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Editorial

Dear Colleagues,

Our journal is about to enter its sixth year of publication, a major goal unhoped for when we presented the first issue of the Rivista Italiana di Ossigeno-Ozonoterapia at the University of Pavia in 2001.

Many things have happened since then, but the best and most important is that our readers have increased from year to year alongside the growing international interest in oxygen-ozone therapy. For this reason, the journal switched to publication entirely in English two years ago to boost its circulation in more than forty nations worldwide from China to Australia over to South America (Venezuela, Brazil, Argentina, Colombia, Uruguay, etc.) to the United States and all over Europe.

This is a major achievement linked to our commitment and passionate dedication to this discipline and its many successful treatment outcomes.

We recently attended the III National Chinese Congress of Ozone Therapy held in Urumqi, the capital of Xinjiang, an international metropolis of oasis in the sea of desert: Urumqi is located in North-West China on the edge of the Gobi desert.

Our visit allowed us to admire the sights of Xinjiang like the Ancient City of Jiaohe and the Kizil Thousand Buddha Caves in the Muroty valley and the Flaming Mountain in Turpan, some of which are shown in the photo-reportage published in this issue.

The occasion offered us the chance to compare our experience in Italy with Chinese practice of ozone therapy which is more recent but scientifically advanced and constantly expanding.

The chairman of the congress, Professor He, a neurosurgeon in Guanzou, was responsible for the impeccable organization of the three day meeting which saw much lively and interesting discussion. We took advantage of our stay in China to lay the groundwork for the I World Congress of the WFOT to be held in Beijing in 2007.

These achievements are a great source of satisfaction. We set out timidly, working with dedication and commitment and have often been the targets of specious criticism. But today ozone therapy is practised successfully the world over and we Italians deserve at least some of the merits!

Thank you.

Matteo Bonetti
Ozone Treatment Inhibits Proliferation in Human Neuroblastoma SK-N-SH Cells

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Key words: neuroblastoma, ozone therapy, O₃, hypoxia

SUMMARY - Tissue hypoxia results from an inadequate supply of oxygen (O₂) that compromises biologic functions. Recent experimental and clinical studies suggest that intratumoral oxygen levels may influence a series of biologic parameters affecting the tumor’s malignant potential. Indeed, sustained hypoxia in a growing tumour may cause cellular changes that can result in a more clinically aggressive phenotype. During the last two decades it has been shown that ozonated autohemotherapy is therapeutically useful in arteriopathic patients because it increases oxygen delivery to hypoxic tissues, leading to normoxia. Although several oxygenation approaches have been tested, none is able to restore normoxia permanently in cancer. Since a prolonged cycle of ozonated autohemotherapy has been postulated to correct tumor hypoxia leading to a less aggressive behavior, we studied whether ozone treatment could affect cell growth and cell cycle perturbations on the human neuroblastoma SK-N-SH cell line. These data indicate that ozone acts as a real chemical drug capable of inhibiting cell growth suggesting its possible antineoplastic role in neuroblastoma cancer.

Introduction

Tissue hypoxia results from an inadequate supply of oxygen (O₂) that compromises biologic functions ¹.

Traditionally, tumour hypoxia has been considered a potential therapeutic problem because it renders solid tumours more resistant to ionizing radiation ²-⁴. More recent experimental and clinical studies ⁵-¹¹ suggest that intratumoral oxygen levels may influence a series of biologic parameters affecting the tumor’s malignant potential. Indeed, sustained hypoxia in a growing tumour may cause cellular changes that can result in a more clinically aggressive phenotype ¹²-¹⁶.

During the process of hypoxia-driven malignant progression, tumour may develop an increased potential for local invasive growth ¹⁷,¹⁸, perifocal tumour spreading ¹²,¹⁹ and regional and distant tumour spreading ¹³,¹⁴,²⁰,²².

Experimental evidence indicates that hypoxia not only induces proteome changes influencing tumour propagation but also drives malignant progression through transient and persistent genomic changes in neoplastic cells ¹⁵,²⁰,²³,²⁴-²⁷. Hypoxia promotes genomic instability (through point mutations, gene amplification, and chromosomal rearrangements) and may unveil pre-existing cryptic genetic variations, thus increasing the number of genetic variants.

Tumour hypoxia, when assessed by polarographic probes, is an independent prognostic factor for response to treatment and/or survival of patients with head and neck ²¹,²⁶,³⁰, uterine cervical tumour ¹²,¹⁴ and sarcoma ¹³,³¹. As demonstrated by Overgaard’s meta-analysis ³², improving tumor oxygenation can lead to better local control and increased overall survival rates following chemo-radiotherapy. Consequently, several strategies have been proposed to enhance tumor oxygenation inducing a constant restoration of normoxia. Ozone therapy is a technique that has been used in the treatment of ischemic syndromes ³³,³⁴.

Bernardino Clavo described a relationship between oxygenation in head and neck cancer and in anterior tibialis muscles ³⁵, and an ozone therapy-induced improvement in the oxygenation of the ‘most-hypoxic’ anterior tibialis muscles, together with improvement in the most hypoxic tumors ³⁶. These data suggested that ozone therapy could have some positive effect during the treatment of head and neck cancer ³⁷.

Nevertheless, the potential usefulness of ozone therapy alone or as an adjuvant in neoplastic
patients receiving chemo–radiotherapy warrants further investigation.

The aim of the present study was to evaluate whether O₃ treatment could affect cell growth and cell cycle in the SK-N-SH human neuroblastoma cell line.

Materials and Methods

Cell Culture

Human SK-N-SH cell line was maintained as a monolayer culture in EMEM supplemented with 10% heat-inactivated FCS (Life Technologies, Scotland, United Kingdom), antibiotics and L-glutamine (2 mM) at 37°C in a 5% CO₂/95% air atmosphere in a humidified incubator.

Ozone Generation

Ozone therapy was administrated using clinical grade O₂, the O₃/O₂ gas mixture was prepared with an ozonosan α plus photonic device 1014/10 (Dr. Hansler, Germany) and sterilized by passage through a sterile 0.20 µm filter.

Treatment and Growth Inhibition Assay

Cells were seeded at a density of 1.0x10⁵ cells per T25 flask and after 24 hours of cell culture were treated at room temperature using an ozonosan α plus photonic device 1014/10 (Dr. Hansler, Germany) and sterilized by passage through a sterile 0.20 µm filter.

Ozone therapy was administrated using clinical grade O₂, the O₃/O₂ gas mixture was prepared with an ozonosan α plus photonic device 1014/10 (Dr. Hansler, Germany) and sterilized by passage through a sterile 0.20 µm filter.

Propidium Iodide Cytotoxicity Assay

The capacity of 6, 12, 25, 50 and 80 µg/ml O₃ used in a single or repeated-regimen, to produce cell death was determined by PI staining exclusion test and flow cytometry. Cytotoxicity was defined as the cellular damage identified by PI staining, which shows the loss of structural integrity of the plasma membrane, a typical event of necrotic cell death. SK-N-SH cell line was stained with 100 µl of the supravital PI (4 mmol/L) for three minutes. After incubation, cells were washed with 5% bovine serum albumin in PBS and kept on ice until flow cytometry analysis.

Results

The oxonation experiments were conducted to investigate whether O₃ could be considered an antineoplastic drug. First we determined the optimal schedule of ozone treatment. We exposed cells to 6, 12, 25, 50 and 80 µg/ml O₃ doses and examined the effects produced by treatment on cell growth and the cell cycle at different times after ozone exposure (4, 24, and 48 hours). At each time, cells were harvested and counted using the trypan blue dye exclusion test. After addition of O₃ given in a single dose (6, 12, 25, 50 and 80 µg/ml), a dose-dependent inhibition of cell proliferation was observed (figure 1A). Four hours after exposure of cells to 80ug/ml, a cell growth inhibition of 58% was observed. The inhibitory effect become 99% after 48 hours, whereas growth inhibitions of 33% and 29% were obtained after 4 hours of exposure to 50 and 25 µg/ml O₃ and persisting during the 48 hours following treatment. When cells were exposed to the lowest O₃ doses of 12 and 6 µg/ml the growth inhibitions were about 24% and 17%, respectively. These latter effects were partially lost during the following days, with cell growth inhibition values evaluated 48 hours after O₃ treatment of about 17% and 10%, respectively.

We also measured the effect of repeated doses of 6, 12, 25, 50 and 80 µg/ml O₃. Figure 1B shows that the different doses of 6, 12, 25, 50 and 80 µg/ml given 24 hours after seeding and repeated three hours after the first treatment (repeated dose-regimen) enhanced cell growth reduction. The
maximum antiproliferative effect was observed at 80 μg/ml dose with similar values to those obtained with the previous regimen of O3 treatment.

Four hours after the start of 50 and 25 μg/ml exposure we already observed cell growth inhibition of about 61% and 50% respectively (versus 33% and 29% obtained with the single-dose regime) and it was maintained up to the last time point of the cell growth experiment (days 3 = 48 hours after start of O3 treatment). The percentage of inhibition was about 37% and 20% in the 12 and 6 μg/ml O3-treated cells in comparison with the single-dose regimen (24% and 17%, respectively) and it was partially recovered during the following 48 hours.

To evaluate whether O3-induced cell growth inhibition could be related to cell cycle perturbations, PI staining and fluorescence-activated cell sorting analysis were done on SK-N-SH cells exposed to 6, 12, 25, 50 and 80 μg/ml O3 in single or repeated-dose regimen (table 1A and B). This analysis, performed at different times after 6, 12, 25 and 50 μg/ml O3 exposure, given in a single-dose regimen (24% and 17%, respectively) and it was partially recovered during the following 48 hours.

To evaluate whether O3-induced cell growth inhibition could be related to cell cycle perturbations, PI staining and fluorescence-activated cell sorting analysis were done on SK-N-SH cells exposed to 6, 12, 25, 50 and 80 μg/ml O3 in single or repeated-dose regimen (table 1A and B). This analysis, performed at different times after 6, 12, 25 and 50 μg/ml O3 exposure, given in a single-dose regimen, revealed an accumulation of the cells in the S-phase (41%, 44%, 46%, 50% and 34% in O3 treated and untreated cells, respectively) suggesting a prolonged stay of the O3-treated cells in S-phase. However, this S-phase accumulation was completely overcome in the 48 hours following 6 and 12 μg/ml O3-treated cells. No detectable changes in the PI percentage toxicity was observed after 6, 12, 25 and 50 μg/ml O3 exposure being less than 10% at 48h. Conversely the PI percentage toxicity was more than 75% after the highest dose indicating 80 μg/ml as a lethal dose.

The O3 repeated dose-regimen induced an accumulation of cells in the G2 phase of the cell cycle compared with untreated cells and evaluated four hours after treatment (the G2 phase were 25%, 33%, 40%, 50% and 19% in 6, 12, 25 and 50 μg/ml and untreated cells, respectively). Cells treated by 6 and 12 μg/ml O3 progressively decreased the G2 phase percentages to 17% and 23%, respectively within 48 hours after O3 treatment and concomitantly increased the G1 phase (49% and 46%, respectively). Conversely, 48 hours after 25 μg/ml treatment, the G2 accumulation was still evident (40%). The cell cycle effect induced by 6 and 12 μg/ml O3 doses was associated with a moderate cytotoxicity, evaluated as percentage of PI stained cells, being <13% and 15%, respectively, while a more consistent toxicity was observed at 25 μg/ml O3 dose (more than 25%) within the same time interval (48 hours from treatment). Twenty-four hours after 50 μg/ml treatment, the cell cycle profile demonstrated that O3 led to irreversible cellular damage promoting cell killing. Consistent with this hypothesis is the increasing toxicity percentages observed, the percentages of PI-positive cells was 50% at 48 hours. Moreover, these data

Figure 1  O3-induced growth inhibition in SK-N-SK human cell line. 1×10^5 cells were seeded in T25 flasks and O3 exposure was administered 24 h later. Cells were seeded at a density of 1.0×10^5 cells per T25 flask and, after 24 hours of cell culture, cells were exposed to increasing doses of O3 6, 12, 25 and 80 μg/ml in a single dose regimen A) and a repeated dose regimen B). The analysis was performed 4, 24 and 48 hours after treatment. At each time point the cells were harvested and counted by trypan blue exclusion test. Cell counts are reported as percentages of control. Data are representative of three different experiments. Values are means of triplicate samples.
confirmed that 80 µg/ml O₃ is a lethal dose. These findings indicate that ozone acts as a real chemical drug capable of inhibiting neuroblastoma cell growth suggesting its possible antineoplastic role in neuroblastoma cancer.

**Discussion**

In solid tumours, oxygen delivery to the respiring neoplastic and stromal cells is frequently reduced or even abolished by a deteriorating diffusion geometry, severe structural abnormalities of tumour microvessels, and impaired microcirculation 39. In an increasing number of reports on tumour oxygenation, the addition, anemia and formation of methemoglobin or carboxyhemoglobin reduce the blood’s capacity to transport O₂. As a result, areas with very low (down to zero) oxygen partial pressure exist in solid tumours, occurring either acutely or chronically. These microregions of very low or zero O₂ partial pressures are heterogeneously distributed within the tumour mass and may be located adjacent to regions with normal O₂ partial pressure. In contrast to normal tissue, neoplastic tissue can no longer fulfil physiologic functions. Thus, tumour hypoxia cannot be defined by functional deficits, although areas of necrosis, which are often found in tumour tissue on microscopic examination, indicate the loss of vital = not evaluable cellular functions. Actually hypoxia (defined as the fraction of measured O₂ partial pressures of <5 mmHg) is a statistically significant adverse prognostic factor of disease-free survival. A Kaplan–Meier analysis showed statistically significantly shorter survival and recurrence-free survival for patients with hypoxic tumors. The results were consistent with the hypothesis that radio-biologically hypoxic tumors (i.e., tumors with a reduced radiosensitivity at critically low O₂ levels) are less curable 40, even though the role of hypoxia in conventional antineoplastic drug resistance could not be excluded.

These data were supported by Cox regression analysis which revealed tumor oxygenation as the strongest independent prognostic factor, followed by FIGO stage 12. Of special interest was the fact that the disadvantage in outcome for patients with hypoxic tumor was independent of primary treatment (radiation therapy or radical surgery). Sundfor et al 41 reported a poor outcome associated with low oxygen tension in 40 patients with advanced squamous cell carcinoma of the uterine cervix. These findings are in agreement with
results obtained by Fyles et Al who found that the pretreatment oxygenation status of tumors can predict disease-free survival in patients with cervical cancer. In addition, Knoke et Al confirmed the prognostic relevance of pretreatment tumor oxygenation status by studying 51 patients with cancer of the uterine cervix after radiation treatment. The pretreatment tumor oxygenation status was also assessed in patients with soft-tissue sarcoma; patients with hypoxic tumor were associated with a poorer survival when compared with patients with normoxic tumor resulting from local treatment failure or distant metastases.

Recent work has led to the hypothesis that tumor hypoxia may be associated with malignant progression by locoregional and distant tumor propagation. This observation was confirmed by Hochel et Al who reported that hypoxia may not only counteract O₂-dependent forms of therapy but may also advance tumor progression per se independently of treatment. Since modification of the levels of hypoxia with therapy has been shown to improve therapeutic outcomes, several strategies have been proposed to enhance tumor oxygenation inducing a constant restoration of normoxia.

A special workshop sponsored by the National Cancer Institute, established the need to investigate methods to overcome tumor hypoxia. Clavo reported that ozone therapy could have an important role in increased oxygenation restoring normoxia in the most poorly oxygenated head and neck tumors.

Ozone (O₃) is the allotropic form of oxygen with three atoms and two unpaired electrons, which has a higher oxidizing capacity than oxygen. Nevertheless, the potential usefulness of ozone therapy as an adjuvant in chemo–radiotherapy for neoplastic lesions warrants further investigation. Our findings demonstrated that O₃ affects cell growth and the cell cycle in the neuroblastoma SK-N-SH cell line. Our data are in agreement with Sweet et Al who reported ozone as a possible antineoplastic drug because of its capacity to inhibit the growth of human cancer cells. Since the therapeutic window for ozone concentration ranges from 20 to 80 µg/ml, we utilized 6, 12, 25, 50 and 80 µg/ml O₃ doses to verify its possible anti-tumoral effect on the SK-N-SH human neuroblastoma cell line.

We found that the 6, 12, 25, 50 and 80 µg/ml O₃ doses produced different cell cycle effects when administered in single or repeated-dose regime. We showed that tumor cells exposed to the lowest O₃ doses of 6 and 12 µg/ml when given in a single dose-regime partially recovered the O₃-induced S-phase accumulation while 6 and 12 µg/ml given in a repeated dose-regimen produced a different cell cycle perturbation, inducing a G2 arrest. However, this G2 accumulation was overcome in the 48 hours following treatment. Whereas the 25 µg/ml O₃ given in the repeated dose-regime abolished the ability of the cells to overcome the G2 block compared to the lowest doses (6 and 12 µg/ml). Indeed, this G2 block seems to be permanent, because at 48 hours after the start of treatment a fraction of the cells was still in G2 phase (40%). On the contrary, 25 µg/ml O₃, in a single dose-regime produced a slight S-phase accumulation. The highest dose of 80 µg/ml in both single and repeated dose-regimens produced irreversible cellular damage, indeed the cells were not able to repopulate the cell cycle and died from necrosis, whereas 50 µg/ml O₃, given in a single dose-regime did not produce any significant toxic effect. Our hypothesis is that different molecular events occur following O₂, which affect the cell cycle and can lead in turn to DNA damage repair, cell cycle perturbation or death. If the damage is too massive it is followed by death in an attempt to eliminate such severely damaged cells.

