Ozone therapy: an overview of pharmacodynamics, current research, and clinical utility

Noel L. Smith[1], Anthony L. Wilson[2], Jason Gandhi[2, 3], Sohrab Vatsia[4], Sardar Ali Khan[2, 5, *]

1 Foley Plaza Medical, New York, NY, USA
2 Department of Physiology and Biophysics, Stony Brook University School of Medicine, Stony Brook, NY, USA
3 Medical Student Research Institute, St. George's University School of Medicine, Grenada, West Indies
4 Department of Cardiothoracic Surgery, Lenox Hill Hospital, New York, NY, USA
5 Department of Urology, Stony Brook University School of Medicine, Stony Brook, NY, USA
*Correspondence to: Sardar Ali Khan, M.D., skysalik@gmail.com.
orcid: 0000-0002-4759-530X (Sardar Ali Khan)

Abstract

The use of ozone (O_3) gas as a therapy in alternative medicine has attracted skepticism due to its unstable molecular structure. However, copious volumes of research have provided evidence that O_3's dynamic resonance structures facilitate physiological interactions useful in treating a myriad of pathologies. Specifically, O_3 therapy induces moderate oxidative stress when interacting with lipids. This interaction increases endogenous production of antioxidants, local perfusion, and oxygen delivery, as well as enhances immune responses. We have conducted a comprehensive review of O_3 therapy, investigating its contraindications, routes and concentrations of administration, mechanisms of action, disinfectant properties in various microorganisms, and its medicinal use in different pathologies. We explore the therapeutic value of O_3 in pathologies of the cardiovascular system, gastrointestinal tract, genitourinary system, central nervous system, head and neck, musculoskeletal, subcutaneous tissue, and peripheral vascular disease. Despite compelling evidence, further studies are essential to mark it as a viable and quintessential treatment option in medicine.

Key words: ozone; ozone therapy; ozone gas; autohemotherapy; oxidative stress; reactive oxidative species; lipid ozonation products; oxidative preconditioning

doi: 10.4103/2045-9912.215752
How to cite this article: Smith NL, Wilson AL, Gandhi J, Vatsia S, Khan SA. Ozone therapy: an overview of pharmacodynamics, current research, and clinical utility. Med Gas Res. 2017;7(3):212-219.

Introduction

Ozone (O_3) gas was discovered in the 1840s, and soon after that, the scientific community began to expand past the notion that it was just another gas of the Earth's atmosphere. Though the migration of O_3 into the medical field has taken a circuitous road since the 19th century, its medicinal value is currently controversial despite compelling research.[1] O_3 is highly water-soluble inorganic molecule composed of three oxygen molecules. O_3's inherently unstable molecular structure, due to the nature of its mesomeric states, tends to make it difficult to obtain high concentrations. O_3 will often experience transient reactions with itself or water. Thus, it was initially problematic to achieve desired levels and even more difficult is to assess the therapeutic effects of such a transient state.[1,2] These mesomeric states create a conundrum within the scientific community. A divide has formed between those who believe the volatile nature of these mesomeric states can foster positive responses and those who are wary of its seemingly dangerous effects.

Despite suspicions, a multitude of O_3 therapies have shown substantial benefits that span a large variety of acute and chronic ailments. O_3 is currently prevalent in dentistry to treat diseases of the jaw.[1] O_3 has also proven itself beneficial as a disinfectant for drinking water and sterilization of medical instruments.[1,3] The function of O_3 shares similarities to that of a prodrug, as it is modified upon reacting with

molecules to create more active substrates, thus stimulating an endogenous cascade of responses. On the other hand, it is hard to classify O_3 as simply a prodrug, due to its capability to directly interact with phospholipids, lipoproteins, cell envelopes of bacteria, and viral capsids. The physiology of these biological responses is herein discussed.

Despite the various benefits, O_3 toxicity and clinical utility depends on the concentration and administration to the appropriate site.[1,2,4,5] One of the major contraindications of O_3 therapy is lung inhalation. O_3 therapy significantly increases airway resistance without changing the compliance or elastic characteristics of the lung.[1] Additionally, direct contact of O_3 with the eyes and lungs is contraindicated because of the low antioxidant capabilities in these specific locations.[6]

Literature Retrieval

A MEDLINE® database search of literature extended from 1980 to 2017 to obtain current information regarding O_3 therapy, its routes of administration, and mechanism of action. Subsequently, trials pertaining to the clinical implications of O_3 therapy were paired by pathology and anatomical system. The most important points refer to the type of pathology, route of O_3 administration, type of research trial, result(s) of the trial, side effect(s), and proposed physiological mechanism(s). Literature retrieval was performed in July 2017 and included the term "ozone therapy" combined with the following search criteria: "routes of administration", "mechanism of action", "cardiovascular", "subcutaneous tissue", "peripheral vascular disease", "neurological", "head and neck", "orthopedic", "musculoskeletal", "gastrointestinal", and "genitourinary". We did not formulate any exclusion criteria.

