

<https://doi.org/10.52645/MJHS.2023.3.08>

UDC: 615.835:546.214+612.014.464



REVIEW ARTICLE



Current affairs in the use of medical ozone. Biological effects. Mechanisms of action

Natalia Cernei^{1*}, Serghei Șandru¹, Serghei Cobîlețchi¹, Ion Grabovschi², Ivan Cîvîrjic¹, Ruslan Baltaga^{1,3}

¹Valeriu Ghereg Department of Anesthesiology and Intensive Care Medicine No. 1, Nicolae Testemițanu State University of Medicine and Pharmacy, Chișinău, Republic of Moldova

²Department of Human Physiology and Biophysics, Nicolae Testemițanu State University of Medicine and Pharmacy, Chișinău, Republic of Moldova

³Department of Anesthesiology and Intensive Care Medicine of the Oncology Institute, Chișinău, Republic of Moldova

ABSTRACT

Introduction. Oxygen-ozone therapy stands as a medically endorsed practice confirmed by numerous international clinical studies. Various authors have illustrated the beneficial clinical outcomes of ozone therapy in terms of its capacity to regulate redox balance, cellular inflammatory responses, and adaptation to ischemia/reperfusion processes. Ozone therapy extends to encompass a range of viral infections, inflammatory disorders, and degenerative ailments, used as both monotherapy and as an adjunct to unified conventional therapies.

Material and methods. Narrative literature review study. Bibliographic search was conducted using the *PubMed*, *Hinari*, and *SpringerLink* databases, as well as the *National Center of Biotechnology Information* and *Medline*. Articles published between 1990 and 2022 were selected using various combinations of keywords, including “ozone”, “ozone therapy”, “mechanisms of ozone action”, “biological effects of ozone”, “antioxidant effect”, “anti-inflammatory effect” and “immunomodulatory effect.” Information regarding ozone’s mechanisms of action was identified and processed. Following the database information processing and search criteria, a total of 475 full-text articles were found. The final bibliography consists of 52 relevant sources that were deemed representative of the materials published on the topic of this synthesis article.

Results. The effects of ozone on oxygen metabolism are explained by changes in the rheological properties of blood, including inhibition of erythrocyte aggregation and stimulation of 2,3-diphosphoglycerate in erythrocytes, favoring the transport and delivery of oxygen to tissues while facilitating the substantial elimination of nitric oxide and increasing blood flow. Intracellular triatomic oxygen enhances the oxidative carboxylation of pyruvate, stimulating ATP production, which also contributes to reducing peripheral vascular resistance.

Conclusions. Ozone generates a moderate oxidative stress. Yet, it can set off several beneficial biochemical mechanisms that reactivate both the intra- and extracellular antioxidant systems and reverse chronic oxidative stress in various inflammatory and degenerative processes. Ozone induces a mild activation of the immune system by triggering neutrophil activation and stimulating the synthesis of certain cytokines (IL-2, TNF- α , IL-6, and IFN- γ), thereby initiating a complete cascade of immune responses. Ozone therapy yields the following biological reactions: optimization of blood circulation and oxygen delivery to ischemic tissue, regulation of cellular antioxidant enzymes, initiation of a slight immune system activation, and enhancing the release of growth factors.

Keywords: ozone, ozone therapy, mechanisms of ozone action, biological effects of ozone, antioxidant effect, anti-inflammatory effect, immunomodulatory effect.

Cite this article: Cernei N, Șandru S, Cobîlețchi S, Grabovschi I, Cîvîrjic I, Baltaga R. Current affairs in the use of medical ozone. Biological effects. Mechanisms of action. *Mold J Health Sci.* 2023;10(3):65-72. <https://doi.org/10.52645/MJHS.2023.3.08>

Manuscript received: 06.02.2023

Accepted for publication: 04.09.2023

Published: 20.09.2023

***Corresponding author:**

Natalia Cernei, MD, Assistant Professor

Valeriu Ghereg Department of Anesthesiology and Intensive Care

Key messages

What is not yet known on the issue addressed in the submitted manuscript

The article summarizes the latest foreign publications on the mechanisms of the antioxidant, anti-inflammatory, regenerative,

Medicine No. 1, *Nicolae Testemițanu* State University of Medicine and Pharmacy,
165, Ștefan cel Mare și Sfânt bd., Chișinău, Republic of Moldova, MD-2004
e-mail: nataliacernei1@gmail.com

Authors' ORCID IDs

Natalia Cernei – <https://orcid.org/0000-0002-2031-5881>

Serghei Șandru – <https://orcid.org/0000-0002-2973-9154>

Ion Grabovschi – <https://orcid.org/0000-0002-7716-9926>

Ivan Čivrić – <https://orcid.org/0000-0002-1360-5485>

Ruslan Baltaga – <https://orcid.org/0000-0003-0659-4877>

and immunomodulatory effects of medical ozone.

The research hypothesis

Systematization and critical analysis of published data on most recent findings regarding ozone therapy's mechanisms of action, including its methodology and optimal timing of application.

The novelty added by manuscript to the already published scientific literature

This comprehensive review reassesses the reported mechanisms of medical ozone, clinical potency and the latest medical approaches towards the effects of medical ozone.

Introduction

Ozone (O₃), a gas discovered in the mid-19th century and composed of three oxygen atoms, represents a highly reactive allotropic form of oxygen. It exhibits high solubility in plasma, extracellular fluids, and water (approximately 10 times more soluble in water than conventional oxygen). At room temperature, it is unstable, causing rapid decomposition into ordinary diatomic oxygen. Notably, its half-life measures 25 minutes at 30°C, 40 minutes at 20°C, and 140 minutes at 0°C [1-10].

