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The Official Journal of
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FIO - Italian Federation of Ozone Therapy,
ACEOOT - Spanish Association of Ozone Therapy,
Hellenic, Indian, Slovach and Chinese National Societies

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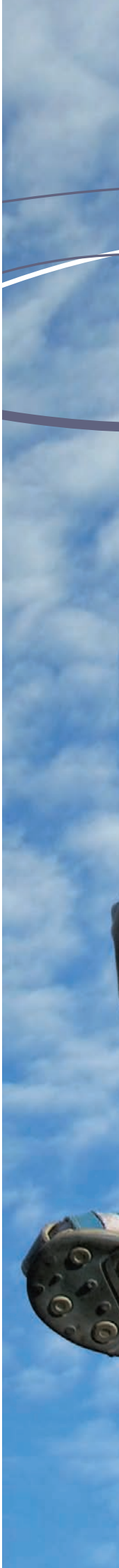

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"It is the only moment you can dream ..."

Bologna, 23 May 2010

Dear Friends,

I invited all neuroradiologists worldwide to take an active part in the 2010 Symposium, suggesting they present at least one paper: the best, the most interesting, or simply dealing with an intriguing topic to be discussed with other colleagues.
We have received more than 800 abstracts.
I believe we have the basis for a very interesting meeting, open to discussion as never before. All the communications have been accepted and will be allocated 15 minutes: 8 for presentation and 6-7 for discussion. This is designed to overcome the usual lack of discussion time, and no moderator will be allowed to ask for "just a burning question"! I am relying very much on the help of SNR moderators to develop a real discussion and analysis of the papers presented.
Posters will be exhibited and electronic posters will be proposed on the Symposium website from September, to allow Symposium participants to read them and possibly prepare for a personal meeting with the author in Bologna.
To organize such a program I plan to set up a large number of parallel sessions. Of course this is a compromise: it would be preferable to have all the works in the main hall to stay together and follow everything, but this would need a congress lasting more than a month! Moreover, we all have our own particular topics of preference to pursue and this organization is the only way to have all the contacts and discussions we desire.
I look forward to welcoming you to Bologna.

Best regards,

Marco

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Oxygen-Ozone Therapy Could Help Patients with Sickle Cell Disease

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Key words: sickle cell disease, oxidative stress, nitrogen monoxide, oxygen-ozone

SUMMARY - *The pathogenesis of sickle cell disease (SCD) was recently reviewed but so far therapeutic options remain limited for reducing the suffering, morbidity and mortality of this devastating disease. Our therapeutic approach was published in 2004 but, regretfully, it was overlooked by recent reviewers. Concomitant clinical experience in chronic limb ischemia has indicated that a judicious ozonated autohemotherapy can be more useful than hydroxyurea and chronic transfusions, which have side-effects and present considerable risks. It is therefore felt that ozone therapy should be evaluated by performing a controlled clinical trial in SCD patients. There are good reasons to believe that the quality of life of these patients can be improved without adverse effects.*

Introduction

The complex pathogenesis of sickle cell disease (SCD) was recently discussed by Aslan and Freeman[1] and by Wood et al.[2] in excellent reviews, correctly pointing out that the available treatments, i.e., hydroxyurea, chronic transfusion and haematopoietic stem cell transplant "*are less than satisfactory*".

Several other approaches such as iron chelation, nitrite therapy, L-arginine and antioxidant supplementation have also been discussed but the use of a combination therapy presents practical problems and may be partially useful. Obviously prevention by genetic counselling is highly recommendable and, whenever possible, bone marrow transplantation from a matched donor represents the only cure for a child as otherwise only 2% of some 120,000 affected babies born in Africa survive to the age of five.

Discussion of the problem

The vascular endothelium is central to disease pathogenesis and Wood et al.[2] emphasized the need for future research but they did not mention our therapeutic approach published[3] in 2004. Having been involved for almost two decades in clarifying the biological mechanisms exerted by ozone in human blood, we have realized that a small and calculated dose of ozone activates a number of biochemical pathways, which, surprisingly, are very useful in vasculopathies, particularly chronic limb ischemia[4-6]. It is an interesting coincidence that SCD manifests itself in large measure as a vasculopathy.

Although it seems paradoxical, a therapeutic small dosage of ozone, a gas with strong oxidant properties, judiciously calibrated against the potent antioxidant capacity of human blood, improves the metabolism of erythrocytes and oxygen delivery into ischemic tissues.

During reinfusion of the ozonated blood into the donor, traces of lipid peroxides enhance the production and release of NO and nitrosothiols, which are very beneficial in inducing vasorelaxation, decreasing platelet aggregation and expression of proinflammatory adhesion molecules by the endothelium.

Moreover the repetition of small acute oxidative stresses, acting as an oxidative preconditioning stimulus, induces an upregulation of antioxidant enzymes while a trace of free haemoglobin (less than 1%) induces heme-oxygenase-1[7].

An increased level of bilirubin and CO is known to exert protective and dilatatory effects on the vascular bed. Today the fact that an oxidant used in therapeutic dosages like other physiological gases such as NO and CO, can act as a useful and atoxic trigger is no longer surprising.

In conclusion, careful blood ozonation appears one of the few effective procedures for correcting chronic oxidative stress[8].

A preliminary evaluation was performed by Cuban physicians in 55 SCA patients: while the control group (25) received only analgesics, vasodilators and IV saline infusion, the experimental group (30) also received an ozone daily dose, five days a week for three weeks [9], via rectal route.

The experimental group displayed a rise in arterial pO_2 and a significantly reduced (by about 50%) frequency and severity of painful crises. No side-effects were recorded. However they used an empirical approach while in our vasculopathic patients we use a precise ozonated autohemotherapy twice weekly [8].

A proposal

In Italy we have very few SCA patients whereas in the United States there are some 100,000 patients so that a well-controlled clinical trial could be performed. The sterile material used for the autotransfusion costs about 12 US dollars, ozone, prepared *ex tempore* by medical oxygen has practically no cost, toxicity is absent [10,11] and the experience gained after millions of treatments all over the world has shown very good patient compliance. I would be only too glad to help any clinical researcher in planning a clinical trial.

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Effect of Ozone Therapy on Redox Status in Experimentally Induced Arthritis

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Key words: ozone, oxidative preconditioning, oxidative postconditioning experimental arthritis

SUMMARY - *Controlled ozone administration has been shown to promote an adaptation to oxidative stress by increasing endogenous antioxidant systems. The present study investigated the effects of O_2-O_3 administration either prophylactically or therapeutically on the alterations of oxidant status in adjuvant-induced arthritis in rats. Seven groups of rats were used: 1) normal control group; 2) control arthritic group (21 days); 3) prophylactic ozone group: arthritic rats received fifteen intra-rectal applications of O_2-O_3 at 0.5, 0.7 and 1 mg/kg b.w. in a 5-6 mL volume starting one day before adjuvant inoculation and continued as five applications/week over 21 days; 4) oxygen group: received oxygen (vehicle of ozone) in a similar schedule to group 3; 5) control arthritic group (24 days); 6) therapeutic-ozone group: arthritic rats received ten intra-rectal applications of O_2-O_3 at 0.5, 0.7 and 1 mg/kg b. w. in a 5-6 mL volume daily for ten days starting 14 days after adjuvant inoculation; 7) oxygen-treated group: received oxygen in a similar schedule to group 6. The effect of O_2-O_3 administration was assessed by measuring: blood glutathione (GSH), erythrocyte glutathione peroxidase and catalase activities, serum levels of protein thiols (PrSH), malondialdehyde (MDA) and nitrite/nitrate (NO_2-NO_3), as well as serum ceruloplasmin activity (CP). The present study showed that adjuvant-induced arthritis in rats caused a significant ($p<0.05$) reduction in blood GSH, serum PrSH levels and erythrocyte antioxidant enzyme activities accompanied by a significant ($p<0.05$) increase in serum levels of MDA, NO_2-NO_3 and CP activity. Ozone administration either prophylactically or therapeutically normalized blood GSH, serum PrSH and MDA levels and restored erythrocyte antioxidant enzyme activities. However ozone did not significantly ($p>0.05$) modify serum NO_2-NO_3 level in induced rats but significantly ($p<0.05$) increased CP activity. In conclusion, NO_2-NO_3 oxidative preconditioning / postconditioning effectively modulated the antioxidant/oxidant balance associated with adjuvant arthritis model in rats.*

Introduction

Ozone therapy as a complementary medical approach has been known for more than four decades. The main areas where this treatment could be useful include resistant infectious diseases, autoimmune diseases, neurodegenerative diseases, orthopedic pathologies and vascular disorders [1]. With the advent of precise medical ozone generators, ozone therapy has met with growing recognition of the use of appropriate and judicious doses making this therapy useful with valuable biological effects [2]. The use of calculated, standardized ozone doses has been found to induce a transient acute oxidative stress condition which is not deleterious but is capable of eliciting multiple useful biological responses. The effect could be seen in activation of an antioxidant defense system, improvement of circulation, oxygen delivery, and trophic processes in tissues as well as enhancement of autocoids, growth factors and cytokine release [3].

Several experimental studies have demonstrated that controlled ozone administration could bring about a state of ozone oxidative preconditioning (O_3OP) or adaptation to oxidative stress, preventing the damage caused by reactive oxygen species (ROS) generated in various experimental models. These include: carbon tetrachloride-induced hepatotoxicity [4], hepatic ischemia-reperfusion injury [5], cisplatin-induced acute renal failure [6], chronic renal failure induced by subtotal nephrectomy [7] and streptozotocin-induced diabetes in rats [8]. More recently it has also been demonstrated that the oxidative postconditioning can be cytoprotective in different experimental model of diseases [9,10]. Experimental arthritis induced by adjuvant is

an experimental model of systemic inflammatory autoimmune disease that shares many features with human rheumatoid arthritis. It involves most of the joints and associated tissues [11]. Although the etiology of rheumatoid arthritis is not fully elucidated, autoimmune destruction of the affected tissues plays a pivotal role in the incidence and progression of the disease [12]. In addition, excessive generation of free radicals and formation of lipid peroxide in target tissues of inflammation are also considered the most common factors implicated in tissue damage in rheumatoid arthritis [13] and experimental arthritis [14]. Thus, a state of oxidative preconditioning such as that achieved with controlled ozone therapy may potentially be able to readjust the redox imbalance in adjuvant arthritis and attenuate the progression of the disease.

The aim of the current work was to investigate the role of ozone as a prophylactic or therapeutic agent in correcting the redox imbalance and the biochemical changes associated with adjuvant-induced arthritis in rats.

Materials and Methods

Animals

Adult male albino rats of Wistar strain, weighing 200-250 g were obtained from the Egyptian Organization for Biological Products and Vaccines (Cairo, Egypt). Rats were housed in plexiglass cages, maintained in an air-filtered and temperature-conditioned (20°C-22°C) room with a relative humidity of 50%-52% and under an artificial light/dark cycle of 12h. Animals were fed with standard laboratory chow and water ad *libitum*. Procedures involving animals and their care were conducted in conformity with the institutional guidelines in compliance with national and international (EEC Council Directive 86/ 609, OJL 358, 1, 12 December 1987; *Guide for the Care and Use of Laboratory Animals*, US National Research Council, 1996) laws and policies.

Chemicals

Complete Freund's adjuvant (Difco laboratories, Detroit, USA) was used for induction of arthritis in rats. It consists of 0.05% heat-killed *Mycobacterium butyricum* suspended in mineral oil. All other chemicals were of analytical pure grade supplied from Sigma-Aldrich St. Louis (USA).

Ozone Generation

Ozone was generated just before application by an ozone generator system [EXT120-T] (Longevity-resources Inc., Canada – ETL approved for proven quality and safety). Ozone obtained from medical grade oxygen represented about 0.4-0.5 % (1 µg/mL–120 µg/mL) of the gas mixture. The ozone concentration was measured using a built-in UV spectrophotometer at 254 nm.

Experimental Design

Adjuvant arthritis induced in rats by a single subcutaneous injection of 0.25 mL of complete Freund's adjuvant into the plantar surface of the right hind foot pad [15]. The peak of adjuvant polyarthritis was reached 14 days after adjuvant inoculation.

