

An Overview About Medical Ozone And Its Possible Effects On Renal Injury

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Abstract

Background: Ozone (O₃) gas discovered in the mid-nineteenth century is a molecule consisting of three atoms of oxygen in a dynamically unstable structure due to the presence of mesomeric states. Although O₃ has dangerous effects, yet researchers believe it has many therapeutic effects. Ozone therapy has been utilized and heavily studied for more than a century. Its effects are proven, consistent, safe and with minimal and preventable side effects. Medical O₃ is used to disinfect and treat disease. Mechanism of actions is by inactivation of bacteria, viruses, fungi, yeast and protozoa, stimulation of oxygen metabolism, activation of the immune system. Medication forms in a gaseous state are somewhat unusual, and it is for this reason that special application techniques have had to be developed for the safe use of O₃. In local applications as in the treatment of external wounds, its application in the form of a transcutaneous O₃ gas bath has established itself as being the most practical and useful method, for example at low (sub-atmospheric) pressure in a closed system guaranteeing no escape of O₃ into the surrounding air. Ozonized water, whose use is particularly known in dental medicine, is optimally applied as a spray or compress. Diseases treated are infected wounds, circulatory disorders, geriatric conditions, macular degeneration, viral diseases, rheumatism/arthritis, cancer, SARS and AIDS.

Keywords: Medical Ozone, Renal Injury

INTRODUCTION

Ozone (O₃), a gas discovered in the mid-nineteenth century, is a molecule consisting of three atoms of oxygen in a dynamically unstable structure due to the presence of mesomeric states. The gas is colorless, acrid in odour and explosive in liquid or solid form. It has a half-life of 40 min at 20°C and about 140 min at 0°C. Its basic function is to protect humans from harmful effects of UV radiation. Ozone occurs at less than 20 µg/m³ from the Earth's surface at concentrations that are perfectly compatible with life. Although O₃ has dangerous effects, yet researchers believe it has many therapeutic effects.[1–3] The beginning of precise medical O₃ generators has only recently allowed the mechanisms, action and possible toxicity of O₃ to be evaluated by clinical trials.[2] Ozone has a capacity to oxidize organic compounds,[4] and has well-known toxic effects on the respiratory tract when present in smog.[5–6] In medical use the gas produced from medical grade oxygen is administered in precise therapeutic doses, and never via inhalation, and advocates that it has excellent health benefits in dental caries, decrease blood cholesterol and stimulation of antioxidative responses, modifies oxygenation in resting muscle and is used in complementary treatment of hypoxic and ischemic syndromes.[7–10]

Ozone therapy has been utilized and extensively studied for many decades altogether. Its effects are proven, consistent and with minimal side effects. Medical O₃, used to disinfect and treat disease, has been around for over 150 years. Used to treat infections, wounds and multiple diseases, O₃'s effectiveness has been well-documented. It has been used to disinfect drinking water before the turn of the last century. Ozone was known to treat as many as 114 diseases.[11] Ozone therapy has been in use since the 1800s and in 1896 the genius Nikola Tesla patented the first O₃ generator in the US, later forming the "Tesla Ozone Company." [12] During the first world war (1914-18) doctors familiar with O₃'s antibacterial properties, and with few other medical resources available to them applied it topically to infected wounds and discovered O₃ not only remedied infection, but also had hemodynamic and anti-inflammatory properties.[13] In the late 1980s, reports had emerged that German physicians were successfully treating HIV patients with O₃-AHT (Autohemotherapy). There was then no pharmaceutical treatment for HIV and a pandemic was feared, so Canadian authorities authorized the study to test safety and efficacy of O₃-AHT in AIDS patients. Ozone had shown promise in *in vitro* testing. Ozone was seen effective at disinfecting extracorporeal blood samples of HIV; unfortunately for AIDS patients, O₃-AHT proved to be an *in vivo* ineffective treatment.