This hypothesis is supported by the capacity of O₃, when administered as autotransfusion in cancer patients, to react with organic compounds (hydro-soluble and lipophilic antioxidants, unsaturated fatty acids, etc) generating a number of messengers acting on various blood components and procuring early (by ROS) and late (by LOP) biological effects. Bocci et Al showed that ozone, via the transitory action of hydrogen peroxide, acts as a mild inducer of cytokines in leukocytes. Therefore, by releasing cytokines in lymphoid microenvironments primed lymphocytes and monocytes may slowly bring about a concerted activation of the immune system usually suppressed by tumor growth.

In contrast to the claim of a possible O₃ toxic effect on blood cells, it has been reported that O₃-induced toxicity is overcome using the appropriate dose. Indeed, the range of the therapeutic window has been determined between 20 and 80 µg/ml per ml of blood cells (0.42-1.68 mM) which did not produce any toxic effect. Indeed, patients undergoing ozone therapy do not have adverse effects and most patients reported a feeling of wellness and euphoria. Normally a cycle of 14-15 treatments (twice weekly) significantly improves visual acuity in about 70% of patients with the atrophic form of age-related macular degeneration: (ARMD) and in most patients with chronic limb ischemia (stage II). Our data are in partial agreement with results obtained by Bocci et Al on O₃ cytotoxicity. We did not find toxicity at doses of 6, 12, 25 and 50 µg/ml when O₃ was administered in a single
dose-regime while toxicity was obtained when O₃ was given in a repeated dose-regime. The highest dose of 80 µg/ml produced an elevated cytotoxicity in both single and repeated-dose regime and was considered a lethal dose. We observed that the O₃ induced a dose-dependent cell growth inhibition and that the highest dose was able to activate necrotic cell death by producing irreversible cellular damage inhibiting the progression of cells through the cell cycle. In agreement with Bocci et Al.⁴⁰ we also believe that ozone acts as a real chemical drug.

**Conclusion**

Ozone directly inhibited neoplastic cell growth following injection into the neoplastic nodule or via reinfusion of ozonated blood in patients bearing neuroblastoma cells. In accordance with Overgaard ³², we think that improving tumor oxygenation can lead to better local neoplastic control, increased patient survival rates and a better quality of life. Indeed ozone therapy could be a new therapeutic approach targeted to specifically bypass the resistance implemented by hypoxic selection reducing the risk of hypoxia-mediated treatment failures and improving survival and disease-free recurrence in neuroblastoma cancer patients. Besides the normalization of hypoxia, ozone therapy displays other interesting biological effects that may enhance the therapeutic outcome.

**Acknowledgements**

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Introduction

It is distressing to note that often ozone therapists are more interested in simply knowing the ozone dosage rather than to understand how ozone acts and why we can avoid toxicity. This behavior reveals a lack of knowledge of the fundamental bases regulating a judicious use of ozone and is the result of a superficial preparation acquired during an occasional ozone therapy’s course of a few hours. This is not surprising because during the last three decades, on the basis of Wolff’s suggestion (1), ozone therapy has been used by practitioners in Europe in an empirical fashion. Unfortunately, even today, most ozone therapists have either a misconception or know only a few technical tips for performing ozone therapy. This problem, associated with the difficulties and cost of performing extensive clinical studies, has hindered real progress, and ozone therapy remains a scarcely known and objected complementary practice. Worst of all, in some countries, often without any medical qualification, quacks continue to inject either ozone intravenously, a procedure prohibited since 1984 in Germany because of the risk of pulmonary embolism and death, or ozonated saline containing a certain toxic amount of hypochloric acid. Moreover, a distinguished American chemist has affirmed the dogma that “ozone is toxic any way you deal with it,” reinforcing the concept that ozone should never be used in medicine. This situation has generated a sort of crusade against ozone therapy in spite of the fact that ozone is considered one of the best disinfectants capable of preventing infection outbreaks. This is becoming a crucial advantage because critically ill patients acquire infections while in hospitals and a number of them die every year as a result.

Table 1 summarizes several good reasons for refusing ozone therapy by orthodox medicine. However, problems 1–5 have now been practically overcome, whereas the remaining 6–9 are stumbling blocks hindering progress. During the last 14 years, we have made a great effort to examine ozone therapy in a scientific fashion both at a basic and clinical level, and we now have some ideas how ozone acts, how and why its toxicity can be controlled and how therapeutic effects can be exerted (2–11). There is no need to invoke philosophical speculations because the mechanisms of action are in the realm of classical biochemistry, physiology and pharmacology.

This review aims to give the reader the essential information and the frame of mind to operate as a real physician. An extensive description is available in three recent books (9–11).

What Is Ozone and How Can We Use It?

Ozone is normally present as a gas made of three atoms of oxygen with a cyclic structure. The medical generator of ozone produces it from pure oxygen passing through a high voltage gradient (5–13 mV) according to the reaction:

\[ 3\text{O}_2 + 68,400 \text{ cal} \rightarrow 2\text{O}_3 \]

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Key Words: Ozone, Antioxidants, Oxidative stress, Ozone tolerance, Ozone therapy.
What Is the Behavior and Fate of Ozone after Coming into Contact with Body Fluids?

The essential concepts to bear in mind are the following: a) as any other gas, ozone dissolves physically in pure water according to Henry’s law in relation to the temperature, pressure and ozone concentration. Only in this situation does ozone not react and, in a tightly closed glass bottle, the ozonated water (useful as a disinfectant) remains active for a couple of days; b) on the other hand, at variance with oxygen, ozone reacts immediately as soon as it is dissolved in biological water (physiological saline, plasma, lymph, urine):

\[ \text{O}_3 + \text{biomolecules} \rightarrow \text{O}_2 + \text{O}^- \]

where atomic oxygen behaves as a very reactive atom. Contrary to the incorrect belief that ozone penetrates through the skin and mucosae or enters into the cells, it is emphasized that, after the mentioned reaction, ozone does not exist any longer.

In order of preference, ozone reacts with polyunsaturated fatty acids (PUFA), antioxidants such as ascorbic and uric acids, thiol compounds with -SH groups such as cysteine, reduced glutathione (GSH) and albumin. Depending upon the ozone dose, carbohydrates, enzymes, DNA and RNA can also be affected.

All of these compounds act as electron donor and undergo oxidation. c) The main reaction:

\[ \text{R-CH} = \text{CH-R}’ + \text{H}_2\text{O} \rightarrow \text{R-CH} = \text{O} + \text{R’-CH} = \text{O} + \text{H}_2\text{O}_2 \]

shows the simultaneous formation of one mole of hydrogen peroxide (included among reactive oxygen species, ROS) and of two moles of lipid oxidation products (LOPs) (12).

The fundamental ROS molecule is hydrogen peroxide, which is a non-radical oxidant able to act as an ozone messenger responsible for eliciting several biological and therapeutic effects (13,14). The concept that ROS are always harmful has been widely revised because, in physiological amounts, they act as regulators of signal transduction and represent important mediators of host defense and immune responses.

Presence of traces of Fe^{++} should be avoided because, in the presence of hydrogen peroxide, via the Fenton’s reaction, they will catalyze the formation of the most reactive OH’ (hydroxyl radical).

\[ \text{Fe}^{++} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{+++} + \text{OH}’ + \text{OH}^- \]

Interestingly, we (15) have also determined the formation of nitrogen monoxide (NO’) in human endothelial cells exposed to ozonated serum. Attention should be paid to the fact that an excess of ROS can lead to the formation of other toxic compounds such as peroxyxynitrile (O=NOO−) and hypochlorite anion (ClO−).

Although ROS have a lifetime of less than a second, they can damage crucial cell components and, therefore, their generation must be precisely calibrated to achieve a biological effect without any damage. This can be achieved by regulating the ozone dose (ozone concentration as µg/mL of gas per mL of blood in 1:1 ratio) against the antioxidant capacity of blood that can be measured and, if necessary, 1/2

### Table 1. Why oxygen ozone therapy has not yet been accepted by orthodox medicine

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<td>2. Lack of standardization</td>
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<td>3. No precise ozone generator</td>
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<td>4. Lack of solid scientific biological and clinical data</td>
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<td>5. Ozone toxicity</td>
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<td>6. The problem of charlatans</td>
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<td>7. Lack of regulation and disinterest of health authorities</td>
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<td>8. Lack of financial support</td>
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<td>9. Skeptical and uninformed scientists</td>
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Consequently, we always collect a gas mixture comprising no less than 95% oxygen and no more than 5% ozone. Air must be excluded because toxic nitrogen dioxide (N2O2) will be formed as well as ozone and it is imperative that generators are made of high quality, ozone-resistant materials such as stainless steel, neutral glass and Teflon.

Ozone is 1.6-fold denser and 10-fold more soluble in water (49.0 mL in 100 mL water at 0°C) than oxygen. Although ozone is not a radical molecule, it is the third most potent oxidant (E°’ = +2.076 V) after fluorine and persulfate. Ozone is an unstable gas that cannot be stored and should be used at once because it has a half-life of 40 min at 20°C.

Ozone is a controversial gas because, although it is very useful in the stratosphere by absorbing dangerous B and C ultraviolet radiations, it is toxic for the pulmonary tract in the troposphere, particularly mixed with carbon monoxide (CO), N2O2 and traces of acids as occurs in photochemical smog.

It must be clear that if we want to use ozone in medicine, we must avoid its toxicity that can be controlled only if we operate cautiously by 1) using a precise ozone generator equipped with a well-standardized photometer, which allows us to determine the ozone concentration in real time, 2) by collecting a precise gas volume with a defined ozone concentration. The total dose is simply calculated by multiplying the ozone concentration with the gas volume. As an example, if we ozonate a blood volume of 225 mL with 225 mL of gas with an ozone concentration of 30 µg/mL, the total dose is equivalent to 6.75 mg of ozone. 3) We must know the optimal dose for achieving a therapeutic effect without any toxicity.

At variance with blood, the eyes and the lungs are very sensitive to ozone because they have minimal antioxidant and neutralizing capabilities and therefore ozone should never contact these organs.

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strengthened by oral administration of antioxidants before and throughout ozone therapy. d) LOPs production follows peroxidation of PUFA present in the plasma: they are heterogeneous and can be classified as lipoperoxides (LOO•), alkoyl radicals (LO•), lipohydroperoxides (LOOH), isoprostanates and alkenals, among which are 4-hydroxy-2,3 transnonenal (HNE) and malondialdehyde (MDA). Radicals and aldehydes are intrinsically toxic and must be generated in very low concentrations. They are in vitro far more stable (6) than ROS but fortunately, upon blood reinfusion, they undergo a marked dilution in body fluids, excretion (via urine and bile), and metabolism by GSH-transferase (GSH-Tr) and aldehyde dehydrogenases. Thus, only submicromolar concentrations can reach all organs, particularly bone marrow, liver, central nervous system (CNS), endocrine glands, etc., where they act as signaling molecules of an ongoing acute oxidative stress (16).

If the stage of the disease is not too far advanced, these molecules can elicit the upregulation of antioxidant enzymes such as superoxide dismutase (SOD), GSH-peroxidases (GSH-Px), GSH-reductase (GSH-Rd) and catalase (CAT). Interestingly, Iles and Liu (17) have just demonstrated that HNE, by inducing the expression of glutamate cysteine ligase, causes an intracellular increase of GSH, which plays a key role in antioxidant defense. Furthermore, LOPs induce oxidative stress proteins, one of which is heme-oxygenase I (HO-1 or HSP-32) which, after breaking down the heme molecule, delivers very useful compounds such as CO and bilirubin (18). Bilirubin is a significant lipophilic antioxidant and a trace of CO cooperates with NO in regulating vasodilation by activating cyclic GMP. Fe2+ is promptly chelated by upregulated ferritin. The induction of HO-1 after an oxidative stress has been described in hundreds of papers as one of the most important antioxidant defense and protective enzyme. Moreover, LOPs exert a neuroimmunomodulatory effect highlighted by a feeling of well being reported by patients during ozone therapy.

Although it remains hypothetical, it is possible that LOPs, throughout the treatments, acting as acute oxidative stressors in the bone marrow microenvironments activate the release of metalloproteinases, of which MP-9 particularly may favor the detachment of staminal cells (11). These cells, once in the blood circulation, may be attracted and home at sites where a previous injury (a trauma or an ischemic-degenerative event) has taken place. The potential relevance of such an event would have a huge practical importance and will avoid the unnatural, costly and scarcely effective practice of the bone marrow collection with the need of the successive and uncertain reinfusion (19).

It is emphasized that submicromolar LOPs levels can be stimulatory and beneficial, whereas high levels can be toxic. This conclusion, based on many experimental data (16), reinforces the concept that optimal ozone concentrations are critical for achieving a therapeutic result: too low concentrations are practically useless (at best elicit a placebo effect), too high may elicit a negative effect (malaise, fatigue) so that they must be just above the threshold level to yield an acute, absolutely transitory oxidative stress capable of triggering biological effects without toxicity.

In conclusion, it must be clear to the reader that the ozonation process either happening in blood, or intradiscal or in an intramuscular site represents an acute oxidative stress. However, provided that it is precisely calculated according to a judicious ozone dosage, it is not deleterious but is actually capable of eliciting a multitude of useful biological responses and, possibly, can reverse a chronic oxidative stress due to aging, chronic infections, diabetes, atherosclerosis, degenerative processes and cancer. Indeed, the ozonotherapeutic act is interpreted as an atoxic but real “therapeutic shock” able to restore homeostasis.

**Which Are the Biological Effects Elicited by ROS and LOPs?**

The ozonation process is therefore characterized by the formation of ROS and LOPs acting in two phases. This process happens either ex vivo (as a typical example in the blood collected in a glass bottle) or in vivo (after an intramuscular injection of ozone) but, while ROS are acting immediately and disappear (early and short-acting messengers), LOPs, via the circulation, distribute throughout the tissues and eventually only a few molecules bind to cell receptors. Their pharmacodynamics allow minimizing their potential toxicity and allows them to become late and long-lasting messengers.

Formation of ROS in the plasma is extremely rapid and is accompanied by a transitory and small ozone dose-dependent decrease (ranging from 5 to 25%) of the antioxidant capacity. Importantly, this return to normal within 15–20 min owes to the efficient recycling of oxidized compounds such as dehydroascorbate to ascorbic acid (20). H2O2 diffuses easily from the plasma into the cells and its sudden appearance in the cytoplasm represents the triggering stimulus: depending upon the cell type, different biochemical pathways can be concurrently activated in erythrocytes, leukocytes and platelets resulting in numerous biological effects. It must be noted that between the plasma and the cytoplasm compartments there is a gradient and the intracellular H2O2 concentration is only about 1/10 of the plasmatic one (21). The rapid reduction to water is operated by the high concentration of GSH, CAT and GSH-Px; nonetheless, H2O2 must be above the threshold concentration for activating several biochemical pathways.

Let us now examine how hydrogen peroxide, now universally recognized as one of the main intracellular signaling molecules (13), acts on the different blood cells. The mass of erythrocytes mops up the bulk of hydrogen peroxide: GSH is promptly oxidized to GSSG and the cell, extremely sensitive to the reduction of the GSH/GSSG...
ratio, immediately corrects the unbalance by either extruding GSSG or reducing it with GSH-Rd at the expense of ascorbate or of the reduced nicotinamide adenine dinucleotide phosphate (NADPH), which serves as a crucial electron donor. Next, the oxidized NADP is reduced after the activation of the pentose phosphate pathway, of which glucose-6-phosphate dehydrogenase (G-6PD) is the key enzyme. We have determined a small but significant increase of ATP formation (10,11), but whether this is due to the activation of the pentose cycle or to phosphofructokinase or to both remains to be clarified. Moreover, for a brief period the reinfused erythrocytes enhance the delivery of oxygen into ischemic tissues because of a shift to the right of the oxygen–hemoglobin dissociation curve, due either to a slight decrease of intracellular pH (Bohr effect) or and an increase of 2,3-diphosphoglycerate (2,3-DPG) levels. Obviously, one AHT treatment has a minimal effect and we need to ozonate at least 2.5–4 L of blood within a period of 30–60 days. During this period, LOPs act as repeated stressors on the bone marrow and these frequent stimuli cause the adaptation to the ozone stress during erythropoiesis with upregulation of antioxidant enzymes. As a consequence, a patient with chronic limb ischemia undergoing ozone therapy can have a clinical improvement due to the formation of successive cohorts of erythrocytes progressively more capable of delivering oxygen to his/her ischemic tissues. However, the final improvement is also due to the localized release of NO, CO and growth factors released from platelets and endothelial cells.