Medical ozone is a blend of oxygen and ozone derived from medical-grade oxygen through the utilization of a medical ozone generator. This medical ozone contains a concentration of 1-5% ozone and 90-95% pure medical oxygen, or 10-80 μg/mL (0.21-1.68 μmol/ml) of ozone per milliliter of blood. Ozone therapy stands as a current and significant avenue of research in contemporary medicine [1, 3-5, 7, 9, 10-15].

Oxygen-ozone therapy is a medically validated practice supported by numerous international clinical studies. Nowadays, many clinical trials have shown its beneficial effects on the modulation of the oxidoreduction balance, cellular inflammation state, and adaptation to ischemia/reperfusion processes. Ozonotherapy is an effective, safe, feasible, and easy-to-perform technique, which finds applications in various inflammatory, infectious, degenerative diseases, as well as in rehabilitation following acute cardiac and cerebral ischemic events. It demonstrates good efficacy both as an independent treatment and, notably, as an adjunct to conventional therapies [3-5, 7, 11, 16-22]. By incorporating this medical practice, patients can attain significant clinical benefits. When combined with standard therapies, it often leads to reduced medication dosages, complication rates, treatment duration, medication toxicity, and medical expenses. It also addresses the issue of bacterial resistance to antibiotics [2, 4, 18, 19, 21, 23].

In the context of the aforementioned, the purpose of this article is to present a synthesis of the most recent findings regarding ozone's mechanisms of action.

Material and methods

To achieve the outlined purpose, an initial search of specialized scientific publications was conducted. These were identified through the Google Search engine, namely,

PubMed, Hinari, SpringerLink, the National Center of Biotechnology Information, and Medline. The article selection criteria encompassed contemporary data regarding the mechanisms of action of ozone therapy, utilizing the following keywords: "ozone", "ozone therapy", "ozone mechanisms of action", "biological effects of ozone", "antioxidant effect", "anti-inflammatory effect", and "immunomodulatory effect." These keywords were employed in various combinations to optimize search efficiency.

For the advanced selection of bibliographic sources, the following filters were used: full-text articles, articles in English, articles published between 1990 and 2022. After a preliminary analysis of the titles, original articles, editorials, articles of narrative synthesis, taxonomy, and meta-analysis were selected, which contained up-to-date information and contemporary concepts regarding the mechanisms of ozone therapy. Furthermore, a search was conducted within the reference lists of the identified sources to highlight additional relevant publications that were not found during the initial database searches.

The information from the publications included in the bibliography was gathered, organized, evaluated, and synthesized, showcasing the key aspects of the contemporary understanding of ozone's mechanisms of action, namely, its antioxidant capacity, vascular and hematological modulation, immune system activation, as well as its anti-inflammatory, bactericidal, virucidal, and fungicidal effects.

To minimize the potential systematic errors (bias) in the study, a meticulous search was conducted within databases to identify a maximum number of relevant publications for the study's purpose. Only studies that satisfy validity criteria were evaluated, rigorous exclusion criteria for articles under consideration were applied, and a comprehensive review was conducted of both positive outcome studies and those that did not highlight the treatment's benefits.

If necessary, additional sources of information were consulted to clarify some concepts. Duplicate publications and articles that did not meet the purpose of the article and were not available for full viewing were excluded from the list of publications generated by the search engine.

Results

Following the data processing, as identified by the Google Search engine and from databases such as *PubMed, Hinari,*

SpringerLink, National Center of Biotechnology Information, and Medline, in accordance with the search criteria, a total 475 articles on the topic of ozone therapy were found. After a primary analysis of the titles, 59 articles were eventually deemed relevant for the given synthesis. Upon repeated review of these sources, a final selection of 52 relevant publications was ultimately made in alignment with the intended purpose. The final bibliography of this work comprises 52 articles that have been considered representative of the materials published on the subject of this synthesis article.

Publications, the content of which did not reflect the relevant topic, despite being selected by the search program, as well as articles that were not accessible for open viewing through the *HINARI (Health Internet Work Access to Research Initiative)* database or available in the scientific medical library of the *Nicolae Testemițanu* State University of Medicine and Pharmacy, were subsequently removed from the list.

Although ozone is the most potent natural oxidant, capable of oxidizing a wide range of organic and inorganic substances, and has the potential for cytotoxicity, researchers believe that under controlled conditions, it possesses numerous therapeutic effects. Moreover, the reactivity to ozone can be effectively mitigated by the blood and cellular antioxidant system [1, 2, 15, 24-28]. Initially used as an empirical approach, oxygen-ozone therapy has now evolved to a stage where the majority of ozone's biological mechanisms of action have been extensively studied and elucidated, these findings being found within the fields of biochemistry, physiology, and pharmacology [18, 22, 25, 27-29].

Ozone is not a pharmaceutical medicine but rather a regulatory molecule capable of generating bioactive mediators. The effects of ozone have been demonstrated to be consistent, safe, and associated with minimal preventable side effects [2, 30]. Chronic oxidative stress, chronic inflammatory processes, and immune overactivation are present and highly detrimental in a wide variety of diseases. The effectiveness of ozone therapy is determined by moderate oxidative stress, resulting from the interaction of ozone with the biological components of the body, triggering an endogenous cascade of biochemical reactions [31].

