The Possible Role of Medical Ozone in Angiogenesis


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Abstract

To elucidate if medical ozone is able to induce angiogenesis, we used three different doses (75,40 & 4µg O3/mlO2) in white albino rats by i.p. injection. Angiogenesis was assessed in both skeletal and cardiac muscle at the end of the study using morphometric method. Both capillary density (cap.dens.) & number of muscle fibers per field were counted and the ratio of cap.dens./ m.fib. (C/F) was calculated. The cap. dens. was highest in the large dose group (which was sacrificed after nearly one week due to marked deterioration), but hemolysis was found in all sera, and in the cardiac muscle fibers which showed marked degenerative and hypertrophic changes. The large dose ozone group showed significant rise in WBCs count which was mainly due to lymphocytes increment. This finding was supported by bone marrow examination. Also, both IFN-γ and TNF-α & fibrinogen levels were significantly raised. Significant decrease of platelet count was confirmed by marked decrease in megakaryocytes in the bone marrow of the large ozone dose group.

The moderate dose group that received (40ugO3/mlO2) for four weeks showed hypertrophy and some degenerative changes in the cardiac muscle fibers, together with mononuclear cell infiltration (MNF) around the newly formed capillaries in the soleus muscle. Also, we found a slight statistically significant rise in cap. density, RBCs count & lymphocytes%, in addition, significant lowering in the
plasma fibrinogen was present. TNF-α level was significantly higher, as compared to their controls and the small dose ozone group, but was lower than in the large dose group.

Analysis of C/F ratio in both cardiac and soleus muscle, peripheral blood & bone marrow samples together with Malondialdehyde levels (MDA), lactate dehydrogenase (LDH), creatine phosphokinase CPK) and other metabolic parameters are in favor that the best angiogenic response occurred when we lowered the dose markedly to (4µgO3/mlO2) and prolonged the duration of the study to 12 weeks. Our data collectively are in favor of occurrence of endogenous induction of angiogenesis in both cardiac and skeletal muscle by medical ozone in the three doses used but the smallest dose and longest duration group was the most efficient and physiologic while the largest dose showed toxic signs.

Conclusion
Repeated medical ozone administration in small dose and over long duration can be encouraged as a physiological therapeutic endogenous angiogenic strategy enhancing all the steps of the multifactorial angiogenic cascade. From this aspect, medical ozone can be superior to the use of several angiogenic factors which are liable to interfere with specific constitutional vascular endothelial growth factor (VEGF) isoforms function and can disturb the special pattern of distribution of its receptors.
Has ozone any effect on leukocytes?

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This was the very question that, by sheer coincidence, posed myself in 1998: after a few years, we discovered that indeed careful ozonation of blood may have immunomodulatory activities due to release of cytokines. Ozone is only the reaction trigger and, among several molecules included in the family of reactive oxygen species, ROS (HOCl, NO*, OH*, O$_2^-$, ROO*, O$_2$−, O=NOO−) H$_2$O$_2$ is one of the most important. Ozone, by reacting with double bonds present in PUFA, generates H$_2$O$_2$ and 2 moles of R-CHO. The sudden increase of H$_2$O$_2$ concentration in the plasma during lipid peroxidation causes the rapid diffusion of H$_2$O$_2$ into the cellular cytoplasm. Here, its increase is checked by antioxidants (GSH, AH− and enzymes such as GSH-Px and catalase) so that its concentration is 20-40% less than in plasma. Nonetheless the sudden increase of H$_2$O$_2$ acts a crucial signal for specific kinases (IKK-1/2) that, by phosphorylating the protein IKBα, causes its detachment from the inactive transcription factor called NF-KB and allows the rapid migration of the heterodimer (p50-p65) into the nucleus where it binds to specific sequences in the promoter regions of several genes, including those inherent to cytokines, haematopoietic growth factors, adhesion molecules and acute phase proteins. This implies that some leukocytes, once activated, can synthetize a variety of proteins including IFNs, ILs, TNFα, chemokines, orosomucoid, C-reactive protein growth factor, MHC proteins etc.

