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Ozone- A Biological Therapy in Dentistry- Reality or Myth?????

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Abstract: The usage of ozone in dentistry has been proposed because of its antimicrobial, disinfectant, biocompatibility and healing properties. In the last decade a number of therapeutic protocols with ozone have been developed to address common dental infections associated with periodontal disease, RCT and caries. Despite these advantages, therapeutic ozone's application in dentistry is limited because of its possible side effects. Hence, dental practitioners need to know the proper usage of ozone therapy that can provide better patient care and considerably cut down the time and cost of the treatment.

Keywords: Antimicrobial, dental applications, Ozone therapy, RCT and caries.

INTRODUCTION

Ozone therapy has been used for therapeutic purposes since the 19th century. The first mention of ozone was made by Dutch physicist Martin van Marun in 1785, but it was Christian Friedrich Schonbein in 1840, a Professor in University of Basel, who demonstrated the changes of the properties of oxygen with the formation of the particular gas called ozone. The word ozone is derived from the Greek word 'OZEIN' which means odor and he also detected for the first time the capacity of the ozone to bind with biological substrates in the double-bond positions [1].

Ozone (O₃) has a characteristic, penetrating odor and is present in small amounts in atmospheric air. The ozone molecules are composed of three oxygen atoms and present naturally in the upper layer of atmosphere in abundance [2, 3]. It protects living organisms by surrounding the earth at altitudes of 50,000 to 100,000 feet from the ultra-violet rays [2, 4]. As it falls downward to earth, being heavier than air, combines with any pollutant it comes in contact with and cleanses the air. This is earth's natural way of self-cleansing [3].

Ozone (O₃) is naturally produced by the photo dissociation of molecular oxygen (O₂) into activated oxygen atoms, which then react with further oxygen molecules. This transient radical anion rapidly becomes protonated, generating hydrogen trioxide (HO₃), which, in turn, decomposes to an even more powerful oxidant, the hydroxyl radical (OH). Ozone gas has a high oxidation potential and is 1.5 times greater than chloride when used as an antimicrobial agent [4].

Ozone therapy became an inherent element of the treatment of infection in such fields as surgery, dermatology, cosmetics, and dentistry [5]. The ozone concentration used may vary between 1 and 100gm/ml (0.05-5%) according to the medical/dental indication and the patient's condition [6]. Controlled ozone application has been found to be extremely safe, free from side effects and far free from most medications, including antibiotics. It also stimulates blood circulation and the immune response [4]. Bactericidal, virucidal, and fungicidal activity of ozone is generally known and has been exploited for years in industry and medicine [3].

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There are three different systems for generating ozone gas. They are:

1. Ultra violet system: produces low concentrations of ozone, used in aesthetics & for air purification
2. Cold plasma system: used in air and water purification
3. Corona discharge system: produces high concentrations of ozone. It is the most common system used in the medical/dental field [7].

HISTORY

1. Christian Friedrich Schönbein (1840), a German Chemist, is regarded as the Father of ozone therapy.
2. In 1857 Joachim Hänsler, a German physicist and physician, along with German physician, Hans Wolff, developed the first ozone generator for medical use [2 - 4].
3. The Institute for Oxygen Therapy- Healing was formed in Berlin and the man credited with founding naturopathy, Dr. Benedict Lust, began practicing in New York, and wrote many articles on ozone.
4. Dr. C. Lender in 1870- purified blood in test tubes by using O₃.
5. In 1881, it was used as a disinfectant in the treatment of diphtheria.
6. Dr. Charles Kenworthy, a Florida physician, in 1885, published the book Ozone in the Florida Medical Association Journal, which details on the use of ozone on therapeutic purposes.
7. In 1896 an Ozone generating system was patented by Nikola Tesla [1].
8. In early 20th century Food and Drug Act, revised its use and effect in the field of medicine.
9. In 1920s Dr Edwin Parr, a Swiss dentist, started to use O₃ as part of his disinfection system [8].
10. A German dentist, Dr. E.A. Fisch, in 1950, used ozonated water for dental procedures and pioneered its use in medicine.
11. 1959; an Ozone machine called "Ozonosan" was patented by Dr. Joachim Hansler which formed the basis of the expansion in German ozone therapy [9 - 13].
12. First Ozone research centre in the world was founded in Cuba [1].
13. 1971; The International Ozone Institute(IOI), now known as International Ozone Association was founded

MODE OF ADMINISTRATION OF OZONE

The European cooperation of medical ozone societies warns from direct intravenous injections of ozone/ oxygen gas that should not be practiced due to possible risk of air embolism.