SARS AND OZONE

Ozone is a naturally occurring energy-rich molecule embodying unique physico-chemical and biological properties suggesting a possible role in the therapy of SARS, either as a monotherapy or, more realistically, as an adjunct to standard treatment regimens. Owing to the excess energy contained within the O_3 molecule, it is theoretically likely that O_3 , unlike organism-specific antiviral options available today, will show effectiveness across the entire genotype and subtype spectrum of SARS.[25]

MECHANISM OF ACTION

Inactivation of bacteria, viruses, fungi, yeast and protozoa: Ozone therapy disrupts the integrity of the bacterial cell envelope through oxidation of the phospholipids and lipoproteins. In fungi, O_3 inhibits cell growth at certain stages. With viruses, the O_3 damages the viral capsid and upsets the reproductive cycle by disrupting the virus-to-cell contact with peroxidation. The weak enzyme coatings on cells which make them vulnerable to invasion by viruses make them susceptible to oxidation and elimination from the body, which then replaces them with healthy cells.[26]

Stimulation of oxygen metabolism: Ozone therapy causes an increase in the red blood cell glycolysis rate. This leads to the stimulation of 2,3-diphosphoglycerate which leads to an increase in the amount of oxygen released to the tissues. Ozone activates the Krebs cycle by enhancing oxidative carboxylation of pyruvate, stimulating production of ATP. It also causes a significant reduction in NADH and helps to oxidize cytochrome C. There is a stimulation of production of enzymes which act as free radical scavengers and cell-wall protectors. Production of prostacycline, a vasodilator, is also induced by O_3 [Figure 1].[25]

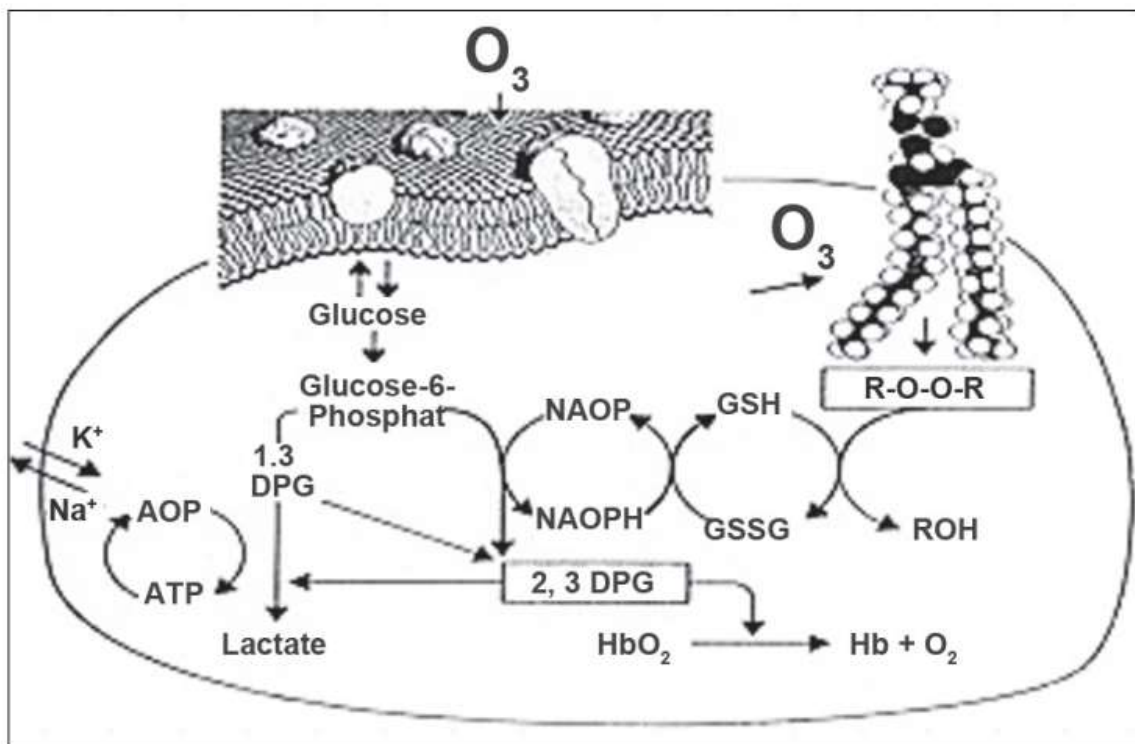


Figure 1: Action of ozone on RBC Metabolism

Activation of the immune system: Ozone administered at a concentration of between 30 and 55 $\mu\text{g}/\text{cc}$ causes the greatest increase in the production of interferon and the greatest output of tumor necrosis factor and interleukin-2. The production of interleukin-2 launches an entire cascade of subsequent immunological reactions. [27]