Routes of Administration

O_3 therapy combines a mixture of oxygen (O_2)-O_3, with a diverse therapeutic range (10–80 μg/mL of gas per mL of blood).[5-7] O_3 therapy administration is variable based on treatment goals and location of therapy. The first and most popular is O_3 autohemotransfusion (O_3-AHT). O_3-AHT has grown in popularity because it allows for a predetermined amount of blood to be taken and thus, using stoichiometric calculations, a precise concertation of O_2-O_3 can be infused. This small amount of blood is subjected to O_2-O_3 *ex vivo* is then administered to the patient.[5,6] Extracorporeal blood oxygenation and ozonation are very similar techniques. However, its goal is to obtain higher blood volume than the 200–300 mL seen in O_3-AHT.[5]

Other modalities of therapies include direct injection *via* the intramuscular, intradiscal, and paravertebral site of administration. Rectal insufflation of O_2-O_3 is another common site of administration. However, insufflation of the nasal, tubal, oral, vaginal, vesical, pleural, and peritoneal cavities have proven to be prudent routes of administration. Cutaneous exposure has also had likely outcomes and can be achieved by sealing the portion of the body in a chamber or bag and insufflating with O_2-O_3 mixture. Saline with O_2-O_3 dissolved is used to avoid the risk of embolism when administered intravenously.[5]

Mechanism of Action

Antioxidant capacity

Upon beginning O_3 therapy, a multifaceted endogenous cascade is initiated and releases biologically active substrates in response to the transient, and moderate, oxidative stress that O_3 induces. O_3 can cause this mild oxidative stress because of its ability to dissolve in the aqueous component of plasma.[8] By reacting with polyunsaturated fatty acids (PUFA) and water, O_3 creates hydrogen peroxide (H_2O_2), a reactive oxygen species (ROS). Simultaneously, O_3 forms a mixture of lipid ozonation products (LOP).[9] The LOPs created after O_3 exposure include lipoperoxyl radicals, hydroperoxides, malonyldialdeyde, isoprostanes, the ozonide and alkenals, and 4-hydroxynonenal (4-HNE). Moderate oxidative stress caused by O_3 increases activation of the transcriptional 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 O_3. 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.[9]

O_3, as well as other medical gases, *e.g.*, carbon monoxide (CO) and nitric oxide (NO), has twofold effects depending on the amount given and the cell's redox status. There is a complex relationship between these three medical gases as O_3 overexpresses HO-1, also referred to as HSPs of 32 kPa (Hsp32),[10] the enzyme responsible for CO formation, and downregulates NO synthase, which generates NO. Furthermore, O_3 upregulates the expression levels of Hsp70 which, in turn, is strictly related to HO-1. O_3 may have a developing role in Hsp-based diagnosis and therapy of free radical-based diseases. HO-1 degrades heme, which can be toxic depending on the amount produced, into free iron, CO, and biliverdin (*i.e.*, precursor of bilirubin), a neutralizer of oxidative and nitrosative stress due to its ability

to interact with NO and reactive nitrogen species.[11,12] Recently, it is becoming clear the heat shock response (HSR) provides a cytoprotective state during inflammation, cancer, aging, and neurodegenerative disorders.[13] Given its extensive cytoprotective properties, the HSR is now a target for induction *via* pharmacological agents.[1] Hsp70 is involved in co- and post-translational folding, the quality control of misfolded proteins,[14] folding and assembly of *de novo* proteins into macromolecular complexes, as well as anti-aggregation, protein refolding, and degradation.[15] HO isoforms are acknowledged as dynamic sensors of cellular oxidative stress and regulators of redox homeostasis throughout the phylogenetic spectrum. The effect of O_3 on these cell activities remains to be evaluated. Hormesis is a potent, endogenous defense mechanism for lethal ischemic and oxidative insults to multiple organ systems.[13] O_3 may have a hormetic role in regulating the anti-inflammatory and proinflammatory effects of CO, including prostaglandin formation akin to NO, which has been shown to exert some of its biological actions through the modulation of prostaglandin endoperoxide synthase activity.[16] Inhibiting HO activity prevents CO biosynthesis and its downstream effects[17]; the effect of O_3 on this cascade is yet to be determined.