There are three basic forms of ozone application:

- 1) ozone gas
- 2) ozonated water
- 3) ozonated oil [4].

MECHANISM OF ACTION OF OZONE

Ozone in the gaseous or aqueous phase has been shown to be a powerful and reliable antimicrobial agent against bacteria, fungi, protozoa and viruses.

Antimicrobial Effect

According to microbiological research data, the ozone is capable of killing all the known types of gram- positive and gram- negative bacteria, including the *Pseudomona aeruginosa* and *Eschericea coli*, both of which are extremely resistant to antibiotics [1]. The anti-microbial effect of ozone is by the result of its action on cells by damaging its cytoplasmic membrane due to ozonolysis of dual bonds and also ozone induced modification of intracellular contents because of secondary oxidants effects. This action is non-specific and selective to microbial cells; it does not damage human body cells, [14] the reason attributed to this is antioxidant ability of mammalian cells.

Thanomsub *et al.* demonstrated the effect of ozone treatment on cell growth and structural changes in bacteria such as *E. Coli*, *Salmonella sp.*, *Staphylococcus aureus* and *Bacillus subtilis* [15]. Ozone is very efficient in antibiotics resistant strains. Its antimicrobial activity increases in liquid environment especially in acidic pH.

In 2003, it was discovered that ozone can be generated *in vivo* in activated neutrophils [16].

This discovery is of striking impact since it shows that ozone has a physiological role, not only as a bactericide agent but rather one that could form part of the physiological amplifying mechanisms of the inflammation and the activation of associated genes [17].

Immunostimulating Action

Ozone influences cellular and humoral immune system. It stimulates proliferation of immunocompetent cells and synthesis of immunoglobulins. It also activates function of macrophages and increases sensitivity of micro-organisms to phagocytosis [18]. Satisfactory results have been reported from applying ozone therapy to patients at a concentration of 30-55 µg/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 [19]. This means that the application of medical ozone is extremely useful for immune activation in patients with a low immune status and/or immune deficit.

Anti-Inflammatory & Analgesic Action

Different data coming from the scientific research recognizes that ozone has a dual action mechanism: analgesic and anti-inflammatory. These effects seem to be due to its way of acting on diverse targets:

1. Decrease the production of mediators of the inflammation
2. The oxidation (inactivation) of metabolic mediators of pain
3. It clearly improves local blood microcirculation, with an improvement in the oxygen delivery to the tissues, essential for the generation of anatomic structures; the elimination toxins and in general to the resolution of the physiological disturbance that generated the pain [1].

i.e., Ozone helps in the synthesis of biologically active substances such as interleukins, leukotrienes and prostaglandins which is beneficial in reducing inflammation and pain. The infection or inflammation is positively charged (acidic) and ozone is negatively charged (basic) so the chemistry of infection and inflammation attracts ozone to the area [14].

A study done on anti-inflammatory effects of ozonated water in an experimental mouse model suggested that ozonated water has anti-inflammatory properties and is a potential therapeutic option for acute inflammation [20].

Anti-hypoxic Action

The effects of ozone on the metabolism of oxygen can be explained from its promoting action of:

1. Changes in the rheological properties of the blood
2. Increase in the speed of glycolysis of the erythrocyte [1].

Ozone brings about the rise of Po₂ in tissues and improves transportation of oxygen in blood, which results in change of cellular metabolism activation of aerobic processes (Glycolysis, Krebs's cycle, B-oxidation of fatty acids) and use of energetic resources. Ozone acts as a super-oxygenator, bringing oxygen to tissues, assisting body in its natural healing process [14].