Ozone Treatment

Ozone was given by intrarectal application using 20 mL silicone-coated disposable syringe and rectal catheter. Fixed volumes of the O_3-O_2 mixture were administered according to the animal weight so as to reach a final O_3 dose. This route of administration was considered the most useful and easy procedure in rats [16]. For studying the prophylactic or therapeutic effects of ozone on the adjuvant arthritis model, the arthritic rats were divided equally into six groups of eight rats each. The first (prophylactic O_3-O_2 group) received 15 intra-rectal applications of O_3-O_2 over three weeks starting one day before adjuvant inoculation. O_3-O_2 mixture was given as five applications per week. It was started with a relatively low dose of ozone as 0.5 mg/kg b.w. /day in the first week, increased to 0.7 mg/kg b.w./day in the second week and ended with 1 mg/kg b. w./day in the third week. The volume of O_3-O_2 mixture administered was 5-6 mL/rat according to the animal's weight. The second arthritic group received oxygen only (as a vehicle for ozone) in a similar schedule to the first group. The third group of arthritic rats was kept without treatment throughout 21 days and served as a control (arthritic 21 days) for the above two groups. The fourth arthritic group (therapeutic ozone group) received ten intrarectal applications of O_3-O_2 mixture starting 14 days after adjuvant inoculation. The treatment was started by a daily dose of 0.5 mg/kg b.w. for three days, followed by 0.7 mg/kg b.w. for another three days and ended with one mg/kg b.w. for four days. The volume of O_3-O_2 mixture administered was 5-6 mL/rat according to the animal's weight. The fifth arthritic group received oxygen only, 14 days after adjuvant inoculation in a similar way to the fourth group. The sixth arthritic group was kept without treatment throughout 24 days and served as a control (arthritic 24 days) for the fourth and fifth groups. A group of normal rats was left without any treatment and served as a control (non arthritic) group for all the above groups. All the groups were kept under the same conditions throughout the experiment.

At the end of the experimental periods, the animals were sacrificed and the blood was collected in heparinized and non-heparinized tubes, an aliquot of heparinized blood was used to assay glutathione (GSH) [17]. Another aliquot of heparinized blood was lysed directly in ice cold distilled water (5% v/v) and used for the determination of catalase activity (CAT; EC 1.11.1.6) [18]. The remaining heparinized blood was centrifuged for ten minutes at 3000 g for the separation of red cells used to measure the glutathione peroxidase activity (GPx; EC 1.11.1.9) [19]. Instead, the non-heparinized blood was allowed to clot and the separated serum was used for the estimation of malondialdehyde (MDA) [20,21]; protein thiols (PrSH) [22] and nitrite/nitrate (NO_2-NO_3) levels [23] as well as ceruloplasmin (CP) activity [24].

Statistical Analysis

Initially the Outliers preliminary test for detection of error values was applied as a first step in the statistical analysis. After this, the homogeneity of variance test (Bartlett-Box) was used. Values are given as means ± SD.

The level of statistical significance was taken at $p<0.05$, using one way ANOVA followed by Tukey-Kramer's multiple comparisons test to judge the difference between various groups. The SPSS software package version 10, 2000 was used for all statistical analyses.

Results

Blood antioxidant levels in arthritic rats subjected to prophylactic and therapeutic intra-rectal application of ozone: The results for these parameters are shown in Tables 1 and 2. Data demonstrated that 21 or 24 days after adjuvant inoculation, arthritic rats exhibited a significant ($p<0.05$) reduction in blood GSH and serum PrSH levels. The reduction also included GPx and CAT activities compared to normal values.

Intrarectal application of O_3-O_2 as prophylactic therapy (Table 1) caused a significant ($p<0.05$) elevation in blood GSH, serum PrSH, erythrocyte GPx and CAT activities compared to the values present in arthritic animals. In the same way, therapeutic intrarectal application of O_3-O_2 mixture (Table 2) successfully restored these blood antioxidants to levels approaching or exceeding normal values.

Serum levels of MDA and NO_2-NO_3 as well as CP activity in arthritic rats subjected to prophylactic or therapeutic intrarectal application of ozone: As indicated in Tables 3 and 4, adjuvant-induce arthritis caused a significant ($p<0.05$) increase in serum levels of MDA, NO_2-NO_3 and CP activity after both 21 or 24 days of adjuvant inoculation. These data, demonstrated that prophylactic intrarectal application of O_3-O_2 mixture (Tables 3) normalized serum MDA level of arthritic induced

Table 1 Blood antioxidant levels in arthritic rats subjected to prophylactic intrarectal application of an O_3-O_2 mixture.

Groups	*GSH mg/dL*	*PrSH µmol/L*	*GPx (nmol NADPH /min/gHb)*	*CAT (µmol H_2O_2/ min/gHb)*
Normal control	23.9±1.16 [a]	346.6 ± 15.3 [a]	274.4±17.4 [a]	124.5 16.7 [a]
Arthritic (21 days)	19.3±2.39 [b]	276.5± 26.4 [b]	175.7±27.3 [b]	87.6±15.34 [b]
Arthritic pretreated with: O_2	19.9±1.56 [b]	280.7± 24.2 [b]	191.6±25.2 [b]	94.6 ±9.07 [b]
Arthritic pretreated with: O_3-O_2 mixture	23.4±1.27 [a]	333.3±65.04 [a]	244±16.3 [a]	130.7±18.3 [a]

Legend: Data are expressed as mean of 7 observations ± SD; Values with non-identical superscripts are significantly different $p<0.05$ / within the same set. Reduced glutathione, GSH; protein thiols, PrSH; glutathione peroxidase, GPx; catalase, CAT.

Table 2 Blood antioxidant levels in arthritic rats subjected to therapeutic intrarectal application of an O3-O2 mixture.

Groups	*GSH mg/dL*	*PrSH µmol/L*	*GPx (nmol NADPH / min/gHb)*	*CAT (µmol H_2O_2/ min/gHb)*
Normal control	23.9±1.16 [a]	346.6±15.3 [a]	274.4±17.4 [a]	124.5±16.7 [a]
Arthritic (24 days)	18.3±3.19 [b]	290.1±13.9 [b]	178.7±39.9 [b]	100.8±8.1 [b]
Arthritic treated with: O_2	18.1±2.2 [b]	295.7±9.44 [b]	193.7±43.7 [b]	104.1±20.1 [b]
Arthritic treated with: O_3-O_2 mixture	24.6±3.9 [a]	338.9±17.9 [a]	249.6±15.4 [a]	128.7±8.8 [a]

Legend: Data are expressed as mean of 7 observations ± SD; Values with non-identical superscripts are significantly different $p<0.05$ / within the same set. Reduced glutathione, GSH; protein thiols, PrSH; glutathione peroxidase, GPx; catalase, CAT.

Table 3 *Serum levels of MDA, NO_2-NO_3 and CP activity in arthritic rats subjected to prophylactic intrarectal application of an O_3-O_2 mixture.*

Groups	*MDA nmol/mL*	*NO_2-NO_3 nmol/mL*	*CP U/L*
Normal control	3.62±0.36 [a]	23.2±1.94 [a]	127.4±17.04 [a]
Arthritic (21 days)	4.83±0.8 [b]	33.4±4.4 [b]	210.6±31.05 [b]
Arthritic pretreated with: O_2	4.26±0.49 [b]	31.6±5.28 [b]	217.9±27.5 [b]
Arthritic pretreated with: O_3/O_2 mixture	3.3±0.51 [a]	35.7±4.36 [b]	282.1±42.4 [b,c]

Legend: Data are expressed as mean of 7 observations ± SD; Values with non-identical superscripts are significantly different p<0.05 / within the same set. Malondialdehyde, MDA; nitrite/nitrate NO_2-NO_3; ceruloplasmin activity CP.

Table 4 Serum levels of MDA and NO_2-NO_3 as well as CP activity in arthritic rats subjected to therapeutic intrarectal application of an O_3-O_2 mixture.

Groups	*MDA nmol/mL*	*NO_2-NO_3 nmol/mL*	*CP U/L*
Normal control	3.62±0.36 [a]	23.2±1.94 [a]	127.4±17.04 [a]
Arthritic (24 days)	4.70±0.66 [b]	33.4±6.29 [b]	199.1±36.4 [b]
Arthritic treated with: O_2	4.12±0.37 [b]	29.7±2.88 [b]	187.6±22.6 [b]
Arthritic treated with: O_3/O_2 mixture	3.43±0.21 [a]	30.1±3.95 [b]	252.0±51.9 [c]

Legend: Data are expressed as mean of 7 observations ± SD; Values with non-identical superscripts are significantly different p<0.05 / within the same set. Malondialdehyde, MDA; nitrite/nitrate NO_2-NO_3; ceruloplasmin activity CP.

rats, but failed to exert any change in serum NO_2-NO_3 level of these rats. Meanwhile, O_3-O_2 pretreatment provided a further elevation of serum CP activity to a level exceeding the arthritic values. Therapeutic intrarectal application of O_3-O_2 mixture (Table 4) caused a significant (p<0.05) reduction in serum MDA level to approach the normal value, together with further elevation of CP activity than the arthritic levels. Meanwhile serum NO_2-NO_3 of arthritic rats was not significantly changed in response ozone treatment.

The results clearly showed that intrarectal application of O_2 (as a vehicle of O_3) in prophylactic and therapeutic treatment did not affect any of the measured parameters compared to the values of arthritic rats.

Discussion

The involvement of ROS in chronic inflammatory conditions such as rheumatoid arthritis and adjuvant induced-arthritis is well documented. ROS once generated provoke deleterious effects on various cellular components, including membrane lipids which are extensively subjected to peroxidation. Aggravation of arthritis was reported to be associated with enhancement of lipid peroxidation [25].

In the present study, overproduction of ROS in an adjuvant arthritis model led to a considerable oxidant stress as indicated by a high serum level of MDA, a marker of lipid peroxidation, as well as consumption of blood antioxidants such as GSH and PrSH. The marked increase in serum MDA was observed in arthritic rats in line with our results in an arthritic rats model [26-28], and in rheumatoid arthritis patients [29]. Increased lipid peroxide in arthritic rats is exacerbated by the decline in blood antioxidants. Similar results on GSH were reported in an arthritic rats model and rheumatoid arthritis patients respectively [30,31]. In the same line, a marked decrease in GSH concentration was observed in the joint articular cartilage of arthritic rats [32]. The reduction of GSH might be attributed to the increased consumption to counteract oxidative stress during inflammation. Increased oxidative stress was reported to enhance the formation and efflux of glutathione disulfides [33]. Moreover, the observed reduction in serum PrSH is in line with previous studies [30,34]. Such reduction could be attributed to the excessive consumption by peroxide [35] and/or to a low serum albumin reported in other studies, since most serum SH (85-90%) are found in albumin [36].

In the current investigation, the decline in blood antioxidants also included erythrocyte GPx and CAT activities. The observation is consistent with a previous paper [26,27]. Defective antioxidant enzyme machinery had been observed in erythrocytes of rheumatoid arthritis patients [37] and in liver, kidney and heart of adjuvant arthritic rats [38]. The increased

production of superoxide anion, H_2O_2 and hydroxyl radicals demonstrated by Ramprasath et al.[39] might be responsible for inhibition of GPx and CAT activities.

The role of NO and other reactive nitrogen species in inflammation has not been conclusively established. However, there is evidence of the implication of NO in the process of inflammation and that NOS inhibitors possess potential anti-inflammatory effects[40,41].

The present results revealed a significant ($p<0.05$) increase in serum NO level in arthritic rats (measured as total NO_2-NO_3). Such result is in line with previous reports[27,41] in arthritic rats and in rheumatoid arthritis patients[42]. The overexpression of iNOS in arthritis might result from increased production of IL-1 and other pro-inflammatory cytokines characteristic of that disease[40].

Moreover, a major systemic event occurring in the rat following the induction of inflammation is the marked change in the level of serum CP, an acute phase protein[43]. In the present study, a marked elevation in serum CP activity was observed. Such elevation is consistent with previous observations[30,34] in arthritic rats. The increase in serum CP activity might be due to an increase in its hepatic synthesis triggered by increased secretion of IL-1, epinephrine and glucocorticoids [44]. Furthermore, such an increase in CP activity has been reported to play a role in downregulating inflammatory mediators and inhibiting lipid peroxidation[43].

In the present study, prophylactic intrarectal application of O_2-O_3 to arthritic rats over three weeks exerted protective effects on some important blood antioxidants (GSH, PrSH, GPx and CAT) and preserved them to pre-arthritic values (Table 1). The present data, demonstrated that therapeutic intrarectal application of O_2-O_3 for ten days after development of adjuvant arthritis attenuated the reduction in blood antioxidants and restored the levels of these defense constituents to values close to or above normal levels (Table 2). Moreover, these stimulant effects of O_2-O_3 therapy on blood antioxidants were accompanied by a decrease in serum MDA level to reach normal levels (Tables 3 and 4). These positive experimental observations could be explained in the light of ozone's ability to up-regulate the antioxidant system, a state reached under controlled use of O_2-O_3 [45]. Ozone post or preconditioning is analogous to other phenomena such as ischemic preconditioning[46], thermal preconditioning[47], chemical preconditioning[48], and ischemic protconditioning[10,49]. All of these processes share the fact that a repeated and controlled stress is able to provide protection against prolonged severe stress.