Animal models have postulated the beneficial effects of prophylactic O_3 therapy in controlling the age-related effects of oxidative stress.[18,19] Evidence was provided to show that low O_3 dose administration provided beneficial effects on age-related alterations in the heart and hippocampus of rats. Additional research has been performed and provided room for speculation that O_3 therapy may provide the mediation of a mechanism involved in rebalancing the dysregulated redox state that accumulates as individuals age.[20] There was an apparent reduction of lipid and protein oxidation markers, lessening of lipofuscin deposition, restoration of glutathione (GSH) levels, and normalization of GPx activity in aged heart tissue. O_3 was demonstrated to decrease age-associated energy failure in the heart and hippocampus of rats. Researchers suspect that the improved cardiac cytosolic calcium and restoration of weakened Na^+-K^+ ATPase activity in the heart and hippocampus, respectively, were associated with the improvements seen.[20]

In hopes of attaining a sense of the possible toxic components of O_3 therapy, a study was done to assess the extent of lesions on human hematic mononucleated cells (HHMC), human thymic epithelium, murine macrophages, mouse splenocytes, and B16 melanoma murine cells. A significant finding of the study was that Hsp70 exhibited an O_3-induced increase in biosynthesis in HHMC. Hsp70s are synthesized in response to thermal shock and other stressing agents to cope with the damage that stimulates their biosynthesis.[21] Additionally, they stimulate several immune system responses in lymphocytes and macrophages. The study provided evidence that O_3 is a stressing agent capable of upregulating the biosynthesis of Hsp70, without toxicity to membranes. However, the membranes of macrophages are highly resistant to the possible toxicity of O_3 at high concentrations; HHMC is less resistant at the high end of the spectrum. The statement above should not discount the effectiveness of O_3 as a therapy because Hsp70s are induced in HHMCs without lesions up to 20 μg/mL— a typical dose given in O_3-AHT.[21]

Cisplatin (CDDP), a treatment used in a variety of cancers has been observed to have nephrotoxicity in 25% of the patients as a side effect. The occurrence of this nephrotoxicity is thought to be secondary to the free radical generation and the inability of ROS scavengers to ameliorate these molecules, leading to acute renal failure. O_2-O_3 therapy was used to increase the antioxidant capacity of rats exposed to CDDP and compared to control groups. Serum creatine levels were significantly reduced compared to control groups, illustrating the decreased nephrotoxicity indirectly in the rats with CDDP and O_2-O_3 therapy. In addition to attenuating the nephrotoxicity, O_2-O_3 therapy also restores the levels of antioxidant defense constituents (GSH, SOD, CAT, and GSH-Px), which are usually decreased by CDDP. Also, thiobarbituric acid reactive substances (TBARS) were reduced, which is a marker of lipid peroxidation in the kidney.[22,23]

Additional human studies examined the beneficial effects of O_3 therapy employed *via* O_3-AHT, in conjunction with coenzyme Q10, administered orally. The study evaluated SOD levels, a powerful antioxidant and catalase enzyme, an additional antioxidant enzyme in a control group, a group of O_3 therapy by itself, and O_3 therapy combined with Q10. Evidence has implied that SOD was significantly increased and catalase enzyme insignificantly increased in the O_3 + Q10 group when compared to the control group. Malondialdehyde, a product of lipid peroxidation, is an indicator of oxidative membrane damage. Malondialdehyde levels were significantly decreased concentrations in the O_3 + Q10 group when compared to the control group. Taken together, this study provides evidence of the beneficial effects of O_3 therapy in combination with Q10 in combatting and the prevention of damage elicited by oxidation.[9]

Multiple studies have provided evidence that O_3 therapy increased activation of the Nrf2 pathway *via* the induction of moderate oxidative stress.[15,24] By doing so, a transient increase in H_2O_2 and LOPs enhances the number of antioxidants and therefore can be used for a longer time frame to re-establish the balance of the redox system. Additionally, the creation of these antioxidant enzymes has effects, not only at the level of O_3 radical metabolism, but on the

whole body.[22,23]

Researchers have argued that knowing the total antioxidant status and plasma protein thiol group levels of a blood sample are indicators of the precise amount of O_3 required to optimize treatments. By developing more accurate antioxidant status indicators, an individual treatment would achieve the correct dosage on a day and case basis.[7,23,25] Systems have been proposed to have a more precise measurement of the redox state of a patient to achieve this goal. One system proposes simultaneously measuring different biological markers in the blood such as GSH, GPx, GST, SOD, CAT, conjugated dienes, total hydroperoxides, and TBARS. Using an algorithm, information can be gathered about the total antioxidant activity, total pro-oxidant activity, redox index, and grade of oxidative stress. Systems like this can provide insights to the correct dosage and response to O_3 therapy based on oxidative stress levels seen in the patient.[7,23,25]

Vascular and hematological modulation

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.[26] 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.[27] With repeated treatment, sufficient enough LOP may be generated to reach the bone marrow acting as repeated stressors to simulate erythrogenesis 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.[1,28]

O_3 has also been shown to improve blood circulation and oxygen delivery to ischemic tissues.[29] 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".[1,30] Also, O_3 increases levels of prostacyclin, a known vasodilator.[1]

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.[29] Since HO-derived bilirubin[31] 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.[29,32]

