Ozonetherapy: a multirole weapon, topical pathway role against SARS-COV-2.

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Abstract

COVID-19 is the respiratory disease caused by the new coronavirus SARS-CoV-2 and is characterized by clinical manifestations ranging from mild, flu-like symptoms to severe respiratory and multi-organ failure. Patients with more severe symptoms may require intensive care treatments and face a high risk of mortality. COVID 19 is characterized by an abnormal inflammatory response similar to a cytokine storm, which is associated with endothelial dysfunction and microvascular complications. To date, no specific treatments are available for COVID-19 and its potentially life-threatening complications.

Ozonetherapy is the administration of a mixture of ozone and oxygen (MO), which produces a series of benefits capable of counteracting a wide range of pathologies, in use for over a century as an unconventional medicine practice.

Ozonetherapy using the techniques of small self-emo-infusion, and the topical application of ozonated oils or irrigation with ozonated water at the nasal level, could help to enhance the innate immune response at the level of the entrance ports in order to decrease the viral load and slow viral growth, especially in the early stages. In fact, recent studies show that nasal transport is likely to be a key feature of transmission, and drugs / vaccines administered intranasally could be highly effective in limiting spread.

Introduction

COVID-19 is a disease caused by SARS-Coronavirus-2 (SARS-CoV-2), a virus belonging to the same family of viruses that causes SARS and MERS, which was first reported in Wuhan in December 2019, in Hubei province, China (1,2). COVID 19 occurs in a wide range of clinical manifestations, ranging from mild, flu-like symptoms to severe interstitial pneumonia.

SARS-CoV-2 infection can be roughly divided into three stages (3):

1. stage I, an asymptomatic incubation period with or without detectable virus;

2. stage II, non-severe symptomatic period with presence of viruses in the upper airways, symptoms flu-like: acute upper respiratory tract infection (fever, fatigue, myalgia, cough, sore throat, runny nose, sneezing) or digestive symptoms (nausea, vomiting, abdominal pain, diarrhea), the virus becomes evident on the swab;

3. stage III, severe respiratory symptomatic stage with high viral load, COVID pneumonia with dyspnoea (frequent fever, cough) without evident hypoxemia/hypoxemia (SpO2 <92%), thoracic CT with lesions: virus in the upper airways;

4. complications that often lead to exitus: critical, complications that often lead to exit: acute respiratory distress syndrome (ARDS) characterized by presenting shock, encephalopathy, myocardial damage, heart failure, clotting dysfunction and acute renal damage.

Patients with more severe symptoms may require intensive care treatment and face a high risk of mortality. COVID 19 can cause an abnormal inflammatory response, which in fact resembles a cytokine storm, which is associated with endothelial dysfunction and microvascular complications.

COVID-19 begins with the arrival of the SARS-CoV-2 viral particles on the surfaces of the human respiratory mucous membranes. The ACE2 SARS-CoV-2 input receptor and the TMPRSS2 viral protease are highly expressed by the hair cells, the epithelial cells that line the mucous membranes, and by the goblet cells that produce the mucus, and which together represent the first defensive barrier (4). If the virus manages to overcome it, the release of warning signs activates the rapid reaction of innate immunity.

We do not yet know if and how many SARS-CoV-2 viral particles are eliminated by this inflammatory reaction, however it is reasonable to assume that the greater or lesser efficacy of this initial reaction is crucial in determining whether the viral infection will be milder or more massive (5). Once the virus has entered the cell, the viral RNA is immediately translated by the infected cell into viral proteins. Subsequently, the infected cell dies releasing millions of new viral particles.

Innate immunity is a first, effective line of defense against microbes. The available evidence indicates that it is able to block more than 90% of microbes invasions and the result of this first line (especially local innate immune response, represented by natural IgA and IgM antibodies, interferons and MBL, will be able to influence, together with the viral load, the response of 10-15 days following infection (6).

If SARS-CoV-2 overcomes innate immunity blockade and spreads from the upper airways to the alveoli in the early stages of infections, it can replicate without local resistance, causing pneumonia and releasing large quantities of antigens. The delayed and strong adaptive immune response (high affinity IgM and IgG antibodies) that follows, causes severe inflammation and triggers its mediator falls (complement, coagulation and cyitokine storm), that may require intensive care treatments and face a high risk of mortality (8).

For adaptive immunity, knowledge is still scarce and based mainly on what has been learned by studying SARS and MERS. As commonly occurs during viral infections, the protective reaction appears to be based on the action of T helper1 lymphocytes which orchestrate a complex immune reaction.