Although ozone is one of the most potent disinfectants, it cannot inactivate bacteria, viruses and fungi in vivo because, paradoxically, the pathogens are well protected, particularly inside the cells, by the powerful antioxidant system. Thus, as I proposed a long time ago (22,23), ozone acts as a mild enhancer of the immune system by activating neutrophils and stimulating the synthesis of some cytokines (2,5–7). Once again, the crucial messenger is hydrogen peroxide, which after entering into the cytoplasm of blood mononuclear cells (BMC) by oxidizing selected cysteines, activates a tyrosine kinase, which then phosphorylates the transcription factor nuclear factor κB (24), allowing the release of a heterodimer (p50+p65). This complex moves on to the nucleus and switches on some hundred genes eventually responsible for causing the synthesis of several proteins, among which are the acute-phase reactants and numerous interleukins. In the past, we have measured the release of several cytokines from ozonated blood upon in vitro incubation (2–7). Once the ozonated leukocytes return to the circulation, they home in lymphoid microenvironments and successively release cytokines acting in a paracrine fashion on neighboring cells with a possible reactivation of a depressed immune system (25). This process, described as the physiological cytokine response, is part of the innate immune system and helps us to survive in a hostile environment.

During ozonation of blood, particularly if it is anticoagulated with heparin, we have noted an ozone dose-dependent increase of activation of platelets (8,26) with a consequent release of typical growth factors, which will enhance the healing of chronic ulcers in ischemic patients. Whenever possible, the use of heparin as an anticoagulant is preferable to sodium citrate because, by not chelating plasmatic Ca²⁺, it reinforces biochemical and electric events.

During reinfusion of the ozonated blood into the donor, the vast expanse of the endothelial cells will be activated by LOPs, resulting in an increased production of NO, plasma S-nitrosothiols and S-nitrosohemoglobin (15,27). Whereas NO has a half-life of less than 1 sec, protein-bound NO can exert vasodilation also at distant ischemic vascular sites with relevant therapeutic effect.

Moreover, on the basis of the phenomenon of ozone tolerance that says the exposure of an organism to a low level of an agent, harmful at high levels, induces an adaptive and beneficial response (28,29), we have postulated that LOPs, by acting as long-distance messengers, can transmit to all organs the information of an acute oxidative stress (10,11). The bone marrow is particularly relevant because it can upregulate antioxidant enzymes during erythropoiesis and allows the release of staminal cells for possibly regenerating infected organs. Moreover, the stimulation of the endocrine and central nervous systems may help to understand why most patients during prolonged ozone therapy report a feeling of euphoria and wellness, probably due to an improved metabolism as well as to an enhanced hormonal or neurotransmitter release.

The paradoxical concept that ozone eventually induces an antioxidant response capable of reversing a chronic oxidative stress is common in the animal and vegetal kingdom and there is good experimental evidence (30–34) that this phenomenon is present in the animal and vegetal kingdom. Moreover, it is already supported by our findings of an increased level of antioxidant enzymes and HO-1 during ozone therapy (10,11). It also suggests that a judicious use of ozone, in spite of acting as an oxidant, enhances the antioxidant capacity, which represents the critical factor for overcoming chronic viral infections, ischemia and cell degeneration.

Which Are the Routes of Ozone Administration?

Table 2 shows that ozone can be administered with great flexibility but it should not be injected intravenously as a gas because of the risk of provoking oxygen embolism, given the fact that the gas mixture contains always no less than 95% oxygen.

So far the most advanced and reliable approach has been the major ozonated AHT because, on the basis of the patient’s body weight, a predetermined volume of blood (200–270 mL) can be exposed to an equal volume of gas
(O₂–O₃) in a stoichiometric fashion, with the ozone concentration precisely determined. Figure 1 shows a schematic drawing of the components necessary to perform AHT with an ozone-resistant glass bottle (plastic bags must be avoided because they are not ozone resistant and contaminate blood with phthalates and plastic microparticles). Blood, drawn from a cubital vein via a G19 Butterfly needle, is rapidly sucked inside the bottle under vacuum via Segment A. Then a precise volume of gas is delivered via segment B. With gentle mixing to avoid foaming, ozonation of blood is completed in 5–10 min and the ozonated blood is reinfused, via suitable tubing with blood filter, into the donor in about 15 min. This simple, inexpensive (all the necessary disposable material costs about 12 US$) procedure has already yielded therapeutic results in vascular diseases superior to those achieved by conventional medicine. Moreover, the therapeutic modalities, until now restricted to major AHT and to the empirical and imprecise rectal insufflation of gas (11), have been extended: they include the quasi-total body exposure to O₂–O₃ (35) and the extracorporeal blood circulation against O₂–O₃ (36). The latter procedure is rather invasive because blood collected from a vein circulates through an ozone-resistant gas exchanger and, with the help of a peristaltic pump, returns to the circulation via a contralateral vein. On the other hand, the partial cutaneous exposure to oxygen-ozone does not need any venous puncture and, owing to the vast expanse of the skin, allows a generalized and beneficial effect. Clearly, today we can select the most suitable method for different pathologies, their stage and the patient’s condition.

A discussion on its own is needed for the minor AHT, which basically consists of withdrawing 5 mL of blood to be immediately and vigorously mixed for 1 min with an equal volume of O₂–O₃ at an ozone concentration ranging between 80 and 100 μg/mL of gas per mL of blood. It has been extensively described in Bocci (11). The strongly oxidized blood, including the foam, is promptly injected into the gluteus muscle without the need of any anesthetic. As an unspecific immunomodulatory approach, I have used this treatment since 1953 and, during the last two decades, several ozone therapists have successfully treated herpetic infections (for review, see Reference 11). I have speculated that blood infiltrated into the muscular tissue will undergo coagulation due to platelet and prothrombin activation.

**Table 2. Routes of ozone administration**

<table>
<thead>
<tr>
<th>Parenteral</th>
<th>Intravenous, intra-arterial, a intramuscular, subcutaneous, intraperitoneal, intrapleural, intra-articular, periarticular, myofascial, intradiscal, intraradial, intraskeletal b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topical or locoregional</td>
<td>Nasal, c tubal, c auricular, oral, c vaginal, urethral and intrabadder, rectal, cutaneous, dental</td>
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a No longer used for limb ischemia. Hepatic metastasis could be embolized via the hepatic artery.

b Intratumoral or via a fistula.

c To be performed during 30–40 sec apnea.
Although patients rarely report a slight swelling and pain at the injection site, a mild sterile inflammatory reaction may take place with infiltration of monocytes and neutrophils scavenging denatured proteins, lysed erythrocytes and apoptotic cells. If plasma contains some free virions (HCV, HBV, HHV, HIV and so on), these will be inactivated by the high ozone concentration and may act as an autovaccine. At the same time a moderate release of cytokines will modulate the physiological response (25), and the abundance of heme will upregulate the synthesis of both antioxidant enzymes and oxidative stress proteins, particularly of heme oxygenase I. It is wonderful that such a simple and autologous treatment can act as a powerful enhancer of several biological responses.

A variant and unnecessarily complicated procedure proposed in the 1990s consists of treating a similarly small volume of citrated blood with ozone, ultraviolet light (obviously generating more ozone and ROS) and heat (42.5°C) for 3 min. To my knowledge, without clarifying the rationale of using three physicochemical stresses, this method appears superfluous because ozone, as an oxidizer, is more than enough and the addition of other stresses makes the interpretation of the response very difficult. A first pilot study by Garber et al. (37) testing this technique in HIV patients was badly conceived and showed neither toxicity nor efficacy, but it has amply discredited the use of ozone. This approach has been subsequently used in patients with either vasculitis (38) or advanced chronic heart failure (39). As might be expected, two biological studies (40, 41) have shown the possibility of controlling a chronic oxidative stress (33) and of activating regulatory T cells for downregulating a chronic inflammation. In conclusion, while I am not using this variant, I systematically couple the major and minor AHT as above described in all patients because I have noticed a potentiation of the biological and therapeutic effects. My opinion is that only by using a double-focused approach (it is less expensive than the variant minor AHT), able to simultaneously expand the interaction of ozonated messengers with both blood and muscular tissue, one can achieve a more rapid and intense therapeutic efficacy.

On the basis of experimental data obtained during the last decade (3–11) and on the average antioxidant capacity of human blood, we have determined the so-called ‘therapeutic window,’ that is the range of ozone concentrations (expressed as μg/mL of gas per mL of blood) within which ozone can exert therapeutic effects without toxicity with regard to major AHT. The range is surprisingly wide: 10–15 μg/mL as a minimum and 80 μg/mL as a maximum. Above 90 μg/mL, an incipient hemolysis (4–5%) warns about toxicity. The threshold level varies between 15 and 20 μg/mL, depending upon the individual antioxidant capacity. The scheme presented in Figure 2 is meant to illustrate the breadth of action expressed by the ozonated blood throughout the whole organism.

It is clear that the ozone oxidative activity is efficiently counteracted by the wealth of plasmatic and intracellular antioxidants so that an ozone concentration of 5–10 μg/mL per mL of blood is practically neutralized: only a trace of ROS and LOPs become detectable and therefore, at this very low level of ozonation, AHT may only have a placebo effect. However, the therapeutic effect is augmented by the double-focused approach, which is coupled with the major AHT as above described.

Figure 2. Ozonated blood, after reinfusion into the donor patient, is distributed throughout the whole organism. Erythrocytes continue to circulate in the vascular system delivering more oxygen into ischemic areas while leukocytes, migrated through post-capillary venules into various organs, slowly induce an immune response. Platelets will release their hormonal contents into the blood and will disappear. The reinfused LOPs undergo dilution into about 3 L plasma and 9–11 L interstitial fluid but will deliver the message of an acute oxidative stress to the whole body.
effect. As we are particularly conscious of ozone toxicity, we always apply the strategy “start low go slow” and, depending on the stage of the disease and the patient’s condition, we usually scale up the concentrations from 15, then 20, 30 and 40 µg/mL, and more when necessary, during the 1st, 2nd, 3rd and 4th weeks, respectively. By using this strategy, after many thousands of autotransfusions, we have never recorded any acute or chronic toxicity. The venous puncture is usually well tolerated because it is performed with a G19 Butterfly needle (quite suitable for withdrawing blood into the glass bottle under vacuum) that remains inserted throughout the 35–40 min treatment. However, a small percentage of women have a very poor venous access: in this case we can select one of the following three options: rectal insufflation of gas, body exposure to gas, or the slow infusion into a visible vein on the hand dorsum, via a G25–27 needle, of an isotonic glucose solution containing a final concentration of 0.03–0.06% (8.8–17.6 mM) hydrogen peroxide (11,14). This last approach cannot be as effective as the classical ozonated AHT, but it is useful. We absolutely discourage the use of ozonated saline because it contains sodium hypochlorite and can cause phlebitis (14).

Normally we perform the treatment bi-weekly but, if necessary, we can do it every day or even three times daily.

When Ozone Therapy Should Be Used?

Whenever orthodox medicine fails to solve the medical problem, the physician has the duty to fully inform the patient of all valid options available before beginning ozone therapy. I dislike antagonizing ozone therapy to orthodox medicine because I believe that there is only good medicine, which is the one that is able to cure the patient.

So far our experience is ample only for chronic limb ischemia (11,42–45), cutaneous chronic ulcers due to ischaemia and diabetes (10,11), and in the atrophic form of age-related macular degeneration (ARMD) (11). In chronic limb ischemia, the orthodox treatment is performed by prostanoid infusions, but the benefit is inferior and far more expensive than ozone therapy. Ozone therapy really helps about 70% of the ARMD (dry form) patients (11) because there is no other conventional option. The neovascular, exudative (or wet form) must be first treated with photodynamic therapy (46) or radiation (47) or with other experimental approaches based on blocking the activity of extracellular vascular endothelial growth factor (48).

I will then enumerate other pathologies where ozone therapy can be proficiently combined with orthodox therapies: 1) Acute and chronic infectious diseases, particularly due to antibiotic or chemoresistant bacteria, virus and fungi (11). Even parasitic infections such as giardiasis and cryptosporidiosis have been treated in children by Cuban physicians after administration of ozonated oil (11). 2) Osteomyelitis, pleural empyema, peritonitis, abscesses with fistulae, bed sores, chronic ulcers, diabetic foot, burns, insect and jellyfish stings, infected wounds, onychomycosis and candidiasis. These infections, often supported by antibiotic-resistant bacteria, like methicillin-resistant Staphylococcus aureus and poor penetration of antibiotics into infected areas, are responsible for too many cases of death occurring in hospitals of even the most advanced countries. In such cases, ozonated olive or sunflower oils allows a rapid disinfection and enhances healing tremendously. Unfortunately, the use of ozonated oils is hardly known and a detailed description of their preparation, application and results is reported in my most recent book (11).

It is most interesting that ozone, an unstable gas, can be stably trapped as an ozonide between a double bond of a PUFA: -(CH₂)₇-O₃-(CH₂)₇CH₃. When the ozonated oil is layered over the ulcer’s exudate at the oil–water interface, the ozone moves slowly into the water and, by reacting with biomolecules, generates a steady flow of H₂O₂. The effects of sterilization and improved oxygenation are responsible for the accelerated cicatrization. In comparison to pharmaceutical creams often containing useless antibiotics and growth factors, once ozonated oil is known and used, it will be extremely beneficial to millions of patients. 3) Herpetic infections (HHVI and II), herpes zoster and papillomavirus infection. The modality of the intramuscular injection of minor ozonated AHT, used as an autovaccine and associated with the topical therapy with ozonated oil, is very effective in preventing relapse of herpetic infections. This approach, particularly when used in combination with the acyclovirs, can cure herpetic infections in the majority of patients (11). It must be mentioned that a new vaccine can significantly reduce the incidence of herpes zoster infection and post-herpetic neuralgia (49). Chronic hepatitis-C and HIV infections, whenever possible, must be basically treated with either PEG-interferon alpha + ribavirin or highly active anti-retroviral therapy, respectively, because these drug combinations usually lower the viral load rapidly. However, ozone therapy could be simultaneously performed as a useful adjuvant treatment (11). 4) Autoimmune diseases (multiple sclerosis, rheumatoid arthritis, Crohn’s disease): results with AHT seem encouraging but are anecdotal. 5) Other chronic ischemic diseases (cerebral and heart ischemia). Ozone therapy exerts beneficial effects because it can a) increase oxygen, glucose and ATP delivery within ischemic tissues, b) enhance neoangiogenesis and possibly facilitate the implantation of bone marrow stem cells, which can provide neovascularization and tissue regeneration, c) induce the preconditioning phenomena by upregulating the expression of antioxidant enzymes and heme oxygenase I and d) trigger a neurohumoral response for improving quality of life. Our preliminary study (11) in end-stage cardiopathic patients, when either transplantation or
surgical revascularization was no longer feasible, has already shown that ozone therapy combined with the conventional best medical therapy can improve a gloomy prognosis. 6) Degenerative disorders: AHT helps patients in the early phase of senile dementia. On the other hand, it is rarely and minimally useful in diabetic retinopathy, retinitis pigmentosa, sudden hearing loss and chronic tinnitus. 7) Pulmonary diseases: emphysema, asthma, chronic obstructive pulmonary disease (COPD) and acute respiratory distress syndrome. COPD is becoming the fourth cause of death in spite of orthodox therapy based on the inhaled combination of corticosteroids plus long-acting β2-agonists and antibiotics, when necessary (50). Unfortunately these drugs, if prolonging the patient’s life, do not arrest the progression of the disease. The rationale for using ozone therapy is briefly based upon a) blood reinfusion, LOPs, present in low concentrations act on the vast endothelial surface and enhance the release of prostacyclin and NO while release of endothelin-1 is depressed (8,15). It is known that the release of NO and S-nitrosothiols represents the physiological mechanism for vasodilation (51,52) and contrasts the release of the anion superoxide, which causes vasoconstriction and deploys negative influences on platelets and endothelial cells. Secondly, the delivery of oxygen in ischemic tissues is enhanced and the progressive increase of antioxidant enzymes and heme oxygenase-1 counteracts the chronic oxidative stress, typical of pulmonary diseases. Moreover, the mild stimulation of the immune system helps to contain recurrent and chronic pulmonary infections. Recently, I have been able to treat advanced COPD patients with very encouraging results demonstrated by a marked improvement of the respiratory parameters and the walking test (11). 8) Terminal nephropathies are progressively worsened by a chronic oxidative stress not yet controllable by orthodox medicine and therefore ozone therapy could stabilize this serious dysfunction and improve the quality of life of these patients (11). 9) In a similar manner, ozonated AHT combined with topical application of ozonated oil is proving to be very useful in the metabolic syndrome well exemplified in patients with type 2 diabetes suffering from chronic ulcers with no tendency to heal (11). There is no doubt that patients prefer ozone therapy to hyperbaric oxygen and local larval (maggot) therapy (53). Needless to say, we must continue to strictly control the glycemic level. 9) Skin diseases (psoriasis, atopic dermatitis): available data seem positive but there are no randomized studies. 10) Chemoresistant metastatic cancer; therapy of cancer-related fatigue: we have reported (11,54) that a 6-month, biweekly, ozone therapy session in preterminal patients previously heavily treated with chemo- or/and radiotherapy does improve their quality of life but is unable to block cancer progression. On the other hand, ozone therapy may be far more useful immediately after surgery, possibly combined with chemo- or/and radiotherapy. Not only could it potentiate the effect of the cytotoxic drugs but by inducing the antioxidant response, it could reduce chemotoxicity (55). It is deplorable that oncologists do not want to cooperate and want to apply only their protocols. Meantime, even if survival is moderately prolonged at the cost of a poor quality of life, the mortality remains very high. Peter Boyle, Director of the International Agency for Cancer Research in Lyon, France has communicated that in Europe, in 2004, new cancer cases amounted to 2.9 million with over 1.7 million deaths. These impressive numbers indicate that the war on cancer remains wide open and that a skeptical attitude against the use of ozone therapy is unjustified. 11) Orthopedic diseases (the problem of backache): the direct intradiscal injection of oxygen-ozone is a great success in about 75% of patients (11,56) and is one of the few modern techniques able to solve the problem of a hernial disc with a mini-invasive approach. The indirect procedure that I defined as a “chemical acupuncture” consists of injecting 10-20 mL of gas into the paravertebral muscle corresponding to the metameres of the disc; it is also effective in about two thirds of patients but, in this case, the mechanism of action is linked to the activation of the antinociceptor system. The gas injection appears also effective in alleviating osteoarthritis and several other joint–tendinitis affections. 12) Chronic fatigue syndrome and fibromyalgia: AHT has been found beneficial in the majority of patients (11). 13) Dentistry and stomatology: ozone has been found very useful for treating primary root carious lesions (57). Moreover, local application of ozonated oil in aphthous ulcers (cold sores) occurring on the tongue, lips and cheeks of many people allows an extremely rapid healing and disappearance of pain (11). 14) Emergency situations such as those occurring after extensive trauma, burns, acute peritonitis and toxic sepsis often lead to multiple organ failure and death. The combination of the best orthodox therapy with three to four daily mild ozonated major AHT can prevent or reduce the worsening of the metabolic impairments and reduce mortality. Moreover, patients waiting for organ (particularly heart) transplantation may improve resistance to infections and immunosuppression (due to anesthesia and surgery) if they could undergo six to eight major and minor AHTs presumably during 6–15 days before surgery. During heart transplantation, organs such as the brain and kidneys may be damaged by the ischemia reperfusion syndrome that can be attenuated by previous adaptation to oxidative stress. A similar concept could be adopted for scheduled complex operation or application of joint implants. This sort of prophylactic ozone therapy, with little effort and expense, may reduce the risk of infections, shorten the hospitalization and save money. However, the implementation of the prophylactic ozone therapy remains a dream in so far as World Health Authorities remain aloof and entangled in economic and political problems.
Hyperbaric Oxygen Therapy (HOT) and Ozone Therapy