The prophylactic role of O_3 has been explored with hepatic ischemia/reperfusion (I/R) injury, a phenomenon associated with liver transplantation. Hepatic I/R is a clinically unsolved problem mainly due to the unknown mechanisms that are the foundations of this ailment. In summary, O_3 oxidative preconditionings (ozoneOPs) were found to protect against liver I/R injury through mechanisms that promote a regulation of endogenous NO concentrations and the maintenance of an adequate cellular redox balance. OzoneOPs are postulated to upregulate endogenous antioxidant systems and generate an increase in NO molecule generation, both of which are protective orders against liver and pancreas damage. The results in this animal model provided evidence that ozoneOPs protected against liver I/R *via* an increase in concentrations of endogenous NO and prime cells to have a more balanced redox system.[32] Additionally, enhanced activation of adenosine A_1 receptors in rat models have been observed with ozoneOPs in liver I/R.[33]

Further studies have expanded upon this postulation by applying O_3 therapy to renal I/R in rats. Renal I/R is a primary cause of acute renal failure after transplantation surgery. The findings of a study by Orakdogen et al.[34] indicated that the ozoneOPs allowed for a protective element when facing I/R. Following an increase in endothelial NO synthase and inducible NO synthase expression, it was concluded that ozoneOPs were intimately related to the increasing NO production as well as reducing renal damage by suppressing endothelin 1.[34]

Cerebral vasospasm after subarachnoid hemorrhage is a significant detriment to the recovery of patients. An animal model examined the effects intravenous O_3 therapy on vasospasms in the rat femoral artery. Histopathological and morphometric measurements provided evidence that O_3 therapy decreased morphometric changes, disruption of endothelial cells, and hemorrhages that are a result of

vasospasm. The study speculated the anti-oxidative and anti-inflammatory effects of O_3 might be a prudent treatment for posthemorrhagic vasospasm.[35]

Pathogen inactivation

When bacteria are exposed to O_3 *in vitro*, the phospholipids, and lipoproteins that are within the bacterial cell envelope are oxidized. As this occurs, the stability of the bacterial cell envelope is attenuated. Moreover, evidence has demonstrated O_3 to interact with fungal cell walls like bacteria. This disrupts the integrity of the cytosolic membrane and infiltrates the microorganisms to oxidize glycoproteins, glycolipids, and block enzymatic function. The combination of these reactions causes inhibition of fungi growth and mortality of bacteria and fungi.[1,3,5] *In vitro*, O_3 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.[1,3,36]

Specifically, animal models have shown that O_3 therapy as an adjunct to vancomycin enhances the animal's capability to eliminate methicillin-resistant *Staphylococcus aureus* mediastinitis.[37]

Immune system activation

In vivo, O_3 therapy has been shown to have multifaceted effects when interacting with PUFA. As stated previously, O_3 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.[36,38] Specifically, increases in interferon, tumor necrosis factor, and interleukin (IL)-2 are seen. The increases with IL-2 are known to initiate immune response mechanisms.[1] Additionally, H_2O_2 activates nuclear factor-kappa B (NF-κB) and transforming growth factor beta (TGF-β), which increase immunoactive cytokine release and upregulate tissue remodeling. H_2O_2 mediates the action of NF-κB by enhancing the activity of tyrosine kinases that will phosphorylate IκB, a subunit of the transcription factor NF-κB.[34,37] Low doses of O_3 have been shown to inhibit prostaglandin synthesis, release bradykinin, and increase secretions of macrophages and leukocytes.[34] Having the correct amount of either of these oxidative markers can be used to create a sufficient rise in H_2O_2 and NO levels to stimulate the most notable increase in IL-8. IL-8 also activates NF-κB, allowing production of ROS scavengers.[7]

Animal models using O_3 have shown to reduce and prevent inflammatory responses steming from the presence of *E. coli* in the renal system.[26,38] Additional studies have provided evidence of the anti-inflammatory effects of O_3. A study by Chang et al.[25] purified rheumatoid arthritis synovial fibroblast cells from human patients and injected them into immunocompromised mouse joints. Using an Ozonsan-α generator to deliver precise gas flows to vessels in the localized area, the authors discovered that 3% and 5% O_3 application significantly decreased the proinflammatory cytokines IL-1β, IL-6, and TNF-α without any toxicity or severe side effects.[25]

Studies have shown that human cancer cells from lung, breast, and uterine tumors are inhibited in a dose-dependent manner by O_3 therapy *in vitro*. O_3 concentrations of 0.3 and 0.5 ppm inhibited cancer cell growth by 40% and 60%, respectively. Furthermore, the noncancerous cell controls were not affected by these levels of O_3. At 0.8 ppm, cancer cell growth was inhibited by more than 90%. However, the control cell growth was less than 50%. Additionally, as control cells aged, they exhibited further growth inhibition and morphological changes. The study speculated that as the healthy cells matured, there was a decrease in growth due to the increased cellular damage incurred by each division.[39]

Clinical Utility

With its ever-growing ubiquity, O_3 therapy is finding a place in many branches of medicine and medical specialties. In fact, its clinical use can be arranged systematically into cardiovascular (**Additional Table 1**), subcutaneous tissue (**Additional Table 2**), peripheral vascular disease (**Additional Table 3**), neurological (**Additional Table 4**), head and neck (**Additional Table 5**), orthopedic (**Additional Table 6**), gastrointestinal (**Additional Table 7**), and genitourinary (**Additional Table 8**). These indications are a product of human clinical trials conducted for specific pathologies related to the aforementioned systems. Despite a lack of direct support of O_3 therapy, the current Food and Drug Administration regulations do not restrict the use of it in situations where it has proven its safety and effectiveness. Nonetheless, there has been support for its safety and effectiveness in multi-international studies.