To summarize, the Ozone therapy can induce the following biological responses: (most frequently ranges between 20 and 40 µg/ml ozone per ml of blood)

1. Improves blood circulation and oxygen delivery in ischemic tissue owing to NO, CO, and increase level of intra-erythrocytic 2,3-DPG
2. Enhances general metabolism by improving oxygen delivery
3. Up regulates cellular antioxidant enzymes and induction of HO-1 and HSP70
4. Induces a mild activation of the immune system and enhances the release of growth factors
5. Does not procure acute or late side effects
6. Procures a surprising wellness in most patients, probably *via* the stimulation of the neuroendocrine system
7. Activates neuroprotective systems [21]
8. Intervenes in the release of autocoids

9. Metabolic regulator
10. Immunological modulator
11. Broad spectrum Germicide [1]

APPLICATIONS OF OZONE IN DENTISTRY

Bacteria are the cause of many problems in dentistry so a powerful agent is needed for effective elimination of these causative agents. In dentistry, Ozone is used as a chair side disinfectant because of its synergistic part of the treatment, both in eliminating bacteria and oxygenating chair side environment.

Role of Ozone on Dental Caries Prevention and Management

Within the past few years, ozone therapy has been launched as a new method for treating caries. It has been suggested that the application of ozone to carious lesions will arrest or reverse these lesions and that the use of ozone will provide an alternative to conventional drilling and filling and also it was showed that Ozone can be used to kill bacteria present in carious lesion, painlessly and even without anaesthetic [22 - 26].

A study conducted by Baysan proved that the number of bacteria in carious root lesions is considerably reduced by ozone therapy, and that the lesions clinically change to stages in which progression of the caries can be considered to have ceased [27].

Two different studies done on ozonated water and gaseous ozone to demonstrate the antimicrobial activity of ozone showed that ozone have strong antimicrobial activity against *E. faecalis* and *S. mutans* infections *in vitro* in bovine dentine as well as in *ex vivo* conditions and it can be used as an adjuvant in caries therapy [28, 29] and it is only suitable for easily accessible surfaces where the caries can also be removed with a toothbrush or other non-operative procedures [30].

A recent Cochrane Review identified 3 randomised controlled trials (RCTs) and two of the three RCTs included in the analysis investigated the effect of ozone therapy on crown lesions, while the third investigated the effect on root lesions and it was concluded that these trials provide no evidence that the application of ozone arrests or reverses the decay process [31] and also ozone and photodynamic therapy(PDT) had a minimal effect on the viability of microorganisms organized in a cariogenic biofilm [32].

Hence, a systematic review of all relevant randomized controlled trials found that there is a fundamental need for more evidence of appropriate rigor and quality before the use of ozone can be accepted into the mainstream primary dental care or can be considered a viable alternative to current methods for the management and treatment of dental caries [31].

Antibacterial Effect of Ozone on Plaque Biofilm

Both caries and periodontal disease are caused primarily by plaque biofilm. Ozone might be useful to control oral infectious microorganisms in dental plaque. Ozonated water strongly inhibited the accumulation of experimental dental plaque.

Ozonated oil is used as a safe therapeutic alternative in patients with Acute Necrotizing Ulcerative Gingivitis. Healing and bactericidal properties make it useful as a subgingival irrigant. The antimicrobial property of Ozone is not only effective in reducing the number of cariogenic bacteria, but also causes significant reduction in the microorganisms present in the root canal. However it was not successful in completely eliminating these bacteria embedded in the biofilm [33].