It appears relevant to briefly clarify the validity and scopes of these two different approaches. In the hyperbaric chamber, the breathing of pure oxygen at 2.6 atmospheres greatly increases the solubilization of oxygen in the plasma (about 5 mL/dL) so that the dissolved oxygen is sufficient to satisfy the cellular requirements even in ischemic tissues. That is the reason why patients with chronic limb ischemia, or with diabetic foot, or ARMD often undergo HOT. Unfortunately, this is only a palliative treatment. Indeed, after 2 h, as soon as the patient comes out of the chamber, hypoxia resumes in the ischemic areas and the therapeutic effect is minimal and temporary. On the other hand, during ozone therapy, while the hyperoxegenaion of the reinfused blood has a negligible relevance, the ozone triggers a series of biological mechanisms that lead to normalizing the delivery of oxygen for several days with consequent therapeutic effects. Two excellent reviews (58–59) clarify the exclusive role of HOT in air embolism, decompression sickness, CO-poisoning and clostridial myonecrosis but, regrettably, do not examine the relevance of ozone therapy. Indeed, they objectively report that HOT may be useful in chronic limb, heart and cerebral ischemia, autoimmunity colitis, sickle cell anemia, chronic osteomyelitis, ARMD, diabetic foot, thermal burns, extensive chronic ulcers and bed sores, but the actual evidence is flawed and anecdotal. All of these latter conditions can instead greatly benefit by the use of parenteral (and when necessary topical) ozone therapy because the multiple mechanisms of action of ozone can correct pathologies linked to ischemia, infections, delayed healing and chronic oxidative stress (reviewed in Reference 11). In conclusion, both HOT and ozone therapy are important, but it is necessary to understand that their respective field of application is different and each approach must be used profitably only in selected pathologies.

Conclusions and Perspectives

I often ask myself if ozone therapy is obsolete or worthwhile being pursued. Our many treated patients answer for me and they loudly say that it is very beneficial. The compliance is excellent and the patients, as soon as the therapeutic effect declines, ask for a new cycle. This is an excellent proof that provided we are using judicious ozone concentrations, there is neither acute nor chronic toxicity. It has been unfortunate that, in the past, the direct intravenous injection of the gas, now prohibited, and misuse of ozone by incompetent quacks has generated the dogma that ozone is toxic and should not be used in medicine. This concept is wrong and has also been based first on non-physiological studies (60) performed in washed erythrocytes, hence unprotected by the plasma antioxidants and second, in not recognizing the profound difference between the endogenous chronic oxidative stress, occurring every day during a lifetime or during a chronic disease, and the calculated, extremely brief and exogenous oxidative stress that we induce on blood by using a precise and small ozone dose. We know that any drug, depending upon its dosage, can be either therapeutic or toxic. The following elementary observation is even more compelling: the normal glucose concentration in the plasma ranges between 0.7 and 1 mg/mL and is essential for survival. However, when this concentration falls below 0.4 mg/mL, the consequent hypoglycemic coma can be deadly. On the other hand, if the glucose concentration remains constantly above 1.3 mg/mL, it induces the metabolic syndrome, as is well exemplified by the current diabetic epidemic. Thus, the dogma about ozone toxicity is futile because, after millions of treatments, we have never observed any acute or chronic toxicity. Moreover, most of the patients report a feeling of wellness. Needless to say, ozone therapy does not “cure” ARMD or other chronic pathologies but, by performing the maintenance therapy, it does improve the condition and maintain a good quality of life. On the other hand, even orthodox medicine, with the exception of several infectious diseases thanks to antibiotics, antivirals, antibodies and vaccines and far less frequently of cancer thanks to surgery/chemotherapy, is unable to “cure” most human diseases such as atherosclerosis, advanced cancer, diabetes, degenerative, metabolic and autoimmune diseases.

We are certainly not blinded by ozone therapy but the great strides of molecular biology and gene therapy during the last decade have not yet been paralleled by comparable advances in therapeutic innovations and many unforeseen difficulties still have to be overcome (61). I do not want to diminish scientific achievements but simply to point out that we are often unable to predict the pitfalls when new treatments are applied from mice to patients. This is probably one reason for the worldwide boom of complementary medicine, not only in underdeveloped countries but also in the U.S. Patients, as human beings, are often disappointed by the high-tech therapist. Moreover, conventional therapy often has side effects, and about 55,000 Americans may have died as a result of taking the now infamous Vioxx (62,63).

Ozone therapy is capturing increasing attention all over the world, since our studies reported in two books (10,11) have clarified the main biochemical mechanisms of action and the real possibility of taming ozone toxicity. We now have the first comprehensive framework for understanding and recommending ozone therapy in a few diseases as a first choice and in combination with orthodox therapy in many others. Indeed, one important characteristic of ozone therapy is that it can be experimentally verified both at the biochemical and clinical levels.

So far, the most advanced and reliable approach has been the major ozonated AHT but today we also have other technical possibilities and we can select the optimal method for different pathologies. As far as chronic diseases are
concerned, the problem is that official medicine tends to treat symptoms rather than the cause(s) of the disease. Besides the fact that the etiology is too complex or remains obscure, the treatment is often limited and remains unsatisfactory. On the other hand, a simple gaseous molecule like ozone, that probably is even produced in vivo (64), by acting on many targets, at least in part can recover functional activities that have gone astray. We have good reasons to believe that the therapeutic power of ozone therapy consists of simultaneously improving circulation and oxygen delivery, in enhancing the release of autacoids, growth factors and cytokines and in reducing the endogenous, chronic oxidative stress. In other words, ozone therapy seems to act as a biological response modifier.

Finally, I cannot omit mentioning some drawbacks. Although the cost of ozone is very low, it represents an impractical drug because it is unstable and cannot be stored in any form. However, by using a portable ozone generator we can perform domiciliary AHT treatments, useful for the elderly and for those patients with chronic diseases. Moreover, rectal insufflation of gas can be easily done by the patient at home, under the ozone therapist’s supervision. Topical therapy of chronic ulcers and infectious wounds with ozonated oil is very practical and easy because we have standard and stable preparations. The last, but certainly not the least, problem is the lack of financial support for performing controlled and randomized clinical trials, whose results are critical and urgently needed to prove the validity and atoxicity of ozone therapy in various diseases. Objective results from clinical studies represent the unique possibility of convincing the biased opponents of this approach. The private ozone therapist, or even the small existing national associations, in comparison to the pharmaceutical industry, in enhancing the release of autacoids, growth factors and cytokines and in reducing the endogenous, chronic oxidative stress. In other words, ozone therapy seems to act as a biological response modifier.

Acknowledgments
The English revision and editorial assistance of Mrs. H. Carter is gratefully acknowledged.

References
Short Communication

Is it true that ozone is always toxic?
The end of a dogma

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Keywords: ozone, lungs, antioxidants, acute oxidative stress; 4-hydroxynonenal, ozonotherapy

Abstract

There are a number of good experimental studies showing that exposure by inhalation to prolonged tropospheric ozone damages the respiratory system and extrapulmonary organs. The skin, if extensively exposed, may also contribute to the damage. The undoubtful strong reactivity of ozone has contributed to establish the dogma that ozone is always toxic and its medical application must be proscribed. Although it is less known, judiciously practiced ozonetherapy is becoming very useful either on its own or applied in combination with orthodox medicine in a broad range of pathologies. The opponents of ozonetherapy base their judgment on the ozone chemistry, and physicians, without any knowledge of the problem, are often skeptical. During the last 15 years, a clear understanding of the action of ozone in biology and medicine has been gained, allowing today to argue if it is true that ozone is always toxic. The fundamental points that are discussed in this paper are: the topography, anatomical and biochemical characteristics of the organs daily exposed to ozone versus the potent antioxidant capacity of blood exposed to a small and precisely calculated dose of ozone only for a few minutes. It is becoming clear how the respiratory system undergoing a chronic oxidative stress can release slowly, but steadily, a huge amount of toxic compounds able to enter the circulation and cause serious damage. The aim of this paper is to objectively evaluate this controversial issue.

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Plasma Concentrations of Thiols and Malondialdehyde in Patients with Age-Related Macular Degeneration Treated by Major Ozonated Autohaemotherapy

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Key words: major autohaemotherapy, age-related macular degeneration, protein SH, malondialdehyde

SUMMARY - We measured the plasma levels of SH protein and malondialdehyde expressed as thiobarbituric acid reactive substances (TBARS) in 15 healthy subjects and in 15 patients with age-related macular degeneration before and after treatment with major ozonated autohaemotherapy. Patients showed an increase in SH protein groups and a decrease of TBARS after 12 sessions of major ozonated autohaemotherapy, with an improvement in quality of life.

Introduction

Age-related macular degeneration (AMD) is an acquired degenerative disease of the retina affecting individuals over the age of 55 years. The disease has a major social impact as it is a leading cause of blindness in the Western world. Changes in central vision at onset and during the disease course cause considerable disability and have a major impact on quality of life. The causes of AMD are multifactorial and in addition to age risk factors include arterial hypertension, obesity and a diet poor in antioxidants.

Oxidative neurotoxic damage to the retinal pigment epithelium plays an important role in AMD pathogenesis with the formation of free radicals in the retina. AMD can therefore be considered a disease caused by chronic oxidative stress in which pathological changes occur following the imbalance between the body’s antioxidant and oxidant substances.

Treatment to date has consisted in the administration of antioxidant supplements, but no studies have yet demonstrated the true benefits of vitamin and mineral intake in patients with AMD. Many AMD patients have been treated at our institution using major ozonated autohaemotherapy (MAHT) with the aim of reducing chronic oxidative stress. To assess possible changes in plasma antioxidant and oxidant levels during ozone therapy, we measured some indices of the redox balance like plasma thiols – the main antioxidants against free radicals, and plasma malondialdehyde expressed as thiobarbituric acid reactive substances (TBARS), deemed an important index of lipid peroxidation.

We report the basal values of thiols and malondialdehyde in healthy subjects and AMD patients and the changes in the redox balance measured after MAHT in AMD patients.

Materials and Methods

Fifteen healthy control subjects (group 1) and 15 patients with age-related macular degeneration (group 2) were enrolled in the study. Control subjects were matched with AMD patients in terms of age, sex and anthropometric characteristics.

Three ml of blood were taken from all subjects in groups 1 and 2 and centrifuged. SH protein groups and malondialdehyde were measured and expressed as thiobarbituric acid reactive substances (TBARS) as reported elsewhere.

Group 2 patients underwent a cycle of 12 twice weekly treatments of MAHT at an ozone concentration of 60 mg/ml. At the end of the twelfth treatment session blood samples were taken to measure SH protein groups and plasma malondialdehyde.

All patients completed the cycle of treatment without no side-effects and with a general improvement in quality of life.
Figure 1 Protein SH values in the control group (group 1) and in AMD patients (group 2): p< 0.05 between the two groups.

Figure 2 Malondialdehyde values (TBARS) in the control group (group 1) and in AMD patients (group 2): p< 0.05 between the two groups.
Figure 3 Comparison between values of SH protein before and after the cycle of major ozonated autohaemotherapy (pre MAHT – post MAHT) in AMD patients: p < 0.05.

Figure 4 Comparison between values of TBARS before and after the cycle of major ozonated autohaemotherapy (pre MAHT – post MAHT) in AMD patients: p not significant.
Statistics

The comparisons between groups before and after MAHT were done using Student’s test for paired data. Values with p < 0.05 were considered significant.

Results

Results are summarised in figures 1-4. Figures 1 and 2 show a significant difference between basal values of protein SH groups and malondialdehyde in controls with respect to AMD patients. Figures 3 and 4 depict the changes in protein SH groups and TBARS before and after MAHT: the SH protein rose significantly at the end of the treatment cycle, whereas the increase in TBARS was not significant given the wide interindividual variability of plasma malondialdehyde which in any case decreased at the end of MAHT.

Discussion

Age-related macular degeneration is universally considered a disease of chronic oxidative stress in which the retina is the main target of free radicals. This study disclosed a clear-cut drop in plasma thiols and an increase in malondialdehyde before the start of MAHT with respect to control values. This difference only reached statistical significance for plasma thiols due to the wide interindividual variability of TBARS.

This basic difference suggests that the antioxidant titre of each individual should be measured before ozone therapy as a presumed value of oxidative indices cannot be used.

The lack of an effective official medical protocol for the treatment of AMD justifies attempts to use MAHT to treat age-related macular degeneration. Our results showed an improvement in quality of life in almost all patients following MAHT, a particularly encouraging outcome since AMD is a highly disabling disease.

The biochemical mechanisms underlying the success of MAHT are probably linked to an increase in endogenous antioxidants and a decreased production of reactive oxygen species (ROS), as confirmed by our measurements of plasma thiols and malondialdehyde before and after MAHT. Alongside the change in the redox balance, MAHT and other ozone therapies change the biological response by interacting with other systems including the immune, inflammatory and blood coagulation systems.

The lack of side-effects and its relative simplicity make major ozonated autohaemotherapy a valid alternative treatment for AMD patients. Further studies are required to assess possible alternative treatment protocols in terms of ozone concentration and treatment duration.

References

CT-Guided Ozone Injection for the Treatment of Cervical Disc Herniation

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Key words: intervertebral disk displacement, ozone, cervical spondylosis, therapy, interventional

SUMMARY - We evaluated the therapeutic outcome of CT-guided ozone treatment for cervical disc herniation. All 86 patients with cervical spondylosis including myelopathy (37 cases), radiculopathy (30 cases), and sympathetic type (19 cases) were treated with ozone injection under CT guidance. The puncture route was anterolateral from the neck to the disk. A total of 2-7 ml of ozone at a concentration of 60 µg/ml were injected into the disk and 5 ml of ozone at a concentration of 40 µg/ml were injected into the paraspinal tissue. Therapeutic outcome was assessed three months after treatment by using a modified MacNab method. After injection of ozone, CT scan showed that ozone was distributed in the disk and extruded disk material in myelopathy and radiculopathy after injection, and distributed in the anterior peridural space and perivertebral body in sympathetic type. The excellent, good and poor clinical efficacy rates were 78%, 16% and 6% respectively three months after treatment. CT guided ozone injection is an accurate, safe and effective method in the treatment of cervical disc herniation.

Introduction

Non invasive procedures, minimally invasive percutaneous injection, and surgery represent the gamut of treatments available in the management of cervical disk herniation. Noninvasive treatments are usually the first choice in most cases, but when patients fail to respond, minimally invasive percutaneous injection or surgery is warranted. Minimally invasive treatments were developed to offer good clinical results combined with a well-tolerated low-cost procedure. In recent years, these procedures were further boosted due to the following drawbacks of traditional surgical therapy: significant soft-tissue injury, extensive hospitalization, and recovery time of six weeks or longer.

