

Ozone Therapy for Periodontal Disease- A Review

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Abstract

Introduction: Interleukins are key modulators of inflammation. They participate in acute and chronic inflammation in a complex network of interactions. Mechanistic explanations for positive and negative interactions between individual interleukins will also depend on new insights into the signal transduction pathways for each interleukin.

Content: Interleukins are the cytokines that act specifically as mediators between leukocytes. Approximately more than 35 interleukins have been described; each having unique biological activity and role in periodontal health or disease.

Summary and Conclusion: It seems quite likely that the lymphocyte may behave in a manner similar to a neuron that receives information from several other neurons and integrates the positive and negative signals, and then corresponds accordingly by initiating or refraining from initiating action potential. Like a neuron, the response of the lymphocyte will depend both on the positive and negative signals and the nature of their individual signal transduction pathways.

Keywords: Cytokines; Osteoblastic Activity; Bone Resorption

Introduction

Microorganisms which colonize the tooth surface constitute the primary etiologic components for chronic destructive periodontal disease. In an electron microscopic examination of the microbial flora on tooth surfaces, Listgarten noted that a distinct microbiota was associated with various clinical periodontal diseases. Culture studies indicate that most subgingival microorganisms are anaerobes [1].

These subgingival microorganisms like *Bacteroides forsythus* and *Porphyromonas gingivalis* are positively associated with attachment loss. *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis* and *Porphyromonas intermedius* are detected more frequently at progressing sites than at non-progressing sites. These anaerobic bacteria differ in regard to their oxygen sensitivity and tolerance to the toxic effects of oxygen [2].

A few studies have reported on the biophysical conditions of the subgingival ecosystem. It has been demonstrated that the redox (oxidation-Reduction) potential (Eh) is lower in periodontal pockets than in healthy sulcus or sites with gingivitis. Measurements of the oxygen tension in the oral cavity indicated that oxygen made up less than 1% of the gas atmosphere in the vicinity of the dentogingival surfaces. The pH in deep periodontal pockets was 7.09 on the average [3].

Association of oxygen tension in human periodontal pockets with gingival inflammation

The spectrum of pO_2 in deep pockets, define the pocket microbiological composition. Thus, the predominance of anaerobes isolated from periodontally diseased sites and the low redox potential in the subgingival plaque suggest that pocket environment is quite anaerobic.

Removal of dental plaque forms an important part of controlling and treating periodontal disease, which brings about both qualitative as well as quantitative changes in the subgingival microflora. The effectiveness of mechanical debridement however, is limited by such factors as individual motivation, inaccessibility to periodontal pockets, concave tooth surfaces, and margins of restorations [4].

Leukocytes play a special role among the host cells concerning oxygen consumption. In response to activation of neutrophils and macrophages by inflammatory mediators, these cells undergo a respiratory burst. This is associated with a 2 - 20 fold increase in oxygen consumption, activation of hexose monophosphate shunt, generation of reactive oxygen derived free radicals (OH), superoxide anion (O_2^-), H_2O_2 of phagocytes etc. These products are essential for the bactericidal activity [1].

Deeper pockets tend to contain less oxygen than shallower pockets and as it is now known that the defense mechanisms of PMN cells are strongly dependent upon certain of their oxidative metabolic processes, so a lower oxygen tension would affect the would affect the bactericidal action of the phagocytic cells. Thus, the gingival tissue oxidative metabolism per se, may also be of importance in the pathogenesis, development and therapy of periodontal disease.

It is known that significant changes in gingival oxygen utilization accompany inflammatory and healing states of gingival tissues [5].

As the role of oxidative metabolism of the PMN is rightfully assigned major importance for its possible influence upon the health of periodontal tissues, it seems appropriate for us to bring together the scattering individual reports on gingival oxidative metabolism.

Oxygen consumption/endogenous respiration

It is possible to judge the character and the intensity of metabolic processes taking place in the tissue. The resulting values of oxygen consumption are mostly expressed as the metabolic quotient Q_{O_2} = the amount of oxygen in micrograms consumed by 1 milligram of dry weight of the tissue per hour ($\mu\text{moles } O_2/\text{mg dry wt/hr}$) [6]. The normal oxygen consumption of gingiva has been measured as 1.6 ± 0.37 (Glickman, Turesky and Hill 1949).