A point that should not be overlooked is that O_3 adaptation caused by judicious use of O_3 is due to the fact that O_3 instantaneously reacts with biomolecules generating ROS e.g. H_2O_2 and lipid peroxidation products (LOP). These molecules can elicit the upregulation of antioxidant enzymes such as SOD, GPx, GSH-reductase and CAT. In bone marrow cells, particularly during erythrogenesis, submicromolar concentrations of LOP can upregulate the synthesis of antioxidant enzymes[2]. Interestingly, Iles and Liu[50] demonstrated that by inducing the expression of glutamate cysteine ligase some LOP cause an intracellular increase in GSH. These findings might account for the generation of biochemically improved erythrocytes during prolonged O_3 therapy. Erythrocytes have been shown to respond to O_3 therapy with activation of glycolysis and the pentose phosphate pathway[51].

In the current study, upregulation of erythrocytes GPx and CAT by O_2-O_3 might be responsible for the preservation of blood GSH and serum PrSH from oxidation by ROS in arthritic rats. Furthermore, the reported activation of the pentose phosphate pathway might play a role in restoring the GSH level from its oxidized form.

On the other hand, prophylactic and therapeutic rectal applications of O_2-O_3 therapy provided further elevation of serum CP activity than the arthritic levels (Tabless 3 and 4). That effect could be explained on the basis that O_3 acts as a mild enhancer of the immune system through activation of gene/regulatory nuclear factor kappa B (NF-κB) by H_2O_2, one of the major decomposition products of O_3. Activation of that transcription factor switches on some genes that are responsible for the synthesis of several proteins including acute phase reactants and numerous interleukins[52]. The increased CP activity might reflect the improved antioxidant status of animals subjected to O_3 therapy. Moreover, the O_3-induced increase in CP activity could be beneficial to prevent against oxidative stress observed in adjuvant-induced arthritic rats.

In the present study, the remarkable enhancement of antioxidant status of arthritic rats provided a protection against ROS and suppressed the process of lipid peroxidation leading to normalization of serum MDA level. Another point which should be considered is the inability of O_3 therapy to change the serum level of NO than was raised in the arthritic induced rats. This effect might be related to the stimulatory effect of O_3 on blood GSH. NO readily reacts with GSH and other cysteine-containing compounds forming S-nitrosothiols with half lives of 5-50 min, in contrast to the very short half-life of NO[53]. Thus, formation of S-nitrosothiols in response to O_3 therapy may allow more pharmacological effects at distant sites.

Conclusions

In conclusion, O_2-O_3 pre or postconditioning effectively improved the antioxidant/oxidant imbalance associated with adjuvant arthritis in rats. These results potentially support the use of ozone therapy as an integrative medical medical approach in rheumatoid arthritis. However, further studies are needed to verify the benefit of O_3 therapy in rheumatoid arthritis at biochemical and clinical levels.

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Effect of Intra-Articular Ozone Injection on Degenerative Knee Cartilage in Rats

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Key words: ozone, osteoarthritis, cartilage, SOD, MDA

SUMMARY - *This study explored the therapeutic effects of medical ozone on cartilage in animal models of osteoarthritis (OA). Forty healthy adult Sprague-Dawley rats were randomly divided into five equal groups: group A (normal), group B (model), group C (air), group D (35μg/ml O_3) and group E (70 μg/ml O_3). OA models were created by transecting the anterior cruciate ligament (ACL) and excising a third of the medial meniscus in groups B, C, D and E. Air was injected into the joints in group C. Ozone with 35 μg/ml O_3 and 70 μg/ml O_3 were injected into the joints in groups D and E respectively every other day, with 1-1.5 ml each time. Injections were made altogether for two weeks at one week intervals. The animals were killed after four weeks to harvest condylar cartilage of femur for HE staining and observation under a transmission microscope. Mankin's indexes were recorded. Douche fluids from the diseased joints were obtained for measurement of superoxide dismutase (SOD) and malondialdehyde (MDA). In OA models of group B, obviously degenerative changes were observed under microscopy, resulting in mostly blue and green color destaining of Masson staining in superficial cartilage. The activity of SOD in the douche fluid from the diseased joints decreased and the quantity of MDA in the serum increased. Mankin's indexes in group D were significantly higher than those in groups B and C (P<0. 05), but not significantly different from those in group A. SOD levels were increased and MDA levels decreased. There were no significant differences between groups E and B in Mankin's indexes. SOD levels decreased and MDA levels increased. 35 μg/ml O_3 can prevent degeneration of articular cartilage and improve the ability to clear free radicals, but 70 μg/ml O_3 may result in peroxidatic reaction of tissue and cells, leading to articular cartilage damage.*

Osteoarthritis (OA) is one of the chronic degenerative diseases of the joint. The chief AO pathological change is destruction of the surface of articular cartilage [1]. With a wide clinical use of O_3 in recent years [2], some orthopedists injected O_3 into the joint to cure OA and have achieved good results. In order to better understand the therapeutic mechanism of O_3 in treating OA, the present study observed the effects of medical ozone injection at different concentrations on the microscopic structure of the knee joint in the rat models with OA, and determined the SOD activity and content of MDA in the joint irrigation fluid

Materials and Methods

Materials

Experimental animals: Forty SPF grade Sprague-Dawley (SD) rats weighing 160 g to 220 g, complex diet fed provided by the Examination Animal Center of Southern Medical University, (animal certification: 2006B023).

Experiment reagents: superoxide dismutase (SOD) and malondialdehyde (MDA) kits provided by Nanjing Jiangcheng Bioengineering Institute; Medical ozone generator (Humazon® Promedic®, Made by HUMARES®GmbH).

Laboratory apparatus: an Olympus optical microscope, an analysis system of histological section, a spectrophotometer of 550 nm, a thermostat water bath pot of 37°C, and a desk centrifuge.

Methods

Model preparation

After the experimental animals were anesthetized [3] with 2 ml of ketamine, the hair was removed from the operating area, cleaned with wet absorbent gauze, disinfected with iodine and protected with normal drape. The skin and joint cavity were

cut open near the medial surface. Then the whirbone was turned over backwards and outwards. The knee joint was made in a maximal bending condition in order to expose the joint cavity better. The medial meniscus was separated with micro-instruments. A third of the meniscus was removed in the medial surface using microscissors and the anterior cruciate ligament was sheared carefully to avoid damaging the cartilage. Then the joint cavity was cleansed with isotonic sodium chloride. The joint capsule and skin were sutured with 0-3 leptonemaa. The wound was cleansed with iodophor cotton balls and covered with dressing for injection. Intramuscular injection of 1/3 of an amikacin was conducted before and during operation. The animals were put into cages and fed routinely. Close observations were conducted to make sure no wound infections or complications occurred. The model group received transection of anterior cruciate ligaments and partial meniscectomy of one third inside with micro-instruments without any other special handling.

Animal grouping

Forty SD rats in a SPF grade were randomly divided into five equal groups: group A (normal group), group B (model group), group C (air group), group D (O_3 group with a concentration of 35 μg/ml) and group E (O_3 group with a concentration of 70 μg/ml). Groups B, C, D and E were made OA models. The model group was sacrificed immediately after animal grouping was finished. Groups D and E received intra-articular injection of 1ml O_3 every two days in the first and third weeks. The O_3 with the concentration of 35 μg/ml was for group D and 70 μg/ml for group E. The air group served as controls, in which 1ml air was injected into the knee joint cavity every two weeks and the rats were sacrificed four weeks later.

Specimen harvesting

Four weeks later, the rats were narcotized and fixed on the operating table. The skin and hypoderma were cut open to show the articular capsule, carefully avoiding cutting open the articular capsule. Then 200 ul of axenic PBS was injected into articular cavity. Lavage fluid was obtained carefully after the joint was moved repeatedly. An opening was made for suction when needed. The irrigating solution was stored at minus 20 degrees. Then the femoral medial condyles and tibial plateau cartilages were harvested as soon as the rats were killed by breaking off their necks. The tissue mass was minced into pieces of 0.5 cm×0.5 cm×0.5 cm in size and the cartilage preparations were fixed with 4% of paraform. The prep was decalcified in 15% EDTA solution for two weeks. The cartilage specimens were embedded with paraffin and observed in HE staining for morphologic changes.

Light microscope observation

After Masson staining, the cartilaginous tissue was graded under light microscope according to Mankin's [4] criteria. The cartilaginous tissue constitution, population of cartilage cells, different shades of Masson coloring and completeness of the Tide Mark were evaluated. The higher the scores were, the more severe the cartilage injury was. The pathologic morphology was observed with HE staining. The expression of superoxide dismutase (SOD) and malondialdehyde (MDA) in joint irrigating solution. Activity of SOD was detected by xanthine oxidase technique and malondialdehyde (MDA) was assayed by TBA method after each joint irrigating solution was diluted using the kits provided by Nanjing Jiangcheng Bioengineering Engineering Institute. The experiment was carried out according to the kit instructions.

Statistical analysis

SPSS 10.0 packages were used to analyze the data. All the data obtained were expressed by $\bar{x}\pm s$ and analyzed by analysis of variance (ANOVA) and the significances were tested by t-test.

Results

Light microscopy of arthrodial cartilage with HE staining:

Normal group

The form and structure of joint cartilage remained normal, without any fissure or infiltrated inflammatory cells. The cells were lined up in order. The sliding layer, transition zone, zona radiata and calcified layer were seen clearly. Masson staining showed the normal bluish-green cartilage cement and red calcified cartilage and bone trabecula collagen.

Model group and air group

The arrangement of the arthrodial cartilage cells was irregular. The surface of the cartilage became rough and contained fissures, deep to the calcified layer. The proliferation of cartilage cells was irregular. Angiogenesis occurred on the articular cartilage surface layer. By Masson staining, loss of bluish-green color could be seen. Flame-like eosin staining rising from the tidemark could be seen as the same as that of the calcified cartilage.

O_3 group with a concentration of 35 μg/ml

The surface of the cartilage looked more regular and there were fewer fissures or fibers on it than

those in the air or model group. A small amount of cartilage cell proliferation could be seen. The form of cells was basically normal and there was less loss of bluish-green color by Masson staining.

O_3 group with a concentration of 70 μg/ml

The arthrodial cartilage cells were irregular and the surface of the cartilage appeared rough. The fissures looked slightly superficial. By Masson staining, the bluish-green loss was more severe.

Knee cartilaginous tissues scoring (Table 1)

The standard Mankin's score was 0.0±0.0 in the normal group. The scores in the model group and the air group were obviously higher than that in the normal group ($p<0.01$). Group D (35 μg/ml) had a lower score than the model group ($p<0.01$). The scores in group E (70 μg/ml) and the air group showed no significant difference ($p<0.05$).

Determination of SOD and MDA in the knee synovial fluid

Determination of SOD

SOD content in the knee synovial fluid was significantly higher in the normal group than those in the model group, air group and 70 μg/ml O_3 group ($P<0.05$, $P<0.01$). There was no significant difference in SOD content between the 35 μg/ml O_3 group and the normal group, but the SOD content in the the 35 μg/ml O_3 group was significantly higher than that in the model group, air group and 70 μg/ml O_3 group ($p<0.05$). This shows that establishment of OA models in SD rats may undermine the capacity of scavenging free radicals in rats. It is consistent with the OA pathological changes and indicates the success of models. On the contrary, the 35 μg/ml O_3 could increase the contents of SOD in the synovial fluid and promote the capacity of scavenging free radicals. The contents of SOD in the synovial fluid could decrease when the O_3 concentration increased to 70 μg/ml. Consequently, the rats could not scavenge the free radicals effectively.

Determination of MDA (Table 2)

MDA content in the synovial fluid was obviously lower in the normal group than that in the model group, air group and 70 μg/ml O_3 group ($P<0.01$). There was no significant difference in MDA contents between the 35 μg/ml O_3 group and the normal group, but the contents of MDA in both groups were lower than those in the air group. Compared with the air group, the MDA contents in the 35 μg/ml O_3 group decreased ($p<0.05$). This shows that the MDA content increased four weeks after establishment of OA model in SD rats suggesting that the MDA contents increased after the attack of oxygen free radicals. The 35 μg/ml O_3 could decrease the MDA content in the synovial fluid but the MDA contents in the synovial fluid could not be decreased when the O_3 concentration increased. Consequently, the free radicals could not be scavenged effectively.

Detection of SOD and MDA in irrigating solution:

Table 1 Mankin's score in each group $\bar{x} \pm s$

Groups	Number of Samples	Mankin's Score
Normal	8	0±0
Model	8	$5.88\pm1.25^{\Delta}$
Air	8	$8.63\pm0.52^{\Delta\Delta}$
35 μg/ml O_3	8	$5.5\pm1.41^{*\Delta}$
70 μg/ml O_3	8	$8.63\pm0.52^{\Delta\Delta}$

Notes: Comparisons between the normal group and the model group, air group, 70 μg/ml O_3 group revealed $^{\Delta}P<0.05$ and $^{\Delta\Delta}P<0.01$. Comparisons between the 35 μg/ml O_3 group and the model group, air group, 70 μg/ml O_3 group revealed $P<0.05$ and $^{*\Delta}P<0.01$.

