



## REVIEW ARTICLE

## Ozone Autohemotherapy: Possible Mechanisms of Anti-Viral Action and Anti Oxidative

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### Abstract

Ozone, because of its special biological properties, has theoretical attributes to make it a viable candidate as a COVID-19 inactivator and treatment of oxidative stress in lung, through a variety of immunological mechanisms and dose dependent.

### Keywords

Antiviral, Ozone therapy, Coronavirus, Immune modulation, COVID-19, Oxidative stress, Cytokine storm

### Introduction

While ozone is the trigger, several blood components such as erythrocytes, lymphocytes, monocytes, polymorphonuclear leukocytes, platelets, and plasma components act as substrates and are responsible for setting in motion a number of biological effects that, directly or indirectly, are responsible for the clinical improvement observed after the autohaemotherapeutic treatment in chronic viral diseases [1].

Upon beginning O3 therapy, a multifaceted endogenous cascade is initiated and releases biologically active substrates in response to the transient, and moderate, oxidative stress that O3 induces. O3 can cause this mild oxidative stress because of its ability to dissolve in the aqueous component of plasma [2]. By reacting with polyunsaturated fatty acids (PUFA) and water, O3 creates hydrogen peroxide ( $H_2O_2$ ), a reactive oxygen species (ROS). Simultaneously, O3 forms a mixture of lipid ozonation products (LOP) [3]. The LOPs created after O3 exposure include lipoperoxy radicals, hydroperoxides, malondialdehyde, isoprostanes, the ozonide and alkenals, and 4-hydroxynonenal (4-HNE). Moderate oxidative stress caused by O3 increases activation of the tran-

scriptional factor mediating nuclear factor-erythroid 2-related factor 2 (Nrf2). Nrf2's domain is responsible for activating 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), NADPH quinone-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 [3].

In its pharmacological effect, medical O3 follows the principle of hormesis: low concentrations (or doses) show a high efficacy, which decreases with increasing concentration [4].

### Antiviral Effect

*In vitro*, O3 has been shown to interfere with virus-to-cell contact in lipid-enveloped viruses via oxidation of lipoproteins, proteins, and glycoproteins, thus interfering with the viral reproductive cycles [5].

*In vivo*, O3 therapy has been shown to have multifaceted effects when interacting with PUFA. As stated previously, O3 reacts with PUFA and other antioxidants,  $H_2O_2$  and varies peroxidation compounds are formed.  $H_2O_2$  readily diffuses into immune cells has been shown to act as a regulatory step in signal transduction and facilitating a myriad of immune responses [5].

Bocci and colleagues have thoroughly investigated the ability of O3 to induce cytokines from blood

via ozonated hemotherapy [6,7]. One of their first endeavors showed that high levels of both IFN- $\gamma$  and TNF- $\alpha$  could be induced from human leukocytes by ozonation at rather low concentrations (30-54  $\mu$ g/ml) [7]. A separate study showed that exposure of whole blood to O<sub>3</sub> (54  $\mu$ g/ml) induced the copious release of IL-1 $\beta$ , TNF- $\alpha$ , GM-CSF and IFN- $\beta$ , as well as IFN- $\gamma$ , IL-2 and IL-6 in smaller amounts [8]. The ability of O<sub>3</sub> to induce production of cytokines is significant; in particular, the induction of TNF- $\alpha$ , IFN- $\gamma$ , IL-2 and IL-8 explain, at least partially, the enhancement of immune function that has been reported following ozone administration [8].

The *in vivo* actions of these cytokines are well established. TNF- $\alpha$ , by definition mediates direct tumor cytotoxic effects. IFN- $\gamma$  is the key cytokine that mediates anti-viral activity of the immune system, mainly by enhancing the activity of natural killer (NK) cells, which can recognize and lyse tumor cells independently of major histocompatibility complex (MHC) restriction. IL-2 is the major proliferative factor for T lymphocytes and also activates NK cells. IL-8 is a chemotactic factor that promotes neutrophil migration to tissues, potently influencing the inflammatory response. Recombinant forms of both IL-2 and IFN- $\gamma$  are approved by the FDA for human use, and have been used to treat patients with various types of cancer, mainly by enhancing immune function [9].