Experimental methods of measurement

Serak's polarographic method

The samples of human gingiva are obtained from extractions and other surgical operations always carried out under local anaesthesia and transported to the laboratory on a cotton pad moistened with cooled saline. After removing the blood, the external part of gingival epithelium is cut in the form of a slice of 0.2 to 0.3 mm in thickness (in contact with cellophane membrane of the oxygen analysator by its keratinized side) which is used to determine the oxygen consumption and the remaining part is histologically examined by means of a common paraffin technique and stained by hematoxylin eosin.

Warburg's manometric method

In the Warburg's method, the tissue under investigation is dipped directly into the medium, containing dissolved oxygen and is continually shaken (unlike the Serak's method). This movement removes the concentration gradient of oxygen at the surface of the slice under investigation, which would virtually make any measurement impossible. It has been seen that canine, bovine, and human gingiva had similar respiration rates. Endogenous respirations studies in human skin revealed that the QO_2 at 32°C ranged from 1.8 - 4.6.

The effects of inflammation, healing and regeneration upon endogenous respiration

Varying observations have been made, i.e. Glickman., *et al.* noted that values increased progressively from a QO_2 of 1.5 on Day 1, to 5.0 between the 12th to 20th days of healing and returned to normal values in 20 - 30 days [6].

Person (1984) suggested that endogenous oxygen utilization by gingiva increased during mild to moderate inflammatory changes but decreased as the inflammatory changes become more severe [7].

Experimental periodontitis and O₂ consumption

The Hb concentration reflects the actual gingival blood volume, and the Hb oxygen saturation depends on blood tissue oxygen extraction. The results of studies showed that changes in gingival oxygen metabolism appear to be characterized by a rapid increase in gingival blood volume and oxygen consumption and therefore a rapid decrease in Hb oxygen saturation during the first seven days. Hence, it would be rational to say that when the increase in blood supply was not enough to meet oxygen demand, it leads to chronic inflammation [8].

The big question - Is there a clinical applicability?

By changing the subgingival environment, which has been shown to be highly anaerobic with a prevailing oxygen tension, the suppression of subgingival bacteria to inhibit their growth, can be carried out as an alternative approach to conventional antimicrobial or antiseptic agents. An increase in tissue oxygen tension, speeds up the process in problem wounds in all parts of the body.

Agents have been applied as molecular oxygen (Box 1937, Hirsh., *et al.* 1981), Hyperbaric oxygenation (Guentherman., *et al.* 1972, Gotsko., *et al.* 1980) and hydrogen peroxide (Salnetz and Brown 1946, Key., *et al.* 1978, Baer., *et al.* 1985) to help treat poorly wound in soft tissues and also in treatment of periodontitis. Recently ozone therapy has also been employed.

In infections caused by anaerobic microorganisms that can lead to progressive soft tissue necrosis, their milieu can be negatively affected to such a degree that even life- threatening infections are easier to deal with. An oxygen rich milieu always inhibits the growth of anaerobic microorganisms, effectively supporting antibiotic and surgical therapy.

In, addition an oxygen rich milieu enhances the function of leukocytes, activating or supporting the body defense mechanism in areas that are already frequently poorly perfused, which in turns speeds up the process.

Regenerative processes are also influenced by the increase in oxygen tension. Tissue capillarity clearly increases, and fibroblastic replication is enhanced. In addition, the healing process in damaged bone can be accelerated by oxygen therapy or even made possible in the first place.

Oxygen can be applied centrally by increasing the oxygen tension in the air breathed in during hyperbaric oxygen therapy (HBO). This leads to an increase in the amount of oxygen physically dissolved in the blood and, thus, to higher oxygen levels in peripheral tissue. The increased concentration of oxygen brings about the change in bacterial milieu and improves the healing process in the wound.

In cases of poorly healing superficial wounds and impaired skin integrity, local external applications can be used instead of central oxygen therapy. The principle behind this type of therapy is occlusion relative to the surrounding area with localized oxygenation. Oxygen can also be applied locally when the oral mucous membrane is diseased, such that the resorption barrier is reduced, and there is only a short distance of diffusion.

In, gingival and periodontal infections also oxygen can be insufflated into the tissues, thus impairing the milieu to reduce the growth of anaerobic microorganisms.