Table 2 Detection of SOD and MDA in synovial fluid $\bar{x} \pm s$

Groups	Number of Samples	Synovial SOD(NU/mgprot)	Synovial A(nmol/L)
Normal	8	27.61±4.05	2.4±0.5
Model	8	$16.42\pm3.02^{\Delta\Delta}$	$4.9\pm1.2^{\Delta\Delta}$
Air	8	$18.47\pm4.31^{\Delta\Delta}$	$4.6\pm1.3^{\Delta\Delta}$
35 μg/ml O_3	8	$26.23\pm3.86^{*\Delta}$	$2.5\pm0.7^{*\Delta}$
70 μg/ml O_3	8	$17.25\pm3.42^{\Delta\Delta}$	$4.8\pm0.9^{\Delta\Delta}$

Notes: Comparisons between the normal group and the model group, air group, 70 μg/ml O_3 group revealed $^{\Delta}P<0.05$ and $^{\Delta\Delta}P<0.01$. Comparisons between the 35 μg/ml O_3 group and the model group, air group, 70 μg/ml O_3 group revealed $P<0.05$ and $^{*\Delta}P<0.01$.

Discussion

As one of the chronic and systemic diseases, OA is characterized by degeneration of arthrodial cartilage and secondary hyperosteogeny. Our experiment established the OA model in rats by combining resection of anterior cruciate ligament and partial resection of meniscus, since it is a conventional practice both at home and abroad to induce OA by destroying joint stability [4], while intra-articular injection of medical O_3 into the knee is a clinical practice. Therefore, our models can simulate the clinical effects and provide direct experimental evidence for prevention and treatment of OA. Moreover, the present experiment is easy to con-

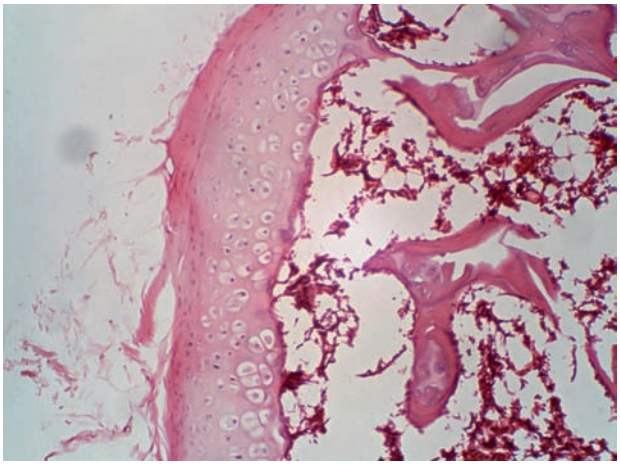

Figure 1 Normal Group (HE Staining ×100). The form and structure of joint cartilage remained normal without any fissure or infiltrated inflammatory cells. The cells were lined up in order. The sliding layer, transition zone, zona radiata and calcified layer were clearly seen.

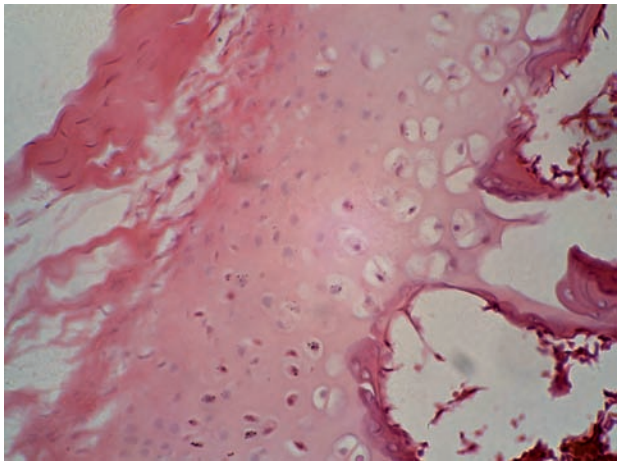

Figure 2 Normal Group (HE Staining ×200). The form and structure of joint cartilage remained normal without any fissure or infiltrated inflammatory cells. The cells were lined up in order. The sliding layer, transition zone, zona radiata and calcified layer were clearly seen.

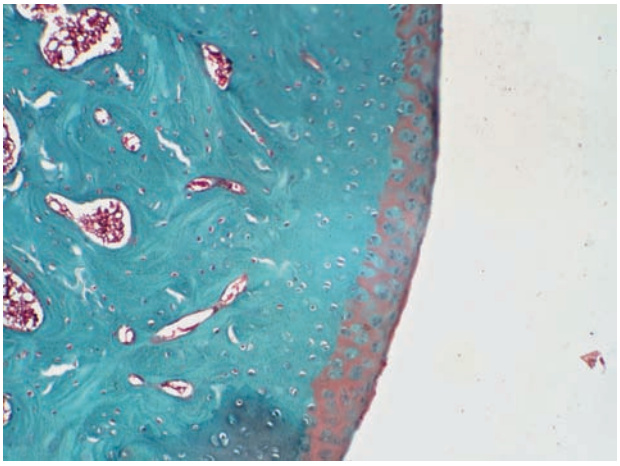

Figure 3 Normal group (Masson stain ×100). Masson staining showed the bluish-green normal cartilage cement. The calcified cartilage and bone trabecula were red.

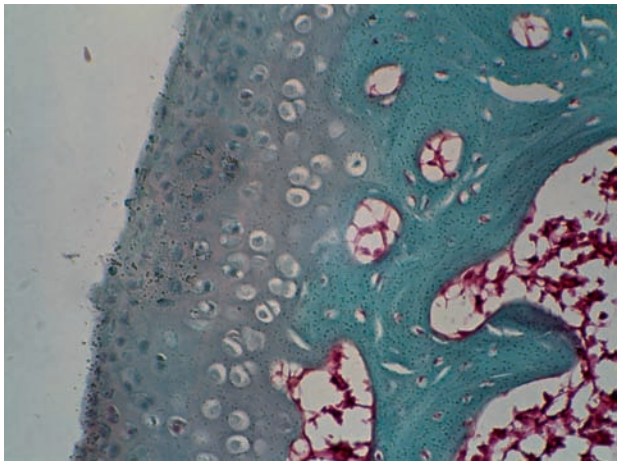

Figure 4 Normal group (Masson stain ×200). Masson staining showed the bluish-green normal cartilage cement. The calcified cartilage and bone trabecula were red.

duct and duplicate in a short cycle, and suits active animals like rats as well. OA can be created in the models four weeks after the operation.

Oxygen-free radicals are one kind of nuclear group which have one or more unpaired electron and very strong reactive activity. Oxygen-free radicals *in vivo* tend to increase in OA patients. The excessive oxygen free radicals in the joint inhibit the composition of proteoglycan and collagen, accelerating degradation of cartilage matrix. It also leads to damage to cartilage cells, apoptosis of cartilage cells [4,5], and a major decrease of cartilage cells. As a result, the arthrodial cartilage becomes atrophic and thin, accelerating damage to the cartilage cells. The free radicals modify the chemical structures of amino acids, polypeptides and proteins, increasing their sensitivity to the proteolytic enzyme, promoting their degradation and causing

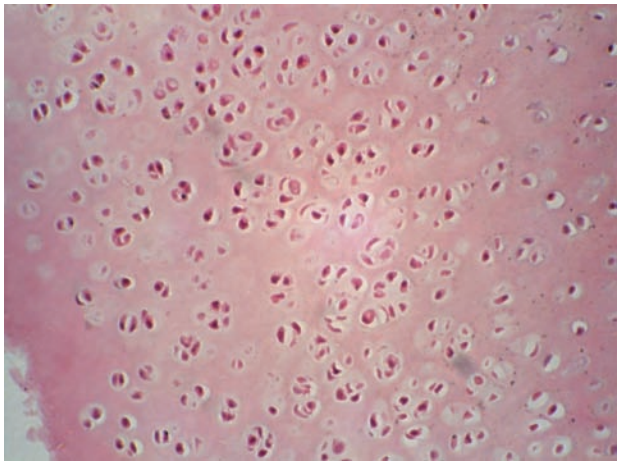

Figure 5 Model group (HE stain×200). The arrangement of arthrodial cartilage cells was irregular. The surface of the cartilage became rough, with fissures on it, deep to the calcified layer. The proliferation of cartilage cells was disorganized. Angiogenesis occurred on the surface layer of articular cartilage. Masson stain showed heavy bluish-green loss. Flame-like eosin rising from the tidemark could be seen, with the same staining on the calcified cartilage.

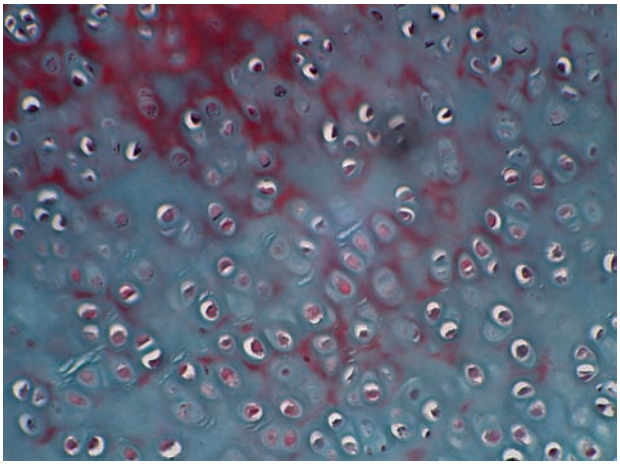

Figure 6 Model group (Masson stain ×200). The arrangement of arthrodial cartilage cells was irregular. The surface of the cartilage became rough, with fissures on it, deep to the calcified layer. The proliferation of cartilage cells was disorganized. Angiogenesis occurred on the surface layer of articular cartilage. Masson stain showed heavy bluish-green loss. Flame-like eosin rising from the tidemark could be seen, with the same staining on the calcified cartilage.

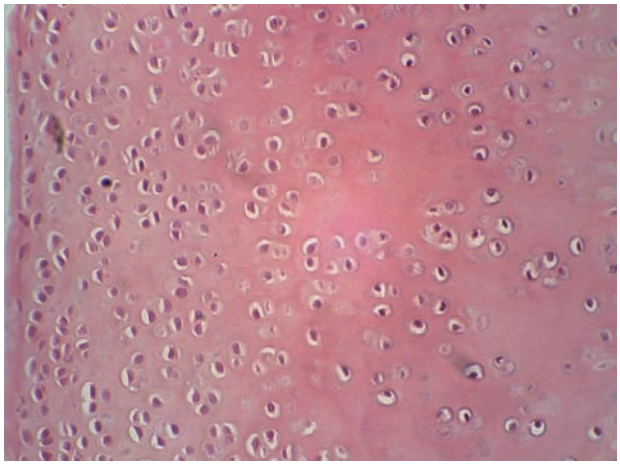

Figure 7 Air group (HE stain×200). The arrangement of arthrodial cartilage cells was irregular. The surface of the cartilage became rough, with fissures on it, deep to the calcified layer. The proliferation of cartilage cells was disorganized. Angiogenesis occurred on the surface layer of articular cartilage. Masson stain showed heavy bluish-green loss. Flame-like eosin rising from the tidemark could be seen, with the same staining on the calcified cartilage.

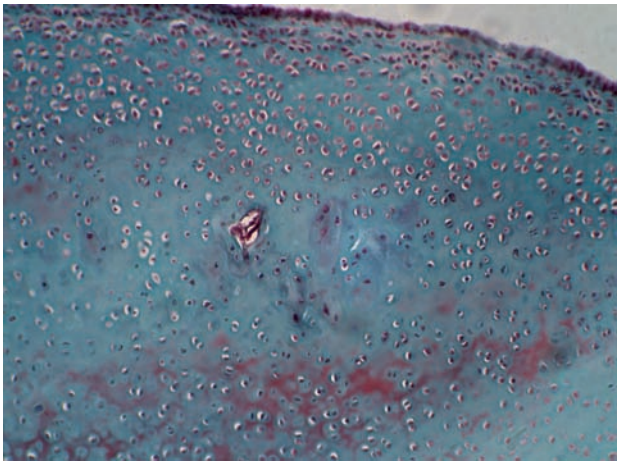

Figure 8 Air group (Masson stain ×200). The arrangement of arthrodial cartilage cells was irregular. The surface of the cartilage became rough, with fissures on it, deep to the calcified layer. The proliferation of cartilage cells was disorganized. Angiogenesis occurred on the surface layer of articular cartilage. Masson stain showed heavy bluish-green loss. Flame-like eosin rising from the tidemark could be seen, with the same staining on the calcified cartilage.

lipid peroxidation in cell membranes. This is the etiology of many diseases.