Ozone can function as an oxidant either directly, when it dissolves in plasma and other biological fluids, immediately reacting with polyunsaturated fatty acids, antioxidants, cysteine-rich proteins, and carbohydrates; or indirectly, by generating reactive oxygen species (ROS) and lipid oxidation products (LOP) [25, 28, 32-37]. At the onset of ozone therapy, an endogenous cascade is triggered, releasing bioactive substances in response to transient and moderately induced oxidative stress by ozone ('oxidative eustress'). Ozone can easily induce this oxidative stress due to its plasma solubility. Reacting with polyunsaturated fatty acids and water, ozone forms ROS in human fluids and tissues. The main molecule among ROS is hydrogen peroxide (H_2O_2) – a non-radical oxidant. Concurrently, ozone also gives rise to LOP – the lipoperoxide radical, hydroperoxides, malondialdehyde, isoprostanes, ozoneides, alkenes, and predom-

inantly, 4-hydroxynonenal. ROS and LOP are the effector molecules responsible for modulating several biological and therapeutic effects in the body following ozone therapy [3, 8, 13, 27, 34, 37-39].

Having reacted with a number of biomolecules, ozone disappears, and hydrogen peroxide, the main molecule of ROS, and other mediators rapidly diffuse into cells, activating various metabolic pathways with numerous biological and therapeutic effects [3, 5, 27, 28, 35, 40]. Therefore, ROS and LOP are "biological messengers of ozone" and are responsible for the biological and therapeutic effects of ozone. ROSs are short-acting early messengers and are responsible for immediate biological effects, while LOPs are important late and long-term messengers [3, 5, 10, 13, 14, 17, 28, 36].

The formation of ROS in plasma occurs extremely quickly (less than a minute), accompanied by a moderate and transient decrease in the antioxidant capacity of the blood (from 5% to 25%). However, this antioxidant capacity returns to normal within 15-20 minutes [3, 9, 17, 28, 31, 40, 41].

Discussion

Although not fully comprehended, the present article will delve into the mechanisms underlying the antioxidant, anti-inflammatory, immunomodulatory, antimicrobial, antiviral, and analgesic effects of ozone.

The **antioxidative capacity** is considered one of the key impacts of ozone therapy. Moderate oxidative stress induced by ozone within the therapeutic range (10-80 $\mu\text{g}/\text{mL}$), most commonly 30-45 $\mu\text{g}/\text{mL}$ (the 'physiological' dose of ozone), physiologically effective and recommended levels for systemic application, elicits controlled low doses of ROS acting primarily as signaling molecules, thereby stimulating the formation of LOP. ROS triggers the activation of nuclear erythroid factor 2 (Nrf2), well known as a pivotal regulator of manifold cytoprotective responses, responsible for up-regulating antioxidant enzyme activity. In response to transient, moderate oxidative stress, the levels of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, glutathione S-transferase, catalase, and heme oxygenase-1 increase. Thus, moderate, transient, and repetitive oxidative stress causes an intense modulation of antioxidants in the body. A multitude of cells across various organs upregulate the synthesis of antioxidants, which are capable of significantly countering excessive ROS, thereby alleviating chronic oxidative stress, which is present and extremely harmful in a variety of diseases. Consequently, ozone, either through oxidative preconditioning or adaptation to chronic oxidative stress, safeguards tissue integrity against ROS-induced damage, fostering a balance between antioxidant and pro-oxidant factors while preserving cellular redox balance [27, 30, 41-45].

Nrf2 and nuclear factor kappa B (NF- κ B) represent the primary signaling pathways through which ozone exerts its effects. Nrf2 activation regulates cell defense and maintains cellular homeostasis [36]. Furthermore, ozone therapy fosters adaptation to oxidative stress by gently triggering the

immune system, releasing growth factors, and/or activating metabolic pathways that contribute to maintaining redox balance [38].

By activating Nrf2, LOP induces oxidative stress proteins, including heme-oxygenase-1 (HO-1), another inhibitor of the NF- κ B pathway and one of the most crucial antioxidant defense enzymes. Through inhibiting the high expression level of hypoxia-inducible factor-1 α (HIF-1 α), it contributes to reducing the production of proinflammatory cytokines, directly activating anti-inflammatory cytokines, enhancing antioxidant protection, and consequently, safeguarding cellular integrity [8, 28, 30, 44-46].

Thus, ozone mimics acute oxidative stress which, when properly balanced, is not harmful, but can trigger several beneficial biochemical mechanisms. It can reactivate the intra- and extracellular antioxidant system, thereby reversing chronic oxidative stress in various inflammatory, degenerative processes, etc. During ozone treatment, cells throughout the body receive gradual and subtle impulses of LOP, significant long-term messengers that play a crucial role in up-regulating antioxidant enzymes in multiple cell types while rebalancing the oxidant/antioxidant system [46, 47].

Vascular and Hematological Modulation. Ozone serves as a catalyst for transmembrane oxygen flow. The increase in cellular oxygen levels resulting from ozone therapy enhances the efficiency of the mitochondrial respiratory chain. Moreover, ozone amplifies the production of prostacyclin, a widely acknowledged vasodilator [2, 6, 8, 26].