Ozonated water had nearly the same antimicrobial activity as 2.5% sodium hypochlorite and also the metabolic activity of fibroblasts was high when the cells were treated with Ozonated water. The aqueous form of Ozone, as a potential antiseptic agent, showed less cytotoxicity than gaseous Ozone or established antimicrobials like chlorhexidine digluconate, sodium hypochlorite or hydrogen peroxide under most conditions. Therefore, aqueous ozone fulfils optimal cell biological characteristics in terms of biocompatibility for oral application. Ozone may be considered as an adjunctive to conventional treatment strategy due to its powerful ability to inactivate microorganisms

In a study on permeability of oral microorganisms and dental plaque, both gram +ve and gram -ve such as *Porphyromonas endodontalis* and *Porphyromonas gingivalis* were more sensitive to Ozonated water than gram +ve oral *Streptococci* and *Candida albicans* in pure culture. Ozonated water proved to have bactericidal activity against bacteria

in plaque biofilm [33].

Role of Ozone in Oral Medicine

Apthous Ulcer

A case report wherein beneficial action of topical application of Ozone in a patient with long standing apthous ulceration involving the lateral border of the tongue has been demonstrated. The use of topical ozone for the treatment of recurrent apthous ulceration requires further investigation before it can be advocated as a valid treatment option for these lesions. However, topical ozone application may be a further option for patients with apthous stomatitis for whom other treatment options have been exhausted or for whom systemic treatment is contraindicated [34].

Mucositis

A case report by Shenberg and Blum [35] demonstrated gaseous and aqueous ozone therapy for treatment of mucositis secondary to chemotherapy/radiotherapy. The treatment protocol involved application of ozone in both aqueous and gaseous forms.

While the evidence base for ozone therapy for secondary effects of chemotherapy or radiotherapy is currently being investigated, studies that used gaseous and aqueous ozone application alone have not been adequately represented in the literature [36].

Temporomandibular Joint Disorders

Intra-articular administration of ozonated water has been found to be a successful alternative therapy for the management of different joint diseases. A randomized control study was conducted by Daif, [37] involving 60 individuals with bilateral internal derangement of the TMJ and disc displacement with reduction. 87% of the patients who received ozone gas injection into the joint space either completely recovered or improved. However, further clinical and experimental studies are required to provide direct evidence for its mechanism of action and to substantiate the results.

Role in Endodontics

Until recently, the dental profession relied on chemical irrigants reaching the main and lateral canals to disinfect and dissolve organic debris where it is impossible to instrument mechanically. In endodontic treatment instead of using irrigation chemicals (NaOCl), Ozonated water can be used for irrigation. Ozone was found to be effective against endodontic pathogenic microorganisms like *E. Faecalis*, *Candida albicans*, *Peptostreptococcus micros* and *Pseudomonas aeruginosa* for disinfecting of root canals and dentinal tubules.

When the specimens was irrigated with sonication, ozonated water had nearly the same antimicrobial activity as 2.5% NaOCl and also it demonstrated the antimicrobial activity of Ozone in root canal treatment without any tissue toxicity [38, 39].

Ozonated oils like Ozonated sunflower oil, olive oil and ground nut oil was efficient in canal sterilization than the conventional irrigation by the Sodium hypochlorite and Sodium peroxide combination [40]. Ozone also eliminates the distinctive anaerobic odor associated with some chronically infected teeth.

The following steps should be added before the final biomechanical preparation:

- The files are coated with ozonated olive oil for lubrication & disinfection.
- The canals are prepared & then irrigated with ozonated water and dried.
- Before filling, a slow insufflations (45-60sec) into each canal with moderate/high concentration of ozone gas. Insufflation of ozone electrochemically travels into the lateral canals and dentinal tubules killing the microbes [14].

Nagayoshi, Kitamura *et al.* examined the effect of ozonated water against *E. faecalis* & *S. mutans* infections and found significant reduction in the viability of these organisms invading dentinal tubules [38]. Ozone will also penetrate through the apical foramen and enter into the surrounding and supportive bone tissue, encouraging healing & regeneration of bone [41].

Role in Bleaching

In discolored non vital teeth, Ozone can be used for bleaching. Once the bleaching agent is placed on the inner aspect of the root canal treated tooth, the crown is irradiated with ozone for minimum of 3-4 mins. This Ozone treatment bleaches the tooth within minutes and provides good esthetic result [4, 42].

