

Oxygen-ozone immunocutaneous therapy in COVID-19 outbreak: facts and figures

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The problems related to the approach to the outbreak of COVID-19 in the world require that all possible effective treatment options be explored. The clinical criterion of the researcher is not to refuse a priori, but to verify and evaluate the proposals that are made. Italian Society of Oxygen Ozone Therapy (SIOOT) proposed to the Italian ISS (Italian Institute of Health) to use oxygen ozone therapy (O₂O₃) in patients with COVID-19. The ISS has said on 24 March 2020 that it is possible to use it in the light of scientific considerations:

Although the proposal is supported by a certain rational basis, the data actually available in relation to the proposed indication consist essentially in demonstrating the effectiveness of ozone in killing SARS virus in monkey cells, and clinical experience of the benefit in patients with bronchopulmonia. However, as the proposal appears to be shared and supported by clinical centers experienced in the treatment of viral pneumonia, treatment could, if deemed appropriate, be carried out under the responsibility of the physician, after obtaining the informed consent of the patient. Considering the "experimental" character of the use this indication, which also requires specific medical experience and the availability of appropriate instruments, it is considered appropriate to acquire also the opinion of the Ethics Committee.

Some hospitals in Lombardy, the region most affected by COVID-19 in Italy, have already started its use with initial positive results.

As little is known about O₂O₃ in the world, although there is a lot of scientific evidence published in the recent years on the anti-inflammatory, immunomodulatory and organ-protective

validity of O₂O₃ therapy, we consider useful to follow the suggestions of the article *Activating Immunity to Fight a Foe - A New path*, by Richard S. Hotchkiss and Steven M. Opal,¹ and proposing the O₂O₃ therapy as immunocutaneous therapy according to the indications of the article. The O₂O₃ therapy has many biological effects, but the most relevant is its ability to modulate the activation of Nrf2 (an important nuclear message transducer), the downregulation of NFκB, and it also modulates the NLRP3 inflammasome.²⁻⁴

In this letter, I wish to present this therapeutic opportunity, outline the important activities of the Oxygen Ozone Therapy (O₂O₃) and explain the rationale for this treatment in COVID-19 patients.

Spike protein and Angiotensin-Converting Enzyme 2 (ACE2) cell receptors have been identified as putative receptors for SARS-CoV-2; they could promote the proliferation of COVID-19 (Figure 1). It is known that these receptors can be blocked with some specific monoclonals but also through the control of Nrf2 that regulates and blocks the activity of Spike and ACE2. Because O₂O₃ acts directly on Nrf2, stimulating them, it seems very likely that this is the most important physiological mechanism to block endogenous COVID-19 reduplication by preventing contact with putative receptors of SARS-CoV-19.

Oxygen-ozone has a high solubility in plasma and induces formation of two second messengers, H₂O₂ and ozonoids and alkenals (Figure 2).⁵ These are the ones who are mainly competent, interacts with the membrane proteins and receptors of the cells, especially the immunocompetent ones, and enter the cells and interact with signal transduction proteins on the nucleus and mitochondria level.⁶⁻⁹ The key action mechanism of O₂O₃ therapy is its action on proteasome and inflammation cascade, to control inflammatory process, by stimulating the nuclear factor Nrf2 and by inhibiting nuclear factor NFκB (Figure 3).^{10,11}

The O₂O₃ therapy can restore the right immune response by stimulating signal transduction molecules via Nrf2 and thus stimulating the nuclear transduction via specific microRNAs restoring the normal antioxidant and immunostimulating reaction.¹² The action mechanism of O₂O₃ therapy has very well summarized by Noel L. Smith *et al.* in 2017:¹³ *By reacting with Polyunsaturated Fatty Acids (PUFA) and water, O₃ creates hydrogen peroxide (H₂O₂), a Reactive Oxygen Species (ROS). Simultaneously, O₃ forms a mixture of Lipid Ozonation Products (LOP).*

The LOPs created after O₃ exposure include lipoperoxyl radicals, hydroperoxides, malonyldialdehyde, isoprostanes, the ozonide and alkenals, and 4-Hydroxynonenal (4-HNE). Moderate oxidative stress caused by O₃ increases activation of the transcriptional factor mediating nuclear factor-erythroid 2-related factor 2 (Nrf2). Nrf2's domain is responsible for activat-

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Received for publication: 2 April 2020.