Conclusions

O_3 therapy can alter the natural history of several disease and disorders, with potentially many more yet untested. A plethora of laboratory studies have provided evidence of O_3's antioxidant capabilities, as well as vascular, hematological, and immune system modulations. This evidence has been further substantiated in clinical trials with O_3 therapy being useful in the cardiovascular, subcutaneous tissue, peripheral vascular disease, neurological, head and neck, orthopedic, gastrointestinal, and genitourinary pathologies. O_3 therapy has proven especially beneficial in the diabetic foot, ischemic wounds, and peripheral vascular disease, areas in which O_3

use is most prevalent. Upcoming laboratory and translational research should begin to develop protocols for O_3-AHT in attempts to establish a dose-response relationship as it has demonstrated high utility in a myriad of pathologies at varying concentrations. Despite the presently compelling evidence, future studies should include more double-blind, randomized clinical trials with greater sample sizes, determination of longevity in benefits produced, as well as methods of measurements and analysis.

Acknowledgments
The authors are thankful to Drs. Kelly Warren, Inefta Reid, Todd Miller, and Peter Brink (Department of Physiology and Biophysics, Stony Brook University School of Medicine, Stony Brook, NY, USA) for departmental support, as well as Mrs. Wendy Isser and Ms. Grace Garey (Northport VA Medical Center Library, Northport, NY, USA) for literature retrieval.

Author contributions
NLS designed, organized, and wrote the article; ALW designed the outline, wrote the article, and solved queries related to scientific publications from the journals; JG performed literature searches, critiqued the literature findings, and wrote the article; SV critiqued and applied logical reasoning to the literature findings; SAK applied clinical concepts, revised the article to add logical reasoning, and cross-checked the referencing. All authors have read and approved the manuscript provided.

Conflicts of interest
The authors have no conflicts of interest to declare.

Plagiarism check
Checked twice by iThenticate.

Peer review
Externally peer reviewed.

Open peer reviewers
Ozan Akca, University of Louisville, USA; Nemoto Edwin, University of New Mexico Health Sciences Center, USA; Mancuso Cesare, Università Cattolica del Sacro Cuore, Italy.

Additional files
Additional Table 1: Cardiovascular indications for O_3 therapy.
Additional Table 2: Subcutaneous tissue indications for O_3 therapy.
Additional Table 3: Peripheral vascular disease indications for O_3 therapy.
Additional Table 4: Neurological indications for O_3 therapy.
Additional Table 5: Head and neck indications for O_3 therapy.
Additional Table 6: Orthopedic indications for O_3 therapy.
Additional Table 7: Gastrointestinal indications for O_3 therapy.
Additional Table 8: Genitourinary indications for O_3 therapy.