O_3, a strong oxidant composed of three oxygen atoms, it is nonpersistent and its half-life period is about 20 min at regular temperature. O_3 decomposes and dissolves in water easily. O_3 can restrain inflammatory cell factors, activate cycloxygenase, and reduce the stress response to histiocytic oxidation, increasing the histiocytic capability of resisting oxidation and free radicals. It can also scavenge the free radicals formed in chronic inflammation [6].

This experiment revealed that the cartilage surface looked more regular and there were fewer fissures and fibers on it in the 35 μg/ml O_3 group than in the air group under the light microscope. But in the 70 μg/ml O_3 group, a mild cartilage cell proliferation could be seen, the arthrodial cartilage cells were irregular, there were more fissures, and

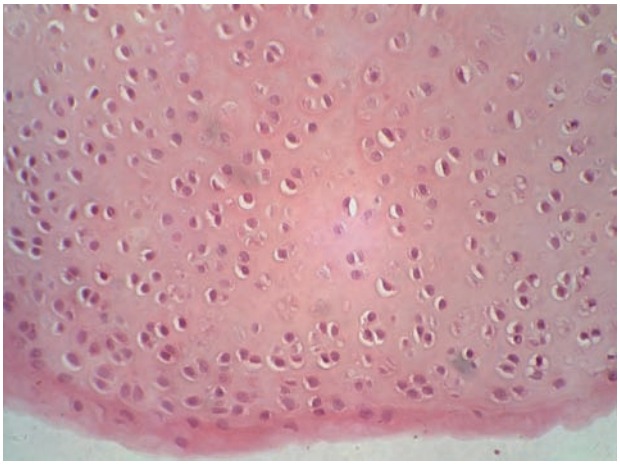

Figure 9 70 µg/ml O_3 group (HE stain ×200). The arthrodial cartilage cells were irregular and the cartilage surface appeared rough. The fissures looked slightly superficial. Masson stain showed more bluish-green loss.

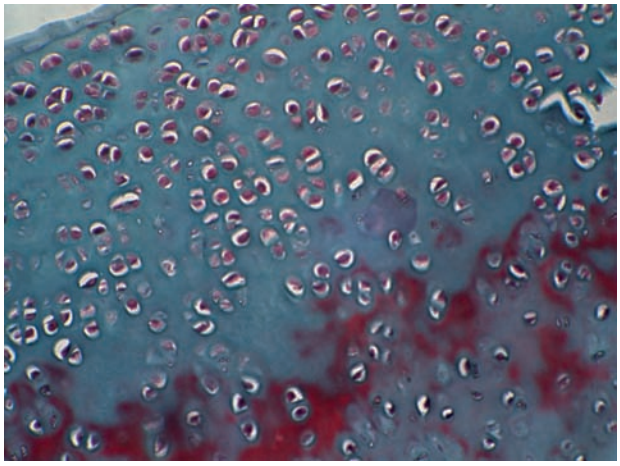

Figure 10 70 µg/ml O_3 Group (Masson stain ×200). The arthrodial cartilage cells were irregular and the cartilage surface appeared rough. The fissures looked slightly superficial. Masson stain showed more bluish-green loss.

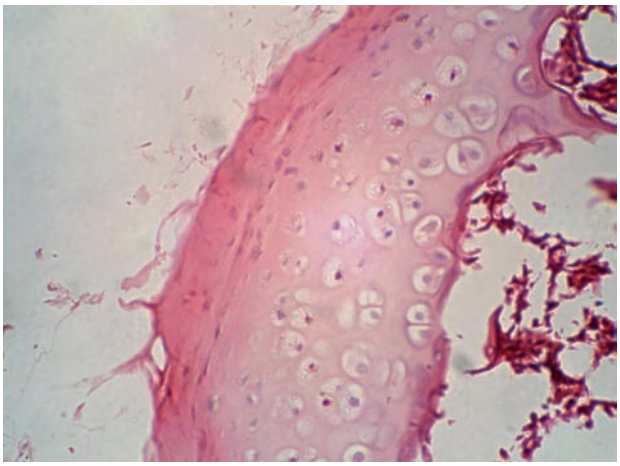

Figure 11 35 µg/ml O_3 Group (HE stain ×200). The cartilage surface looked more regular and there were fewer fissures and fibers on it than in the air group. A small amount of cartilage cell proliferation could be seen. The form of cells was basically normal and Masson stain showed less bluish-green loss.

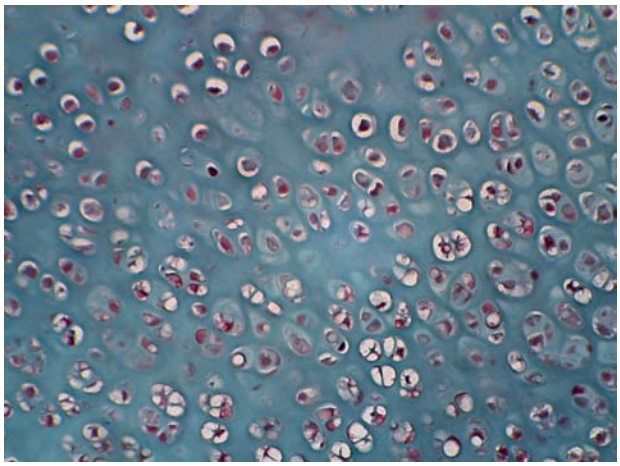

Figure 12 35 µg/ml O_3 Group (Masson stain ×200). The cartilage surface looked more regular and there were fewer fissures and fibers on it than in the air group. A small amount of cartilage cell proliferation could be seen. The form of cells was basically normal and Masson stain showed less bluish-green loss.

the fiber components increased in the cartilage. Mankin's score was 5.88 when the OA models were established. Four weeks later, the control group deteriorated to 8.63, while the 35 µg/ml O_3 group improved to 5.50. The SOD content in the synovial fluid in the model group, air group, 70 µg/ml O_3 group was obviously lower than that in the normal group ($P<0.05$, $P<0.01$). The SOD content in the 35 µg/ml O_3 group was not significantly different from that in the normal group ($P>0.05$), but significantly higher than that in the model group ($P<0.01$). The MDA content in the synovial fluid was significantly higher in the normal group than that in the model group, air group and the 70 µg/ml O_3 group ($P<0. 01$). There were no significant differences between the 35 µg/ml O_3 group and the normal group in MDA content ($P>0.05$), but the MDA contents in these two groups were both lower than in the air group ($P<0.01$). The results show that the 35 µg/ml O_3 injection enhanced the local SOD content in the joint and the capacity of scavenging free radicals. On the one hand, O_3 can reduce the oxidative damage to DNA [6] and apoptosis of cartilage cells. It maintains the intact structure and function of the cartilage cells, promoting synthesis of cartilage matrix and inhibiting decomposition. On the other hand, O_3 can restrain inflammation of the synovium, reduce the release

of inflammatory substances, and block damage to the cartilage cells, matrix and synovium by the inflammatory synovial fluid. In this way, O_3 plays a role in protecting the arthrodial cartilage, preventing and treating knee osteoarthritis.

Research shows that 30 μg/ml O_3 has a role of regulation but 70 μg/ml O_3 can destroy tissue structures due to its powerful oxidization. usually When the joint surface is attacked by the antigen-antibody complex and complement components, many free radicals, such as superoxide anion free radicals, are released, leading to degeneration of articular cartilage and synovial fluid induced by vivid hydroxy radicals. By enhancing the SOD content, O_3 may promote the scavenging of oxygen free radicals, reduce damage to articular cartilage enzyme, neutralize reactive oxygen metabolites, antagonize mediators of inflammation and relieve inflammation in the synovium and surrounding tissues. The present experiment shows that O_3 can interrupt injury to arthrodial cartilage by improving the internal environment of the articular cavity, facilitating reparative regeneration of arthrodial cartilage and delaying joint retrogression. In a word, 35 μg/ml O_3 is effective in treating OA patients, but 70 μg/ml O_3 may be too high a concentration because it may injure the protein and DNA, induce cell necrosis and suppress the reproductive activity of cartilage cells and matrix.

This study reports a preliminary observation on the effects of O_3 on the range of motion of osteoarthritic joint, structural change in cartilage and oxygen free radicals in rats. Biochemical indexes and the long-term clinical efficacy of osteoarthritis should be further investigated for a more comprehensive study of O_3 in treatment of the disease. Further research is needed on the mechanism underlying the role of O_3.

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Can Oxygen–Ozone Injections in Sport Overuse Tendinopathies Be a Valid Alternative to Cortisone Therapy?

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Key words: sport, tendinopathies, O_2O_3, cortisone

SUMMARY - *Intra-articular cortisone injections (CI) are widely used to treat sport overuse tendinopathies even though this is considered a doping practice with many side-effects and its efficacy has been questioned in the literature. Oxygen-ozone injections (O_2-O_3) are not considered doping and have proved effective but are underused with respect to their potential. Can O_2-O_3 be a valid future alternative to CI?*

Oxygen-ozone injections (O_2-O_3) are commonly used in frequent pathology of the cervical and lumbar spine and are to be considered the elective treatment. The use of O_2-O_3 is low in overuse tendinopathy (OT) typical of practising sports. Cortisone injections (CI) are widely used in this type of pathology because they are considered a rapid and cheap method to quickly return to competitions. Although many reviews demonstrate the doubtful effectiveness of this pratice not only in sport injuries but also in common traumatology of sedentary people, and the damage that frequent CI can cause on tendons and joints, this method is frequently used. There are instead several reviews in scientific literature that demonstrate the effectiveness of O_2-O_3 on OT in athletes although this practice is less used. There are also the protocols to be followed in overuse tendinopathy, the dosage in ml, the concentration, the type of needle and the minimum number of treatments to yield benefits. But there are few reviews on this practice because OT is inadequate for experimental study because the recovery time must be short. It is so difficult to convince the athletes to undergo experimental research without certain guarantees to promptly return to competitions. Many efforts are also made to demonstrate the effectiveness of O_2-O_3 in spine pathology but little effort has been dedicated to the potential of this method on OT in athletes. The effectiveness of O_2-O_3 in OT has also not been extensively demonstrated as a valid alternative to CI.

The most common OT in atletes are:

Pathology

Sport with highest frequency; Impingement syndrome of rotator cuff; Sopraspinatus (Swimming); Infraspinatus/Teres minor (Tennis and Volley); Epicondylitis; Tennis; Epitrocleitis; Golf; Adductor/ Rectus Abdominis Entesopathy; Soccer; Entesopathy Medius/Minimus Gluteus; Running (Walking); Tendinopathy of Rotuleus/Quadricipital tendon; Cycling, Soccer, Basketball, Volley and Running; Entesite Achillea; Running, Soccer, Basketball and Volley; Plantar Fascitis; Running, Soccer and Basketball.

Anti-inflammatory effect of CI

CI have anti-inflammatory and anti-allergenic effects because they induce the production of the lipocortine enzyme (LC). LC inhibits the phospholipase enzyme that converts the phospholipids of cellular membrane in arachidonic acid that through lipoxygenase and ciclooxgyenase is converted into inflammatory mediators. The foremost anti-inflammatory action of the CI is different from NSAID drugs that inhibit cyclooxygenase I (always present) and II (present only in inflammatory status).

Advantages and disadvantages of CI

The advantages of CI are:

1) Quick regression of symptoms sometimes with only one treatment. This effect is related to mild OT of recent onset.
2) Lack of side-effects if the pathology is solvable with one treatment.

The disadvantages of CI are:

1) It is rated as a doping treatment and it is therefore essential to demonstrate the ineffectiveness of non doping treatments and to give adequate

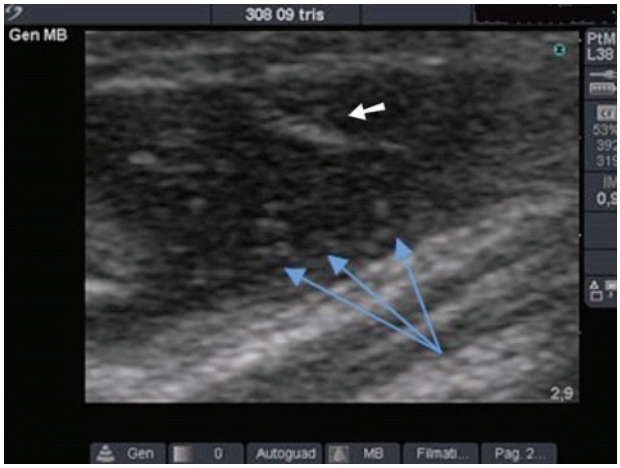

Figure 1 The needle (white arrow) introduces O_2-O_3 (blue arrows).

documentation to the committee for the exemption to therapeutic use (CEFT).