Ozone's ability to inactivate cysteine dependent proteins was reported as an ozonide attack on cysteine-dependent papain, believed to inactivate the enzyme by oxidizing the active sulfhydryl group to sulfenate or sulfenic acid. Furthermore, coronavirus spike protein is also rich in tryptophan, which is second to cysteine in vulnerability to oxidation [10].

### Oxidative Stress and Cytokine Storm Can Be Corrected By Using Ozone-Therapy in High Dose

The axiom: "start low, go slow" proves to be ideal and under these conditions, ozone behaves as an acceptable stressor. Indeed O<sub>3</sub> therapy induces a series of graduated small oxidative stress acting on all organs and able to reactivate the potent defense system, which counteracts the deleterious chronic oxidative stress induced by COPD.

The high reactivity and solubility of ozone in the water of plasma allows its exhaustion in one min while it generates two crucial messengers such as H<sub>2</sub>O<sub>2</sub> and 4-hydroxynonenal (4-HNE) finally responsible for eliciting the well defined biochemical and molecular reactions responsible for the biological activities. 4-HNE readily forms an adduct with the Cys34 of albumin or with glutathione and this compound is consequently be able to reach most of cells of the body and to reactivate the antioxidant defenses.

The real molecular mechanisms of antioxidant activation Alkenal adducts are able to react with the Kelch-like ECH-associated protein 1 (Keap 1)-NF-E2-related factor 2 (Nrf2) system that is present in the cell cytosol with the role of antagonizing oxidative and electrophilic insults. In detail, keap1 is a protein molecule with many -SH groups that are important for the repression of Nrf2 activity.

Normally, the complex Nrf2-Keap1 has a half life of about 20 minutes because keap 1 is readily ubiquitinated and digested in the proteasome. However, the alkenal interaction with both Cys 272 and 288 of Keap-1 allows the release of Nrf2, which escapes proteosomal degradation and traslocates into the nucleus, heterodimerizes with a small Maf protein and binds to the anti-oxidant Response Element (ARE or EHRE) on DNA [11].

On this basis, it is clear that Nrf2 is now correctly recognized as the key cellular defense system against oxidative and xenobiotic stresses. Such a crucial event is able to upregulate the synthesis of relevant enzymes. The reactivation of innate defense system leads to the synthesis of a number of antioxidant enzymes able to counteract the chronic oxidative stress:

- a) Activation of the gamma-glutamyl-cysteine ligase and of GSH synthase allows a marked increase of the GSH intracellular level. The enhanced GSH/GSSG ratio allows an important protection against ROS;
- b) Activation of the synthesis of antioxidant enzymes able to detoxify an excess of ROS such as catalase, SOD, GSH-peroxidases, GSH-reductase, NADPH-quinone oxidoreductase (NQO1), cytochrome P450 monooxygenase system and HSP70;
- c) The upregulation of Heme-oxygenase 1 (HO-1) is also very protective and the trace of CO in combination with NO allows vasodilatation of ischemic tissues;
- d) The enhancement of the synthesis and levels of phase II enzymes such as GSH S-transferases, UDP-glucuronosyltransferases, N-acetyltransferases and sulfotransferases;
- e) Inhibition of cytokine mediated inflammation via the induction of leukotriene B4 reductase;
- f) Reducing iron overload and subsequent oxidative stress induced via elevated ferritin and bilirubin as a lipophilic antioxidant;
- g) The repetition of graduated small oxidative stress induces a multiform adaptative response. Moreover, during the ozone therapy sessions there is an increased release of adrenocorticotropic hormone, followed by cortisol from the cortex of the adrenal glands as a consequence of the liberation of corticotrophin releasing factor from the hypothalamus. Such a response is responsible for an improved feeling of well being reported by the majority of patients.

The efficacy of the mentioned orthodox drugs associated with the safe and valid support of the ozonated autohaemotherapy proves that the concept of integrated medicine is the best combination because it correctly associates suitable drugs with the critical stimulus of reactivating the natural defenses. The absolute lack of toxicity of ozone-therapy Gaseous ozone can be harmful at even low concentrations of part per million, affecting especially the eyes and respiratory systems. Administration of ozone by aerosol is toxic and must be avoided, as well as the intravenous administration of the oxygen-ozone gas mixture. On the contrary, very small and precisely determined ozone dosages during ozone therapy do not procure any acute or late side effects. In fact addition of ozone to blood happens *ex vivo* and the minimal amount of ozone acts as a pro drug and within 2-3 min is exhausted in small parts by the plasma antioxidants by generation of  $H_2O_2$  and alkenals [12].