Although this is a significant mechanism due to an acute and transitory oxidative stress, in leukocytes is not necessarily the only one because there are other transcription factors that can be either activated via diacylglycerol (DAG activates protein kinase C), inositol 1,4,5-trisphosphate (IP$_3$, opens Ca$^{2+}$ channel) or adenylate cyclase or inhibited by ceramide via activation of sphingomyelinase. However they still need to be investigated.
Another uncertain issue is the role played by other ROS and particularly compounds such as $\text{OCl}^-$, $\text{OH}^-$, and $\text{O}=\text{NOO}^-$ that can be responsible of cytotoxic effects when ozone concentrations overwhelm the antioxidant capacity. Similarly, we have just started to investigate the relevance of one typical aldehyde such as 4-hydroxy-2,3-trans-nonenal (4-HNE), while the activity of the heterogeneous group of other lipid oxidation products (LOP) remains unknown.

The effects of these compounds is likely quite different if the problem is examined in the whole blood, or in isolated leukocytes resuspended either in their own plasma, or in protein-free tissue culture media and, most critically, if the problem is examined in vitro or in vivo. Owing to the dogma that “ozone is always toxic”, orthodox medicine is very much against ozonetherapy and therefore the problem of cytotoxicity must be seriously addressed. We have taken the view that, although ozone is potentially toxic, low concentrations can be tamed by the physiological antioxidant capacity and can have beneficial effects. LOP have a relatively long half-life and in vivo the re-infusion of the blandly ozonated blood implies a considerable dilution in body fluids, an extensive breakdown by aldehyde-, alcohol-dehydrogenases and GSH S-transferase and a marked excretion via urine and bile. In this way LOP’s uptake by cells is minimized (probably at submicromolar levels) and while atoxic, it can serve as the crucial signal for inducing oxidative stress proteins (OSP), hence the adaptation to chronic oxidative stress and possibly activation of staminal cells. In recent years, even the most implacable supporters of free radical toxicity, have to admit that very low concentrations of ROS and LOP behave as physiological messengers and can be beneficial, while higher concentrations can obviously provoke detrimental consequences culminating in inflammation and cell degeneration. Our recent studies with Jurkat cells, maintained in media with variable antioxidant capacities, when tested against a very wide range of ozone and 4-HNE concentrations have beautifully demonstrated the validity of this thesis. Nonetheless there are still many outstanding scientists, who maintain that ozonetherapy is “a barbaric, unsafe procedure” and could be substituted by the injection of “a little lipid peroxide or LPS”. Obviously the problem of contraposition between molecular and “natural” medicine is a fascinating issue but it cannot be discussed here.
In order to finally understand whether the exposure of whole blood to oxygen-ozone can lead to immune modulation and therapeutic effects, we have addressed the following problems:

1. How important is the maintenance of the plasmatic Ca\(^{2+}\) level during blood ozonation?
2. Is there any advantage in ozonizing a large number of isolated leukocytes rather than whole blood?
3. Within the “therapeutic window” is there a range of either immunostimulating or immunosuppressive O\(_3\) concentrations?
4. On the experimental basis that ozone can act as a modest cytokine inducer, does reinfusion of ozonized blood modify the plasma cytokine in vivo?
5. Is O\(_3\)-AHT effective and is there an optimal schedule?
6. Does the induction of OSP and of adaptation to chronic oxidative stress have an immunomodulatory effect?
7. Which is the immunomodulatory role and relevance of granulocytes?
8. Can we select tests suitable for evaluating modifications of the immune status during ozonotherapy?

Unfortunately, owing to several reasons (lack of cooperation of oncologist and clinicians, lack of funds, scepticism towards ozonotherapy, etc.), so far we have only a few hints that repeated administration of ozonated blood can be therapeutically useful but, as now we have been able to open an ozonotherapy center at our University polyclinic, there is a hope to answer this question in the next couple of years.