2) Many irreversibile side-effects are described in continued use of CI. The most common collateral effects are:
   a) Allergenic effect.
   b) Irreversible damage to treated tendons (even rupture in some cases), joints, cartilage and ligaments.
   c) Osteoporosis of the treated joint.
   d) Skin atrophy and and depigmentation of the treated part.
   e) Risk of hemarthrosis with consequent persistent damage to the cartilage.
   f) Osteonecrosis (bone infarction) in some cases that is however to be considered an uncommon but always possible event.
   g) Worsening of some preexisting diseases, especially diabetes mellitus, arterial hypertension and gastritis.
   h) Risk of infections by opportunist germs caused by CI immunosuppressive effect.

Anti-inflammatory effect of O_2-O_3

The anti-inflammatory effect is linked to the reduced production of prostaglandins (PGE) and to the improvement of the use of glucose thanks to the increased action of the glucose 6-P dehydrogenase enzyme that favours the repair process.

Advantages and disadvantages of the O_2-O_3

The advantages are:

1) Possibility to use O_2-O_3 without asking for special authorization from the CEFT because it is not considered a doping practice.
2) No side-effects especially of allergic type and no damage to joints, tendon and cartilage.
3) Impossibility to worsen preexisting diseases like diabetes mellitus and arterial hypertension.
4) Repeatibility of diabetes even after a short interval without significant side-effects.
5) Low risk of infection by opportunist germs thanks to the bactericidal effect of O_2-O_3.

The disadvantages are:

1) Frequent need for more than one treatment. It is possible to use other types of therapies at the same time like mesotherapy to accelerate the return to competition.
2) Higher cost than CI because it is necessary to utilize an ozone generator of good quality and because only one treatment is often insufficient.

Injection method of O_2-O_3

The injection method that I personally favour, making reference to the available reviews in scientific literature and to my personal experience, is the peritendon method. The amount utilized is 5-10 ml depending on the tendon to be treated and the degree of the overuse. Five ml are enough for epicondylitis and epitrocleitis, whereas for the Achilles tendon or for the rotator cuff a greater quantity may be needed. The concentration of ozone that I use, always making reference to the available protocols and to my personal experience is 14-16 µg. The number of treatments needed to obtain a significant benefit are two to four. Treatment frequency is two treatments a week. Combining mesotherapy is useful even if it is often considered an alternative practice with respect to O_2-O_3 and not a useful adjunct. O_2-O_3 can be practised even by the mesotherapic route but more treatments are needed and with greater concentrations of ozone and not many reviews are available in scientific literature.

Potential damage of O_2-O_3

The damage arising from O_2-O_3 is essentially linked to a incorrect injection method. The tendons, ligaments and cartilage can be damaged only if they are erroneously pierced and not for a potential harmful effect of O_2-O_3. I prefer to use ultrasound guidance to avoid this potential complication also because the O_2-O_3 can cause damage due to a mechanical effect on tendons, ligaments, nerves and cartilage. This fact is more evident if the tendon is already damaged. The introduction of the O_2-O_3 into a knee bursa can be seen in the Figure 1 with ultrasound guidance to be sure to inject in certain zones.

No particular side-effects are described if the injection technique is correct and if the amount and concentrations in µg are respected. The patient can at most note pain of the needle injection and the mechanical effect of the O_2-O_3.

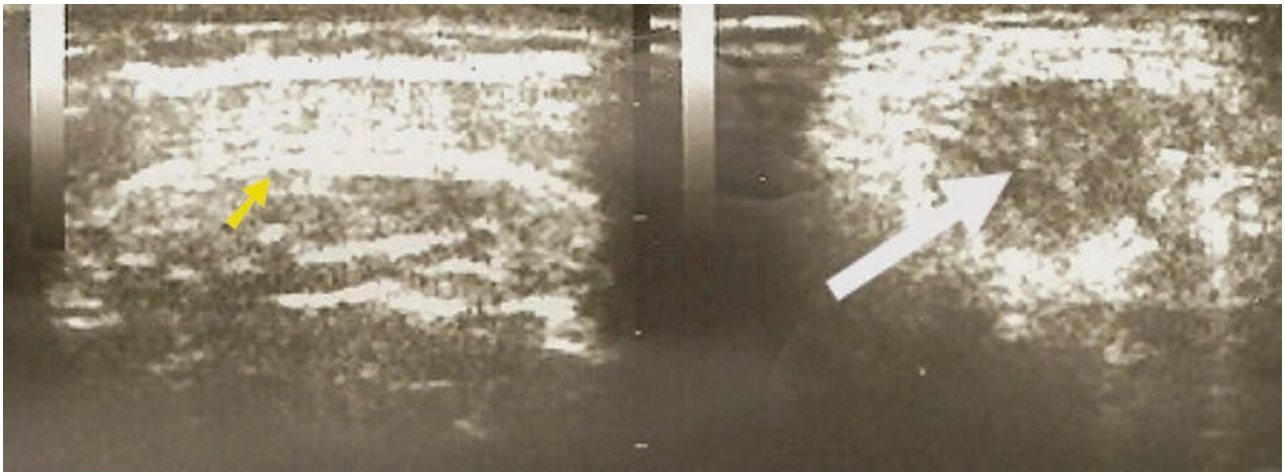

Figure 2 Scans on the long axis of the healthy patellar tendon (yellow arrow and of the damaged tendon (white arrow).

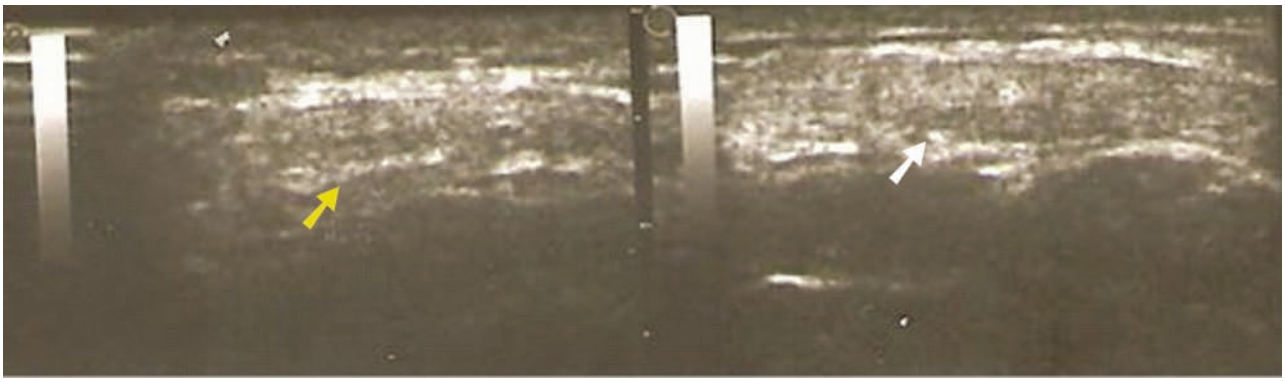

Figure 3 Scanning the long axis after three sessions of O_2-O_3. The damaged tendon (white arrow) is indistinguishable from the healthy tendon (yellow arrow).

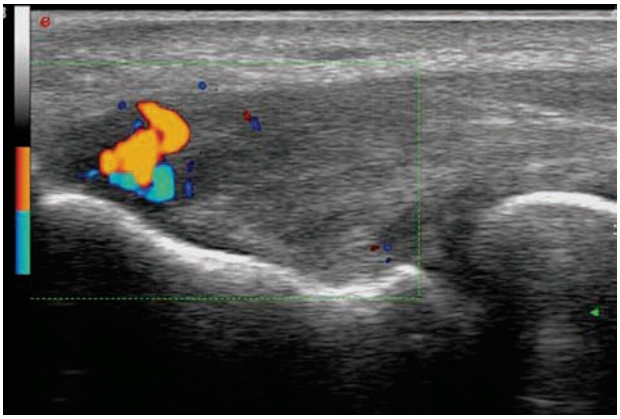

Figure 4 Ultrasound scan before O_2-O_3 treatment.

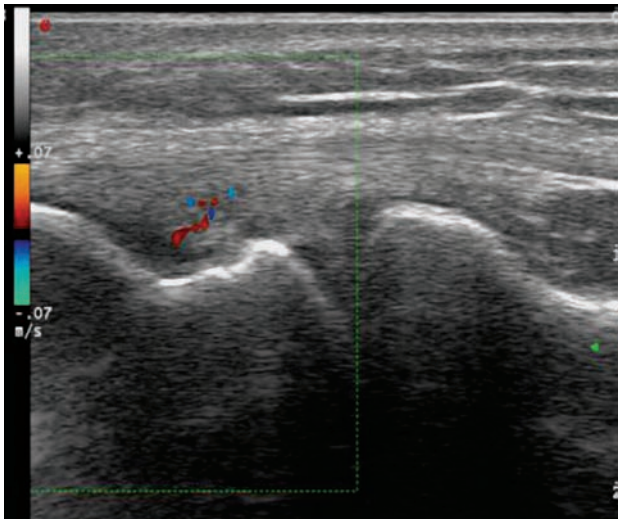

Figure 5 Ultrasound scan of the tendon after the first treatment with O_2-O_3.

Sport OT curable with O_2-O_3

Every OT normally cured with CI can be treated with O_2-O_3. Every tendon can be treated by a peritendoneal or mesotherapic route. I prefer to use ultrasound guidance to avoid damage to tendons, nerves and ligaments. The ulnar nerve, for example, can be damaged in the treatment of the epitrocleitis.

Clinical cases examples cured with O_2-O_3

Two clinical cases are described to illustrate how O_2-O_3 has proved a valid support for these athletes treated without benefit with CI and traditional physical therapy (ultrasound, laser and iontophoresis)

***First Case**: Rotuleus tendinopathy in a basketball player*

The patient reported knee pain for over six months unsuccessfully treated with traditional physical therapies (laser, ultrasound and iontophoresis and CI. Before treatment with O_2-O_3 ultrasound examination, as shown in Figure 2, the right patellar tendon (white arrow) appeared severely impaired compared to the healthy left tendon (yellow arrow). In the scans you can see how the damaged patellar tendon shows a clear increase in thickness and the typical fibrillar and oval structure is lost. Having received no results with previous treatments and needing to return quickly to competitions, the patient was therefore treated with three sessions of O_2-O_3 with 5 ml at 15 µg/ml. A 25 gauge needle was used. After three sessions there was benefit not only for clinical symptom reduction but it can be observed in the ultrasound scans that the treated patellar tendon (white arrow) has returned to normal conditions becoming essentially indistinguishable from the healthy tendon (yellow arrow) as seen in Figure 3.

***Second Case**: Epicondylitis in a tennis player*

The patient, an amateur tennis player, complained of epicondylitis treated for over six months without success, as in the previous case with traditional physical therapy and CI. The tendon was as seen in Figure 4 with a loss of typical fibrillar structure, increased thickness and increased vascularity that should not be present in a tendon under normal conditions. Increased vascularity and thickness are indicative of a state of severe inflammation. The patient was treated with three sessions of O_2-O_3 infiltration at 5 ml to 14 µg/ml and 25 G needle. The patient, again, has not only achieved a clinical benefit but, as shown in Figure 5, demonstrates a significant reduction in abnormal vascularization already with the first infiltration of O_2-O_3 and a recovery of the fibril structure. Also in this case the patient not only had a clinical benefit but, thanks to ultrasound examination, significant anatomical changes can also be observed.

Conclusion

CI are widely used in sports OT despite presenting many potential complications and side-effects and although it is considered a doping practice. As already described in previous publications the benefits achievable by the infiltration of O_2-O_3 in the most common diseases of overload in sports is desirable to produce more works that demonstrate unequivocally that this technique gives results comparable or even superior to cortisone with the advantage of not incurring the use of doping practices and without significant complications and potential side-effects.

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Effectiveness of Treatment with Oxygen-Ozone and Hyaluronic Acid in Osteoarthritis of the Knee

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Key words: oxygen ozone, knee hyelmann, osteoarthitis injection

SUMMARY - *Osteoarthritis (OA) is an increasingly common disease in Western society characterized by progressive degeneration of joint cartilage with a consequent increase in social spending. The onset of OA is supported by several risk factors such as obesity, advanced age and job function. The traditional conservative therapies prove ineffective and by arthroscopic surgery does not seem to give real benefits over conservative treatment. Ozone Therapy (O_2-O_3) is proving an effective treatment for OA as well as combined with viscosupplementation with hyaluronic acid (HA). We evaluated a sample of patients of various ages and with varying degrees of OA to establish whether concomitant O_2-O_3 and intra-articular injection yields concrete benefits by reducing the costs and side-effects of traditional therapy.*

Introduction

Osteoarthritis (OA) is a disease characterized by progressive degeneration of joint cartilage, with hard-elastic tissue consistency and very smooth, providing some ability to scroll across the joints subjected to constant trauma due to the movements made during the day. Cartilage is formed by 2% cells called chondrocytes, 20 to 40% of extracellular matrix and the 60 to 80% water. Chondrocytes produce and maintain the extracellular matrix which consists of 60% collagen (mostly type II collagen) and 40% proteoglycans (PG) which in turn are made up of glycosaminoglycans (or mucopolysaccharides) linked to a protein chain. The most representative are glycosaminoglycans chondroitin-4 sulfate, chondroitin-6 sulfate, keratan sulfate and dermatan sulfate. PGs are interconnected by hyaluronic acid (HA). In humans there are three types of cartilage:

1. *Hyaline cartilage*: is a white-blue type of cartilage longest lasting in the body. Is the mostly found in the fetal skeleton and gradually it is almost completely replaced by bone. In the adult hyaline forms the costal cartilages, nasal, tracheal, bronchial and laryngeal and covers the joint surfaces.
2. *Elastic cartilage*: is a yellow cartilage with the particular characteristics of elasticity. This cartilage constitutes the framework of the ear, the epiglottis, the eustachian tube and some laryngeal cartilage.
3. *Fibrous cartilage*: is a off-white cartilage particularly resistant to mechanical stress. It is found in the insertion of some tendons on the skeleton in the intervertebral discs, menisci in some joints (knee) and the pubic symphysis.