The most promising method to date in terms of simplicity and minimal invasiveness is ozone therapy. Ozone is used in medicine to treat different conditions based on its biologic effects: oxidization, bactericide, fungicide, and virustatic, immuno-modulating action, analgesic and anti-inflammatory effects. A vast bibliography on the topic can be found in a recent study on how ozone therapy works. As for herniated disk, a reduction in volume is one of the therapeutic aims of intradiscal administration of medical ozone, as disk shrinkage may reduce nerve root compression. Another reason for using medical ozone to treat disk herniation is its analgesic and anti-inflammatory effects.

This article assesses the results obtained in treating 86 patients with ozone injection under CT guidance, the guiding modality, puncture route, ozone concentration and dose, imaging changes in post-treatment and outcome of combination of various treatments.

Material and Methods

Patient Population

From January 2002 to December 2005, 86 patients (57 males, 29 females) aged 36-72 years (mean 52 years) received CT guided ozone injection. The levels of involvement were 11 at C3-4, 17 at C4-5, 32 at C5-6, 23 at C6-7, and 3 at C7-T1. The cases were classified into myelopathy type (37 cases), radiculopathy type (30 cases), and sympathetic type (19 cases); vertebral arterial type was not included in this study. The indications for injection were: (a) neck pain with radiation down the arm; (b) symptoms and signs of sensory loss, tingling, numbness, muscle weakness, and/or decreased deep tendon reflexes; (c) MRI or CT findings of no cervical spinal canal bony stenosis or lateral recess stenosis, no ossification of poste-
rior longitudinal ligament (OPLL) and ligament flavum and no malacia of spinal cord; (d) positive electromyography and/or nerve conduction studies; and (e) no improvement after 12 weeks of conservative therapy. According to the policies of our hospital review board, approval was not required for this retrospective analysis.

**Procedure**

**Puncture approach**

Before the procedure, all patients were fully informed about the anticipated benefits and potential risks of the procedure, including the possibility of recurrence of radicular symptoms during injection and/or transient exacerbation after treatment. The patients were placed comfortably in a supine position on the CT table with their arms at their sides and an anterolateral approach was used. Two-millimeter, axial, contiguous scans were obtained to locate and mark the puncture site. The distance from this point to the disc, puncture angle and puncture depth were subsequently measured. After the injection site was disinfected and local anesthesia applied using 0.1% lidocaine hydrochloride, a 22-gauge spinal needle was introduced by an anterolateral approach (with the vertebral artery located to avoid it) and gently pushed into the herniated vertebral disc under CT control. The puncture route differed at different levels of cervical disc, for cervical disc 3-4, the needle was introduced between the parapharyngeal space and the medial margin of the cervical arterial sheath; for cervical disc 4-5, the needle was introduced between the lateral margin of lamina of thyroid cartilage and the medial margin of the cervical arterial sheath; for cervical discs 5-6 and 6-7, the needle was introduced between the lateral margin of thyroid gland and the medial margin of the cervical arterial sheath. In addition, ozone injection into paraspinal muscles was performed on 11 patients with neck pain one week later after intradiscal injection.

**Dose and concentration of ozone**

After CT scanning to check correct needle placement, 2 mL 60 µg/ mL ozone with 5 mL syringe from an ozone generator (CHY-11- ozone generator, YiDeKang medical Technology Co., Ltd., Shandong province, China and Hyper-Medozon Comfort ozone generator, Herrmann Apparatebau GmbH, Germany) were pumped and injected into the nucleus pulposus of the disc repeatedly until the correct distribution of the gas was checked by CT scanning. The needle was then pulled out, the puncture site was sterilized and dressed. The concentration of ozone injected into paraspinal muscles was 40 µg/ mL and the total dose was not more than 10 mL. At the end of treatment, patients were discharged with collar securing and antibiotics administration for three to five days.

Clinical outcome was assessed three months after treatment by applying the modified MacNab method (table 1). Results were evaluated using a questionnaire and direct patient interviews.

**Table 1: Modified MacNab method for assessing clinical outcome after ozone therapy**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excellent</td>
<td>Disappearance of symptoms, complete recovery of working and sports activities</td>
</tr>
<tr>
<td>Good</td>
<td>Occasional episodes of low back pain or sciatica or no limitations of occupational activities</td>
</tr>
<tr>
<td>Poor</td>
<td>Insufficient improvement of symptoms or periodic administration of drugs or limitation of physical activities</td>
</tr>
</tbody>
</table>

**Results**

All patients were punctured successfully guided by CT with intradiscal injection dose of 3-7 ml ozone (mean 4 ml). CT scan showed that only a small amount of ozone accumulated in the disc and a great deal of ozone flowed out of the cranny of the annulus fibrosus and into the paraspinal space, perivertebral artery and peridural space. Hypoattenuation gas was shown in the extruded disc material on CT scan after injection in myelopathy type (figures 1-2), peri-extruded disc material and nerve root in radiculopathy type (figure 3), anterior peridural space and perivertebral body in sympathetic type (figure 4), and intramuscular space and posterior peridural space in cases receiving posterolateral paraspinal injection of ozone (figure 5). The excellent, good and poor clinical efficacy rates were 78%, 16% and 6% respectively three months after treatment.

**Discussion**

The initial pathologic change of spondylosis is disk degeneration, and then crack formation of annulus fibrosis. This results in displacement of the nucleus pulposus through a crack in the annulus
Figure 1  Images in a patient with C4-C5 midline disk herniation (myelopathic type). A) Precontrast CT image at the C4-C5 level shows disk herniation (white arrow): the needle (black arrow) into the disk before ozone injection. B) After ozone injection, CT shows ozone dispersion into the herniated disk material (black arrow) with local intact annulus fibrosus (black arrowheads). After intradiscal injection, ozone is injected into the paraspinal tissue (white curved arrow).

Figure 2  Images in a patient with C5-C6 midline disk herniation (myelopathic type). A) Precontrast CT image at the C5-C6 level shows disk herniation (black arrow head): the needle was introduced into the disk (white curved arrow) between the lateral margin of the thyroid gland and the medial margin of the cervical arterial sheath before ozone injection. B) After ozone injection, CT shows ozone distribution into the disk (white arrow), the herniated disk material (white arrowheads) and right paraspinal tissue and perivertebral arterial space (white curved arrow).

fibrosis, and then compression on the nerves or spinal cord causing radiculopathy and myelopathy. Repeated chronic trauma, injury and accumulation of local metabolic products and inflammatory factors may irritate the radicular and sympathetic nerves and cause clinical syndromes. With further degeneration, the uncinate process and facet joints hypertrophy and ossification of the posterior longitudinal ligament also occur, forming osteophytic bars, hypertrophy of ligaments and stenosis of the spinal canal. Clinically, cervical spondylosis may be classified as cervical type, myelopathic type, radic-
ulopathic type, sympathetic type and vertebral arterial type. These syndromes may be overlapping or distinct. Patients are often reluctant to receive traditional surgical therapy due to the following drawbacks: significant soft-tissue injury, extensive hospitalization, and recovery time of six weeks or longer. Ozone injection is commonly used for early and medial stages of cervical spondylosis. The proper concentration and dose of ozone injected into the disk can oxidize the nucleus pulposus and reduce pressure on the disk. This may lead to a return of protruded disk material or a reduction of nerve root compression. Ozone injected into perispinal tissue may exert its analgesic and anti-inflammatory effects, and improve local microcirculation, increasing the supply of oxygen due to reduced venous stasis caused by disk compression of vessels, at the same time, reducing hypoxia due to deoxidization of ozone into oxygen. Ozone injection, due to its minimal invasion, simple procedure,
efficacy and safety, is widely focused. The various imaging modality guidance techniques offer different advantages. C-armed X-rays fluorescence has a real-time visualization but cannot display the distribution of ozone. CT provides accurate guidance by measurement of software and experienced manipulation by the operator but has no real-time visualization. Cervical intradisk ozone injection through an anterolateral approach requires a two-degree cephalic tilt of the needle so as to puncture the disk precisely and requires manipulator skill and has a good spatial vision for fear of puncturing the endplate. A 5 to 15 degree cephalic tilt of the needle should be made according to the supine degree of the patient's head, and the CT gantry should be tilted parallel to the axial position of the disk and the needle should penetrate the disk parallel to its level. Due to the needle insertion into the disk between the medial margin of the cervical arterial sheath and the midline, the angle of puncture is determined by distance between the medial margin of the cervical arterial sheath and the lateral margin of the vertebral body. A 45 degree cephalic tilt of the needle should be made for cervical disks 4-5 and 5-6; a greater degrees cephalic tilt should be made for cervical disk 6-7 due to greater distance between the medial margin of the cervical arterial sheath and the lateral margin of the vertebral body; a lesser degree from 35 to 40 cephalic tilt should be made for cervical disk 3-4 due to the shorter distance between the parapharyngeal space and the medial margin of the cervical arterial sheath, and the puncture should be more cautious for fear of piercing the pharyngeal mucous membrane and causing infection of the paraspinal space. The cervical arterial sheath should be manually displaced laterally with the middle finger and first finger (the apophysis of the bulging disc can be touched in thin patients), and then the needle is inserted dorsally to the middle finger and the first finger into the disk. When the needle reaches the annulus fibrosis, there is a resistance, and then the needle is gently pushed into the disk to a depth of 3 mm for fear of piercing the posterior part of the annulus fibrosis. CT scanning should be performed to determine and adjust the tip of the needle in the central part of the disk.

The ozone capacitance of the cervical disk is relatively small due to its smaller volume compared to the lumbar disk. Injection into the central part of the disk may help the dispersal of ozone to protruded disk material. After injection, there is hypoattenuating gas pervading into the protruded disk material, then the annulus fibrosis can be displayed clearly at CT. However, the injection pressure should be gentle for fear of the iatrogenic rupture of the annulus fibrosis. In this study, ozone pervading into the protruded disk material was clearly displayed at CT in the cervical disk herniation of myelopathic type and radiculopathic type. If the annulus fibrosis is ruptured, the injection pressure will be relatively lower and a small quantity (2 mL) and repeat injection should be made with intermittent CT scanning observing the distribution of ozone. A small deposit of ozone in the disk with a great deal of ozone dispersing out of the disk will occur in such cases, but it will not reduce the treatment efficacy because the intradisk nucleus pulposus has been oxidized in the process of dispersion of ozone. For the extruded nucleus pulposus, ozone should be pervaded into it in order to obtain a satisfactory efficacy. In this study, the needle tip was positioned adjacent to protruded disk material and post CT scanning showed that ozone diffused into it for patients with radiculopathic type. In the puncture process, the needle may frequently penetrate into the superior and inferior endplate due to narrow cervical disk, which has the same handle of penetration into the annulus fibrosus. This can be verified by difficult injection of ozone and CT scan. But C-armed fluoroscopy usually cannot differentiate it and display the distribution of ozone, so that the injection dose of ozone cannot be determined correctly. In this study, CT showed no ozone distribution into the disk after initial injection in 37 patients, and satisfactory distribution of ozone was shown at CT after adjusting the needle tip adjacent to the herniated disk material. Paraspinal injection of 40 µg/ml ozone for the sympathetic type obtained a satisfactory efficacy in 16 out of 19 patients in one week.
and three patients in one month after injection. It is very important for obtaining a satisfactory efficacy to strictly select the indications for injection. CT findings of osteophytes, ossification of posterior longitudinal ligament and ligament flavum and T2-weighted magnetic resonance imaging showing malacia of the spinal cord should be considered contraindications. Ozone injection should be used for early and medial stage cases with slight or medial symptoms and without bony spinal stenosis and spinal cord injury.

The concentration and dose of ozone is crucial for a satisfactory efficacy. Generally speaking, the degree of oxidation is positively related to the ozone concentration, too low a concentration lacking therapy efficacy and an exorbitant concentration causing injury to adjacent tissue. The dose of ozone administered must not exceed the capacity of antioxidant enzymes (superoxide dismutase and catalase) and glutathione to prevent accumulation of the superoxide anion (O2\(^-\)) and hydrogen peroxide (H\(_2\)O\(_2\)), which can cause cell membrane degradation. Free radicals are mainly formed by ozone in a medium with a pH higher than 8, whereas at a pH below 7.5 the ozonolysis mechanism prevails, mainly leading to the formation of peroxides. In our study, the concentration of ozone injected into the disc was 60 µg/ml, and the concentration injected into peri-nerve roots was 40 µg/ ml so as not to cause injury. The dose of ozone is determined by the degree of dispersion of ozone shown at CT.

This study had several limitations, including the fact that it was uncontrolled. Since it is well known that medical treatment may need time to be effective in cervical spondylosis, it would be important to design randomized controlled trials to compare the efficacy of medical treatment and injection. However, prospective studies comparing treatment regimens are bound to fail because a double-blind study design would be hard to apply. The small patient population did not permit us to evaluate the efficiency of a second injection in patients for whom the first procedure did not improve the symptoms.

Lastly, our follow-up period was only three months, which hindered long-term assessment of periradicular corticosteroid injections in cervical disk herniation.

**Conclusion**

CT-guided ozone injection is a minimally invasive, accurate, safe, and effective method in the treatment of cervical disc herniation of early and medial stages.
Editorial Comment

The paper by Dr. Xiao and colleagues and their therapeutic results are interesting, but the article lends itself to some criticism in the light of the international literature.

Their study specifies that treatment was administered injecting ozone into the disc at a concentration of 60 µg/ml and into the paraspinal tissues at a concentration of 40 µg/ml.

Most authors deem these concentrations to be extremely high, especially with reference to experimental studies (Muto, Iliakis) in which administration of concentrations over 50 µg/ml to animals risked damaging the anulus fibrosus (Muto’s paper described anatomo-pathological preparations showing delamination of the anulus to necrosis in swine disc).

In the light of these findings, the quality of equipment is crucial.

Nowadays all ozone devices must be certified and fitted with photometric detectors of ozone concentration in the gas mixture.

We advise against administering very high ozone concentrations at more than one session to ensure full recovery of any microlesions caused during treatment.

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Ozone Therapy to Resolve Disc Space Infection Spondylodiscitis

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Key words: spondylodiscitis, lumbar puncture, ozone therapy

SUMMARY - We describe three cases of disc space infection, spondylodiscitis, treated by ozone therapy. One case of spontaneous spondylodiscitis and the other two cases were postoperative complications. The predominant clinical feature was low back pain. The ozone treatment produced very good results.

Introduction

Mixter and Barr presented their classic paper on intervertebral disc herniation in 1934. Since then, laminectomies have been performed with increasing frequency for the treatment of this condition. Inevitable complications of such surgical treatment have been recognized and they include disc space infection known as spondylodiscitis. However, the first description of intervertebral disc space infections did not come from surgical patients. In 1940 Ghormley and colleagues presented 20 cases of spontaneous disc space infection and differentiated this condition as a clinical separate entity, different from other pathologies, particularly vertebral osteomyelitis. It was not until 1953, when Turnbull presented a series of three patients with post operative disc space infection, that this condition became a recognized clinical entity. Infection of the intervertebral space is frequently caused by disc surgery, but there are other causes like spinal puncture, spinal catheterism, alcoholism, drugs, HIV and others which can cause the disease.

Incidence

The true incidence of postoperative disc infection is difficult to determine for several reasons. The condition can be mild and self-limiting and it resolves spontaneously, the patient may be misdiagnosed or labelled as functionally disabled or psychoneurotic, some patients operated on at one institution go to another for assessment and treatment of the disc space infection.

Pathophysiology

The disc annulus is composed of elastic fibrous layers, which surround the semi-liquid gelatinous nucleus and provide the disc with its inherent strength. The fluid properties of the nucleus pulposus provide incompressibility and shock-absorbing potential. The plate of hyaline cartilage at each end of the disc, from which the annulus arises, constitutes the interface between the bony vertebra and the rest of the disc, and acts as a barrier between one vertebra and the contiguous one. This plate and the epiphyseal ring of the vertebra are in intimate association during embryological development, and are analogous to the epiphysis of a long bone. The disc itself, prior to its later avascular state, is the most distant element supplied by the intersegmental artery.

Initially, the disc material is vascularized by small arteries from the cartilaginous plate. Between childhood and the third decade these vessels gradually disappear, and the disc becomes totally avascular. These changes are the basis for haematogenous post operative intervertebral disc space infections.

Based on these premises, childhood disc infection is thought to be of haematogenous origin, with bacteria spreading directly to the intervertebral disc through the vessels from the epiphyseal ring and cartilaginous plate. Adult hematogenous infection, on the other hand, is a hematogenous infection not of the disc substance itself but of the epiphyseal vertebral ring plate region.

Postoperative disc space infections occur by direct inoculation of microorganisms into the disc.
space during a surgical procedure. The surgeon performs the operation on an avascular structure and leaves behind small pieces of degenerated disc, necrotic tissue, and hematomas of various sizes, all these elements strongly predispose to an eventual infection. In spite of the use of a scrupulously sterile technique, organisms may occasionally be introduced in most operations in the “locus minoris resistentiae”, as Pilgaard named these structures. These types of infections have been reported after lumbar puncture myelography, paravertebral injections, discography and recently in several cases after percutaneous discectomy.