Accepted for publication: 8 April 2020.

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Ozone Therapy 2020; 5:9014

doi:10.4081/ozone.2020.9014

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ing the transcription of Antioxidant Response Elements (ARE). Upon induction of ARE transcription, an assortment of antioxidant enzymes gains increased concentration levels in response to the transient oxidative stress of O₃. The antioxidants created include, but are not limited to, Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), Glutathione S-Transferase (GST), Catalase (CAT), Heme Oxygenase-1 (HO-1), NADPHquinone-Oxidoreductase (NQO-1), Heat Shock Proteins (HSP), and phase II enzymes of drug metabolism. Many of these enzymes act as free radical scavengers clinically relevant to a wide variety of diseases.

Masaru Sagai *et al.* in 2011¹⁴ described the biological responses induced via the activation of Nrf2/ARE with mild oxidative stress (O₂O₃ therapy) that can be summarized in:

- i) Increasing the levels of direct antioxidants, such as GSH, CO, and bilirubin;
- ii) Stimulating GSH regeneration via glutathione and thioredoxin reductase;
- iii) Increasing the levels of enzymes that detoxify oxidants and electrophils (*i.e.* catalase, SOD, GPx, GSTr, NADPH-Quinone Oxidoreductase (NQO1), HO-1, HSP70, *etc*);
- iv) Increasing the levels of phase II enzymes;
- v) Inhibiting cytokine-mediated inflammation via the induction of leukotriene B4 reductase;
- vi) Reducing iron overload, and subsequent oxidative stress induced via elevated ferritin;

- vii) Recognizing, repairing, and removing damaged proteins;
- viii) Protection from apoptosis induced via oxidative stress;
- ix) Increasing DNA repair activity.

In addition, Jacqueline Diaz-Luis *et al.* in 2015¹⁵ demonstrated that ozone was able to modulate the phagocytic cells in peripheral blood and the mechanisms on how messengers can activate immunological response leading to the therapeutic biological effects. Furthermore, it was demonstrated that there is a range of ozone concentrations where we can obtain the highest positive results, while lower doses are ineffective and higher doses can produce lower effects. Accordingly, ozone, in a dose-dependent behavior, may stimulate the phagocytic function of the peripheral blood cells.

Another important effect of O₂O₃ therapy than can explain its effects in improving the therapeutic approach to COVID-19 infected patients is its important action on NLRP3 inflammasome that is recognized to play a crucial part in the initiation and continuance of inflammation in various diseases. Gang Yu *et al.* in 2016 demonstrated that the protective effect of ozone therapy was achieved by its anti-inflammatory property through the modulation of the NLRP3 inflammasome. Ozone-oxygen mixture at low concentration could effectively improve organ ischemia-reperfusion that is what happens in the lungs of patients affected by COVID-19 infection.¹⁶

Ischemia-Reperfusion Injury (IRI) is a major cause of lung dysfunction during many pathological diseases. Zhiwen Wang *et al.* in