Cartilage is available in four layers:

1. Shallow (3%)
2. Intermediate (5%)
3. Deep (90%)
4. With calcified cartilage (2%)

Classification of OA

There are several classifications that assess the degree of OA. Currently one of the most widely used is the "staging" of chondral damage and was devised by the International Cartilage Repair Society (ICRS). This classification includes:

- Grade 0 (normal: no damage)
- Grade 1 (nearly normal, superficial lesions)
- Grade 2 (abnormal: lesion extended to <50% of the thickness of cartilage)
- Grade 3 (very abnormal: fault> 50%)
- Grade 4 (severe: osteochondral lesion)

Epidemiology

OA is very common in people aged over 50 years and affects women more than men. In the US 43,000,000 people are affected by OA and an

estimated 60,000,000 in 2020 will be affected by this condition resulting in enormous social costs. In Europe the situation is equally alarming. It is estimated that in a country like France OA affects approximately 12,000,000 people with an incidence of 18% of men and 30% of women below 60 years and 58% of men and 65% of women above this age threshold. OA mainly affects the spine, knee and hip. OA of the knee in particular is about 23% of all OA. It affects 27% of the population under 70, whereas over 80 years OA reaches 44%. The factors weight and age in this form of OA are fundamental.

Etiology

OA is a multifactorial disease. The factors predisposing to this condition are:

Familiarity and genetics: Asians are more affected than Caucasians who, in turn, are more affected by OA than blacks. OA of the hand is certainly more affected by genetic predisposition.

Sex: under 50 years men have a prevalence of OA in some locations but above that age there is a prevalence for women in all forms, probably because of reduced estrogenic activity and increased incidence of certain forms of OA, particularly in the hand, and recognized genetic factors.

Age: aging is not necessarily a "conviction" to OA but there is no doubt that the resistance of chondrocytes to oxidative stress is reduced with age, which alters the equilibrium between metal and metal proteinase inhibitor and that alters the ultrastructure of PG.

Occupation: a job involving heavy or repetitive movements and sports activities are two strong a risk factors for excessive wear in the cartilage, particularly the large joints. Physical exercise of moderate intensity seems to be rather a protective factor.

Metabolic diseases: diabetes (although in fact insulin appears to be a protective factor), dyslipidemias, chondrocalcinosis and fluorosis have a clear association with OA.

Obesity: it is clear that overuse causes premature wear of the cartilage so that it is estimated that the possibility to maintain their body weight in a BMI within the limits results in a 25% reduction in the risk of developing OA.

Smoking: smoking seems to have a role, even though nicotine appears to represent a protective factor.

Diagnosis of osteoarthritis

The diagnosis of OA generally poses no particular problems and is based on:

Clinical criteria: The patient with OA refers pain, stiffness, swelling, crepitation noises and functional impotence. The pain and resulting functional impairment are worse under load and stiffness usually occurs after a period of inactivity (e.g. in the morning on awakening) but disappear fairly quickly after the joint is mobilized. The differential diagnosis must be made against arthritis but the history is usually sufficient to distinguish the two conditions.

Radiological imaging examinations: The patient-reported symptoms are usually sufficient to suspect OA. However, it is necessary to determine the severity of OA to refer the patient for conservative therapy or surgery x-ray is better and typically shows bone thickening, decreased joint space, presence of geodes and, in severe cases, also deformation of the joint heads. CT, or better MRI, are the examinations of choice for assessing the severity of OA.

Traditional treatment of knee OA

Traditionally, the treatment is to drain the affected joint, weight reduction, rehabilitation, use of tutors and classical drug therapy based on NSAID and intra-articular infiltration with cortisone. Traditional drug therapy often gives short-term benefits with the aggravating circumstance that it often has significant side-effects. When traditional treatments do not work, surgical intervention is usually performed arthroscopically. However, a recent study reported in the British Medical Journal showed that the effectiveness of conservative treatment of knee OA based on movement therapy provides results comparable to the measures undertaken by arthroscopical surgery (the cases of OA associated with severe meniscal tears were excluded). The obvious consequence is that arthroscopy should be limited to severe cases that do not respond in any way to conservative treatment. In severe cases not responding to conservative therapies prosthetic knee implants are possible but have high costs.

Alternatives to traditional therapy

An alternative to traditional drug therapy is viscosupplementation (VS) based on hyaluronic acid (HA) and on oxygen-ozone therapy (O_2-O_3) by intra-articular infiltration applicable to any joint. The use of HA was also approved by the Food and Drug Administration (FDA) in 1997. It was initially used almost exclusively for several years for the knee, but is now also used for the hip,

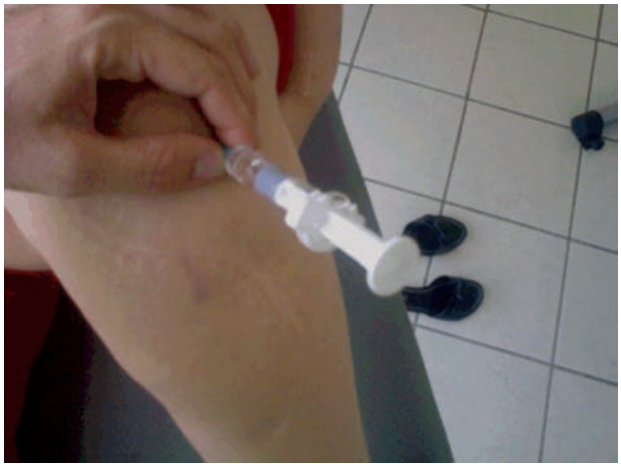

Figure 1 The image shows the route of choice for front access of O_2-O_3 inoculation before HA.

shoulder and ankle. The use of HA, however, not only has significant anti-inflammatory effects and can still only partially reduce the cartilage damage. O_2-O_3, on the other hand, certainly has a significant anti-inflammatory effect but it can act on limited cartilage damage.

The aim of the study

OA of the knee is a common and highly debilitating disease and has been the subject of several studies on the effectiveness of VS and recently of O_2-O_3. There are no scientific literature reports demonstrating the efficacy of combination therapy with O_2-O_3 and VS with HA. For this reason, we recruited a sample of patients with OA at different levels of severity to evaluate the efficacy of combination O_2-O_3 therapy with VS and HA.

Materials and Methods

We enrolled in the study 40 male and female patients with symptomatic OA disabling at least six months that had not responded to traditional physical therapy, drug treatment with NSAID and corticosteroids and movement therapy and manipulation. For infiltration we used 5 ml syringes with a 22 G needle, the E30 ozone generator (Medica s.r.l, AI, Jointex pre-filled syringes of 16 mg/2 ml Chiesi Farmaceutica). The severity of OA was previously evaluated by MRI of the affected knee. MRI was performed not earlier than six months. Patients with severe meniscus or previous fractures were not included. After signing the informed consent and having explained to the patient what the study involved, we proceeded to start treatment. Pain was assessed before starting treatment, at the end of treatment and after three months to assess the benefit obtained with treatment. Patients were not subjected to any treatment within three months after treatment. Pain pre-treatment, after treatment and after three months was evaluated on the NRS numerical scale where grade 0 represented absence of pain and grade 10 maximum pain imaginable by the patient. Before infiltration the knee was first cooled with an ice pack for five minutes and then was disinfected with alcohol and betadine. This was followed by infiltration with access to the front knee angle of 90° as shown in Figure 1. We first introduced slowly 5 ml of ozone at 15 µg/ml and, after 40-50 seconds after the end of infiltration, HA was inserted into the syringe leaving the needle over which was "screwed" into the vial containing HA. HA was always injected slowly to limit the pain as much as possible. After infiltration the bag of ice was applied in place for another five minutes. Patients treated with HA should only be treated with five sessions according to the protocols available, but it was decided to assess whether three sessions of OT and HA combined was sufficient to obtain a significant benefit.

Results

The 40 patients enrolled in the study comprised 27 women and 13 men. The results are shown in Table 1. The average age of the sample was 54.4±13.7 years, the level of OA according to the ICRS classification was 2.7±0.7. Pain with classification CR before therapy with three sessions of infiltration and HA-based O_2-O_3 was 7.9±1.4, 2.8±0.8 after three sessions and after three months without additional pharmacological aids of 3.3±1.5.

Figure 2 shows the histogram trends before

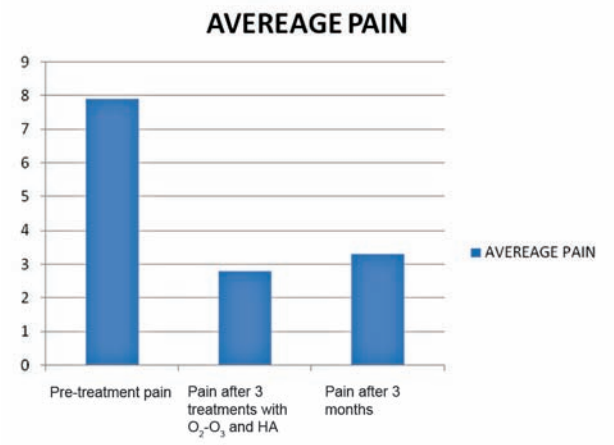

Figure 2 Evolution of pain before treatment, after treatment and after three months.

Table 1 Results of the sample analyzed

	Age (years)	*Level of OA (ICRS)*	*Pain pretreatment*	*Pain after 3 treatments of O_2-O_3 and HA*	*Pain after 3 months*
AVERAGE	54	2.7	7.9	2.8	3.3
SD	13.7	0.7	1.4	0.8	1.5

treatment with OT and AI, at the end and after three months.

Discussion

The results indicate that the sample showed a prevalence of females, according to what is already described for the epidemiology of OA. The age of the sample analyzed was still young also influenced by the fact that some subjects evaluated were under the age of 40 years. These patients were athletes and had an OA caused by sport overload. The women studied were almost all overweight. The pain perceived by the average sample before treatment appeared to be quite substantial and prevented normal daily activities. As can be seen, the pain after the third session of O_2-O_3 and HA was already greatly reduced and in 16 of those surveyed even almost disappeared. In any case, no patient reported a significant benefit from therapy. Although the resumption of pain after three months is still quite insignificant on average and only two subjects in the sample reported an increase in pain that could somehow still affect normal daily life. Furthermore patients were subjected to only three of the five sessions of protocols of treatment with HA and then only with saving money. Indeed HA cannot be purchased on prescription in agreement with the NHS (Except for Hyalgan that however has alow molecular weight) and has an average price of about € 50 which must be added to the cost of the O_2-O_3 session which itself costs around €50 to 60. The savings for the patient who underwent only three sessions instead of five was then about €200 compared to a protocol that provides for the use of HA only or just O_2-O_3. Furthermore, patients did not need to use the classic NSAIDs thus avoiding the unpleasant collateral-effects from this therapy taken chronically as well as substantial costs to the NHS.

Conclusions

The combination of O_2-O_3 and HA has certainly proved an effective therapy against OA of the knee in cases of first, second and third degree. Moreover, the combined use of O_2-O_3 and HA rather than the traditional conservative therapies has almost immediate effect, no significant side-effects, affordable cost and maintains the effect over time. For these reasons, O_2-O_3 should definitely be proposed in first aid guidelines for treatment of knee OA. However, wider testing and larger studies are required to prove the efficacy of O_2-O_3 vs. placebo and vs. traditional drug therapies.