Box advocated the use of oxygen insufflation for the treatment of periodontal pockets with the rationale of stimulation of periodontal tissues by a surplus of oxygen. It leads to a direct exposure of oxygen sensitive anaerobic periodontal pathogens to artificially - elevated oxygen tension levels within the periodontal pocket.

Also, Manhold (1974) showed through an experiment that some commercially available oxygenating agents demonstrated shorter healing times when applied on inflamed gingiva [9].

The therapeutic effects of Ubiquinone Q10 (topical and systemic), a hydrogen carrier in the Krebs cycle, which increases the availability of oxygen, has already been advocated in literature and is now available in gel form [8].

Ozone therapy for periodontal disease

What is ozone?

Ozone is an allotropic form of oxygen. It possesses unique properties which are being defined and applied to biological systems as well as to clinical practice. As a molecule containing a large excess of energy, ozone, through incompletely understood mechanisms, manifests bactericidal, viricidal and fungicidal actions which may make it a treatment of choice in certain conditions and an adjunctive treatment in others.

Ozone, known for its protective role in the earth's ecological harmony, and for its interaction at ground level with industrial pollutants, has unique biological properties which are being investigated for applications in various medical fields. As early as the First World War, ozone's bactericidal properties were used to treat infected wounds, mustard gas burns and fistulas. Medical ozone generators have been developed and refined. They differ from industrial generators in their capacity to deliver the purest ozone-oxygen mixtures in precise dosages.

In the last few years ozone treatment has seen growing interest from diverse medical disciplines, and research is in progress to delineate its effects on biological systems and to define its clinical applications.

Physico-chemical and biochemical properties

The oxygen atom exists in nature in several forms:

1. As a free atomic particle (O), it is highly reactive and unstable
2. Oxygen (O_2), its most common and stable form, is colorless as a gas and pale blue as a liquid
3. Ozone (O_3), has a molecular weight of 48, a density one and a half times that of oxygen and contains a large excess of energy in its molecule ($P3—$) $3/2 O_2 + 143 \text{ KJ/mole}$. It has a bond angle of 127, which resonates among several forms, is distinctly blue as a gas and dark blue as a solid
4. O_4 is a very unstable, rare, nonmagnetic pale blue gas which readily breaks down into two molecules of oxygen.

It is calculated that the dose of ozone administered will perform its therapeutic functions without disrupting blood constituents. Since there are a variety of lipid components in whole blood, it is of more than theoretical interest to determine the end products of ozone per oxidation and their effects, not only on physiological systems but on the integrity of ambient pathogenic organisms, since one of the mechanisms of viral inactivation is thought to be through this modality.

Mechanism of action

1. **Inactivation of bacteria, viruses, fungi, yeast and protozoa:** Ozone disrupts the integrity of the bacterial cell envelope through oxidation of the phospholipids and lipoproteins. In fungi, ozone inhibits cell growth at certain stages. With viruses, the ozone damages the viral capsid and disrupts 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.
2. **Enhancement of circulation:** In circulatory disease, a clumping of red blood cells hinders blood flow through the small capillaries and decreases oxygen absorption due to reduced surface area. Ozone reduces or eliminates clumping and red cell flexibility is restored, along with oxygen carrying ability. Oxygenation of the tissues increases as the arterial partial pressure increases and viscosity decreases. Ozone also oxidizes the plaque in arteries, allowing the removal of the breakdown products, unclogging the blood vessels.

- 3. Stimulation of oxygen metabolism:** Ozone causes an increase in the red blood cell glycolysis rate. This leads to the stimulation of 2, 3-diphosphoglycerate (2, 3-DPG) which leads to an increase in the amount of oxygen released to the tissues. There is a stimulation of the production of the enzymes which act as free radical scavengers and cell wall protectors: glutathione peroxidase, catalase, and superoxide dismutase. Ozone activates the Krebs cycle by enhancing oxidative carboxylation of pyruvate, stimulating production of ATP. Ozone also causes a significant reduction in NADH and helps to oxidize cytochrome C. Prostacycline, a vasodilator, is also induced by ozone.
- 4. Formation of peroxides:** Ozone reacts with the unsaturated fatty acids of the lipid layer in cellular membranes, forming hydro peroxides. There is a synergistic effect with cellular-formed H_2O_2 . Lipid peroxidation products include alkoxy and peroxy radicals, singlet oxygen, ozonides, carbonyls, alkanes and alkenes.
- 5. Dissolution of malignant tumors:** Ozone inhibits tumor metabolism. In addition, ozone oxidizes the outer lipid layer of malignant cells and destroys them through cell lysis. Phagocytes produce H_2O_2 and hydroxyl to kill bacteria and viruses. The generation of hydroxyl by killer cells is critical to their cytotoxic capability. Ozone stimulates conversion of L-arginine to citrulline, nitrite by phagocytes, acting on tumors.
- 6. Activation of the immune system:** Ozone administered at a concentration of about 50 pg/CC causes the greatest increase in the production of interferon. Higher or lower concentrations have a correspondingly lower effect. Interleukin tumor necrosis factor (TNF) is released in the greatest quantities between 30 and 55 ug/cc.