The effects of ozone on oxygen metabolism can be explained by promoting (1) changes in the rheological properties of blood (reversal of erythrocyte aggregation, increased flexibility and elasticity of red blood cells, favoring the transport and delivery of tissue oxygen), leading to enhanced blood flow in microcirculation; (2) increasing the speed of glycolysis in erythrocytes; and (3) the release of substances (adenosine triphosphate, nitric oxide, and prostaglandins) that may contribute to reducing peripheral vascular resistance and increasing oxygen supply to tissues [18, 25, 35, 37, 40, 48-50].

Hydrogen peroxide (H₂O₂) diffuses from the plasma into the cellular cytoplasm and serves as the triggering stimulus. Depending on the cell type, various biochemical pathways can be simultaneously activated in red blood cells, white blood cells, and platelets, leading to a multitude of biological effects [10, 28, 32].

The Impact of Ozone on Erythrocytes. Erythrocytes are the focus of ROS. During erythropoiesis, submicromolar concentrations of LOP positively regulate the synthesis of antioxidant enzymes. Consequently, ozone therapy increases the glycolytic rate by enhancing intracellular adenosine triphosphate production. This approach intensifies erythrocyte generation, yielding metabolically enhanced erythrocytes (super-endowed erythrocytes) capable of more effectively transporting and delivering oxygen to tissues, including ischemic tissues, thereby correcting hypoxia in vascular diseases [7, 12, 25, 27, 28, 39, 48]. Coupled with increased nitric oxide synthase activity, there is a significant increase

in nitric oxide, an essential element in maintaining optimal levels of vasodilation and blood perfusion [1, 6, 8, 40].

Ozone therapy, through careful regulation of ozone dosage, stimulates the production of antioxidant enzymes within the system (catalase, glutathione peroxidase, and superoxide dismutase) while mitigating excessive formation of ROS, thereby reducing chronic oxidative stress [1, 6, 12, 14, 39, 43, 49].

The impact of ozone on leukocytes. Ozone acts as a mild cytokine and serves as a cytokine inducer by lymphocytes and monocytes, thereby enhancing the immune system's activity. This stimulation fosters intercellular matrix synthesis and contributes to the healing process [1, 12, 32, 35, 37, 39].

The Impact of ozone on platelets. Hydrogen Peroxide (H₂O₂) and other ROS generated through blood ozonation initiate a cascade of enzymatic reactions. These reactions gradually elevate intracellular Ca levels and trigger the release of prostaglandins (F2a and E2), leading to irreversible platelet aggregation. Increased levels of growth factors released from platelets, mobilization of endogenous stem cells, and stimulation of neoangiogenesis promote tissue regeneration, as well as healing of injuries and wounds [27, 49, 51].

Thus, the impact of ozone on oxygen metabolism is explained by how it alters the blood's rheological properties (reversing red blood cell clumping, enhancing the flexibility and elasticity of red blood cells, promoting the transport and delivery of oxygen to tissues). This, in turn, facilitates blood flow in the microcirculation, increases glycolysis in red blood cells, and triggers the production of substances (such as adenosine triphosphate, nitric oxide, and prostaglandins) that help reduce peripheral vascular resistance.

Activation of the immune system. Ozone promotes an increase in the production of interferon- γ (IFN- γ) and some cytokines, with interleukin-2 (IL-2) being the primary one, subsequently triggering a whole cascade of immunological reactions [1, 2]. It has been shown that ROS, including H₂O₂ and LOP generated by ozone therapy, can easily diffuse into plasma cells and activate NF- κ B, inducing the production of immunoreactive cytokines in normal cells (IL-2, tumor necrosis factor alpha - TNF- α , IL-6 and IFN- γ), thereby enhancing the immune response [9, 13, 14, 26, 32, 40, 44].

Ozone indirectly activates the innate (non-specific) immune system by enhancing phagocytosis and promoting the synthesis of cytokines and interleukins in neutrophils and leukocytes. It also triggers the components of both cellular and humoral immunity [8, 26, 33, 39]. Within mononuclear cells, ozone stimulates immune responses by modulating the NF- κ B transcription factor, thereby reactivating the suppressed immune system [27, 28].

Furthermore, ROSs trigger the activation of the immune system, which acts through monocytes and lymphocytes, promoting the production of a variety of cytokines (IL-1, IL-2, IL-6, IFN- β , IFN- γ , TNF α) [6, 49].

Thus, ozone induces mild immune system activation by stimulating neutrophils and initiating the synthesis of cer-

tain cytokines that trigger a whole cascade of immunological responses.

Bactericidal, virucidal and fungicidal action of ozone.

Ozone used in vitro acts directly on the membrane of bacterial cells (direct oxidative effect), disrupting and damaging the integrity of bacterial cell membranes, oxidizing phospholipids and lipoproteins, thereby impeding their enzymatic function. Additionally, ozone damages the viral capsids, disturbing their structure and interfering with the virus-cell interaction, leading to disruption in the reproductive cycle. When it comes to fungi, ozone inhibits cell growth by perturbing intracellular homeostasis, resulting from the compromised barrier properties of the plasma membrane [2, 6, 12, 16, 26, 44, 48, 50].

Although ozone is one of the most potent disinfectants, used in various ways, it cannot deactivate any pathogens (bacteria, viruses, and fungi) in vivo. This is because pathogens are well protected, especially within cells, by the cell's powerful antioxidant system. Consequently, ozone acts as a gentle enhancer of the immune system by activating neutrophils and stimulating the synthesis of certain cytokines [1, 10, 19, 22, 28, 39, 46].