## Ozone: Improves Blood Rheology, Oxygen Delivery, Oxygen Utilization, Endothelial Nitric Oxide Production

$O_3$  is a stimulator of the transmembrane flow of  $O_2$ . The increase in  $O_2$  levels inside the cell secondary to  $O_3$  therapy makes the mitochondrial respiratory chain more efficient [13]. In red blood cells,  $O_3$  -AHT may increase the activity of phosphofructokinase, increasing the rate of glycolysis. By enhancing the glycolytic rate, there is an increase in ATP and 2,3-diphosphoglycerate (2,3-DPG) in the cell. Subsequently, due to the Bohr effect, there is a rightward shift in the oxyhemoglobin dissociation curve allowing for the oxygen bound hemoglobin to be unloaded more readily to ischemic tissues. Combined with the increase in NO synthase activity, there is a marked increase in perfusion to the area under stimulation by  $O_3$  -AHT [14]. With repeated treatment, sufficient enough LOP may be generated to reach the bone marrow acting as repeated stressors to simulate erythropoiesis and the upregulation of antioxidant enzyme upregulation.  $O_3$  also causes a reduction in nicotinamide adenine dinucleotide (NADH) and assists in the oxidation of cytochrome c [15,16].

$O_3$  has also been shown to improve blood circulation and oxygen delivery to ischemic tissues [17]. Multiple studies have provided evidence that the correction of chronic oxidative stress via the increase of antioxidant enzymes in  $O_3$  can increase erythroblast differentiation. This leads to a progressive increase in erythrocytes and preconditions them to having resilience towards oxidative stress. This is known as "oxidative preconditioning" [15-18]. Also,  $O_3$  increases levels of prostacyclin, a known vasodilator [15]. Additionally, it was speculated that  $O_3$ 's oxidative capabilities would interfere with the endothelial production of NO and thus hinder vasodilation. However, studies have provided evidence that because NO is not substantially transported in the vasculature of the blood,

a deleterious interaction is unlikely [16]. Since HO-derived bilirubin31 has been demonstrated to interact with NO,11,12  $O_3$  -induced HO upregulation could modify NO production and alter vasodilation.

Unpredictably, studies have shown an increase of NO, which led to speculation of  $O_3$ 's ability to activate genes associated with NO synthase expression to further promote higher levels of NO formation. Moreover,  $O_3$ 's stimulation of antioxidant enzymes are also speculated to increase NO levels. While endothelial generation of superoxide disrupts the activity of NO,  $O_3$  upregulates the enzymes to ameliorate the downstream effects of ROS responsible for deleterious vasoconstriction [16-19].

## Summary and Conclusions

Covid-19 pan-inflammatory multi-system syndromes caused by coronavirus species. These virions incorporate novel RNA genomes and lipid bilayered envelopes. The Covid-19 viruses possess high mutation rates, allowing any one infected individual to harbor numerous quasispecies, all with variable infectivity and lethality.

$O_3$  is an energy-rich naturally-occurring molecule that embodies unique physico-chemical and biological properties suggesting a possible role in the systemic therapy of Covid-19, either as a monotherapy or, more realistically, as an adjunct to standard treatment regimens.

This paper outlines possible mechanisms by which  $O_3$  may exert its antiviral actions, correction of oxidative stress, inhibit cytokine storm in lung, and increased oxygen delivery via vascular and hematological modulation. Due to the excess energy inherent in the ozone molecule and supported by the vast scientific literature attesting to its pan-microbial powers, it is quasi-certain that ozone can demonstrate effectiveness across the entire coronavirus spectrum.

As our world becomes increasingly challenged by viral adversaries, the need for rapidly developing specific vaccines adapted to each viral species becomes evident. Yet, in parallel, research also needs to center on finding new methods of relieving the biological stress caused by onslaughts of viremic invasions that are common to many families of pathogenic viruses.

In conclusion, a proposal is herewith made that oxygen/ozone systemic therapies are granted research consideration for Covid-19 treatment. Such therapeutic approaches may then be found useful not only in these specific coronavirus conditions, but also in a number of human lipid-enveloped viral pathogenic infections, and importantly for the future coronavirus epidemics that are certain to emerge.

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