THE INTENSIVE CARE OF LOWER LIMB DIABETIC WOUNDS: OUR 5 YEARS EXPERIENCE IN 121 PATIENTS TREATED TOPICALLY WITH OZONE AS AN ADJUNCTIVE AGENT.

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The purpose of this study was to critically assess the efficacy of topical OZONE treatment of chronic diabetic and PVD ulcers of the lower limb as an adjunctive modality to serial debridements of the wounds; topical and systemic antimicrobial agents and, frequent dressing changes.

Patients: 121 patients were treated between 6/1997 to 8/2001. 3 were excluded from the study. 66/118 (56%) were diabetic, while 38/66 (57.6%) suffered also of peripheral vascular disease. 15/66 (22.7%) were discontinued due to physiological disturbances. 52/118 suffered of venous ulcers, and 8/52 were discontinued.

Modality of treatment: consisted of – repeated wound cultures (X 3/week), topical and systemic antimicrobial agents according to wound colonization, serial wound and osteomyelytic bone debridements, dressing changes X 3/day in the presence of our Resident and, topical Ozone X 3/week.

Results: 21 patients who were previously subjected to b/k amputation in another hospital - healed completely. The mean wound duration prior to treatment was – 14.1 months. The mean healing time was – 49 days. The mean number of Ozone treatments was 25.7/patient. 23/118 (19.5%) - patients were discontinued from the trial. 79/95 (83.16%) - were fully healed, 29/79 (36.7%) – were skin grafted, 16/95 (16.8%) – were diagnosed as non-response patients, 4 of them underwent b/k limb amputation.

Conclusions: Topical Ozone might be considered an optimal adjunctive agent for the treatment of chronic diabetic; arterial and venous leg ulcers.
The DISCOSAN method and the Italian experience.
Dr William Gamba - Dr Luigi Cursio

The point of the situation on the lumbar and cervical paravertebral treatment of the Back Discal Deformation with Discal Radicular Conflict. The importance of the diagnosis. Evaluation between invasive methods and intradiscal O2-O3 therapy.

Thought out from Dr Cesare Verga, the Discosan method defers for executive conceptuality from the methodology of treatment used at present from many Doctors and Specialists. Some doctors, however, continued his school and obtained very satisfactory resultants with high percentages of recovery.
The importance of the diagnosis and why it works so well.
The method was upgraded in the time with some eventual modification.
The comparison of the results with the other methodologies puts in evidence the validity, united to the simple applicability.
Hepatitis "C" is a medical problem in Egypt. The usual line of treatment is very expensive with major side effects and low efficacy especially in type 4, which is common in Egypt. The aim of this study is to evaluate the role of ozone as a safe line of treatment. This study included 60 patients' type 4 hepatitis "C" patients, 45 males and 15 females. Their age ranged between 34 and 65 years. Investigations including C.B.C., liver function tests, A.F.P., serological tests for Bilharziasis, P.C.R. quantitative for H.C.V., prothrombin time and concentration and abdominal ultrasonography were done before and 8 weeks, 24 weeks after treatment with ozone. Patients received combined treatment of Major AutoHaemotherapy in a dose range from 4mg to 9 mg and rectal insuflation in a dose range from 6mg to 14 mg per visit. The number of visits was three times per week for 8 weeks followed by twice per week for 16 weeks. The general condition in 95% of cases improved. There was a decrease in the quantitative P.C.R. (viral load) in 91.66% of cases that reached zero (no viraemia) in 18.33 % of cases after 8 weeks treatment. The number of -ve cases for HCV virus increased to reach 36.67 % of cases after 24 weeks treatment. Ozone therapy was found to be an effective, safe and less expensive method in Hepatitis "C" patients.
**Herpes Zoster – a Treatment Concept with Ozone**

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**Abstract**

The trigger of the zoster or shingles condition is the varicella zoster virus. It manifests itself as a local recurrence through a weakened immune condition, mainly arising during childhood.