Effects of ozone on bone and soft tissues

In oral surgery ozonated water is suitable for prophylactic applications against infections after osteotomies. In a prospective study involving 250 patients, the application of ozonated water during surgery as a cooling and rinsing medium in the osteotomy of third molars reduced the occurrence of infectious complications after the operation. In another prospective study, the positive effect of ozone water in oral soft tissue healing could be demonstrated clinically and histologically. Apart from the microbiologic effect, a therapeutic one must also be assumed. Several experimental studies on blood treated with ozone showed that the contact with ozone led to an increased release of interferons (IFN-alpha, beta, gamma), interleukins (IL-1alpha, IL-2, IL-6, IL-8). Tumor necrosis factor (TNF-alpha), as well as transforming growth factor (TGF-beta 1).

Particularly after radiotherapy in the maxilla or mandible, oxygen supply may be considerably reduced in the affected area. Apart from numerous intraoral side effects like xerostomia, mucositis or loss of the sense of taste, the obliteration of intraosseous vessels is caused, resulting in a deficient vascular supply of the spongy medullary spaces. The consequences are fibrosing and aseptic osteonecrosis.

Such compromised bone as that after surgical interventions like tooth extractions or implant dentistry heals later than does healthy bone with a good blood supply. Also, surface wounds, e.g. caused by denture pressure, frequently heal much later. Such cases always carry the risk of a persisting osteoradionecrosis. Ozone might possibly be successfully used to treat such wound-healing impairments after radiotherapy.

Routes of administration:

1. Intravenous injection- a direct injection of the ozone gases into the vein.
2. Intraarticular injection- a direct, injection into the painful joint.
3. Direct injection into a tumor- ozone gases can be injected into a tumor or a cyst.
4. Autohemotherapy- 50 - 200 ml of blood drawn into a bottle. The bottle is turned upside down and ozone is infused into the bottle and then transfused back to patient.
5. Intracutaneous blistering- ozone is injected into the skin.
6. Intramuscular- a direct injection of the ozone gases into the muscle.

7. Subcutaneous- an injection of ozone gas performed just under the skin.
8. Uterine insufflation- a catheter is placed into the uterus where ozone is slowly infused.
9. Bladder insufflation- a catheter is placed into the bladder where ozone is slowly infused.
10. Dental use of ozonated water- water that is bubbled through ozone gases and kept near freezing.
11. Rectal insufflation- ozone is passed into the rectum through a catheter.
12. Vaginal insufflations- ozone is passed into the vagina through a catheter.
13. Drinking water- water that is bubbled through ozone gases and drank nearly immediately.
14. In the ear- a slow flow of ozone gases is run through a small tube into the ear.
15. Ozonated water enema- ozonated water is passed through a catheter into the rectum.
16. Breathing ozonated olive oil- ozonated olive oil is breathed into the lungs.
17. Deep lymphatic massage with ozonated olive oil- olive oil that has been bubbled through ozone gas until nearly solidified.
18. Ozonated bath Epsom salts and sea salt- bathing in ozonated water.
19. Body suit- suit that is sealed off wrists, ankles, and neck. Humidified ozone is pumped into the body suit and lasts for 15 - 30 minutes.
20. Steam cabinet- a cabinet filled with ozone gases you can sit in with your head protruding for 15 - 30 minutes.
21. External limb bagging- the leg is placed over the affected area. Once the ozone is humidified it is pumped into the bag. It will kill any bacteria, viruses, fungus, or molds infecting the open wound.