The first experimental study was done by Tessier *et al.* to evaluate, in an experimental model of growing rats, the efficacy of using ozone to lighten tetracycline- stained incisors and it was demonstrated that ozone can be successfully used for lightening the yellowish tinge of tetracycline-stained rat incisors. But further studies are required for its potential use in the dental clinic [43].

A study was conducted to compare the beaching effect of ozone with office bleaching and it was concluded that the hydrogen peroxide gel has a more powerful whitening effect than ozone; in addition, ozone has no synergistic effect when is used simultaneously with hydrogen peroxide [44].

Role in Desensitization of Sensitive Root

Quick relief from root sensitivity has been documented after ozone spray for 60 seconds followed by mineral wash onto the exposed dentine in a repetitive manner. This desensitization of dentine lasts for longer period of time. Smear layer present over the expose root surface prevents the penetration of ionic Calcium and Fluorine deep into the dentinal tubules. Ozone removes this smear layer, opens up the dentinal tubules, broadens their diameter and then Calcium and Fluoride ions flow into the tubules easily, deeply and effectively to plug the dentinal tubules, preventing the fluid exchange through these tubules. Thus, ozone can effectively terminate the root sensitivity problem within seconds and also results last longer than those by conventional methods [45 - 47].

Ozgul [48] conducted a study to evaluate the hypersensitivity observed in MIH (Molar incisor hypomineralization)-affected teeth and the effect of desensitizing agents applied with or without ozone to incisors affected by MIH and it was demonstrated that desensitizing paste effectively reduced the hypersensitivity of teeth with MIH. CPP- ACP paste was found to be more effective, and ozone therapy prolonged the effect of CPP-ACP paste.

Role in Wound Healing

Ozone has been reported to accelerate the healing of soft tissue conditions *i.e.*: aphthous ulcers, herpes labialis, ANUG and other gingival infections because ozone encourages physiological healing rate as well as control opportunistic infections [49]. Application of ozonated water and ozonated oil daily accelerate the healing rate thus effective in the treatment of alveolitis. It also reduces the post extraction healing time by forming a pseudo membrane over the socket and protecting it from any physical and mechanical insults [50, 51].

In alveolitis, there is accelerated healing by irrigation with ozonated water after removal of the necrotic plug and debris under antibiotic coverage [52].

After radiotherapy in the jaw, oxygen supply may be considerably reduced in the affected area due to the obliteration of intraosseous vessels. Such compromised bone heals slowly compared to healthy bone, after surgical interventions like tooth extractions or implants. Ozone might be successfully used to treat such wound healing impairments after radiotherapy.

Role in Periodontics

Dental biofilm makes it difficult for antibiotics in targeting putative periodontal pathogens. Higher concentration of antibiotics is required to kill these organisms which are inevitably associated with toxic adverse effect on the host microbial flora.

Ozonated water (4mg/ml) strongly inhibited the formation of dental plaque and reduced the number of subgingival pathogens both gram positive and gram negative organism. Gram negative bacteria, such as *P. endodontalis* and *P. gingivalis* were substantially more sensitive to ozonated water than gram positive oral streptococci and *C. albicans* in pure culture [38, 53]. The application of ozone therapy in chronic gingival and periodontal diseases, showed subjective and objective improvement of their status, as well as patients with periodontal abscess, with no exudation was observed [54].

Huth, *et al.* compared the effectiveness of ozone with that of the established antiseptic CHX, against periodontal microorganisms. There were no significant differences in the effectiveness of aqueous Ozone (20 µg ml [-1]) or

gaseous ozone (≥ 4 g [-3]) compared with 2% CHX but they were more effective than 0.2% CHX. Therefore, high-concentrated gaseous and aqueous ozone merit further investigation as antiseptics in periodontitis therapy [55].

Dodwad *et al.* compared the effect of oral irrigation with ozonated water, 0.2% Chlorhexidine and 10% Povidone iodine in patients with chronic periodontitis and it was concluded that local Ozone application can serve as potent atraumatic, antimicrobial agent to treat periodontal disease nonsurgically both for home care and professional practice. It may also serve as good tool during supportive periodontal therapy [55, 56].