The ***anti-inflammatory effect*** is revealed in ozone's ability to influence the inflammatory cascade by oxidizing biologically active substances (arachidonic acid and its derivatives - prostaglandins), which participate in the development and sustenance of the inflammatory process. Additionally, ozone significantly reduces the levels of pro-inflammatory cytokines (IL-1 β , IL-6, IL-8, and TNF- α) without any signs of toxicity or recorded side effects [8, 26, 30, 31]. These cytokines induce the prostaglandin E₂ pathway, which causes pain or increases the sensitivity of nerve roots to other algogenic substances (such as bradykinin) [31].

Severe oxidative stress, triggered by high concentrations of ozone, along with proinflammatory cytokines (IL-1 β , IL-6, IL-8, TNF- α), activate NF- κ B, a key regulator of the inflammatory response and muscle atrophy. This contributes to an increased inflammatory response and tissue damage, including the release of other inflammatory factors that enhance the migration of eosinophils and neutrophils [9, 13, 17, 47, 49].

On the contrary, mild oxidative stress induced by precise and small doses of ozone activates Nrf2. The latter indirectly inhibits the pro-inflammatory mechanism driven by the NF- κ B pathway. As a result, there is a reduction in NF- κ B activity along with a modification in the expression of inflammatory cytokines associated with NF- κ B activity. This triggers an anti-inflammatory effect, leading to a decrease in IL-1, IL-2, IL-6, IL-7, and TNF α , as well as an increase in interleukins such as IL-4, IL-10, IL-13, and the transforming growth factor beta - TGF- β [11, 13, 19, 38, 43, 49, 50].

Nrf2 also plays an important role in intracellular inflammatory signaling pathways. Triggering the Nrf2-antioxidant signal can dampen NF- κ B activity, leading to the downregulation of the inflammatory response by suppressing essential inflammatory mediators and cytokines (IL-6, IL-8, and TNF- α) [31, 38, 42, 50].

Moreover, a small amount of H₂O₂ stimulates the NF- κ B pathway, which is typically balanced out by the Nrf2's blocking action, resulting in an immunomodulatory effect [11].

The ***analgesic effect*** of ozone is ensured by the oxidation of the byproducts of albuminolysis, known as algopeptides, which act on the nerve endings in the damaged tissue and determine the intensity of the pain response. Additionally, the analgesic effect is attributed to the restoration of the antioxidant system and, subsequently, the reduction of harmful molecular byproducts from lipid peroxidation [26]. Recent preclinical studies have elucidated the role of ROS in hyperalgesia by activating N-methyl-D-aspartate receptors [11].

Following ozone therapy, there has been a demonstrated increase in antioxidant molecules (serotonin and endogenous opioids), which induce pain relief by stimulating antinociceptive pathways [31, 39].

Data from scientific research acknowledge that the mechanisms of action of ozone are due to: (1) a decrease in the production of inflammatory mediators; (2) oxidation (inactivation) of metabolic mediators of pain; (3) improvement of local blood microcirculation leading to improved oxygen delivery to tissues; (4) elimination of toxins and resolution of physiological disorders that generate pain [42, 52].

Therefore, ozone exhibits pleiotropic properties, extending beyond its exclusive role as an antioxidant, anti-inflammatory, or immunomodulatory one. It also encompasses the capacity to employ ROS as a signaling molecule rather than merely as intracellular toxic substances. In existing experimental models and clinical studies, the anti-inflammatory, antioxidant, regenerative and immunomodulatory effects of ozone therapy have been associated with several molecular mechanisms, the main ones being the NF- κ B/Nrf2 balance and IL-6 and IL-1 β expression. NF- κ B and Nrf2 are the most studied and important transcription factors and regulatory proteins that control the expression of a wide range of genes, encoding proteins involved in a multitude of vital biological functions, including those associated with redox status, immunity, and inflammatory responses. Additionally, indirectly through these pathways, LOP initiates the HIF-1 α , HO-1, and NO/iNOS pathways.

The main pharmacological effects of medical ozone through ozone-produced peroxides are as follows: (1) increased oxygen release by erythrocytes due to activated metabolism; (2) immunomodulation due to leukocyte activation; and (3) regulation of cellular antioxidants via Nrf2 signaling. Ozone therapy can elicit the following biological reactions: (a) improved blood circulation and oxygen delivery to ischemic tissue; (b) optimization of overall metabolism by improving oxygen delivery; (c) regulation of cellular antioxidant enzymes and induction of HO-1; (d) triggering a mild immune system activation and intensified release of growth factors; (e) providing a state of well-being in most patients, probably due to stimulation of the neuroendocrine system.