References

1. Elvis AM, Ekta JS. Ozone therapy: A clinical review. *J Nat Sci Biol Med*. 2011;2:66-70.
2. Zanardi I, Borrelli E, Valacchi G, Travagli V, Bocci V. Ozone: a multifaceted molecule with unexpected therapeutic activity. *Curr Med Chem*. 2016;23:304-314.
3. Azarpazhooh A, Limeback H. The application of ozone in dentistry: a systematic review of literature. *J Dent*. 2008;36:104-116.
4. Bocci VA. Tropospheric ozone toxicity vs. usefulness of ozone therapy. *Arch Med Res*. 2007;38:265-267.
5. Bocci VA. Scientific and medical aspects of ozone therapy. State of the art. *Arch Med Res*. 2006;37:425-435.
6. Bocci V. Autohaemotherapy after treatment of blood with ozone. A reappraisal. *J Int Med Res*. 1994;22:131-144.
7. 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;7:313-317.
8. Bocci V, Larini A, Micheli V. Restoration of normoxia by ozone therapy may control neoplastic growth: a review and a working hypothesis. *J Altern Complement Med*. 2005;11:257-265.
9. Inal M, Dokumacioglu A, Ozcelik E, Ucar O. The effects of ozone therapy and coenzyme Q(1)(0) combination on oxidative stress markers in healthy subjects. *Ir J Med Sci*. 2011;180:703-707.
10. Bocci V, Aldinucci C, Mosci F, Carraro F, Valacchi G. Ozonation of human blood induces a remarkable upregulation of heme oxygenase-1 and heat stress protein-70. *Mediators Inflamm*. 2007;2007:26785.
11. Mancuso C, Capone C, Ranieri SC, et al. Bilirubin as an endogenous modulator of neurotrophin redox signaling. *J Neurosci Res*. 2008;86:2235-2249.
12. Barone E, Trombino S, Cassano R, et al. Characterization of the S-denitrosylating activity of bilirubin. *J Cell Mol Med*. 2009;13:2365-2375.
13. Dattilo S, Mancuso C, Koverech G, et al. Heat shock proteins and hormesis in the diagnosis and treatment of neurodegenerative diseases. *Immun Ageing*. 2015;12:20.
14. Martínez-Sánchez G, Delgado-Roche L, Díaz-Batista A, Pérez-Davison G, Re L. Effects of ozone therapy on haemostatic and oxidative stress index in coronary artery disease. *Eur J Pharmacol*. 2012;691:156-162.
15. Bocci V, Valacchi G. Nrf2 activation as target to implement therapeutic treatments. *Front Chem*. 2015;3:4.
16. Mancuso C, Pistritto G, Tringali G, Grossman AB, Preziosi P, Navarra P. Evidence that carbon monoxide stimulates prostaglandin endoperoxide synthase activity in rat hypothalamic explants and in primary cultures of rat hypothalamic astrocytes. *Brain Res Mol Brain Res*. 1997;45:294-300.
17. Navarra P, Dello Russo C, Mancuso C, Preziosi P, Grossman A. Gaseous neuromodulators in the control of neuroendocrine stress axis. *Ann N Y Acad Sci*. 2000;917:638-646.
18. Onal O, Yetisir F, Sarer AE, et al. Prophylactic ozone administration reduces intestinal mucosa injury induced by intestinal ischemia-reperfusion in the rat. *Mediators Inflamm*. 2015;2015:792016.
19. Kal A, Kal O, Akillioglu I, et al. The protective effect of prophylactic ozone administration against retinal ischemia-reperfusion injury. *Cutan Ocul Toxicol*. 2017;36:39-47.
20. El-Sawalhi MM, Darwish HA, Mausouf MN, Shaheen AA. Modulation of age-related changes in oxidative stress markers and energy status in the rat heart and hippocampus: a significant role for ozone therapy. *Cell Biochem Funct*. 2013;31:518-525.