Antibodies to SARS-CoV-2 virus have been isolated from the blood of SARS, MERS and COVID-19 patients, antibodies that are able to neutralize its infectious capacity. Coronaviruses, however, are extremely effective viruses in suppressing various immune response protective mechanisms. They suppress the production of interferons and block the presentation of antigens by Class I and Class II major histocompatibility complex (HLA) glycoproteins.

In this scenario, it is essential to find new therapeutic treatments, possibly able to react even in the early stages of the disease and in this work we will examine the reasons for the usefulness of topical

administration of MO, which has indication in treating COVID-19 in all its stages. In the present work we will examine the reasons for the usefulness of topical administration of MO in stage I and II.

Ozone is an allotropic form of oxygen, the molecule of which is made up of 3 oxygen atoms. Ozone in nature is a powerful oxidant, which acts directly on cells through lipid lipid peroxidation, oxidation of amino acids and irreversible DNA damage that leads to cell death. In fact, despite being one of the best disinfectants, it is extremely toxic; ozonetherapy consists in the preparation of an extemporaneous mixture of ozone and oxygen, so-called MO (MO).

MO has various actions capable of contrasting a wide range of pathologies, in use for over a century as an unconventional medicine practice. MO can be administered systemically by adding it to a patient's blood sample, which is then reinfused (self-emo-infusion), or adding it to the saline solution. It can also be administered locally, by subcutaneous injection, by inhalation of ozonated water or nasal and rectal insufflation or by exposing the skin to a mixture of air containing MO or by adding ozonated oils.

Ozone reacts mainly with biological liquids that are a constituent part of our body, both as intracellular and extracellular liquids. The complexity and the number of biochemical reactions generated during the exposure of blood to MO involve, in the space of milliseconds, a very wide range of substrates that interact in different metabolic pathways.

Nasal oxygen-ozone insufflation and fight against SARS-COV-2

The antiseptic, antiphlogistic, antiviral, hemoreological and trophic-tissue properties, due to MO contact with mucous membranes, find significant indication in acute and chronic nasopharyngeal pathologies such as allergic rhinitis, vasomotor rhinitis, atrophic rhinitis, sinusitis (8,9). Through this specific technique you can help cold and sinus sufferers to heal, but also to prevent these diseases (10,11,12,13).

The technique is fundamental, as the patient during the insufflation, which the doctor will carry out with a syringe without a needle, taking care to close the nostril with the hand at that moment not treated, must remain in apnea, in order not to inhale the mixture of oxygen-ozone, very irritating to the airways. The treatments have a biweekly frequency, for a total of about 10 sessions, but it can also be greater.

It is at the level of the mucosa of the upper airways that MO intervenes to block or limit the aggression of SARS-COV-2, thus reducing the viral load and helping to enhance the innate immune response, at the level of the gates entered in a way to slow viral growth, especially in the early stages: since nasal transport is likely to be a key feature of transmission, intranasally administered drugs/vaccines could be highly effective in limiting the spread.

A. To successfully penetrate cells, many viruses require membrane glycoproteins to be in the reduced form of RSH rather than oxidized (RSSR). MO directly inactivates many viruses. Norwalk, MS3 collofagus, hepatitis A and poliovirus depend on sulfhydryl groups reduced (14,15,16,17,18). "Reflecting on the reduction" of critical disulfide bonds for cellular entry of the vaccine virus, Ryser found that disulfide isomerase protein inhibitors limited HIV entry -1 in T cells (19).

Mirazmi, et al. found that cytomegalovirus loses infectivity if its thiol groups are oxidized. Reducing the oxidized thiols (with dithiothreitol) allowed the virus to regain 65% of infectivity. HIV depends on reduced groups of sulfhydryl for infectivity, as reported also for the Ebola virus that enters the cells (20).

Like Ebola, the structure of SARS-COV-2 also has regions rich in cysteine, including peak and envelope proteins. Cysteine is an amino acid that carries a sulfhydryl residue (RSH), also called "thiol" group. Alterations of these residues have been found to "paralyze" the growth properties of the virus

The thiol bonds SH are much weaker than the OH bonds in alcohols and vulnerable to oxygen-based oxidants and the ozone itself immediately oxidizes the SH groups on contact and even if it practically extinguishes instantly on contact with the blood, it will create ozonides which are oxidants in their own right.

They have an extended life according to Bocci and Menendez works, providing continuous protection after a single treatment. These molecules are less reactive than ozone, but still possess an oxidizing power and act as biochemical signaling molecules that modulate the immune system. By creating a more "oxidized" environment, MO can help the body inactivate thiols in blood and tissue viruses.