Conclusions

1. Ozone induces both mild and moderate oxidative stress. When appropriately balanced, this stress poses no harm; instead, it can initiate several beneficial biochemical mechanisms. These mechanisms, in turn, reactivate the intracellular and extracellular antioxidant systems, effectively countering long-term oxidative stress in various inflammatory and degenerative processes, etc. Cells throughout the body receive small and gradual bursts of lipid oxidation products, important late and long-term messengers that are responsible for activating antioxidant enzymes in many cell types to rebalance the oxidant/antioxidant system.
2. The impact of ozone on oxygen metabolism is explained by changes in the blood's rheological properties. This involves reversing red blood cell aggregation, enhancing the flexibility and elasticity of hemoglobin, and promoting the efficient transport and delivery of oxygen to tissues. This process also facilitates blood flow within microcirculation, speeds up glycolysis within red blood cells, and triggers the release of substances like adenosine triphosphate, nitric oxide, and prostaglandins, which help to reduce peripheral vascular resistance.
3. Ozone triggers a slight activation of the immune system by up-regulating and activating neutrophils and promoting the synthesis of cytokines (IL-2, TNF- α , IL-6, and IFN- γ), setting off a chain reaction of immune responses.
4. Ozone therapy induces the following biological responses: enhanced blood circulation and oxygen delivery to ischemic tissue, regulation of cellular antioxidant enzymes, mild immune system activation, and intensified release of growth factors.
5. Ozone is an inherently toxic gas that should never be inhaled, cannot be stored, and must be handled with caution. Generally, no toxic effects were reported, and only the respiratory tract was found to be highly sensitive to inhaled ozone since the respiratory mucosal cells contain a minimal amount of antioxidants and are extremely susceptible to oxidation.
6. Although ozone ranks among the most potent disinfectants, being employed in various ways, it cannot neutralize any pathogens (bacteria, viruses, and fungi) *in vivo*, since pathogens are effectively shielded by the strong blood and cellular antioxidant system. Furthermore, elevated ozone concentrations induce severe oxidative stress, prompting increased inflammatory responses and tissue damage.

Competing interests

None declared.

Authors' contribution

NC and RB conceived the study, participated in the study design and assisted in drafting the manuscript. SŞ and IC

performed the analysis and data interpretation. IG drafted the manuscript. SC conceived the significant revision of the manuscript and provided significant intellectual involvement. The authors have read and approved the final version of the manuscript.

References

1. Cakir R. General aspects of ozone therapy [Internet]. In: Atroshi F, editor. Pharmacology and nutritional intervention in the treatment of disease. London: IntechOpen; 2014 [cited 2023 Apr 12]. Available from: <http://dx.doi.org/10.5772/57470>
2. Elvis AM, Ekta JS. Ozone therapy: a clinical review. *J Nat Sci Biol Med.* 2011 Jan;2(1):66-70. doi: 10.4103/0976-9668.82319.
3. Bocci V. Ozone: a new medical drug. 2nd ed. New York: Springer; 2011. 315 p. <https://doi.org/10.1007/978-90-481-9234-2>.
4. Allorto N. Oxygen-ozone therapy: an extra weapon for the general practitioners and their patients. *Ozone Ther.* 2019 July;4(2):8424. doi: 10.4081/ozone.2019.8424.
5. Baeza-Noci J, Cabo-Soler JR, Moraleda-Gómez M, et al. Revisión WFOT sobre Ozonoterapia Basada en Evidencias. Comité Científico Asesor de la WFOT – 2015 [WFOT Review on Evidence-Based Ozone Therapy. WFOT Scientific Advisory Committee – 2015]. Brescia: World Federation of Ozone Therapy; 2015. 117 p. Spanish. <https://semdor.es/wp-content/uploads/2021/10/WFOT-OZONE-2015-ESP.pdf>.
6. Scassellati C, Ciani M, Galoforo A, Zanardini R, Bonvicini C, Geroldi C. Molecular mechanisms in cognitive frailty: potential therapeutic targets for oxygen-ozone treatment. *Mech Ageing Dev.* 2020 Mar;186:111210. doi: 10.1016/j.mad.2020.111210.
7. Yousefi B, Banihashemian S, Feyzabadi Z, Hasanpour S, Kokhaei P, Abdolshahi A, et al. Potential therapeutic effect of oxygen-ozone in controlling of COVID-19 disease. *Med Gas Res.* 2022 Apr-Jun;12(2):33-40. doi: 10.4103/2045-9912.325989.
8. Smith N, Wilson A, Gandhi J, Vatsia S, Khan S. Ozone therapy: an overview of pharmacodynamics, current research, and clinical utility. *Med Gas Res.* 2017 Oct 17;7(3):212-9. doi: 10.4103/2045-9912.215752.
9. Sagai M, Bocci V. Mechanisms of action involved in ozone therapy: is healing induced via a mild oxidative stress? *Med Gas Res.* 2011 Dec 20;1:29. doi: 10.1186/2045-9912-1-29.
10. Bocci V, Borrelli E, Travagli V, Zanardi I. The ozone paradox: ozone is a strong oxidant as well as a medical drug. *Med Res Rev.* 2009 Jul;29(4):646-82. doi: 10.1002/med.20150.
11. Hidalgo-Tallón F, Torres-Morera L, Baeza-Noci J, Carrillo-Izquierdo M, Pinto-Bonilla R. Updated review on ozone therapy in pain medicine. *Front Physiol.* 2022 Feb 23;13:840623. doi: 10.3389/fphys.2022.840623.
12. Manjunath R, Singla D, Singh A. Ozone revisited. *J Adv Oral Res.* 2015 May-Aug;6(2):5-9. <https://doi.org/10.1177/222941122015020>
13. Cenci A, Macchia I, La Sorsa V, Sbarigia C, Di Donna V, Pietraforte D. Mechanisms of action of ozone therapy in emerging viral diseases: immunomodulatory effects and therapeutic advantages with reference to SARS-CoV-2. *Front Microbiol.* 2022 Apr 21;13:871645. doi: 10.3389/fmicb.2022.871645.
14. du Plessis LH, van der Westhuizen FH, Kotzé HF. The protective effect of plasma antioxidants during ozone autohe-