Mechanism of action of O_3 on the human lung: Ozone exposure induces a significant mean decrement in vital capacity. It significantly increases mean airway resistance and specific airway resistance but does not change dynamic or static pulmonary compliance or viscous or elastic work. It also significantly reduces maximal transpulmonary pressure. And further more significantly increases respiratory rate and decreased tidal volume. [27]

CLINICAL TRIALS

The study to evaluate effect of bimosiamose on O_3 -induced sputum neutrophilia: Bimosiamose is an anti-inflammatory glycomimetic and selectin inhibitor.[28] It is found effective against disease states involving inflammatory cells like for example for asthma.[29] This drug, as per last updation, was in phase 2 trials and being evaluated for its efficacy and safety in treating chronic pulmonary obstructive disease (COPD), the study is sponsored by Revotar Biopharmaceuticals AG and was carried out by NCT00962481 (ClinicalTrials.gov Identifier).[30]

Evaluate the effects of the drug (SB-656933-AAA) on the body after a single dose in subjects who have inhaled O_3 : Drug SB-656933-AAA was developed by GlaxoSmithKline which was found to exhibit good activity in treating COPD as well as cystic fibrosis. This action was found to be enhanced by administration of a single dose of O_3 before administration of the

aforementioned drug. This drug until latest updated data was in phase 1 stage, study was carried out by NCT00551811.[31] Intraarticular O₃ therapy for pain control in osteoarthritis of the knee: Ozone is being currently tested for its effectiveness in relieving the pain in patients suffering from osteoarthritis of the knee. The current status of the study is phase 2 which is sponsored by Ben-Gurion University of the Negev and the study being conducted by NCT00832312.[32] The Effect of Ozone Therapy for Lumbar-Herniated Disc: Ozone is also being evaluated for its efficacy infiltration and its effectiveness in comparison with microdiscectomy in the treatment of lumbar-herniated disc with criteria for surgery. The study is currently in its phase 2 studies, which is sponsored by Kovacs Foundation and trials being carried out by NCT00566007. The study also evaluates the efficacy of infiltration in presence of corticoids, anesthetics, which is being compared by replacing O₃ by oxygen.[33–35]

ADVANTAGES OF OZONE THERAPY

Diabetic complications are attributed to the oxidative stress in the body, O₃ was found to activate the antioxidant system affecting the level of glycemia. Ozone prevented oxidative stress by normalizing the organic peroxide levels by activating superoxide dismutase.[36–37] Ozone was found to completely inactivate the HIV *in vitro*, this action of O₃ was dose-dependent. Concentration used for inactivation was found to be non-cytotoxic. The inactivation was owing to the reduction of the HIV p24 core protein.[38] Ozone was also found to increase the host immunity by increasing the production of cytokine.[39] In an *in vitro* study, it was observed that O₃ is very effective in reducing the concentrations of *Acinetobacter baumannii*, *Clostridium difficile* and methicillin-resistant *Staphylococcus aureus* in dry as well as wet samples, hence it can be used as a disinfectant. Oxygen/ O₃ mixture was also found to prolong the appearance of arrhythmia induced by potassium chloride, aconitine, etc., in laboratory animals like rats.[40]