The ability of ozone to inactivate cysteine-dependent proteins has been reported as an attack of ozone on cysteine-dependent papain, which is believed to inactivate the enzyme by oxidizing the sulfhydryl group active in sulfate or sulfenic acid. In addition, the coronavirus peak protein is also rich in tryptophan, which is second to cysteine in vulnerability to oxidation.

Finally, it is well documented in the scientific literature that MO inactivate microorganisms including bacteria, fungi and viruses, wrapped and unwrapped viruses, including herpes simplex virus type 1 (HHV-1, McIntyre strain), Indian vesicular stomatitis virus (VSIV), vaccinia virus (VACV, Elstree strain), type 2 adenovirus (HAdV-2) and PR8 strain of influenza A virus (FLUAVA / PR / 8/34 / H1N1; FLUAV) (12).

B. It’s well known that MO stimulates cellular and humoral immunity through the activation of the pathways linked to the transcriptional factors NFAT (nuclear factor activated T-cells) and AP-1 (Activated Protein-1) (21). NFAT and AP-1 are crucial factors responsible for inducing the transcription of cytokine-related genes: Interleukine- (IL) -2, IL-6, IL-8, Tumor necrosis factor-α (TNF-α), Interferon-γ (IFN -γ). These cytokines perform potent pro-inflammatory functions, have chemotactic ability to promote aggregation and infiltrations and improve the phagocytosis of neutrophils, lymphocytes, macrophages and other inflammatory cells to inactivate local pathogens (22,23).

T cells, like soldiers, defend our body from foreign pathogens: a tyrosine-phosphorylation response takes place immediately in the ZAP-70 molecule when the T cell antigen receptor (TCR) recognizes any invaders, and therefore activates the phospholipase Cγ1 (PLCγ1). The membrane lipid phosphatidylinositol-4,5-bisphosphate (PIP2) can be hydrolyzed by activating PLCγ1, thus producing two critical second messengers including inositol triphosphate (IP3) and diacylglycerol (DG). Hence, IP3 binds to its receptor (IP3R) located in the membrane of the endoplasmic reticulum (ER), which leads to Ca +2 transformed by ER into the cytosol. High levels of Ca +2 in the cytosolic will activate calcineurin (CN), a Ca +2 / calmodulin dependent phosphatase, which dephosphilizes nuclear factor activated T cells (NFAT) and transports them to the nucleus. NFAT therefore induces the

transcription of cytokines, such as IL-2, TNFα, IL-6 and IFNγ, participating in the immune response of our body.

These nuclear factors produced in mild oxidative stress are induced by ozone therapy and therefore activate the immune functions. Several studies have been conducted using normal human blood treated with adequate ozone concentrations and ozone works well without any toxicity. In numerous researches several cytokines have been found including IFNγ, TNFα, IL-2, IL-6 and IL-8, synthesized and released by immune cells, showing a dose dependent on the concentration of ozone. Torossian et al. showed that TNFα level was significantly improved with ozone pretreatment in septic rats (24).

Interestingly, the amount of lymphocytes and monocytes present in the blood exposed to ozone *ex vivo* was activated only for about 4% during each session of MO, which suggested that it is the small portion of immunocytes activated by H2O2 *ex vivo*. It is assumed that NF-κB can play a vital role in transmitting the activation effect in vivo after infusing ozonated blood into the donor patient and thus activating other cells, balancing NF-kb effects in sophisticated loop. In fact, in line with the MO antimicrobial effects in acute and chronic bacterial and viral infections, MO can serve as an ideal therapeutic method without any toxicity in the appropriate dosages.

On the other hand, a recent study conducted by Frank A. et al. (25) showed obviously decreased IgE and HLA-DR levels in patients with asthma treated with systemic MO. Lung function and symptom testing have been significantly improved. This study demonstrates the effectiveness of MO in reducing IgE and inflammatory mediators together with the induction of antioxidant elements through its immunomodulatory and oxidative stress regulation properties. The study showed that MO improved the expression levels of innate immune surface proteins CD14, CD11b and TLR4, CD80 antigen presentation markers, CD86e HLA-DR receptors and immunoglobulins CD23, CD16 and FcεR I. At the same time, MO increased oxidative burst and phagocytosis, so MO exposition could increase checked body inflammatory environment improving the response to biological agents.

