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Is Hyperbaric Oxygen an Effective Treatment for the Prevention of Complications in SARS-CoV-2 Asymptomatic Patients?

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Abstract: Is hyperbaric oxygen therapy (HBO2T) useful to counteract severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in positive asymptomatic patients? Asymptomatic persons seem to account for approximately 45% of SARS-CoV-2 infections, and they can transmit the virus to others for an extended period, perhaps longer than 14 days. In patients dying from SARS-CoV-2 infection, the mean number of concomitant diseases was 3.6 (median 3, SD 2.1). Many of these diseases are correlated with the nitric oxide synthase (NOS) genetic polymorphism and reduced NO synthesis [risk for coronary heart disease: OR (95% CI) = 2.74 (1.78-3.85)]. HBO2T significantly increases the production of nitric oxide and free radicals which, in laboratory tests, inhibit the replication of the SARS-CoV. HBO2T upregulates hypoxia inducible factor, which promotes the expression of human antiviral peptides: defensins and cathelicidins, both effective to block the virus. Thus, HBO2T regulates the inflammatory response. We share our pilot study conclusions as a basis for clinical trials.

Keywords: free radicals; hyperbaric oxygen; infectious diseases; inflammation; nitric oxide; SARS-CoV-2

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Introduction

As of March 21, 2021 there have been 3,402,290 coronavirus disease 2019 (COVID-19) cases (5,704.57 cases per 100,000 inhabitants) in Italy, of which 56.2% are asymptomatic.\(^1\) Asymptomatic persons can transmit the virus to others for an extended period of time, perhaps longer than 14 days. Asymptomatic infections may be associated with subclinical lung abnormalities as detected by computed tomography.\(^2\) After being identified, these patients must observe quarantine. There are no generally recognized effective treatments for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) positive asymptomatic patients. Hyperbaric oxygen therapy (HBO2T) was hypothesized as a clinically available treatment to increase oxygenation of tissues.\(^3\)

Nitric oxide (NO) inhibits virus replication in vitro.\(^4,5\) Birds and some bats (frugivores) like to eat fruit, seeds and pollen that help increase NO production in their bodies.\(^6\) In birds, the NO produced by macrophages inhibits viral infections like in low pathogenic avian influenza and in avian infectious laryngotracheitis.\(^7,8\) The severe acute respiratory syndrome coronavirus (SARS-CoV) is inhibited by normal NO concentrations.\(^9,10\) NO and its derivatives cause a reduction in the palmitoylation of nascently expressed Spike (S) protein, which affects the fusion between the S protein and its cognate receptor, angiotensin converting enzyme 2 (ACE2). They also cause a reduction in viral RNA production in the early steps of viral replication, which is possibly due to an effect on one or both of the cysteine proteases encoded in open reading frame 1a (Orf1a) of SARS-CoV.\(^8,9\) The NO S-Nitroso-N-AcetylPenicillamine donor's efficacy to inhibit SARS-CoV replication is directly correlated to the NO concentration.\(^11\) The SARS-CoV-2 genome is closely related to the genome of SARS-CoV, with 88% identity. Protein E membrane alterations and the Orf3 genetic site modifications make the virus inactive.\(^12\)

Alterations in the level and activity of inducible nitric oxide synthase (iNOS) might be responsible for the susceptibility/severity and outcome of infectious diseases.\(^13\) The virus killing efficacy might be reduced in case of an NOS gene polymorphism in concomitance with other comorbidities and conditions triggering oxidative stress (aging and age-related conditions).\(^14\) The oxidative stress, through human DNA hypomethylation, determines the up-regulation (over-expression) of ACE2, used by SARS-CoV-2 to access the cells.\(^15\) The mean age of patients dying from SARS-CoV-2 infections in Italy was 81 years (median 83, range 0-109, IQR 75-88), which was 30 years higher as compared with the national sample diagnosed with SARS-CoV-2 infections (median age 48 years). The mean number of comorbidities was 3.6 (median 3, SD 2.1), with 85.1% of the patients that died having 2 or more comorbidities.\(^16\) There is a significant relation (odds ratio >1, range 1.24-2.74) between NOS gene polymorphisms and the comorbidities associated to COVID-19 deaths.\(^13,17,18\)