Depending on the degree of immune weakness, it can spread from one to several segments (*i.e.*, zoster generalisatus).

The duration of healing, severity of disease, and post-zoster neuralgia also depend on the immune condition prevailing.

Older patients often indicate an immune weakness. This is why zoster illness with the complications mentioned above occur frequently in their case.

Zoster is normally treated with viralstatic agents as the preferred method. In comparison to viralstatic agents, ozone therapy also shows good results. Yet it shows the comparative advantage of an immune system regulating or stimulating effect. Furthermore, it can already be used at an early stage in case of slight diagnostic suspicion, since – in contrast to viralstatic agents - practically no side effects are anticipated. Normally ozone therapy is more economical than treatment with viralstatic agents. Beginning treatment early improves the chances of avoiding the dreaded post-zoster neuralgia.

Zoster patients in the pilot study were treated with ozone systematically as well as locally. In case of strong neuralgia, the related ganglia were injected with local anesthesia and ozone at the same time.

Enzymes, zinc, and vitamins E, C, B1, B6, and B12 were administered as added measures.

**Keywords:** Ozonotherapy, Herpes zoster, Medical ozone.
ABSTRACT
The overall aim of this study was to assess the ability of ozone therapies to reverse the clinical severity of primary root carious lesions (PRCLs). In addition, novel methods to detect PRCLs using the Electrical Caries Monitor (ECM) and the Resilience Caries Monitor (RCM) were used. 200 PRCLs were examined in vitro to relate the ECM and RCM readings to clinical criteria used to detect PRCLs. Significant differences in ECM values were found for colour, hardness and all five classes of severity of PRCLs, in addition to sound root tissues, but not for the RCM values.

In the second part of this study, the use of ozone was considered. Initially, the anti-microbial effects of ozone on PRCLs were tested, and a significant reduction in total colony forming units (tcfus) was observed in the ozone-treated groups. Secondly, a significant reduction in tcfus was observed in ozone-treated samples for Streptococcus mutans and Streptococcus sobrinus. A further in vivo study demonstrated a significant reduction in tcfus. In order to assess the safety of ozone during these treatments, the maximum ozone detectable levels (ppm) adjacent to the point of the application were measured in vivo and in vitro. These investigations revealed that the mean maximal detectable levels of ozone were all within EU and FDA guidelines. A further longitudinal study assessed the safety
and efficacy of ozone either with or without a root sealant, for the management of PRCLs. In this longitudinal study, the ECM, DIAGNOdent and clinical detection criteria were used and there were four experimental groups involved:

**Group 1.** Ozone application only was performed for a period of 10 s on PRCLs

**Group 2.** Neither ozone nor root sealant was applied to the PRCLs

**Group 3.** Both ozone treatment and a root sealant were applied to PRCLs

**Group 4.** A root sealant only was applied to PRCLs

At baseline, and after 1 and 3 months, ECM and DIAGNOdent readings were obtained and each PRCL was then clinically assessed. The longitudinal study revealed that at 1 month recall, 26.5% of PRCLs had reversed from severity index 2 to 0 (i.e., hard) in the ozone group, whilst in the control group, 1.5% of PRCLs got worse ($p < 0.001$) and 54.4% of lesions reversed from severity index 2 to 1 in the ozone group compared to those in the control group ($p < 0.001$). After 3 months, 13.5% of PRCLs reversed from severity index 1 to 0 (i.e., hard) in the ozone group, whilst none of the lesions reversed in the control group when compared to the baseline results ($p < 0.001$). Furthermore, 23.1% of lesions reversed from severity index 2 to 1 in the group receiving ozone alone compared to only 5.9% in the control group ($p < 0.001$). The ECM and DIAGNOdent readings showed improvements in the ozone only group when compared to the control group after 1 and 3 months ($p < 0.001$). The ozone and sealant group also had greater improvements in the ECM and DIAGNOdent values when compared to the sealant only group after 1 and 3 months ($p < 0.001$). In addition, Modified USPHS criteria after 3 months showed that there were 53% of intact sealants in the ozone and sealant group, whilst 40% of intact sealants were present in the sealant only group.
The use of the ECM has promise for accurately detecting PRCLs in clinical practice. Leathery lesions can be treated non-operatively either by using ozone. The root sealant showed a reduced retention at 3 months but was retained better on ozone treated PRCLs.
Local, deep insufflation of an oxygen-ozone mixture in the prevention and treatment of infections in the locomotor system