21. Cardile V, Jiang X, Russo A, Casella F, Renis M, Bindoni M. Effects of ozone on some biological activities of cells in vitro. *Cell Biol Toxicol*. 1995;11:11-21.
22. Gonzalez R, Borrego A, Zamora Z, et al. Reversion by ozone treatment of acute nephrotoxicity induced by cisplatin in rats. *Mediators Inflamm*. 2004;13:307-312.
23. Valacchi G, Bocci V. Studies on the biological effects of ozone: 11. Release of factors from human endothelial cells. *Mediators Inflamm*. 2000;9:271-276.
24. Re L, Martínez-Sánchez G, Bordicchia M, et al. Is ozone preconditioning effect linked to Nrf2/EpRE activation pathway in vivo? A preliminary result. *Eur J Pharmacol*. 2014;742:158-162.
25. Chang JD, Lu HS, Chang YF, Wang D. Ameliorative effect of ozone on cytokine production in mice injected with human rheumatoid arthritis synovial fibroblast cells. *Rheumatol Int*. 2005;26:142-151.
26. Madej P, Plewka A, Madej JA, et al. Ozonotherapy in an induced septic shock. I. Effect of ozonotherapy on rat organs in evaluation of free radical reactions and selected enzymatic systems. *Inflammation*. 2007;30:52-58.
27. Bocci VA, Zanardi I, Travagli V. Ozone acting on human blood yields a hormetic dose-response relationship. *J Transl Med*. 2011;9:66.
28. Brigelius-Flohé R, Flohé L. Basic principles and emerging concepts in the redox control of transcription factors. *Antioxid Redox Signal*. 2011;15:2335-2381.
29. Bocci V, Zanardi I, Huijberts MS, Travagli V. Diabetes and chronic oxidative stress. A perspective based on the possible usefulness of ozone therapy. *Diabetes Metab Syndr*. 2011;5:45-49.
30. León Fernández OS, Ajamieh HH, Berlanga J, et al. Ozone oxidative preconditioning is mediated by A1 adenosine receptors in a rat model of liver ischemia/reperfusion. *Transpl Int*. 2008;21:39-48.
31. Mancuso C. Bilirubin and brain: a pharmacological approach. *Neuropharmacology*. 2017;118:113-123.
32. Ajamieh HH, Menendez S, Martinez-Sanchez G, et al. Effects of ozone oxidative preconditioning on nitric oxide generation and cellular redox balance in a rat model of hepatic ischaemia-reperfusion. *Liver Int*. 2004;24:55-62.
33. Chen H, Xing B, Liu X, et al. Ozone oxidative preconditioning protects the rat kidney from reperfusion injury: the role of nitric oxide. *J Surg Res*. 2008;149:287-295.
34. Orakdogen M, Uslu S, Emon ST, Somay H, Meric ZC, Hakan T. The effect of ozone therapy on experimental vasospasm in the rat femoral artery. *Turk Neurosurg*. 2016;26:860-865.
35. 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;29:646-682.
36. Gulmen S, Kurtoglu T, Meteoglu I, Kaya S, Okutan H. Ozone therapy as an adjunct to vancomycin enhances bacterial elimination in methicillin resistant Staphylococcus aureus mediastinitis. *J Surg Res*. 2013;185:64-69.
37. Bocci V. Does ozone really "cure" cancer? *Int J Cancer*. 2008;123:1222; author reply 1223.
38. Caliskan B, Guven A, Ozler M, et al. Ozone therapy prevents renal inflammation and fibrosis in a rat model of acute pyelonephritis. *Scand J Clin Lab Invest*. 2011;71:473-480.
39. Sweet F, Kao MS, Lee SC, Hagar WL, Sweet WE. Ozone selectively inhibits growth of human cancer cells. *Science*. 1980;209:931-933.
40. Hernández F, Menéndez S, Wong R. Decrease of blood cholesterol and stimulation of antioxidative response in cardiopathy patients treated with endovenous ozone therapy. *Free Radic Biol Med*. 1995;19:115-119.
41. Wainstein J, Feldbrin Z, Boaz M, Harman-Boehm I. Efficacy of ozone-oxygen therapy for the treatment of diabetic foot ulcers. *Diabetes Technol Ther*. 2011;13:1255-1260.
42. Martínez-Sánchez G, Al-Dalain SM, Menéndez S, et al. Therapeutic efficacy of ozone in patients with diabetic foot. *Eur J Pharmacol*. 2005;523:151-161.
43. Bertolotti A, Izzo A, Grigolato PG, Iabichella ML. The use of ozone therapy in Buruli ulcer had an excellent outcome. *BMJ Case Rep*. 2013;2013:bcr2012008249.
44. Moore G, Griffith C, Peters A. Bactericidal properties of ozone and its potential application as a terminal disinfectant. *J Food Prot*. 2000;63:1100-1106.
45. Shah P, Shyam AK, Shah S. Adjuvant combined ozone therapy for extensive wound over tibia. *Indian J Orthop*. 2011;45:376-379.
46. Tafil-Klawe M, Wozniak A, Drewa T, et al. Ozone therapy and the activity of selected lysosomal enzymes in blood serum of patients with lower limb ischaemia associated with obliterative atheromatosis. *Med Sci Monit*. 2002;8:CR520-525.
47. Romero Valdés A, Menéndez Cepero S, Gómez Moraleda M, Ley Pozo J. Ozone therapy in the advanced stages of arteriosclerosis obliterans. *Angiologia*. 1993;45:146-148.
48. Verrazzo G, Coppola L, Luongo C, et al. Hyperbaric oxygen, oxygen-ozone therapy, and rheologic parameters of blood in patients with peripheral occlusive arterial disease. *Undersea Hyperb Med*. 1995;22:17-22.
49. Giunta R, Coppola A, Luongo C, et al. Ozonized autohemotransfusion improves hemorheological parameters and oxygen delivery to tissues in patients with peripheral occlusive arterial disease. *Ann Hematol*. 2001;80:745-748.
50. Di Paolo N, Bocci V, Garosi G, et al. Extracorporeal blood oxygenation and ozonation (EBOO) in man. preliminary report. *Int J Artif Organs*. 2000;23:131-141.
51. Di Paolo N, Bocci V, Salvo DP, et al. Extracorporeal blood oxygenation and ozonation (EBOO): a controlled trial in patients with peripheral artery disease. *Int J Artif Organs*. 2005;28:1039-1050.
52. Molinari F, Rimini D, Liboni W, et al. Cerebrovascular pattern improved by ozone autohemotherapy: an entropy-based study on multiple sclerosis patients. *Med Biol Eng Comput*. 2017;55:1163-1175.
53. Molinari F, Simonetti V, Franzini M, et al. Ozone autohemotherapy induces long-term cerebral metabolic changes in multiple sclerosis patients. *Int J Immunopathol Pharmacol*. 2014;27:379-389.
54. Lintas G, Molinari F, Simonetti V, Franzini M, Liboni W. Time and time-frequency analysis of near-infrared signals for the assessment of ozone autohemotherapy long-term effects in multiple sclerosis. *Conf Proc IEEE Eng Med Biol Soc*. 2013;2013:6171-6174.
55. Clavo B, Santana-Rodriguez N, Gutierrez D, et al. Long-term improvement in refractory headache following ozone therapy. *J Altern Complement Med*. 2013;19:453-458.