Ozonated water can be used in the ultrasonic water reservoir, also as a pre treatment rinse before scaling, root planning and the sulci, pockets are irrigated using syringe and canula in non surgical pocket curettage. This process will reduce the initial pathogenic load on the patient locally and systemically. After treatment, each pocket & sulcus is insufflated with ozone gas which directly goes into tissues, sterilizing the area.

Role in Prosthodontics

Microbial plaque accumulating on the denture is composed of several oral microorganism, mainly *C. albicans*. Denture plaque control is essential for the prevention of denture stomatitis. This can be controlled by topical application of ozonated oil over tissue surface and over denture surface. The disinfecting action of ozone is also used to clean denture.

Arita *et al.* demonstrated that the rinsing of dentures with flowing Ozonated water (2 or 4 mg/l) for 1 minute, might be useful in reducing the number of *C. albicans* on denture bases [51].

Role in Implantology

When Ozone therapy is used in implants, prevention of infection and enhancement of bone regeneration is seen when Ozone is bubbled into the socket for about 40sec, followed by placement of implant into the socket. Matsamura K *et al.* treated implants with ozone and found that there was regeneration of periodontal cells similar to that around natural teeth [57].

El Hadary *et al.* evaluated that short-term administration of cyclosporine A, when administered with topical ozonated oil, may influence bone density and the quality of dental implant osseointegration [58].

Role in Pedodontics

Most of the child patients have fear and anxiety towards dental treatment.

Dahnhart JE *et al.* evaluated the anxiety level of children (and their parents) treated with ozone and found that all children & parents reported significant anxiety prior to ozone treatment. However, following the treatment, the children reported they would be pleased to return for future treatments [59].

Abu Naba'a *et al.* conducted a split mouth randomized clinical trial. Using the anxiety questionnaire, they found that ozone treatment provoked the least state of anxiety comparing to traditional dentistry [60].

Lynch demonstrate the effect of Ozone in pediatric dentistry for treating primary carious lesions and it was shown that the tooth with caries treatment with ozone- oxygen for 60 secs, sterilizes the dental lesions and allows the remineralization of tooth [61]. It must be stressed that larger lesions are not those to be treated with ozone alone; most will require a combined approach of traditional therapy, as well as Ozone. Where the lesion extends deep into the dentine, the action of Ozone will take a longer time period of treatment, or may require several treatment periods over time.

OZONE TOXICITY

Even though ozone has certain benefits like non-invasiveness, simplicity, less time consumption and elimination of dental phobia, the inhalation of ozone can be toxic to the pulmonary system and other organs. European cooperation of Medical Ozone Societies prohibited the intravenous injections of Ozone gas due to risk of air embolism. Complications caused by ozone therapy are infrequent at 0.0007 per application.

Known side-effects are:

1. Epiphora,
2. Upper respiratory irritation

3. Rhinitis
4. Cough
5. Headache,
6. Occasional nausea, vomiting
7. Shortness of breath
8. Blood vessel swelling
9. Poor circulation
10. Heart problems [62].
11. The patient must be placed in the supine position
12. Treatment with vitamin E and n-acetylcysteines [4].

In the event of an ozone intoxication the patient must be placed in the supine position, inhale humid oxygen, and take ascorbic acid, vitamin E, and n-acetylcysteine. Because of ozone's highly oxidative power, all materials that come in contact with the gas must be ozone resistant, such as glass, silicon, and teflon [4, 63].

CONCLUSION

Dentistry is changing as we are now using modern science to practice dentistry. Ozone therapy has been more beneficial than present conventional therapeutic modalities that follow a minimally invasive and conservative application to dental treatment. Ozone is a promising treatment modality for various dental problems in future. But, it has to be kept in mind that presently ozone is an adjunct to other conventional treatment modalities and should be used in combination until more research shows benefits in independent usage.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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