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Summary

Background: Ozone therapy – i.e. the treatment of patients by a mixture of oxygen and ozone – has been used for many years as a method ancillary to basic treatment, especially in those cases in which traditional treatment methods do not give satisfactory results, e.g. skin loss in non-healing wounds, ulcers, pressure sores, fistulae, etc.

Material and methods. In the period from January 2000 until September 2002, 68 patients with extensive injuries to the locomotor system and septic complications after surgical procedures were treated with ozone at the Department of Physiotherapy of the 2nd Medical Faculty and the Department of Orthopedics and Traumatology of the Locomotor System at the Medical Academy of Warsaw. The ozone therapy was administered using an authorial technique of deep ozone application. Two groups of patients were treated: posttraumatic patients at risk for primary deep infections (e.g. compound fractures without skin loss) and patients diagnosed with postoperative infections in the locomotor system. The first group involved 41 patients; the second group, 27.

Results. In the first group, five patients had septic complications despite mixed treatment, including three case of osteitis. In the second group, all the patients experienced much faster than normal wound healing with inhibition of septic processes. In six cases, the septic process was reactivated after 6 and 9 months respectively, and these patients are still undergoing treatment.

Conclusions. Our data confirm the advantages resulting from the deep application of ozone in the prevention and combined treatment of septic complications in the locomotor system. Our technique of
deep ozone application makes it possible to reduce the risk of post-traumatic infections and promotes quicker healing of post-surgical complications and chronic septic infections. This method is also cost-efficient, shortens the duration of required antibiotic therapy, and is sometimes the only available auxiliary technique to support surgical procedures.

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OSTEOMIELITE POST-ESTRATTIVA TRATTATA CON OZONOTERAPIA

Dr. A. SCUCCIMARRAS.R., di anni 74, un anno prima era stato sottoposto ad estrazione di un molare inferiore destro. Tale intervento si complicava con una osteomielite di entità tale da costringere il paziente a sottoporsi a diverse terapie tra cui la camera iperbarica. Egli però doveva interrompere tale trattamento perché colto da infarto del miocardio. La situazione sembrava piuttosto seria in quanto il paziente lamentava una sintomatologia ingravescente data da dolori lancinanti alla mandibola erosa dal processo osteomielitico. Tale processo patologico aveva causato una grossa area osteolitica mandibolare (fig.1) che aveva indebolito l’osso a tal punto da richiedere, una volta guarita l’osteomielite, un intervento di chirurgia maxillo-facciale. Tale intervento presentava d’altro canto un elevato rischio perché il paziente era coronaropatico.

Fig.1                                                Fig.2

Sembrava che il paziente fosse entrato in un circolo vizioso senza nessuna possibilità di uscirne. Come estremo tentativo veniva consigliato dal suo medico curante, il dr Evandro Tascione, a sottoporsi ad Ozonoterapia.

Il ciclo veniva effettuato con una serie di 10 sedute consistenti in una semplice iniezione di 3cc di Ozono a 20microgr./ml fatta con ago 27G di 20mm alla superficie dell’osso mandibolare.

Dopo 6 sedute spariva il dolore e ad una NMR dopo 1 mese dalla fine del trattamento si aveva un quadro incredibile in cui oltre alla guarigione dell’osteomielite si assisteva ad una così’ consistente formazione di osso da rendere superfluo l’intervento di chirurgia maxillo-facciale (Fig.2).