- motherapy. *Afr J Biotechnol.* 2008 Aug;7(14):2472-7. doi: 10.4314/ajb.v7i14.59039.
15. Rowen RJ. Ozone and oxidation therapies as a solution to the emerging crisis in infectious disease management: a review of current knowledge and experience. *Med Gas Res.* 2019 Oct-Dec;9(4):232-7. doi: 10.4103/2045-9912.273962.
 16. Tizaoui C. Ozone: a potential oxidant for COVID-19 virus (SARSCoV-2). *Ozone Sci Eng.* 2020 Jul;42(5):378-85. <https://doi.org/10.1080/01919512.2020.1795614>.
 17. Zanardi I, Borrelli E, Valacchi G, Travagli V, Bocci V. Ozone: a multifaceted molecule with unexpected therapeutic activity. *Curr Med Chem.* 2016;23(4):304-14. doi: 10.2174/0929867323666151221150420.
 18. Radzimierska M, Śmigielski K. Ozone and products of ozonation in medical use. *Biomed J Sci Tech Res.* 2019 Apr;17(1):002959. doi: 10.26717/BJSTR.2019.17.002959.
 19. de Sire A, Agostini F, Lippi L, Mangone M, Marchese S, Cisari C, et al. Oxygen-Ozone therapy in the rehabilitation field: state of the art on mechanisms of action, safety and effectiveness in patients with musculoskeletal disorders. *Biomolecules.* 2021 Feb 26;11(3):356. doi: 10.3390/biom11030356.
 20. Wen Q, Liu D, Wang X, Zhang Y, Fang S, Qiu X, et al. A systematic review of ozone therapy for treating chronically refractory wounds and ulcers. *Int Wound J.* 2022 May;19(4):853-70. doi: 10.1111/iwj.13687.
 21. Mendes C, Cunha M, Ferreira A, Santos E. Ozone therapy as a coadjuvant in wound healing and pain reduction. *Millennium.* 2021 Sep;2(9):131-8. <https://doi.org/10.29352/mill029e.25237>.
 22. Smith A, Oertle J, Warren D, Pratoet D. Ozone therapy: a critical physiological and diverse clinical evaluation with regard to immune modulation, anti-infectious properties, anti-cancer potential, and impact on anti-oxidant enzymes. *Open J Mol Integr Physiol.* 2015 Aug;5(3):37-48. doi: 10.4236/ojmip.2015.53004.
 23. León Fernández OS, Oru GT, Hansler RV, Cabreja GL, Espinosa IS, Polo Vega JP, et al. Medical Ozone: the pharmacological mechanisms accounting for its effectiveness against COVID-19/SARS-COV-2. *J Med Clin Res Rev.* 2021 Mar;5(3):1-10. doi: 10.33425/2639-944X.1199.
 24. Bocci V. Biological and clinical effects of ozone. Has ozone therapy a future in medicine? *Br J Biomed Sci.* 1999 Apr;56(4):270-9.
 25. Di Mauro R, Cantarella G, Bernardini R, Di Rosa M, Barbagallo I, Distefano A, et al. The biochemical and pharmacological properties of Ozone: the smell of protection in acute and chronic diseases. *Int J Mol Sci.* 2019 Feb 1;20(3):634. doi: 10.3390/ijms20030634.
 26. Maslennikov OV, Kontorshchikova CN, Gribkova IA. Ozone therapy in practice. *Health manual [Internet]. Nizhny Novgorod (Russia); 2008 [cited 2023 Feb 23]. 42 p. Available from: <https://absoluteozone.com/wp-content/uploads/2022/05/Ozone-Therapy-in-Practice.pdf>.*
 27. Tricarico G, Travagli V. The relationship between Ozone and human blood in the course of a well-controlled, mild, and transitory oxidative eustress. *Antioxidants (Basel).* 2021 Dec 4;10(12):1946. doi: 10.3390/antiox10121946.
 28. Borrelli E, Bocci V. Basic biological and therapeutic effects of ozone therapy in human medicine [Internet]. In: Munter R, editor. *Ozone science and technology.* Oxford, UK: Encyclopedia of Life Support Systems; 2010 [cited 2022 Apr 18]. Available from: <https://www.eolss.net/sample-chapters/c07/E6-192-15-00.pdf>
 29. Inal M, Dokumacioglu A, Özcelik E, Ucar O. The effects of ozone therapy and coenzyme Q₁₀ combination on oxidative stress markers in healthy subjects. *Ir J Med Sci.* 2011 Sep;180(3):703-7. doi: 10.1007/s11845-011-0675-7.
 30. Chirumbolo S, Valdenassi L, Simonetti V, Bertossi D, Ricevuti G, Franzini M, et al. Insights on the mechanisms of action of ozone in the medical therapy against COVID-19. *Int Immunopharmacol.* 2021 Jul;96:107777. doi: 10.1016/j.intimp.2021.107777.
 31. de Sire A, Marotta N, Ferrillo M, Agostini F, Sconza C, Lippi L, et al. Oxygen-ozone therapy for reducing pro-inflammatory cytokines serum levels in musculoskeletal and temporomandibular disorders: a comprehensive review. *Int J Mol Sci.* 2022 Feb 25;23(5):2528. doi: 10.3390/ijms23052528.
 32. Bocci V, Zanardi I, Travagli V. Has oxygen-ozonotherapy a future in medicine? *J Exp Integr Med.* 2011 Jan;1:5-11. doi: 10.5455/jeim.161210.ir.002.
 33. Néri J, Lomba E, Karam A, Reis S, Marchionni A, Medrado A. Ozone therapy influence in the tissue repair process: a literature review. *J Oral Diagn.* 2017 Jul;2:e20170032. doi: 10.5935/2525-5711.20170032.
 34. Bocci V, Valacchi G, Corradeschi F, Fanetti G. Studies on the biological effects of ozone: 8. Effects on the total antioxidant status and on interleukin-8 production. *Mediators Inflamm.* 1998 Jul;7(5):313-7. doi: 10.1080/09629359890820.
 35. Bocci V. *Oxygen-ozone therapy: a critical evaluation.* Dordrecht: Kluwer Academic Publishers; 2002. 440 p. <http://doi.org/10.1007/978-94-015-9952-8>.
 36. Viebahn-Haensler R, León Fernández O. Ozone in Medicine. The low-dose ozone concept and its basic biochemical mechanisms of action in chronic inflammatory diseases. *Int J Mol Sci.* 2021 Jul 23;22(15):7890. doi: 10.3390/ijms22157890.
 37. Bocci V. Ozone as Janus: this controversial gas can be either toxic or medically useful. *Mediators Inflamm.* 2004 Feb;13(1):3-11. doi: 10.1080/0962935062000197083.
 38. Scassellati C, Galoforo A, Bonvicini C, Esposito C, Ricevuti G. Ozone: a natural bioactive molecule with antioxidant property as potential new strategy in aging and in neurodegenerative disorders. *Ageing Res Rev.* 2020 Nov;63:101138. doi: 10.1016/j.arr.2020.101138.
 39. Bocci V, Zanardi I, Travagli V. Ozone acting on human blood yields a hormetic dose-response relationship. *J Transl Med.* 2011 May 17;9:66. doi: 10.1186/1479-5876-9-66.
 40. International Scientific Committee of Ozonotherapy (ISCO₃). *Madrid Declaration on Ozone Therapy [Internet]. 2nd ed. Madrid: ISCO₃; 2012 [cited 2023 Feb 23]. 50 p. Available from: <https://borncclinic.com/wp-content/uploads/2020/12/OZONE-THERAPY.pdf>.*
 41. Thorp J, Thorp K, Thorp E, Viglione D. Ozone preconditioning in viral disease. *Virol Curr Res [Internet]. 2022;6(S1):001 [cited 2023 Feb 23]. Available from: <https://www.hilarispublisher.com/open-access/ozone-preconditioning-in-viral-disease.pdf>.*
 42. Chirumbolo S, Varesi A, Franzini M, Valdenassi L, Pandolfi S, Tirelli U, et al. The mito-hormetic mechanisms of ozone in the clearance of SARS-CoV2 and in the COVID-19 therapy. *Biomedicines.* 2022 Sep 12;10(9):2258. doi: 10.3390/biomedicines10092258.
 43. Pivotto AP, Banhuk FW, Staffen IV, Daga MA, Ayala TS, Menolli RA. Clinical uses and molecular aspects of ozone therapy: a review. *OnLine J Biol Sci.* 2020 Feb;20(1):37-49. <https://doi.org/10.3844/ojbsci.2020.37.49>
 44. Obeid BM. Ozone autohemotherapy: possible mechanisms of anti-viral action and anti oxidative. *J Infect Dis Epidemi-*