Listed contraindications to ozone treatment:

- Acute alcohol intoxication
- Recent myocardial infarction
- Hemorrhage from any organ
- Pregnancy
- Thrombocytopenia and
- Ozone allergy

Application of ozone in periodontitis

A study was conducted to examine the effect of ozonated water on oral microorganisms and dental plaque. The antimicrobial properties of ozonated water were assessed on experimental dental plaque and also on the formation of plaque. The effect of ozonated water was examined on *S. mutans*, *S. sobrinus*, *S. sanguis*, *S. salivarius*. Gram negative bacteria such as *P. gingivalis*, *P. endodontalis*, *A. actinomycetemcomitans* and the fungus, *Candida albicans*. The results were assessed using Live/Dead® Bac Light™ Bacterial viability kit, fluorescence microscopy and electron microscopy. The results showed that the number of viable *S. mutans* remarkably decreased when treated with ozonated water and also it inhibited the accumulation of experimental dental plaque in vitro. When the dental plaque samples from human subjects were exposed to ozonated water in vitro, almost no viable bacterial cells were detected. Hence these results suggest that ozonated water should be useful in reducing the infections caused by oral microorganisms in dental plaque.

Immunohistochemical the Periodontal cells which are capable of proliferation were studied on extracted human teeth after 2-minutes irrigation with saline or ozonized water and marking of proliferating cell nuclear antigen (PCNA). 23 completely erupted 3rd molars without antagonists were extracted for prophylactic reasons in patients aged 20 - 35 years and immediately treated randomly with intensive irrigation of ozonated water for 2 minutes of the concentration of 2.5 - 3.5 mg/lit or irrigated with sterile isotonic saline solution as a control group. The specimens were fixed, decalcified, histologically processed, stained immunohistochemically and visualized for proliferating cells

using PCNA antigen under light microscope of 250 magnification. The results showed that the teeth irrigated with ozone descriptively show a higher cell marking rate in comparison to the control group. The authors suggested that ozonated water leads not only in a mechanical cleansing, but also decontaminates the root surface.

Karin C Hutch., et al. investigated whether gaseous ozone and aqueous ozone exerted any cytotoxic effect on human oral epithelial cells (BHY) and gingival fibroblasts (HGF-1) compared with established antiseptics chlorhexidine gluconate 2%, 0.2%, sodium hypochlorite (NAOCL), 5.25%, 2.25%, hydrogen peroxide H₂O₂ 3%, over a time of 1 minute and compared with Metronidazole over 24 hr. Ozone gas was found to have toxic effects on both cell types. No cytotoxic signs were observed for aqueous ozone. CHX (2%, 0.2%) highly toxic to oral epithelial cells and slightly (2%), non-toxic (0.2%) to HGF-1 cells. H₂O₂, NAOCL resulted in markedly reduced cell viability (BHY, HGF-1). Metronidazole showed mild toxicity to BHY cells. Taken together aqueous ozone showed the highest level of compatibility [11].

Ripolles De Ramon J., Colmenero Ruiz C., Gallut Ruiz J., et al. 2004, Analyzed the periodontal response from a clinical, microbiological and immunological point of view of a population with moderate-severe periodontal pockets and its comparison with the scraping and smoothing periodontal technique. A total of 72 quadrants with at least more than four teeth in each quadrant and more than 6 mm pocket depth probe were studied in a population of 43 average age. A cross mouth study was done, in which half were treated with ozone and the rest with scraping technique. The clinical, microbiological and immunological response were evaluated. The authors concluded that the periodontal treatment with ozone produces statistically significant reduction in the amount of gingival bleeding, in the microbiological periodontal parameters, as also in the immunological patterns of IL1beta, TNF-alfa [13].



Figure 1: Kent ozone unit.



Figure 2: Subgingival irrigation with ozonated water.

Conclusion

Studies have suggested that ozonated water should be useful in reducing the infections caused by oral microorganisms in dental plaque. There is positive effect of ozone water in oral soft tissue healing which has been demonstrated clinically and histologically. In gingival and periodontal infections also oxygen can be insufflated into the tissues, thus impairing the milieu to reduce the growth of anaerobic microorganisms. Future perspective would be to include ozone preparations for periodontal and oral and maxillofacial healing and conduct more studies to evaluate the efficacy of ozone therapy histologically.

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