ozonated-saline injections to prevent out-of-body ozonation concerns and incompatibilities with heparin. Oxygen-ozone therapy seems to be an effective treatment for COVID-19 patients with severe respiratory failure. The rectal ozone protocol is intrarectal ozone, which can cause clinical improvement of oxygen saturation, biochemical improvement (fibrinogen, D-dimer, urea, ferritin, LDH, IL-6, and CRP), and 1–2 degree improvement of radiological symptoms which are assessed based on the Taylor Radiology Scale. Rectal ozone is a safe, effective, inexpensive, and simple option that can affect the SARS-CoV-2 virus and is offered as an adjunctive treatment choice in the management of COVID-19 severe bilateral pneumonia.

Ozone prevents virus replication and kills the virus, increases oxygenation to the blood and tissues, stimulates immune function through Nrf2, inhibits inflammatory pathogens (interleukins, cytokines, and TNFs), prevents the formation of small thrombosis, increases the expression of heme oxygenase-1 in endothelial cells, stimulates 2–3 diphosphoglycerates, so more oxygen to the delivers tissues.

It is important to note that medical ozone should not be administered directly into the bloodstream and should not be mixed in solution with other drugs (other than saline) due to its oxidizing effect. There are also no studies on OT and pregnancy. Therefore, it should be avoided in this case. Another absolute contraindication is its systemic administration to people with glucose-6-phosphate dehydrogenase deficiency. Medical ozone administration in patients with hyperthyroidism can also be harmful to the lungs. In general, many studies have shown the positive effect of OT as a complementary therapy in the recovery of patients with viral diseases including SARS-CoV-2. The results indicate that systemic OT

is not toxic and has no side effects in patients with severe COVID-19. Oxygen–ozone therapy seems to be effective in COVID-19 patients with severe respiratory failure. OT is more effective for severe patient's conditions, and oxygen is an oxidizer that acts stronger the higher the temperature, so it can be more effective than existing treatments in critically ill patients with fever.

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Author contributions
ME investigate and supervised the findings of this work, wrote the article, supervised the project, contributed to the interpretation of the results; BY designed the study, helped supervise the project and conceived the original idea; SZB and ZKF developed the theoretical framework; SH and PK helped supervises the project, conceived the original idea; AA and AE were responsible for manuscript revision. All authors discussed the results and commented on the manuscript, provided critical feedback, and helped shape the research, analysis, and manuscript. The authors contributed to the final version of the manuscript.

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The authors have no conflict of interest.

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