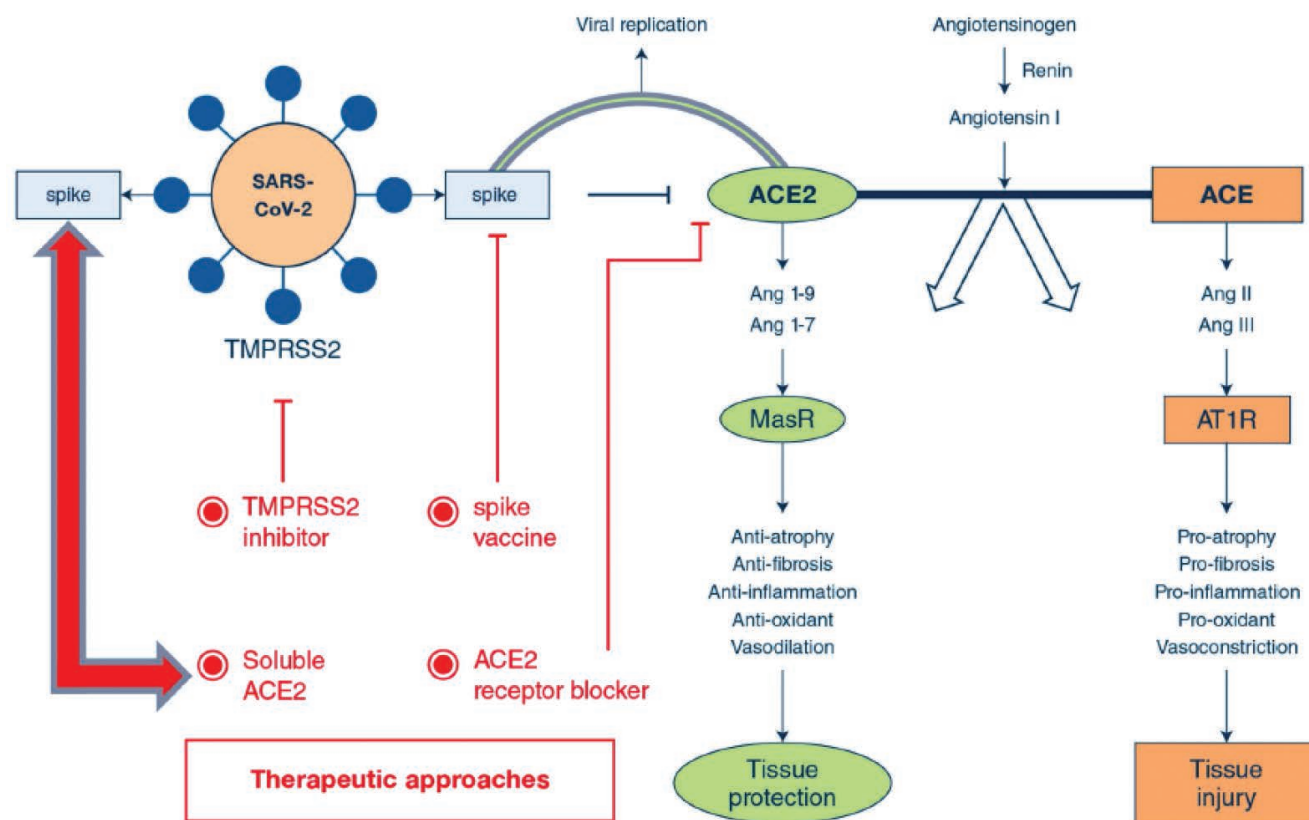


Figure 1. Potential approaches to address ACE2-mediated COVID-19 following SARS-CoV-2 infection. *Intensive Care Med* 2020;46:586-90. doi: 10.1007/s00134-020-05985-9. Creative Commons licence (<http://creativecommons.org/licenses/by-nc/4.0/>).

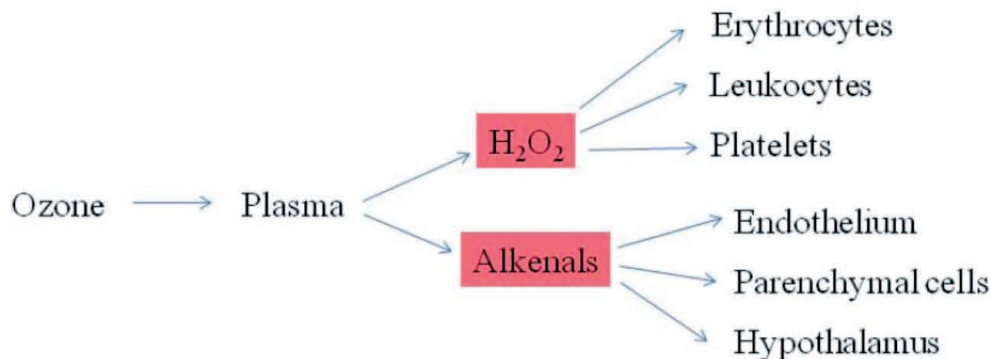


Figure 2. A scheme showing the reaction of ozone with plasma. The generated hydrogen peroxide triggers biochemical pathways in blood cells, while alkenals, after the infusion of ozonated blood into the donor, act on a variety of cells, upregulating the synthesis of many antioxidant proteins. *Med Gas Res* 2011;1:29. doi: 10.1186/2045-9912-1-29. Creative Commons licence (<http://creativecommons.org/licenses/by-nc/4.0/>).