Clinical Manifestations

Postoperative disc space infection has a characteristic clinical presentation.

The most common presenting symptom is severe back pain that can radiate towards the leg, buttock, groin or testis, mimicking a root compression syndrome, or into the perineum or abdomen. The pain can appear at any time from five days to three months after an uneventful operation. More than 85% patients suffer excruciating paravertebral muscle spasms which are relieved only by immobilization.
The appearance of the operative incision does not usually indicate an underlying infection. Although fever is not a uniform finding, it is present in 30% of patients.

**Diagnosis**

A post operative disc space infection can be suspected on the basis of very few typical findings like severe low back pain with severe paravertebral muscle spasm and elevated ESR. CT findings are very useful as they show early narrowing and destruction of the disc space and erosive changes in the vertebral end plates. CT scan also demonstrates any soft tissue involvement or paravertebral abscess.

The procedure of choice for diagnosing a disc space infection is MRI which shows disc space changes consistent with an infection as early as five days after operation.

These changes include a reduction in signal intensity on T1-weighted images and an increase in signal intensity on T2-weighted images from the involved disc, plus a blurring of the disc margin on the T1-weighted images.10-11.
Case 1

A 40-year-old woman with systemic lupus erythematosus was evaluated in our out-patient clinic in September, 2005. She complained of severe lumbar pain for the last six months, which was irradiated to both buttocks and lower limbs. The pain increased with movements, strain and rotation movements of the spine.

MRI showed an image consistent with spondylodiscitis. A prescription for a lumbo-thoracic corset to immobilize the spine was issued, and 25 sessions of ozone therapy were performed through paravertebral taps, each of them twice a week with a concentration of 20 micrograms of ozone.

A control MRI in April 2006 showed a complete resolution of the spondylodiscitis (figures 1-2) and a complete improvement of the clinical complaints. No antibiotics were used because of the patient’s SLE.

Case 2

A 64-year-old man had a surgical intervention in October 2004 for a radicular compression at L5-S1 level. In November 2005 he complained of severe lumbar pain, irradiated to the right low limb. CT scan showed an intensification of the cortical plate at the level of L5-S1 (figure 3). The MRI showed an image of spondylodiscitis (figure 4 A). Ozone treatment was instituted with 20 paravertebral taps, with an ozone concentration of 20 micrograms and immobilization of the spine with a corset.

There was a 100 % clinical improvement, and a control MRI performed six months later showed a complete resolution of the spondylodiscitis (figure 4 B).

Case 3

A 35-year-old man underwent surgery in March 2005 for a spine herniated disc at L4-L5 level. One week after the surgical intervention, he complained of intense lumbar pain, irradiated to the right low limb. MRI study showed a spondylodiscitis at L4-L5 (figure 5 A).

Ozone therapy was prescribed with an intra discal tap at L4-L5 level with an ozone concentration of 30 micrograms to desinfected the disc infection, followed by ten paravertebral injections of oxygen-ozone with a 20 micrograms concentration of ozone. In June 2005, three months later, there was a complete remission of the clinical complaints and an improvement of the clinical picture in the control MRI (figure 5 B).

Discussion

Until now the treatment of spondylodiscitis has ranged from incision and drainage to the use of specific antibiotics and spinal immobilization. Nowadays, there is controversy over the use of antibiotics to treat disc space infection. Some authors use immobilization for definitive treatment and do not recommend open drainage or fusion.
later on (Wilkins)\textsuperscript{12}. There is no doubt that ozone can play an important therapeutic role in various types of infections because it generates ROS (O\textsubscript{2}, OH, H\textsubscript{2}O\textsubscript{2}, NO and HOCl), which are also produced by granulocytes and macrophages during an infectious process (Badwy and Karnowsky, 1990; Chanock et Al, 1994 Anderson et Al, 1997; Saran et Al, 1999; Titheradge, 1999; Babior, 2000).

Moreover, neutrophils have a variety of antimicrobial proteins in their granules and release pro-inflammatory cytokines whose numerous effects can cause tissue damage. Witko-Sarsat et Al, (2000) and Nieva and Wenworth (2004) postulated that ozone might produce in vivo, via antibodies, catalyzed water-oxidation pathways through a dihydrogentrioxide (H\textsubscript{2}O\textsubscript{3}) intermediate. To scientists’ surprise, it appears that nature is able to generate gaseous and reactive molecules (CO, NO and O\textsubscript{3}) which, in adequate amounts, may discharge critical physiological roles, but during inflammation, excessive amounts of these substances can cause a continuous damaging oxidative stress. This strengthens our conviction that ozone, used at appropriate doses, can be therapeutically useful (Bocci)\textsuperscript{13}. Nowadays, antibiotic resistant bacteria are rapidly expanding, but some physicians continue to use expensive, and often ineffective antibiotics, while it is possible to use ozone that acts as a potent antibacterial drug.

Ozone is a useful alternative, not only for poor countries like Bolivia, but also for European countries like in Italy, Germany and Spain, where it is widely used. For this reason, ozone therapy should be the first alternative treatment in disc space infection. The effectiveness of ozone shows that very good results can be obtained with this method, with minimum surgical trauma and without any complications.

References


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Care amiche e amici,
questo è il primo annuncio del nostro Congresso 2007, che vi chiedo di lasciarmi aprire con una considerazione personale. Sono stato professionalmente fortunato. Ho scelto tanti anni fa, da neolaureato, una specialità sconosciuta, la neuroradiologia, solo per caso.
Ho così vissuto nell’ultimo quarto di secolo una delle più straordinarie rivoluzioni nella storia della medicina. I neuroradiologi hanno reinterpretato, con l’aiuto del progresso tecnologico, tutta la patologia neurologica, anche definendo nuove entità nosologiche, introducendo tecniche terapeutiche impensabili solo pochi anni prima. Prendendo in prestito una frase famosa, penso che poche volte nella storia (della medicina) tanti (pazienti) abbiano dovuto così tanto a così pochi (specialisti).
Tutto ciò è stato possibile perché in un unico specialista sono racchiuse sia le capacità d’analisi diagnostica, strutturale e funzionale di una lesione, sia la diretta conoscenza, che può venire solo dalla diretta esecuzione, delle possibilità e dei rischi del trattamento; questa sintesi, unitamente all’osmosi di capacità e conoscenze interna alla singola équipe neuroradiologica, consente una visione completa e ragionata della patologia, unica garanzia del successo terapeutico. Tutto ciò ora rischia di andare disperso, in un’assurda frammentazione in specialità diverse delle capacità e delle conoscenze del neuroradiologo. Sono stato fortunato anche perché nella neuroradiologia italiana ho trovato tanti amici ma soprattutto una qualità professionale assoluta, una scuola di rilievo mondiale. Voi mi avete voluto onorare della Presidenza del 23° Congresso Nazionale della nostra Associazione, che si terrà quindi a Bergamo dal 20 al 23 Giugno 2007. Esso sarà dedicato alla nostra volontà di restare attori principali di quella rivoluzione, entusiasmante per chi l’ha vissuta in prima persona, e una ricchezza straordinaria da trasmettere ai giovani. Il filo rosso scientifico sarà quindi costituito da sessioni comuni per diagnostica e interventistica, nelle quali renderci conto della nostra identità unitaria, per mettere a fuoco tutto l’aiuto che le tecniche diagnostiche possono e devono fornire ad una corretta pratica terapeutica neuroradiologica.
Che altro posso dirvi, oltre che spero di avervi tutti?
Bergamo, per chi abbia la fortuna di poterla ancora scoprire, è un gioiello architettonico. Giugno è il mese più bello, il sabato del villaggio. E io credo che la medicina sia anche gioia, come quando negli occhi dei pazienti leggiamo la gratitudine per aver dato per loro il meglio di noi stessi. Quindi mia moglie Marina (neuroradiologa ad honorem) e io c’impegniamo a che il Congresso sia anche un’occasione d’allegria e divertimento per tutti.
Vi aspettiamo.

Beppe Bonaldi

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Epidural Steroid Injection vs Paravertebral \( \text{O}_2\text{O}_3 \) Infiltration for Symptomatic Herniated Disc Refractory to Conventional Treatment
A Prospective Randomized Study

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*** San Rocco Orthopaedic Medical Centre; Montichiari  
**** Sant’Antonio Abate Hospital; Gallarate

Key words: peridural, low back pain, sciatica, ozone therapy, cruralgia, corticosteroids, herniated disc

SUMMARY - The medical management of patients with symptomatic herniated disc of the lumbar spine to treat low back pain, sciatica and cruralgia includes epidural injection of steroids and intramuscular paravertebral infiltration of an \( \text{O}_2\text{O}_3 \) mixture. Our study compared the short (three weeks) and long-term (six months) efficacy of the two treatments after failure to response to conventional medical management (steroids and muscle relaxants). 351 patients were enrolled: 171 (Group A) were treated by epidural steroid injection, while 180 (Group B) underwent paravertebral administration of an \( \text{O}_2\text{O}_3 \) mixture. In the short-term 59% of patients treated by epidural injection and 88.2% (p<0.05) of patients treated with \( \text{O}_2\text{O}_3 \) had a total or near total remission of pain. Long-term outcome remained excellent or good in 47.3% of patients treated by epidural injection and 77.1% (p<0.05) of patients treated with \( \text{O}_2\text{O}_3 \). Given the relative simplicity of treatment administration, limited contraindications and the lack of side-effects, ozone therapy is the first choice treatment in patients refractory to conventional medical management.

Introduction

Backache, with or without sciatica nerve or femoral involvement, affects 90% of the population at least once in their lives and is one of the major causes of working days lost in the western world\(^1\).

Until 15 years ago surgery was the treatment of choice. Nowadays the trend is to adopt conservative treatments given the many limitations of surgery in terms of short and long-term pain resolution\(^2\). The different methods used to treat low back pain and sciatica caused by disc and non-discal disease (osteoarthritis, facet joint syndrome, canal stenosis, etc.) include epidural steroid injections and paravertebral infiltration of \( \text{O}_2\text{O}_3 \) gas mixtures\(^3\)\(^,\)\(^4\)\(^,\)\(^5\)\(^,\)\(^6\)\(^,\)\(^7\).

The aim of this study was to compare the therapeutic efficacy of these two procedures in a randomized prospective study of patients with acute or chronic low back pain with sciatica/cruralgia caused by herniated disc.

Materials and Methods

Between January 2002 and January 2006 we treated 351 patients. All patients presented irradiating low back pain over the sciatic nerve lasting less than 180 days and failure to respond to medical management with steroids, NSAIDs, tramadol and muscle relaxants. After giving their informed consent, patients were randomly assigned to one of two groups.

Patients in the first group (A) were treated by epidural injection of steroid (80 mg, triamcinolone acetonide; Kenacort, Bristol-Myers Squibb, Italy), diluted in 20 ml saline solution, into the intervertebral space of the herniated disc or into the space immediately above it. A maximum of three injections were given at weekly intervals after no or only partial response to treatment.

Patients in the second group (B) were treated with a gas mixture of \( \text{O}_2\text{O}_3 \) (5 ml \( \text{O}_2\text{O}_3 \), at a concentration of 10-20 microg/ml injected bilaterally into
the paravertebral muscle 2 cm from the spinous apophysis of the herniated disc and into the space immediately above and below. In case of failure to respond to the randomized treatment, a cross-over to the other group was planned after four weeks of treatment. In addition to personal details, the type of pain, irradiation, paraesthesias, presence of Lasegue’s sign, any sensory and/or motor deficits and osteotendon reflexes were recorded for each patient. The type of treatment each patient received (epidural steroid or paravertebral) was also recorded but the colleagues evaluating therapeutic response were blinded to this information.

Patients with clinical or electromyographic features of neurogenic or denervating pain were excluded from the study. Each patient enrolled was asked to produce CT or MR imaging documentation not more than six months old.

All infiltrations were carried out using a device (CE class 1B equipment, Alnitec, Cremosano, Italy) for the production of O₂O₃ fitted with photometric detectors of O₃ concentration in the gas mixture (the machine automatically adjusts the change in concentration occurring when the syringe is filled) at a constant pressure during O₃ filling.

Injections in both groups were performed by a team of anaesthetists (A.Z., M.M.B., B.F.) belonging to three hospitals, whereas follow-up monitoring was undertaken by three doctors (R.P., G.T., L.M.) blinded to the type of treatment administered. Clinical outcome was assessed in the short (three weeks) and long-term (six months) using a modified version of the McNab method.

Clinical results were considered excellent with a complete resolution of pain and a return to previous activities; good with a 50-75% reduction of pain and a return to previous activities; satisfactory with a reduction of pain below 30-50% and partial return to previous activities; poor with no response to treatment or a response below 30%.

Statistical analysis was performed using Student’s t-test, the chi-squared test and Fisher’s test when necessary, and results processed using the SPSS 8.0 package for Windows.

Results

Patient details are summarised in table 1.

Table 1 Patient details

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex *</th>
<th>Age (average ± sd)§</th>
<th>Response to conventional treatment °</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>F</td>
<td></td>
</tr>
<tr>
<td>Group A</td>
<td>171</td>
<td>91</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>48 (±3.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>120</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>51</td>
</tr>
<tr>
<td>Group B</td>
<td>180</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>51 (±6.1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>136</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>44</td>
</tr>
</tbody>
</table>

*, §, ° Differences not statistically significant (p >0.05)

Table 2 Short-term outcome

<table>
<thead>
<tr>
<th>No. Patients</th>
<th>Excellent</th>
<th>Good</th>
<th>Excellent/ Good</th>
<th>Satisfactory</th>
<th>Poor</th>
<th>Satisfactory/ Poor</th>
<th>Cross-over</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>171</td>
<td>77 (45.0%)</td>
<td>48 (28.0%)</td>
<td>125 (73.0%)</td>
<td>22 (12.8%)</td>
<td>24 (14.2%)</td>
<td>46 (27.0%)</td>
</tr>
<tr>
<td>Group B</td>
<td>180</td>
<td>131 (72.7%)</td>
<td>28 (15.5%)</td>
<td>159 (88.2%)</td>
<td>14 (7.9%)</td>
<td>7 (3.9%)</td>
<td>21 (11.8%)</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
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</tr>
</tbody>
</table>

Treatment outcome is summarised in tables 2 and 3.

In the short-term 59% of patients treated by epidural injection and 88.2% (p<0.05) of patients treated with O₂O₃ had a total or near total remission of pain (33.3% in the epidural group, 72.7% in the O₂-O₃ group; p<0.05).

Long-term outcome remained excellent or good in 47.3% of patients treated by epidural injection and 77.1% (p<0.05) of patients treated with O₂O₃. 61.1% of patients in the O₂-O₃ group reported excellent results (21.6% in the epidural group; p<0.05).

The patients in the O₂-O₃ group subjected to a
Table 3 Long-term outcome

<table>
<thead>
<tr>
<th>Control of pain</th>
<th>N° Patients</th>
<th>Excellent</th>
<th>Good</th>
<th>Excellent/Good</th>
<th>Satisfactory</th>
<th>Poor</th>
<th>Satisfactory/Poor</th>
<th>Cross-over</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>171</td>
<td>54 (31.5%)</td>
<td>40 (23.5%)</td>
<td>94 (55%)</td>
<td>43 (25.1%)</td>
<td>34</td>
<td>77 (45.0%)</td>
<td>38 (22.2%)</td>
</tr>
<tr>
<td>Group B</td>
<td>180</td>
<td>126 (70.0%)</td>
<td>14 (7.7%)</td>
<td>140 (77.7%)</td>
<td>22 (11.8%)</td>
<td>18</td>
<td>40 (22.3%)</td>
<td>11 (4.4%)</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

Table 4 Short-term outcome after cross-over treatment

<table>
<thead>
<tr>
<th>Control of pain</th>
<th>N° Patients</th>
<th>Excellent</th>
<th>Good</th>
<th>Excellent/Good</th>
<th>Satisfactory</th>
<th>Poor</th>
<th>Satisfactory/Poor</th>
<th>Cross-over</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidural (post O2O3)</td>
<td>11</td>
<td>3 (9.1%)</td>
<td>4 (27.3%)</td>
<td>3 (36.4%)</td>
<td>4 (27.3%)</td>
<td>4</td>
<td>7 (63.6%)</td>
<td></td>
</tr>
<tr>
<td>post epid.</td>
<td>38</td>
<td>17 (35.4%)</td>
<td>17 (35.4%)</td>
<td>34 (70.8%)</td>
<td>9 (18.7%)</td>
<td>5</td>
<td>14 (29.2%)</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>ns</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

ns = not significant

Table 5 Short-term outcome after cross-over treatment

<table>
<thead>
<tr>
<th>Control of pain</th>
<th>N° Patients</th>
<th>Excellent</th>
<th>Good</th>
<th>Excellent/Good</th>
<th>Satisfactory</th>
<th>Poor</th>
<th>Satisfactory/Poor</th>
<th>Cross-over</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidural (post O2O3)</td>
<td>11</td>
<td>2 (18.2%)</td>
<td>2 (18.2%)</td>
<td>4 (36.4%)</td>
<td>4 (36.4%)</td>
<td>3</td>
<td>7 (63.6%)</td>
<td></td>
</tr>
<tr>
<td>post epid.</td>
<td>48</td>
<td>24 (50.0%)</td>
<td>11 (22.9%)</td>
<td>35 (72.9%)</td>
<td>9 (18.7%)</td>
<td>4</td>
<td>13 (27.0%)</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>ns</td>
<td>&lt;0.05</td>
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</tr>
</tbody>
</table>

ns = not significant

Discussion

The origin of sciatic or crural pain is only partly due to mechanical nerve root compression while an aspecific inflammatory reaction plays a major role. Migration of the disc through its natural barrier of the anulus fibrosus exposes the autoantigen-reactive immune system of the mucopolysaccharide matrix on the disc surface. The inflammatory reaction is linked to the release of lytic enzymes like phospholipase A2 and prostaglandins E2. In fact the quantity of these enzymes in the peridiscal epidural space is a hundredfold higher in patients with herniated disc compared to those with only a bulging disc, thereby confirming the inflammatory origin of pain. The inflammatory reaction stimulates the macrophage activity favouring degeneration of disc fragments. The resulting process involves the root ganglion and mainly the nociceptive C fibres, enhancing their...
mechanical sensitivity and generating a painful stimulus\(^4\).