C. Equally noteworthy is the fact that MO modulates the processes of inflammation through molecular pathways linked to Nf-kB (Nuclear Factor-kB) and Nfr2 (Nuclear Factor (erythroid-derived 2) -like 2). NF-κB and Nrf2 are part of an important network of transcription factors and regulatory proteins that modulate the expression of a wide range of genes, including those associated with inflammatory responses. Activation of Nf-kB promotes the transcription of genes linked to pro-inflammatory cytokines, while MO exerts its anti-inflammatory and consequently anti-apoptotic capacity, blocking the action of NF-κB (26,27).

Finally, MO acts as an anti-inflammatory but also an antioxidant, through the activation of the Nfr2 pathway. MO activates Nrf2 (Galiè et al., 2018) in the cytoplasm, which in turn moves to the nucleus, dimers and binds to the ARE (Anti-oxidant Response Elements) regions of genes that encode different anti-oxidant enzymes: Superoxido dismutase (SOD), Glutathione (GSH), Glutathione -S- Transferase (GST), catalase, glutamate-cysteine ligase (GCL), heme oxygenase 1 (HO-1), NADPH: quinone oxidoreductase 1 (NQO1), phase II enzymes of drug metabolism and heat shock proteins (HSP). HO-1 is an enzyme that encodes the gene that catalyzes the degradation of heme to carbon monoxide (CO), which in turn inhibits the NF-kB pathway, causing a reduced expression of pro-inflammatory cytokines. HO-1 directly activates anti-inflammatory cytokines (28,29).

The Cuban Menendez team found that preconditioning animals with ozone is as powerful as dexamethasone in reducing tumor necrosis factor α and subsequent endotoxic shock. This could be

exceptionally valuable as a means of safely suppressing the "cytokine storm", often the cause of the final mortality from viral lung infection, including coronavirus (30,31,32).

MO promotes immune system homeostasis by normalizing the parameters that have increased and increasing those that have decreased. In this case, the increase in antioxidant enzymes prevents free radicals from damaging vital structures and blocking the production of new oxidizing molecules. As the cells move from an oxidizing environment to a reducing environment, they maintain the integrity of the membrane and maintain their specific functions which, in the case of cells of the immune system, defend against pathogenic microorganisms (33).

The functioning of the immune system is strongly influenced by the redox balance, in particular in cells that have cytotoxic and phagocytic functions, which, due to their microbicidal activity, are linked to the generation of free radicals and deteriorate under oxidative stress.

The importance of these transcription factors in the immune response is based on their participation in intracellular signaling pathways for the activation of T lymphocytes whose adequate function is essential for defense against infections.

Finally, the significant increases in IgG and IgM found after two cycles of MO, by large autoheminfusion, show its effect on these immunological parameters, favorably influencing the clinical status of patients who had a good therapeutic response by reducing recurrence of infection. It should also be noted that the stimulating effect of humoral immunity is maintained even in the period of 6 months after the end of the treatments in immunocompromised patients.

In this perspective, a phase II controlled and randomized clinical trial was conducted: IgA improved, with a significant difference ($p = 0.04$) between the MO group and the control group, the blood IgG levels are significantly increased ($p = 0.03$) in the ozone-treated group compared to the control group. Leukocyte activity improved significantly ($p = 0.04$) a month later, reaching 97% of normal values (34).

D. Finally, it should be noted that MO induces Nitric Oxyde synthetase (NOS) at the molecular level with the consequent formation of nitric oxide (NO) and other RNS. NO is produced continuously by the epithelial cells of the paranasal sinuses and nasopharynx and from here it can spread to the bronchi and lungs, where it induces vasodilator and bronchodilator effects (35).

Furthermore, NO activates ciliary movement and mucus secretion which can increase the removal of dust and viral particles from the respiratory tract and produces antimicrobial effects against a wide range of microbes including bacteria, fungi, helminths, protozoa and viruses and in particular SARS-VOC (36).

In humans, higher basal levels of exhaled NO are associated with fewer common cold symptoms (37), also interfering with the interaction between SARS-COV-2 S protein and cellular ACE-2 receptor (38).

Furthermore, NO is a well-characterized signaling molecule that mediates various physiological effects, influencing the functional states of transcription factors and other signaling molecules and modulating gene expression, which leads to activation of the KEAP1-NRF2 pathway and increasing HO-1 levels through its upregulation in transcription mediated by the activation of Nrf2 (39).

In turn HO-1 is an enzyme that codes the gene catalyzing heme to carbon monoxide degradation (CO), which in turn inhibits the NF-kB pathway, causing a reduced expression of pro-inflammatory cytokines. HO-1 directly activates anti-inflammatory cytokines: there are many cytokine signaling

circuits involving HO-1 activity or expression, including a positive feedback circuit between HO-1 and IL-10 (anti-inflammatory) and a circuit negative feedback between HO-1 and TNF-α (pro inflammatory) with inhibiting cytokines and chemokines such as IL-1β, IL-8, IL-33, MCP-1 and MIP-1β.