DISADVANTAGES OF OZONE THERAPY

An array of ill-effects are observed owing to the reactivity of O₃ viz oxidation, peroxidation or generation of free radicals and giving rise to cascade of reactions like peroxidation of lipids leading to changes in membrane permeability,[41] lipid ozonation products (LOP) act as signal transducer molecules.[42] The main reason for this being presence of unsaturated fatty acids in both lung lining fluid and pulmonary cell bilayers, O₃ reacts with unsaturated fatty acids to give their specific products i.e., LOP, which activates the lipases triggering the release of endogenous mediators of inflammation.[43] The loss of functional groups in enzymes leading to enzyme inactivation. These reactions further results in cell injury or eventual cell death. Combinations of O₃ and NO₂ occur in photochemical smog, have hazardous effects on lung alveoli and act additively or synergistically. Dietary antioxidants or free radical scavengers like vitamin E, C, etc., can prevent aforementioned effects of O₃. [44–45] In an *in vitro* study it was observed that arachidonic acid was oxidized in presence to O₃ to give peroxides, viz. arachidonic acid peroxides (AAP), having activity comparable to prostaglandin endoperoxides. These peroxides were found to show following actions contraction of rabbit aortic strips and rat fundus strips in presence on indomethacin and Vane's mixture of vasoactive hormones at doses comparable to naturally formed prostaglandin peroxides. AAP also caused aggregation of human platelets in platelet-rich plasma, but these effects were not observed in presence of indomethacin and vitamin E, which indicated that these can be used to treat such toxicity of O₃. [46]

RECENT DEVELOPMENT

Ozone was effectively used as an antibacterial agent to treat oral infections caused by *Actinomyces naeslundii*, *Lactobacilli casei* and *Streptococcus mutans*. Exposure of about 60 s exhibited 99.9% killing efficiency, but exposure for such a long period showed degradation of saliva proteins. So exposure of 10 s to 30 s was proved effective to kill significant number of bacteria.[47] A single subcutaneous injection of O₃ in mouse with spared nerve injury of the sciatic nerve was found to decrease the neuropathic pain-type behavior. Mechanism of this action is yet unclear but O₃ was observed to regulate the expression of the genes that play vital role in onset and maintenance of allodynia.[48]

Effect on Renal Ischemia:

Ischemia (cessation of blood flow), followed by reperfusion (reestablishment of blood flow), causes morphological and functional damages, which increases during the reperfusion phase. Reperfusion of kidney increases the effects of early ischemic injury by release of oxygen-derived reactive oxygen species (ROS), such as superoxide (O₂⁻), hydrogen peroxide (H₂O₂), and hydroxyl radical (·OH), or nitrogen-derived reactive nitrogen species (RNS), such as nitric oxide (NO) and peroxynitrite (ONOO⁻). The consequences of oxidative and nitrosative stress are multiple and include lipid peroxidation, resulting in the destruction of membrane lipids and oxidative DNA damage, collectively leading to the loss of cell viability, either by necrotic or apoptotic pathways [49]

The intracellular and molecular mechanisms involved in the development of renal ischemia/reperfusion injury (IRI) regarding ROS/RNS are complex and not yet fully understood, but have been extensively discussed and reviewed. Several investigations have demonstrated the beneficial effects of pharmacological interventions, which prevent ROS generation, inhibit the enzymes responsible for ROS generation, and in administration of antioxidant enzymes, which degrade ROS and ROS scavenging molecules [48].

Ozone administration significantly reduced the severity of IRI in rats' kidneys. After administration, ozone is dissolved in

biological fluids such as plasma, lymph, and urine; it immediately reacts with macromolecular glycoproteins comprising the carbohydrates and polypeptide chains, namely proteoglycans and collagen types II and IV. All of these compounds act as electron donors and undergo oxidation, resulting in the formation of H₂O₂ and lipid oxidation products (LOPs). H₂O₂, an essential ROS molecule, is able to act as an ozone messenger for eliciting several biological and therapeutic effects. In contrast to the conventional idea that H₂O₂ is harmful, it has been widely accepted that it acts as a regulator of signal transduction and is an important mediator of host defense and immune responses. Although H₂O₂ acts immediately and disappears (early and short-acting messenger), LOPs, via the circulation, distribute throughout the tissues and become late and long-lasting messengers. This process stimulates the innate immune system and helps the cell to survive when an injury occurs. In light of recent pharmacological knowledge, our consideration is that ozone acts as a pro-drug and induces a rearrangement of the biochemical pathways with the activation of a second messenger in a cascade with a multiple system action. [49]

So in Conclusion; OT might protect kidneys against IRI by modulating a moderate oxidative and nitrosative stress, which, in turn, increases the antioxidant endogenous systems. [49]

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