56. Clavo B, Catalá L, Pérez JL, Rodríguez V, Robaina F. Ozone Therapy on Cerebral Blood Flow: A Preliminary Report. *Evid Based Complement Alternat Med*. 2004;1:315-319.
57. Clavo B, Suarez G, Aguilar Y, et al. Brain ischemia and hypometabolism treated by ozone therapy. *Forsch Komplementmed*. 2011;18:283-287.
58. Bocci V, Travagli V, Zanardi I. Randomised, double-blinded, placebo-controlled, clinical trial of ozone therapy as treatment of sudden sensorineural hearing loss. *J Laryngol Otol*. 2009;123:820; author reply 820.
59. Ragab A, Shreef E, Behiry E, Zalat S, Noaman M. Randomised, double-blinded, placebo-controlled, clinical trial of ozone therapy as treatment of sudden sensorineural hearing loss. *J Laryngol Otol*. 2009;123:54-60.
60. Clavo B, Ruiz A, Lloret M, et al. Adjuvant ozonetherapy in advanced head and neck tumors: a comparative study. *Evid Based Complement Alternat Med*. 2004;1:321-325.
61. Clavo B, Pérez JL, López L, et al. Ozone therapy for tumor oxygenation: a pilot study. *Evid Based Complement Alternat Med*. 2004;1:93-98.
62. Menéndez S, Del Cerro A, Alvarez T, Hernández F. Application of ozone therapy in the vestibulocochlear syndrome. *Rev Recent Clin Trials*. 2012;7:321-328.
63. Borrelli E, Bocci V. Visual improvement following ozonetherapy in dry age related macular degeneration; a review. *Med Hypothesis Discov Innov Ophthalmol*. 2013;2:47-51.
64. Steppan J, Meaders T, Muto M, Murphy KJ. A metaanalysis of the effectiveness and safety of ozone treatments for herniated lumbar discs. *J Vasc Interv Radiol*. 2010;21:534-548.
65. Paoloni M, Di Sante L, Cacchio A, et al. Intramuscular oxygen-ozone therapy in the treatment of acute back pain with lumbar disc herniation: a multicenter, randomized, double-blind, clinical trial of active and simulated lumbar paravertebral injection. *Spine* (Phila Pa 1976). 2009;34:1337-1344.
66. Oder B, Loewe M, Reisegger M, Lang W, Ilias W, Thurnher SA. CT-guided ozone/steroid therapy for the treatment of degenerative spinal disease--effect of age, gender, disc pathology and multi-segmental changes. *Neuroradiology*. 2008;50:777-785.
67. Magalhaes FN, Dotta L, Sasse A, Teixera MJ, Fonoff ET. Ozone therapy as a treatment for low back pain secondary to herniated disc: a systematic review and meta-analysis of randomized controlled trials. *Pain Physician*. 2012;15:E115-129.
68. Al-Jaziri AA, Mahmoodi SM. Painkilling effect of ozone-oxygen injection on spine and joint osteoarthritis. *Saudi Med J*. 2008;29:553-557.
69. Bonetti M, Fontana A, Albertini F. CT-guided oxygen-ozone treatment for first degree spondylolisthesis and spondylolysis. *Acta Neurochir Suppl*. 2005;92:87-92.
70. Bocci V, Paulesu L. Studies on the biological effects of ozone 1. Induction of interferon gamma on human leucocytes. *Haematologica*. 1990;75:510-515.
71. Zaky S, Kamel SE, Hassan MS, et al. Preliminary results of ozone therapy as a possible treatment for patients with chronic hepatitis C. *J Altern Complement Med*. 2011;17:259-263.
72. Zaky S, Fouad EA, Kotb HI. The effect of rectal ozone on the portal vein oxygenation and pharmacokinetics of propranolol in liver cirrhosis (a preliminary human study). *Br J Clin Pharmacol*. 2011;71:411-415.
73. Clavo B, Ceballos D, Gutierrez D, et al. Long-term control of refractory hemorrhagic radiation proctitis with ozone therapy. *J Pain Symptom Manage*. 2013;46:106-112.
74. Peretyagin SP, Vorobyov AV, Martusevich AK, et al. Ozonotherapy of the gastrointestinal tract stressinjuries at urgency patients and biocristalloscopic monitoring its effectiveness. *Revista Ozonoterapia Rev*. 2008;1:24-28.
75. Neimark AI, Nepomnyashchikh LM, Lushnikova EL, Bakarev MA, Abdullaev NA, Sizov KA. Microcirculation and structural reorganization of the bladder mucosa in chronic cystitis under conditions of ozone therapy. *Bull Exp Biol Med*. 2014;156:399-405.
76. Gu XB, Yang XJ, Zhu HY, Xu YQ, Liu XY. Effect of medical ozone therapy on renal blood flow and renal function of patients with chronic severe hepatitis. *Chin Med J (Engl)*. 2010;123:2510-2513.
77. Clavo B, Gutiérrez D, Martín D, Suárez G, Hernández MA, Robaina F. Intravesical ozone therapy for progressive radiation-induced hematuria. *J Altern Complement Med*. 2005;11:539-541.
78. Bonforte G, Bellasi A, Riva H, et al. Ozone therapy: a potential adjunct approach to lower urinary tract infection? A case series report. *G Ital Nefrol*. 2013;30:gin/30.34.16.