NO gas Inhalation is currently being studied as a preventive measure and treatment against COVID-19, given its use as a rescue treatment to improve arterial oxygenation against acute respiratory distress syndrome (ARDS) 40).

Discussion

From this point of view, it seems fundamental to study how to manage MO at the level of the nasal mucosa, taking into account the technical rudeness and the possibility of putting dedicated healthcare personnel at risk, due to the huge production of pressure aerosols. Help could come

- from the nasal instillation of ozonated water, which has the only disadvantage of having to be produced daily and adequately preserved (with all the precautions and appropriate equipment, given the toxicity of volatile ozone), given its poor duration, or a few days if suitably refrigerated;
- from affixing ozonated oil based ointments, widely tested at the mucous/skin level for the resolution of infected wounds/ulcers and Herpes virus expressions.

Ozonated oil born from the encounter between an oil and ozone: through a bubbling process (with accurate processes for the potential production of volatile ozonides), products are created, called OZONIDS, in which the double bonds of the fatty acids contained in the oil are saturated with 3 oxygen molecules. In this way, oxygen is imprisoned, stabilized and made available and ready for use for topical applications. The ozonated oil does not induce the adaptation or habituation phenomena typical of drugs and can therefore be applied for long periods without contraindications, indeed with a positive effect added over time.

Its range includes herpetic, fungal, bacterial, dermatosis, acne infections, Herpes simplex genital treatment, vulvovaginitis and irritative vulvitis, breast fissure prevention, bedsores, post radiotherapy treatments, treatment of skin ulcerations, hemorrhoidal syndromes, bruises and muscle-joint injuries (41,42).

Finally, to remember as the possibility of local administration of ozonated oil in the form of mouthwash (43).

In addition to topical use, the small self emotherapy has relevance in which 10 ml of venous blood is taken from the patient then mixed with Ozone and reintroduced intramuscularly. Indications include: asthma, acne, particular allergic conditions. The immune modulating properties of ozone are of primary importance in the treatment of chronic diseases, such as chronic bronchitis and bronchial asthma and it could play an important role especially in the paucisymptomatic stage II.

Conclusions

MO administration with insufflation/inhalation of ozonized distilled water, appopsition of ozonated oli and small autoheminfusion could help to regulate the immune response, help slow down viral growth, and avoid or limit the use of the intubation and, ultimately, allow a shortening of healing times with the possibility of a greater replacement in intensive care, up to now the real limiting factor.

Furthermore, since there are no important side effects and can be synergistic with other therapies, it is a candidate to be an essential therapy in home care, given its ease of execution and low cost.

Finally, from the prevention, individuals at stage I and asymptomatic carriers are the least manageable because, at least on some occasions, they spread the virus unknowingly: lower the viral load in everyone and limit COVID-19 to stages I and II, by means of endonasal insufflation or similar technique, would allow us to save time pending the synthesis of molecules capable of arresting the virus or the fielding of the much required vaccine.

In addition, studies on the involvement of the S.N. are multiplying in COVID-19: Netland et alt [44] using transgenic mice for human ACE2 have shown that the SARS-COV virus enters the brain through the olfactory bulb and uses a rapid transneuronal spread to reach the olfactory cortex, basal ganglia etc.: in analogy the viral neurotropism of SARS-CoV-2 could cause invasion of the olfactory nerve. the olfactory brain and thus the brain stem causing irreversible respiratory failure typical of severe COVID-19. [45]

Therefore, on the one hand, a human respiratory pathogen may reach the central nervous system through different pathways and induce short-term disease and, on the other hand, may persist in resident cells of the human central nervous system and may become a factor or co-factor in neuropathogenesis associated with long-term neurological sequelae in genetically or otherwise predisposed individuals, as happened in late Parkinsonism that occurred among survivors of lethargic encephalitis [46] during the 1918-1920 influenza pandemic and, more recently, the increased risk of narcolepsy observed in 2009-2010 after the swine flu pandemic [47].

This suggests that better surveillance, diagnosis and in-depth studies of virus-host interactions are warranted in order to gather more knowledge that will make possible the development of therapeutic strategies to prevent or treat the events and in this perspective these latter considerations should motivate a serious experimentation that ascertains the potential of MO by topical pathway in preventing or limiting SARS-COV-2 infection at the first airway level, especially at the nasal level, and thus also block or limit the invasion of the Nervous System.

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