- ol. 2020 Apr;6:117. doi: 10.23937/2474-3658/1510117.
45. Galiè M, Covi V, Tabaracci G, Malatesta M. The role of Nrf2 in the antioxidant cellular response to medical ozone exposure. *Int J Mol Sci.* 2019 Aug 17;20(16):4009. doi: 10.3390/ijms20164009.
46. Martínez-Sánchez G, Schwartz A, Donna V. Potential cytoprotective activity of ozone therapy in SARS-CoV-2/COVID-19. *Antioxidants (Basel).* 2020 May 6;9(5):389. doi: 10.3390/antiox9050389.
47. Ranaldi G, Villani E, Franza L. Rationale for ozone-therapy as an adjuvant therapy in COVID-19: a narrative review. *Med Gas Res.* 2020 Jul-Sep;10(3):134-8. doi: 10.4103/2045-9912.289462.
48. Bodrug N, Barba D, Istrati V, Botezatu A. Eficacitatea terapiei cu ozon în medicină [Effectiveness of ozone therapy in medicine]. Chișinău: Medicina; 2012. 116 p. Romanian.
49. Dias EN, Andrade KF, Silveira RS, Machado RRP. [The acting of ozonotherapy in wounds, neuropathies, infections and inflammations: a systematic review]. *Braz J Dev.* 2021;7(5):48604-29. Portuguese. <https://doi.org/10.34117/bjdv.v7i5.29786>.
50. Borges F, Meyer P, Jahara R, de Moraes Carreiro E, Antonuzzo P, Picariello F, et al. Fundamentals of the use of ozone therapy in the treatment of aesthetic disorders: a review. *J Biosci Med.* 2021 Dec;9:40-70. doi: 10.4236/jbm.2021.912005.
51. Fitzpatrick E, Holland O, Vanderlelie J. Ozone therapy for the treatment of chronic wounds: a systematic review. *Int Wound J.* 2018 Aug;15(4):633-44. doi: 10.1111/iwj.12907.
52. Franzini M, Valdenassi L, Pandolfi S, Tirelli U, Ricevuti G, Simonetti V, et al. The biological activity of medical ozone in the hormetic range and the role of full expertise professionals. *Front Public Health.* 2022 Sep 16;10:979076. doi: 10.3389/fpubh.2022.979076.