The virus acts following two distinct stages. During stage 1, after the incubation period, the SARS-CoV-2 virus activates mild symptoms and protective immune responses.\(^18\) During stage 2, a serious and damaging inflammatory response is activated. This affects mainly the lungs due to the triggering of a cytokines storm and the macrophage monocyte system.\(^18\)
successful elimination of the virus depends on the infected persons' human leukocyte antigen haplotype, their health condition and the therapy promptness. Our recommendation is to administer HBO2T (Figure 1) during stage 1 of SARS-CoV-2 infection. HBO2T has in fact shown to reduce the expression of inflammasome nucleotide-binding oligomerization domain-like receptor family pyrin domain containing 3 (NLRP-3), which activates the interleukins IL-1β and IL-18 and, in general, regulates the inflammatory response (Table 1). The iNOS is a key enzyme in the macrophage inflammatory response, which activates the production of reactive oxygen and reactive nitrogen species. Both play a key role in virus elimination. This enzyme is absent in the cytosol of inactive macrophages, but its synthesis is rapidly induced by the combined action of lipopolysaccharide and proinflammatory gamma interferon cytokine (IFN-γ). The iNOS catalyzes the conversion of arginine into citruline with consequent release of diffusible NO, which, at phagosomal level with acid pH, can combine with superoxide (O₂⁻) or hydrogen peroxide (H₂O₂) generated by the oxidase system, resulting in the production of highly reactive peroxynitrite radicals that effectively neutralize the virus. In addition, HBO2T upregulates hypoxia inducible factor (HIF) by reactive species and the extracellular regulated kinases (ERK1/ERK2) pathway. HIF induces the expression of NOS and virus killing peptides (defensins and cathelicidins such as cathelin-related anti-microbial peptide; CRAMP) with consequent neutrophil and monocyte phagocytosis of the virus. Increased cathelicidins in mice lungs provide a better response to the flu virus. Cathelicidin-deficient mice show higher susceptibility to viral damage.

The antiviral response in lab tests is effective in the range between 30 µM (lipopolysaccharide-induced damage) and 50 µM (CpG DNA-induced damage). It is on the other hand ineffective at values <15 µM (RNA single cell virus, ssRNA, such as SARS-CoV-2). The NO synthesis in healthy persons occurs halfway of maximum speed [Michaelis-Menten constant (Km)] for partial pressure oxygen (ppO₂) in arterial blood of about 5.33 kPa (40 mmHg equivalent to 50 µM of oxygen). In case of concomitant pathological alterations, such as comorbidities, in order to stabilize NO synthesis the ppO₂ needs to be raised to the range 196.15-245.17 kPa.

However, an excessive ppO₂ can trigger the formation of peroxynitrite free radicals that are derived from NO, which under selective pressure can facilitate SARS-CoV-2 mutagenesis, making it resistant to the therapies. HBO2T allows, contrary to other NO donors or to oxygen-ozone therapy, to finely regulate both NO and ROS concentrations.

HBO2T could be a suitable treatment of asymptomatic patients, which would be interesting to test in a clinical trial. Some critical issues for such a trial are a steep learning curve and that a well-designed randomized-controlled multi-center trial is necessary to obtain level 1 evidence supporting HBO2T application. On the other hand, HBO2T does reduce inflammation by impacting cytokine production and action, reduces D-dimers, with consistent reports showing that it improves the feeling of well-being. Moreover, HBO2T
procedures are safe for patients when hyperbaric chambers are properly disinfected and precautions are made during sessions. All said, we must have a vaccine.

Conclusions

Based on the laboratory evidence, we assume HBO2T may be effective in patients with reduced NO availability due to NOS gene polymorphisms, comorbidities and oxidative stress. The authors' intention is to share this study as a short communication to contribute to potential clinical trials.

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References


Figures and Legends

**Figure 1.** View of the interior of the hyperbaric chamber at the Hyperbaric Center of Ravenna (Italy), during the COVID-19 pandemic period.

![Image](https://via.placeholder.com/150)

**Table 1** HBO2T acts as substrate regulating the mitochondrial energetic metabolism, the oxidative burst and the expression of human antiviral peptides (defensins and cathelicidins) by the NO pathway. In addition it acts as messenger regulating inflammatory responses and triggers antiviral peptides' synthesis by reactive species, the ERK 1-2 pathway and the HIF pathway.

<table>
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<th>Oxygen as substrate</th>
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ERK, extracellular-signal regulated kinases; HIF, hypoxia inducible factor; NLRP-3, nucleotide-binding oligomerization domain-like receptor family pyrin domain containing 3; NO, nitric oxide; TNFα, tumor necrosis factor alpha.