Indirect vessel-mediated mechanical factors are also involved. These may be ischaemic factors linked to compression of the feeding arteriole with impairment of perineural microcirculation and demyelination due to anoxia of nerve fibres, or venous factors causing oedema and partial or total blockage of venous reflux. These mechanisms explain the efficacy of both epidural anti-inflammatory treatment aimed at reducing inflammation and thereby favouring recovery of the ganglionic myelin sheath and hence nerve function \(^15\), and also paravertebral infiltration of an \(\text{O}_2\text{O}_3\) gas mixture. The \(\text{O}_2\text{O}_3\) mixture is claimed to favour the normalization of the level of cytokines and prostaglandins, increase levels of superoxide dismutase and improve the perineural and periganglionic microcirculation with a eutrophic effect on the nerve root, thereby combating the hypoxia linked to both arterial compression and above all to venous stasis. The mixture also seems to have a reflex therapy effect able to break the chain of chronic pain by stimulating antinociceptive antalgic mechanisms \(^11\).

Epidural steroid injections have been widely used in the conservative treatment of symptomatic herniated lumbar disc. Some studies \(^16\) have reported an efficacy between 44% and 77%. Our study had significant short-term results (73.9% excellent/good outcome) with a worsening of symptoms in the follow-up assessment at six months (53.1%).

In recent years treatment by \(\text{O}_2\text{O}_3\) infiltration has yielded significant results with a positive outcome in 65-75% of cases. Our findings are in agreement with literature reports with an excellent/good remission of pain in 88.2% (\(p<0.05\) with respect to the epidural group) of patients.

Only a slight decrease was found at long-term follow-up (77.1%) (\(p<0.05\) with respect to the epidural group). The comparison between the two therapeutic procedures showed that \(\text{O}_2\text{O}_3\) infiltration was significantly more successful in terms of the number of patients with an excellent outcome with short (45.0% vs 72.7%; \(p<0.05\)) and long-term (31.5% vs 70.0%; \(p<0.05\)) remission of pain, vis-à-vis the number of patients reporting satisfactory/poor results in the short (26.9% in the epidural group) and long-term (22.2% vs 4.4%; \(p<0.05\)).

A similar but less successful treatment outcome was found in the cross-over patients. Patients treated by \(\text{O}_2\text{O}_3\) obtained excellent/good short-term results in 70.8% of cases vs 30.4% in the epidural group (\(p<0.05\)), and a similar long-term outcome (72.9% vs 36.4%; \(p<0.05\)).

These findings fit the number of patients who subsequently underwent surgery: 15 patients in the epidural group (8.7%) and three in the \(\text{O}_2\text{O}_3\) group (1.6%; \(p<0.05\)).

**Conclusions**

Most patients with low back pain with or without sciatic or femoral nerve involvement are treated with painkillers (NSAIDs or tramadol) and steroids, sometimes in association with muscle relaxants. Outcome is often satisfactory with complete clinical remission. Nonetheless a variable number of patients fail to respond to medical management, irrespective of whether the choice of associated treatment is appropriate. Until a few years ago, the only remedy available for these patients was surgery. Back surgery effectively attenuates symptoms but its effect over time is limited and tends to cease around four years after surgery \(^17\). About thirty thousand operations a year are performed in Italy and most patients are between the ages of 30 and 50 years. The wide variability among different regions (the majority are performed in Lombardy) suggests that many of these operations are inappropriate \(^17\). Bearing in mind the limitations of surgery and the fact that herniated disc will very often regress spontaneously, it is important for patients to be well informed and involved in the choice of treatment \(^17\). Surgery is however indicated in patients presenting signs of neural injury which could give rise to permanent damage if all other treatments fail \(^17\).

Peridural steroid injection is a valid treatment for patients refractory to medical management. However, this procedure carries the risk of potentially serious complications (post-dural puncture headache, subarachnoid haematoma, infection) although none occurred in our series or in the group receiving \(\text{O}_2\text{O}_3\).

Paravertebral infiltration of an \(\text{O}_2\text{O}_3\) gas mixture is a simpler alternative treatment in terms of administration and more limited side-effects and contraindications. Ozone therapy also has a higher success rate and is the first choice of treatment in patients refractory to conventional management. The possibility of a cross-over from one treatment to the other is a further means of reducing the number of patients referred to surgery.
References


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The Medical Association of Kefalonia and Ithaca

THE HYPERBARIC OXYGEN IN THE TREATMENT OF INFLAMMATORY DISEASES

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Argostoli Kefalonia - Greece

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Intradiscal Ozone Treatment of Nerve Root Compression in the Lumbar Spine

Prof. Marco Leonardi:
Ozone Treatment of Nerve Root Compression in the Cervical Spine

Dr Matteo Bonetti:
Ozone Therapy in Italy

The conference aims to inform the medical community about the possible uses and applications of the ozone in medicine, in particular against the inflammatory diseases, and also to disclose the latest therapeutic methods.

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Administrative Assistant: P. G. Politis
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e-mail: ppolith@biol.uoa.gr
Oxygen-Ozone Treatment of Buruli Ulcer

A. BERTOLOTTI, A. IZZO
InterMed Onlus, Africa Sahariana

Key words: Buruli’s ulcera, ozone therapy

SUMMARY - The Authors introduce turn out to you preliminary of the treatment with oxygen-ozone-terapia of the ulcera of Buruli. Buruli ulceri is a tropical necrotizing skin disease caused by the alcohol-acid resistant mycobacterium Mycobacterium ulcerans. In January 2006 “Intermed Onlus”, an organization working in Africa for several years, first proposed and tested oxygen-ozone therapy using an extremity bag. The results obtained were positive and the ulcers were cured without recourse to antibiotics.

Buruli ulceri is a tropical necrotizing skin disease caused by the alcohol-acid resistant mycobacterium Mycobacterium ulcerans.

The disease is almost certainly transmitted by the bite of an aquatic insect of the naucoridae family (beetle) since a 2002 French study reported the presence of this mycobacterium in the salivare glands of these insects (REF). Mycobacterium ulcerans contains a toxin responsible for the necrosis of subcutaneous tissue.

The mycobacterium was isolated for the first time by MacCallium et Al, in Australia in 1948. In 1958 a number of cases were described in Buruli County, Uganda from where the disease takes its name.

Clinically the disease tends to manifest with a nodule, in some cases with oedema or plaques commonly located on the limbs. Ulceration occurs within two weeks and can be extensive if left untreated.

The current treatment of Buruli ulcer in some areas of Sub-Saharan Africa consists of washing the lesion with sodium hypochlorite, treatment with the antibiotics streptomycin and rifampicin, surgical resection with extensive scarring and functional loss.

In January 2006 “InterMed Onlus”, an organization working in Africa for several years, first proposed and tested oxygen-ozone therapy using an extremity bag.

The results obtained were positive and the ulcers were cured without recourse to antibiotics.

# Health Cooperation

*InterMed Onlus* is an international non-profit organization involved in health care cooperation.

The main aim of InterMed is to promote the human personality through emergency or ongoing health care programs in permanent or emergency way.

# Our Programs

InterMed plans and implements social and health projects in developing countries. Many projects have been implemented to date with good results for the lives and development of many people.

# Aims and Operating Methods

Team-working with public and private nonprofit organizations, InterMed is engaged in improving the quality and efficiency of local health services by promoting basic social health care, building appropriate facilities, training medical, nursing and technical personnel. InterMed projects always respond to specific requests from local communities which have been participating in strategic and operative activities from the outset.

InterMed acts independently from local political or religious institutions showing respect for indigenous cultures in a frame of self-development and self-determination.

# Activities

Here is a list of some projects implemented by InterMed in Africa, Latin America, Asia and Eastern Europe:

- hospitals, city and country dispensaries, laboratories and diagnostic multifunction centers linked to the local national health services (Burkina Faso, Burundi, Congo, Randa, Togo);
– basic health joint projects for community growth, environmental health, health education and training of primary health operators (Benin, China, Avory Coast, Somalia, Tanzania);
– health schools for the education and training of local specialists (Burikina Faso, Congo, Togo);
– emergency interventions to tackle natural or man-made disasters (Albany, Congo, Eritrea, Kosovo, Randa, Democratic Republic of Congo).

Human Resources

InterMed’s staff includes experts in health project planning and management, basic social medicine, health education and training, tropical medicine, emergency aid.

InterMed can use a network of cooperators for:
– feasibility studies and preliminary projects;
– selection and training of outsourced personnel;
– implementation and management of health structures;
– supply of biomedical technology;
– health education and training of local personnel;
– supervision and validation of the operations.

How to Help Us

What You Can Do

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Ask for information from InterMed
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www.intermed-onlus.it; e-mail: info@intermed-onlus.it

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Choose the method you prefer:
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  Onlus, to be sent in a sealed envelope to InterMed
  Onlus - Viale Venezia, 20 - 25123 Brescia.

Bequests and Legacies

All gifts issued to InterMed are tax free.

Figure 1  Nodules: initial manifestation of the disease: appearance of skin nodules.
Figure 2 A,B Full-blown disease with cutaneous ulceration: A) ankle; B) hand.

Figure 3 Ozone treatment.
Figure 4  Patient 1: A woman. Before treatment.

Figure 5  Pazient 1: A woman. Treatment with only two session of O,O_3 administration.
Figure 6: Pazient 2: A woman. Before treatment.

Figure 7: Pazient 2: A woman. Before treatment.
Figure 8  Pazient 2: A woman. Treatment with only two session of $O_2O_3$ administration.

Figure 9  Pazient 2: A woman. Treatment with only two session of $O_2O_3$ administration.

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Oxygen-Ozone Treatment of the Knee, Shoulder and Hip
A Personal Experience

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Key words: oxygen-ozone therapy, medical ozone, hip, knee, shoulder

SUMMARY - This paper describes the author’s experience of treating acute and chronic disease of the large joints (knee, shoulder, hip) by intra and peri-articular injections of microdoses of an oxygen-ozone gas mixture. Three illustrative case reports are given. The patients were assessed before and after treatment. In addition to a resolution of joint pain, patients had a good functional recovery of their daily activities and the treatment was well-tolerated.

Introduction

Ozone is a highly soluble gas with great oxidizing activity. In contact with biological fluids ozone forms lipid oxidation products and reactive oxygen species. These substances react with white blood cells triggering the production of cytokines, proteins strongly conditioning inflammatory reactions, and red blood cells enhancing the oxygen supply to tissues.

Ozone is used to treat many painful syndromes affecting the joints, muscles and tendons even when peripheral neurological impairment is present. Its painkilling mechanism is thought to be based on stimulation of the antinociceptive apparatus mediated by endogenous opioids and serotonin thereby raising the pain threshold. In addition, the marked anti-inflammatory properties of ozone reduce oedema, joint swelling and compression on nerve root structures. Ozone favours tissue hyper-oxygenation following increased vascularization due to neoangiogenesis improving local tissue trophism and the inhibitory capacity of inflammatory metabolites.

There is a consensus in literature reports that the sum of the beneficial effects of ozone reduces local pain favouring the recovery of joint function and motion lost during the painful phase (walking, going up and down stairs, washing, dressing and feeding). Ozone injections (intra-articular, peri-articular or percutaneous) are considered a good treatment with a high success rate, little risk of complications, easy execution, repeatability and stable outcome.

Administration and Dose

After thorough examination of the patient and obtaining signed informed consent to oxygen-ozone injection, the gas mixture is administered. Ordinary sterile conditions are maintained for disinfection of the patient’s skin as well as the syringe, needles and hands. The increasing use of oxygen-ozone injections to treat inflammatory-degenerative diseases of the joints has led to a specific treatment protocol defining the gas concentrations, doses and infiltration techniques to follow. Some authors favour a single intra-articular injection with or without instrumental guidance. Others prefer the peri-articular approach with injection into certain standardized painful points for each district. Experience has shown that there is no single effective dose for all patients: the ozone probably needs to reach an activation threshold to exert its potent pharmacological effects. The latest indications of the Italian Federation of Oxygen-Ozone Therapy clearly recommend smaller amounts of gas than in the past with slightly higher concentrations of ozone. The gas mixture should be injected slowly, always after aspiration. The protocol includes an initial cycle of at least five injections.
at weekly intervals. The infiltration is usually painless. The route of administration and ozone dose are reported in clinical cases for each of the main joints treated.

**Knee**

The knee is the intermediate joint of the lower limb. It is mainly a joint with only one degree of freedom – flexion-extension – even though it also has a second accessory degree of freedom: rotation around the longitudinal axis of the leg which occurs only when the knee is flexed. The knee essentially works in compression, under the effect of gravity. Even though the knee joint has a good mobility under the considerable body load it must sustain, it is composed of two refined joints (one could say three). The first is the inferior distal face of the femur with two distinct joint regions and articular parts and the superior proximal face of the tibia which meets the femur with two articular parts. This anatomical arrangement suggests two separate joints each working independently and placed parallel to one another. The second joint composing the knee is the femoral-rotular (or patellar) 10. During flexion, an unstable position, the knee is particularly exposed to injury to the ligaments and meniscus. In extension, when the knee is in a highly stable joint position, joint fractures or ligament rupture are more common. Knee pain is the main symptom leading patients to consult a specialist. Pain may be invalidating and progressive, exacerbated by movement (while walking or going up and down stairs). Swollen knee is the second symptom: it reflects the synovial membrane reacting to joint inflammation (caused by cartilage degeneration, trauma, overloading joint structures) producing synovial fluid to better lubricate the joint.

Oxygen-ozone therapy is used to treat patients with gonarthrosis (initial and late), inflammatory disease due to overloading, patellar tendinitis, goosefoot bursitis of the knee, synovitis during inflammatory arthritis, bursitis, knee pain caused by joint cartilage degeneration due to femoral-rotular chondropathy, knee pain following surgery.

The patient is placed in a supine position with the knee in extension. After having located the patella its medial margin is pressed to subluxation in the lateral margin to increase the articular rim as much as possible. A 21G needle is inserted along the superolateral margin of the patella (to the superior third). After thorough sepsis and aspiration, 10-15 cc of the gas mixture is injected slowly at variable concentrations depending on the disease and in any case not more than 25 µg/ml. The patient is invited to perform some flexion-extension movements of the knee to better distribute the drug throughout the joint including the recesses.

**Case Report**

A 62-year-old businessman with bilateral knee pain (measured as VAS 8) prevalent on the left and with swelling of both knees (extensive on the left) with increased tactile knee temperature and no skin reddening. Physical examination disclosed painful knee joint movements throughout the range of movements with flexion possible up to 90° on the left and 110° on the right, and complete extension. Painful swelling with increased temperature and skin rubor was evident in the right metatarsal phalange probably due to gout arthritis (the patient was receiving allopurinol treatment). The patient was limited in climbing stairs (up and down) and required a support, in dressing (putting on his socks) and in walking which he managed successfully for 200-300 metres. In addition he complained of pain at night resulting in insomnia not controlled by current anti-inflammatory treatment. On medical examination he produced a knee CT scan and orthopaedic report (predating the onset of joint swelling) noting largely normal findings (“meniscal degeneration, arthrosis of the patella”) and no indication. He underwent blood tests which disclosed acute gout (uricaemia 8.1, Pcr 7.60) with probable polyarticular involvement. He then started treatment with bilateral intra-articular oxygen-ozone injection into the knees of 10 ml gas mixture at a concentration of 20 µg/ml (a clear synovial fluid was aspirated from the joint cavity but we decided not to perform arthrocentesis and to proceed with the infiltration). One week after the first treatment session there was a rapid reduction of joint swelling and pain (VAS 5) with a recovery of bilateral joint function (flexion 120°). At the end of the five weekly injections knee swelling had subsided with almost total remission of pain (VAS 2) and a complete recovery of function (going up and down stairs, walking, putting on socks). The patient was prescribed medical management for the gout (metatarsal phalange joint) per os: a full dose of allopurinol and NSAIDs. The patient did not attend the first follow-up visit one month later and let us know he was busy in a sailing competition. At a recent visit knee joint function was normal with no swelling. The painkilling effects of the oxygen-ozone treatment have persisted a long time after suspension of NSAIDs (previously during pain), the patient readily goes up and down stairs and walks normally. Further follow-up visits have been scheduled, the first in four months’ time.
Shoulder

The shoulder has great freedom of movement and serves to orientate the hand in space and hence to perform most daily activities. The gleno-humeral joint is a highly mobile but not very stable ball-and-socket joint. Its stability is ensured by the glenohumeral fibrous capsule. Joint motility depends on the rotator cuff muscles: supraspinatus, infraspinatus, teres minor and subscapularis.