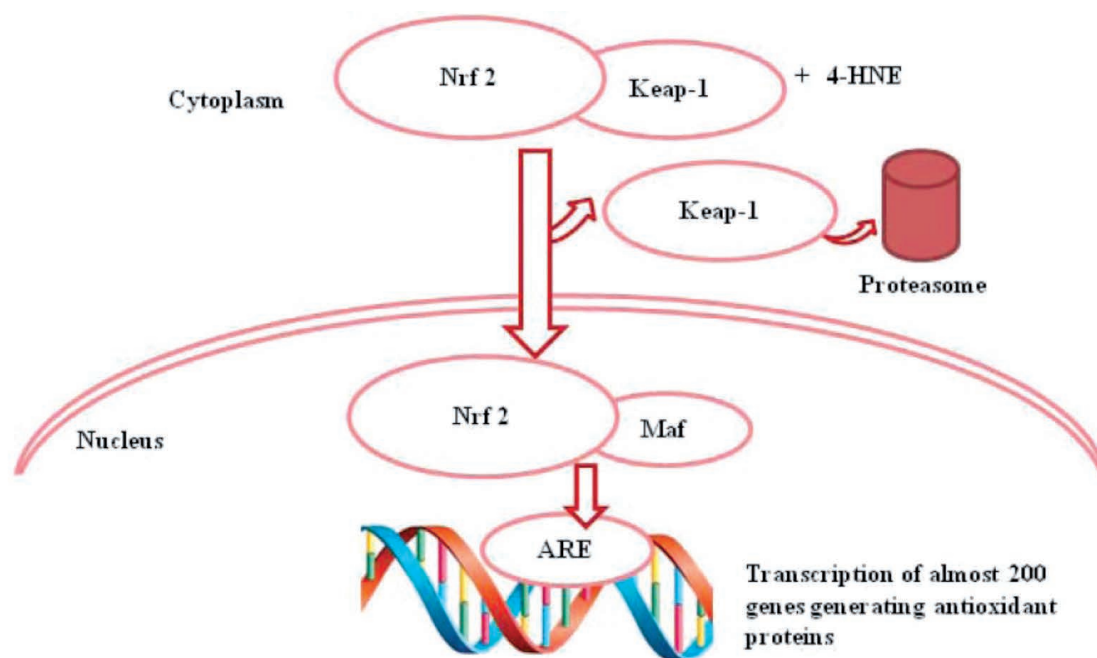


Figure 3. The transcription factor Nrf2 bound to Keap-1 activated by alkenals. The released Nrf2 translocates into the nucleus and, after binding to Maf, docks on ARE and activates a number of genes leading to the synthesis of antioxidant proteins. *Med Gas Res* 2011;1:29. doi: 10.1186/2045-9912-1-29. Creative Commons licence (<http://creativecommons.org/licenses/by-nc/4.0/>).

2018 demonstrated that ozone oxidative treatment protects the lung from IRI by attenuating nucleotide-binding oligomerization domain-like receptor containing pyrin domain 3 (NLRP3)-mediated inflammation, enhancing the antioxidant activity of Nrf2 and inhibiting apoptosis.¹⁷

In conclusion, as systemic oxygen therapy has all these positive effects: control of inflammation, stimulation of immunity, antiviral ability, protection from ischemia-reperfusion damage, action on proteasome and inflammation.^{7,9} Oxygen-ozone therapy can be said to be a new method of immunocutaneous therapy and therefore its use in combination with other treatments in COVID-19 positive patients may be justified, helpful and synergistic.

Further studies and tests are needed, but we hope to soon have confirmation that O₂O₃ therapy is synergistic and effective in controlling COVID-19-infection.

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