Figure 1  A-D) MR study of the knee before oxygen-ozone therapy: the scan shows femoral-rotular arthrosis with erosion of the joint cartilage pannus and marginal osteophytic appositions. Erosion of the cartilage pannus of the patella of the trochlear groove apparently without signs of subchondral injury. Continuous collateral ligaments with marked infiltrate of the lateral and medial capsuloligamentous compartments. Extensive bilateral degeneration of the meniscus with barely recognizable meniscal fibrocartilage and residual material extruded from the joint margin. The cartilage pannus appears thin and eroded on both condylar surfaces with signs of subchondral injury at the third middle anterior surface of the medial condyle. Fluid within the joint and infiltration of Hoffa’s fat pad.
The suprhumeral joint is not a proper joint but plays a functional role protecting the head of the humerus. It is made up of the coracoacromial arch (coracoid process, acromion and coracoacromial ligament) and the glenoid cavity. This space houses the subacromial and subcoracoid bursae, the supraspinatus muscle and its tendon, the gleno-humeral capsule and the tendon of the long head of the biceps muscle. The acromioclavicular and scapulocostal joints regulate scapular movement.
(gliding of the scapula on the rib cage) whose scapular part depends on the trapezius and dentate muscles. The synergic action of these two muscles lowers the inferior part of the scapula and rotates it externally making it rotate around the acromioclavicular joint. In turn this movement raises the glenoid cavity. All this occurs during abduction and raising of the arm.

The biceps muscle does not play an active part in glenohumeral movement and its gliding on the bicipital ridge during arm abduction is passive: it is the ridge that moves, whereas the muscle stays still. When this mechanism is hampered shoulder function will be impaired to some extent. When a person complains of pain in the shoulder it is important to rule out an irradiated pain (e.g. from the cervical region) or a visceral pain manifesting in the shoulder (e.g. of cardiac, pulmonary, splenic or hepatic origin).

History-taking, physical examination and con-
control of active and passive movements yield useful information on the origin of pain. It is important to investigate the features of pain such as its origin and extension, bearing in mind that pain arising from the shoulder seldom extends beyond the elbow. It should also be noted how the patient presents to the doctor, the position the painful arm is kept in and how s/he undresses. This should be observed from all angles. In cases of extreme pain caused by microcrystalline bursitis, examination is virtually impossible because of the pain (pain usually subsides after about two weeks of treatment).

The patient must be examined bare chested to search for any swelling, asymmetry and differences in level between one limb and the other and to assess muscle trophism.

Oxygen-ozone therapy is used to treat patients with inflammatory rotator cuff tendinopathy (also due to overloading), tendinitis of the supraspinatus muscle caused by impingement, tenosynovitis of
Oxygen-ozone therapy is used to treat hip pain due to functional overload, pain caused by coxarthrosis (initial and late), trochanteric bursitis, without pain (abduction and flexion 60°, rotations 1/3 of the movement). At the end of the treatment cycle the patient reported an almost total reduction of pain (VAS 2) and a recovery of active shoulder function: abduction and flexion more than 90°, rotation possible for more than 2/3 of the joint excursion, combined abduction and rotation movements with consequent recovery of full autonomy in her daily life. After oxygen-ozone therapy the patient started a series of exercises designed to strengthen the scapulo-humeral muscles and further regain movement in the shoulder joint.

**Hip**

The hip joint is a ball-and-socket joint like the shoulder. It therefore has wide possibilities of movement but whereas the arms are attached to the trunk adding weight to the body, the legs receive 80% of body weight through the femoral heads. Hip movements are achieved only through the coxofemoral joint which is highly stable and well seated. Unloading body weight onto the femoral heads is a stabilizing element facilitating the congruence of joint parts and also contributes to the ground reaction allowing maintenance of an upright posture and under dynamic conditions triggering the force required for walking and running. This explains how the particular function and structure of the hip expose it to ongoing mechanical stress which may be the first cause on disease onset. Patients are often not aware of a hip inflammation because they complain of pain in the anterior thigh and knee rather than the hip where the pathological process is underway. This is known as referred pain called FABER (Flexion, ABduction, External Rotation). The Faber test is simpler to perform than to describe. The patient lies down placing the heel of one leg on the knee of the other. In this position it should be possible to lower the knee to the same level of the other leg so that both knees touch the bed. If there is inflammation the Faber manoeuvre will be painful and in case of consolidated coxarthrosis then the manoeuvre cannot be performed or is only partial due to restriction of movement. When the hip starts to be limited in movements the remote symptoms are manifested not only in the thigh and knee but also in the pelvis and back. When a patient has backache or pains in the side of the pelvis the origin may lie in hip malfunction. Unable to perform certain hip movements, the patient overloads the pelvic joints and/ those of the lumbar spine.

**Case Report**

A 58-year-old housewife with extremely painful shoulder and severely limited joint movement presented for outpatient assessment. She had previously undergone many surgical interventions for “painful shoulder” and in April 2003 she had also undergone shoulder mobilization under narcosis in an attempt to recover even partial joint movement but without success. Before orthopaedic surgery the patient had been treated with physical therapy and steroid injections into the right shoulder without benefit. At physical examination the right shoulder showed diffuse hypertrophy of the muscles of the whole scapulo-humeral joint. Joint movement was only possible for a few degrees in abduction and flexion due to the reduction of movement caused by pain. Pain was assessed as VAS 10 and was present also at night. The patient required help getting dressed and was limited in feeding and personal hygiene movements.

She produced an MR scan report of the right shoulder (July 2004) stating the following key findings: “recurrent rupture of the shoulder rotator cuff ... extensive uncovering of the head of the humerus ... involution of the supraspinatus muscle...”. Oxygen-ozone therapy was prescribed by intra-articular injection (posterior access: under the posterolateral margin of the acromion) and peri-articular infiltration (subacromial bursa + stretch of the long head of the biceps) during the same session using a total of 10 ml gas mixture at a concentration of 15 µg/ml. The patient had only a reduction of nocturnal pain one week after the first treatment session. She subsequently reported a further decrease of shoulder pain (measured as VAS 5) after another two weekly treatment sessions and an initial recovery of active shoulder motion
hip tendonitis and pain following hip surgery. Whenever possible oxygen-ozone therapy should be flanked by a period of rehabilitation with individually prescribed exercises.

The lateral approach is indicated in oxygen-ozone therapy of the hip. After having located the cranial margin of the greater trochanter and after sterilizing the area a 25G needle is inserted in a direction perpendicular to the greater trochanter and after aspiration slowly injecting 5 cc of an oxygen-ozone mixture at a concentration of 25 µg/ml.

Case Report

This method of hip infiltration is called peritrochanteric (lateral approach, perpendicular needle, single injection of 5 cc gas mixture at a concentration of 25 µg/ml) and has been successfully used to treat many patients with hip pain of different origin (coxarthrosis, muscle and tendon disease of the hip, trochanteric bursitis). In particular, we treated a 54-year-old sportsman whose hobby was running. He had already received medical management of hip pain and physical therapies (ultrasound, laser) and functional rehabilitation cycles consisting of masotherapy and joint mobilizations without benefit.

Muscle and tendon ultrasound scan at the start of the patient’s diagnostic work-up showed “right trochanteric bursitis”. Initial physical examination disclosed swelling in the right greater trochanter with increased tactile temperature. Right hip flexion reached 90° with painful arrest, rotations were painful (> intrarotation already at the first degrees of movement), hip pain was present on applying pressure to the greater trochanter and on contrasted abduction of the hip. In addition the patient referred nocturnal hip pain, difficulty putting on his socks and limp on walking with deambulation for about 50 metres. Initial pain assessment was estimated as VAS 9. The patient was treated by a cycle of five ozone therapy sessions (5 cc oxygen-ozone at a concentration of 25 µg/ml) at weekly intervals.

After the first treatment session joint pain subsided with a marked improvement in trochanteric swelling. However, the patient’s hip pain had a fluctuating course during the five treatment sessions with phases of complete remission and episodic reacutization. At the end of the treatment cycle nocturnal and daytime pain assessed on the VAS score had disappeared. MR scan of the right hip at the subsequent follow-up visit five weeks after treatment ruled out the previous bursitis, thereby confirming the clinical finding. This outcome was confirmed at the MR follow-up scan four months later. The patient has had a complete recovery of hip joint movement and resumed walking without limping and going up and down stairs without difficulty. He had also resumed the sports activities he had previously abandoned. He currently attends the ozone therapy outpatients’ clinic for follow-up visits and any injections to consolidate the results obtained.

Our experiences are in line with current literature on the topic and demonstrate the efficacy and versatility of ozone as an agent counteracting joint pain, inflammation and swelling also when administered in small amounts at weekly intervals. Ozone also proved able to spread through tissues even after percutaneous periarticular administration with no adverse reactions whatsoever.
References


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Xinjiang is a fine place

"Our Xinjiang is a fine place, with beautiful scenery around Xinjiang...". It is the beginning of the popular song Xinjiang is a Fine Place sung all over China.

The song eulogizes Xinjiang’s charm and fertility as well as splendid prosperity.

Located in China northwest, Xinjiang Uygur Autonomous region covers land of 1.66 square kilometers, one sixth of the country’s total area. It borders on the eight countries of Mongolia, Russia, Kazakhstan, Kirgizstan, Tajikistan, Afghanistan, Pakistan and India, with a border-line of 5,000 kilometers.

Xinjiang is a multi-national compact community denominated Uygur nationality, with a population of 19.25 million.

Urumqi is the capital of the region.

Located in the inner-land of Euro-Asian continent, Xinjiang is featured by splendid mountains and rivers, charming scenery, particular and primitive topography, with the Altay Mountains in the North, the Kunlun Mountains, Karakorum Mountains and the Altun Mountains in the south; the Tianshan mountains towering in the middle, which separates Xinjiang into southern and northern areas and forms the Tarim Basin and the Junggar Basin. The natural beauty is made up of imposing glaciers and snow ridges. Our visit allowed us to admire the sights of Xinjiang like the Ancient City of Jiaohe and the Kizil Thousand Buddha Caves in the Muroty valley and the Flaming Mountain in Turpan.

Xinjiang is a fine place, a straordinary experience for me and Prof. Leonardi.
I SABATI DI OZONOTERAPIA
16 Settembre 2006
Istituto Clinico Città di Brescia

Reportage

Dr.ssa Pacico

Dr Gjonovich

Dr Morosi

Dr Chimenti e Dr Parodi

Segreteria Organizzativa:
X-RAY SERVICE:
Tel +39.030.3197173 - Fax +39.030.3197171 - E-mail: info@xrayservice.it
Conoscere e capire la terapia con ossigeno-ozono

Docenti: Dr Gian Luca Maria Alati Dr Alberto Alexandre Dr Emma Borrelli Dr Antonio Gjonovich Dr Luca Rigobello

21 ottobre 2006 - ore 9.00

Sala Riunioni - Euganea Medica
Via Colombo, 13 Albignasego (Padova)

Info: 049.86.50.374/371
formazione@datamedica.it

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Federazione Italiana di OSSIGENO-OZONOTERAPIA
Il Corso Itinerante in Ossigeno-Ozonoterapia, viene effettuato allo scopo di divulgare la pratica clinica dell’Ossigeno-Ozono secondo le linee guida prefissate dalla Federazione Italiana di Ossigeno-Ozonoterapia. Il corso viene diviso in tre sezioni: La prima dedicata al razionale della terapia, la seconda alle linee guida di trattamento e la terza alla dimostrazione pratica delle varie applicazioni.

Il Corso si terrà presso Hotel River Chateau, Roma ed è riservato a 90 medici.

Il corso di aggiornamento si svolge in una giornata della durata di 9 ore, al termine della quale verrà effettuato test di valutazione ECM. Al termine verrà consegnato un attestato di partecipazione.

L’iscrizione è aperta fino a 1 settimana prima del corso e prevede una quota di 75,00 Euro per i soci FIO; 100,00 Euro per i non soci, comprensiva di materiale didattico e colazione di lavoro.

**PROGRAMMA**

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**Relatori**

- Alberto Alexandre - Treviso
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- Velo Bocci - Siena
- Matteo Bonetti - Brescia
- Luigi Brina - Bari
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- Marco Leonardi - Bologna
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FIRST ANNOUNCEMENT & CALL FOR ABSTRACTS

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Sincerely yours,
Dr. A. Fontana
W.F.O.T. (World Federation of Oxygen - Ozone Therapy)
World Federation of Oxygen - Ozone Therapy

First name ........................................... Family name ...........................................

Institution ..........................................................

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Street and number .............................................

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City - State - Post-code ......................................

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mail: info@xrayservice.it
<table>
<thead>
<tr>
<th>Option</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 year Italy</td>
<td>Euro 50,00</td>
</tr>
<tr>
<td>1 year European Countries</td>
<td>Euro 55,00</td>
</tr>
<tr>
<td>1 year non-European Countries (air mail)</td>
<td>Euro 60,00</td>
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Al Presidente della FIO

Il sottoscritto/a .................................................................

Residente in via .................................................................

CAP .......... Città .................................................................

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Allega un breve curriculum vitae (una pagina)

Data ..................... Firma .................................................................

Mi impegno al versamento della quota sociale annua di 125,00 €.
Di cui 85,00 € come iscrizione alla FIO e 40,00 € come abbonamento alla Rivista Italiana di Ossigeno-Ozonoterapia, organo ufficiale della FIO. Banca Carige agenzia 2 di Brescia, CIN: k abl 06172 cab 11202 C/C 000000624780, oppure inviare con bollettino postale C/C nr. 43650316, intestato a F.I.O. (Federazione Italiana di Ossigeno-Ozonoterapia).

Dr Matteo Bonetti
Segretario FIO

Presso: X-Ray Service Srl
Sede amministrativa: Via Leonardo da Vinci, 20 - 25100 Brescia

Sede operativa: c/o Casa di Cura Sant'Anna, Via del Franzone 31 - 25100 Brescia

Tel.: 030.3197173 - Fax: 030.3197171
e-mail: segreteria.fio@cdcsantanna.it

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Dear Colleague,

This is a reminder that the Association membership fee for 2006 is €125,00, inclusive of a subscription to the Rivista Italiana di Ossigeno-Ozonoterapia, payment by bank draft to Banca Carige - agenzia 2 - Brescia, Italia.

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Yours sincerely,

Dr Matteo Bonetti
FIO Secretary

Objeto: cuota de asociación

Estimado Colega,

quería recordarte que la cuota de asociación por el año 2006 es de €125,00, la que incluye la subscripción a la Rivista Italiana di Ossigeno-Ozonoterapia, con un pago en la Banca Carige - agencia 2 - Brescia, Italia.

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Te agradezco desde ahora por el pago de la cuota.

Cordialmente

Dr Matteo Bonetti
Segreteria FIO

Oggetto: quota associativa

Caro Collega,

desidero recordarti che la quota sociale della FIO è per il 2006 di €125,00, comprensiva dell’abbonamento alla Rivista Italiana di Ossigeno-Ozonoterapia, con un bonifico alla Banca Carige - agenzia 2 - Brescia, Italia.

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oppure inviare con bollettino postale: c/c nr. 43650316, intestato a F.I.O.
(Federazione Italiana di Ossigeno-Ozonoterapia)

Ti ringrazio fin da ora per il pagamento.

Cordialmente

Dr Matteo Bonetti
Segreteria FIO
INSTRUCTIONS TO AUTHORS

Rivista Italiana di Ossigeno-Ozonoterapia is a clinical and practice journal documenting the current state of neuroradiology practice. The journal publishes original clinical observations, descriptions of new techniques or procedures, case reports and articles on the ethical and social aspects of health care. Papers are accepted on the understanding that they are subject to peer review, editorial revision and, in some cases, comment by the editors. Manuscripts are examined by independent anonymous reviewers. All authors remain anonymous to the reviewers, in line with international standards. Manuscripts submitted in English will be edited and corrected if necessary. Articles and other material published in the journal represent the opinions of the authors and should not be construed to reflect the opinions of the publisher.

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GENERAL GUIDELINES – Original articles should be organized in the customary format of: Summary, Introduction, Methods, Results, Discussion and Conclusion. Case reports should be concise, clear and well documented. Technical notes will offer brief descriptions of techniques with possible applications. Summaries and captions will be published in English and Italian.

REFERENCES – References should be prepared carefully. Journal names should be abbreviated according to Index Medicus using the following format:

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