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Non-Invasive Approaches to Back Pain in Patients with Somatization

A. BARISELLI

Oberdan Surgery; Brescia. Italy

Key words: back pain, psychosomatic, somatization, I.S.T.D.P., S.C.L.90

SUMMARY - *In our work at the Oberdan Surgery we have noted that patients treated for back disorders, namely low back pain, present diseases that often cannot be fully explained by a general medical condition or diagnostic tests even though they have painful symptoms resistant to treatment. It is assumed that a certain percentage of these patients may present strong somatoform pain disorders associated with anxiety. The hypothesis is linked to the fact that chronic disorders are often correlated to somatization and hypochondria. This study set out to demonstrate that the use of non-invasive techniques like short-term dynamic psychotherapy and relaxation techniques can yield satisfactory outcomes in terms of quality of life linked to the underlying disorder and general state of health.*

Material and Methods

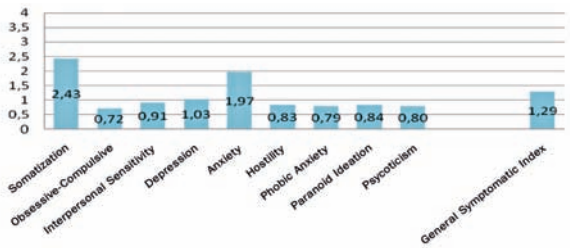
We studied a cohort of 32 patients, mostly women (28 women and four men) who presented at the Oberdan Surgery in Brescia for low back pain. After history-taking and neurological examination by Dr. Matteo Bonetti, patients were offered a psychological consultation combined with administration of a psychological questionnaire.

The instrument used, the Symptom Checklist-90-Revised (SCL-R) created by Leonard R. Derogatis is a method to evaluate psychological problems and identify symptoms [1]. This instrument is also used by psychologists, psychiatrists, mental health, medical, and educational professionals for monitoring patients' progress or treatment outcome. Participants are required to respond to a 90 minute test using a five-point rating scale. Approximately 12-15 minutes are necessary for completion. Testing can be done with a computer, audiocassette, or paper and pencil. Individuals aged 13 years or older are recommended for accurate test results. Another name for the SCL-90 is the Global Severity Index. The SCL-R is an established instrument and has over 1,000 independent studies supporting its reliability and validity [2]. The internal consistency coefficient rating ranged from 0.90 for depression to 0.77 for psychoticism. Test-retest reliability has been reported at 0.80 to 0.90 with a time interval of one week [3]. All nine primary subscales are well correlated with the Minnesota Multiphasic Personality Inventory. The SCL-R was also correlated with the IIP, 0.73, and the SAS, 0.69 (Pearson).

Testing was undertaken adopting a test-retest approach with a three-month interval between the two tests. In this interval patients attended ten psychology sessions combined with learning the autogenous training relaxation technique. The technique is a self-relaxation method designed to attenuate physical and mental tension, and through a complete control of the body allows patients to reach a high level of mental relaxation and spontaneous changes in muscle tone, vascular function, breathing, heart rate and internal organs. Applied in specific regions and apparatuses, the technique yields major benefits in the treatment of pain and spinal rigidity.

Results

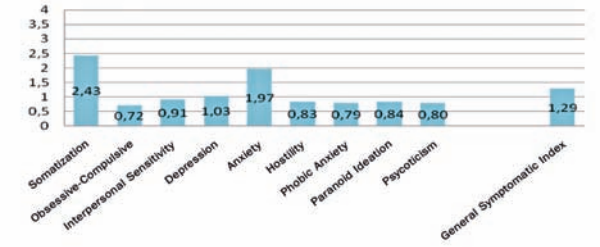
The first test administration yielded the following results:



Additional scales:

General neuroticism	1.11
Sleep disorders	3.33
Distress	1.41
Difficulty in cognitive performance	1.38

The second test administration yielded the following results:



Additional scales:	
General neuroticism	0.82
Sleep disorders	1.23
Distress	0.81
Difficulty in cognitive performance	0.64

Discussion

An immediate outcome was a marked reduction in anxiety (from 1.97 points to 0.85) and somatization (from 2.43 to 1.56), together with a considerable decrease in most of the remaining indices.

It is also noteworthy that the "Sleep disorders" scale showed a marked improvement: most patients reported that their quality of sleep improved notably following the psychology sessions thanks to the identification of psychological components making up the unresolved neurotic core giving rise to conflict. This underlying unresolved situation is readily discharged through the expression of physical distress sometimes becoming chronic. These components are discussed in the work of Fishbain et al.[3] and Bacon et al.[8]. As confirmed by these studies, patients with chronic pain may present underlying major psychological dynamics that impact on compliance with treatment (cf. the "Hostility" scale see in the Results section above) and a successful outcome.

Our patients presented strong somatic components associated with major anxiety/depression. Despite this none of the patients were administered drugs to manage the psychological impairment, and treatment focused on psychology and relaxation sessions. After treatment, 11 of the 32 patients enrolled in the study (34.37%) decided to continue the treatment with short-term dynamic psychotherapy, currently ongoing.

Currently, the pain presented by the patients is not such as to prescribe further clinical-diagnostic tests. For this reason, it is assumed that their symptoms are in fact merely psychological, but for most patients are expressed in the form of requesting medical treatments, as demonstrated in the study by Lipowski[9].

In these conditions, anxiety, distress or traumatic experiences are immediately somatized into a physical disorder. Hence the first choice treatment for this type of problem (or conversion) must be integrated, as shown by Kriegler and Ashenberg[4] who combined psychological therapy with appropriate non-invasive medical intervention.

Somatization may be transient or persistent and may or may not be accompanied by a medical or psychiatric disease subject to diagnosis. Most of the components linked to somatization are the result of an affective disorder (neurosis), anxiety and, to a lesser extent, pure somatoform disorders. For this reason, it is confirmed that chronic entrenched somatization raises serious clinical, social and economic problems. Early diagnosis and treatment of such disorders are therefore crucial.

The aetiology of somatization disorders is multidisciplinary ranging from initial medical diagnosis to psychological therapy, neither of which ever rule out the other.

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Congress Location

Dr Fabris, Dr De Monte, Prof. Tomei

Dr Cardelli

Dr Zambello

COMUNE DI BRESCIA

Federazione Italiana di
OSSIGENO-OZONOTERAPIA

Word Federation Oxygen
Ozone Therapy

III WORLD CONGRESS OF Oxygen-Ozone Therapy

V CONGRESSO NAZIONALE F.I.O.

Museo della Mille Miglia

from 14th to 16th April 2011

Brescia Italy

SCENTIFIC SECRETARIAT
Chairman: Prof. Matteo Bonetti

ORGANIZING SECRETARIAT
Koinè Eventi snc
Via Fontane, 24
25133 Brescia
Tel 030.2002844 - Fax 030.2096783
info@koineeventi.com
www.koineeventi.com

Dear Colleagues,

The 3rd World Congress of the Ozone Therapy Federation which follows those held in Beijing and Madrid wants to offer a further scientific value in a sector where approximation and vague knowledge lead - let me say - to a mere justification of a much easier indifference.
This is not acceptable and this congress is another important meeting occasion to give validating certainties to physicians operating in this discipline.
Recent clinical results of national and international case history show a need for a bigger effort in the research of rationality in the way this matter is dealt with. This 3rd World Congress then gives itself ambitious targets. It wants to fight deception and re-establish incontestable truth which is only possible with concrete knowledge. It wants to give credit to all those around the world who have been able to give certainty to this therapy.
It wants to give the opportunity of a correct approach and give an occasion of deep analysis.

For all this I feel honoured to organize the WFOOT 3rd World Congress of Ozone Therapy for the first time in Italy. A big opportunity for all those who want to collaborate, compare, give the results of their own knowledge and experience, but above all for those who are humble enough to ask themselves "why?"

Thank you all

Matteo Bonetti

The Francesco Riccardo Monti prize for life time achievements is a recognition for scientific work done to spread Oxygen-Ozone Therapy practice in Italy and around the world.
The great artist Francesco Riccardo Monti, who is now represented by his heirs, was a sculptor and an architect from Cremona and he created many monumental works of art. At the end of 1928 after winning a commission and being prevented from completing it he had an argument with a fascist party official and left Italy. He moved first to France and then to Manila in the Philippines where within a short time he became the most important sculptor and architect in the country. There he carried out great works of art merging the style of the European school with the local tradition. He came back to Italy only for short periods between 1930 and 1932 to finish some uncompleted works.
His style is full of poetic symbolism and leads to works of art rich in grace and imagination. His devotion is the same of those physicians who have always believed in the effectiveness of Oxygen-Ozone Therapy and have made possible for this very successful therapy to develop all over the world.

Organizer Committee

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TOPICS

1 - Ozone Therapy

State of the art
Velio Bocci

2 - Ozone Therapy in the Musculo-Skeletal pathology

State of the art
Marco Leonardi
The Future
Yves Bergeron

3 - The Research

State of the art
Emmanuel Iliakis

4 - The Great Autoemotherapy

State of the art
Amato De Monte

5 - Applications in Cosmetic Medicine

State of the art
Mario Sirito

6 - Other Applications

State of the art
Lamberto Re

7 - Veterinary Medicine

Cows
Paolo Scrollavezza
Horses
Ettore Ballardini
Dogs
Helen Giuliano

8 - Technology

Thursday April 14th

16.00-16-30 **Opening Ceremony**

16.30-17-30 V.Kumar
Ozone therapy today

17.30-18.00 "Francesco Riccardo Monti"
Awards ceremony

18.00-19.00 G. Pellicanò
"History of Rock"

19.00 **visit of "Mille Miglia" Museum**

HOW TO REACH THE MUSEUM

LOCATION OF THE MUSEUM

The Museum is situated inside the Monastery of Saint Eufemia, founded in years 1008.
Saint Eufemia is a district lying on the east side of Brescia, on the 45Bis "Gardesana Occidentale" Main Road:Viale della Rimembranza that is between Via Indipendenza and Via della Parrocchia.

GPS NAVIGATOR:

TomTom Navigator and similar:
Lat= 45.52411 N - Lon= 10.26783 E - Garmin, eTrex e simili:
Lat= 45° 31'.446 N - Lon= 10° 16'.0698 E

How to reach us:

By car:

A4 and A21 Highway "Brescia Centro" exit

Follow direction to S. Eufemia, Proceed along Via Maggia, Via Mensi, Via Fiorentini, Via Gatti, Via Zammarchi, Viale S. Eufemia.

A4 Highway: "Brescia Est" exit

Follow direction to Brescia along the South ring road S. Eufemia exit. Follow direction to S.Eufemia. Proceed along Via Serenissima, Viale Sant'Eufemia

From the city centre of Brescia:

Piazzale Arnaldo, Viale Venezia della Mille Miglia, Viale della Bornata, S. Eufemia

By bus: From the city: n. 3 Urban line (Rezzato direction) and n. 11 from Garda Lake bus stop "Eufemia" Others extra urban lines: station bus stop of Brescia and then urban lines.

By train: Brescia Station, Bus line n. 3 (Rezzato direction) and n. 11, Taxi

By plane: G. D'Annunzio di Montichiari Airport, Shuttle bus from/to Bus Station, Line bus n. 3 (Rezzato direction) and n. 11

GENERAL INFORMATION

CONFERENCE DATE AND LOCATION

The conference will take place in S. Eufemia (BS) at the Mille Miglia Museum Viale della Rimembranza, 3 on 14th, 15th and 16th April 2011.

ABSTRACTS

Submitted abstracts related to the topics of the conference will be selected for presentation as poster or oral communications.
Abstracts will be published on the International Journal Of Ozone Therapy.
All abstracts must be in English and must mention authors and their affiliations. They must contain title, goals, materials and methods, results and conclusions.
Maximum length allowed for each abstract is 3000 characters including spaces.
Please send your abstracts to info@koineeventi.com

Deadline for abstracts submission is February 1st 2011

REGISTRATION FEE

	Before March 15th	After March 15th
Regular fee:	❑ 250 €	❑ 350 €
F.I.O. Members:	❑ 100 €	❑ 200 €
W.F.O.O.T Members:	❑ 100 €	❑ 200 €

Registration fee includes:
congress kit
coffee break
lunch

HOW TO PAY FOR REGISTRATIONS

It is possible to pay by cheque or bank transfer in favour of Koinè eventi (see attached registration form).
Koinè eventi will send the invoice to the participant or to the paying company/institution.
Note: in case of reservation paid by public or private institution you are kindly requested to provide us with an authorization written on the institution letterhead with all invoicing details (name, address, VAT No.).
Please send the authorization along with the reservation form

HOTEL RESERVATIONS

(Prices are available in the reservation form).
Koinè eventi has reserved a certain number of rooms at the Villa Fenaroli Palace Hotel especially for the conference. To make a reservation please fill in the attached form and send it to Koinè eventi to allow them to reserve the room/rooms depending on availability and according to your request. After the reservation has been made successfully Koinè will send you a voucher with the full address of the hotel.

English will be the official language of the conference

III WORLD CONGRESS OF OXYGEN – OZONE THERAPHY
V CONGRESSO NAZIONALE F.I.O.

Title _____ Name _____ Surname _____

Date of birth _____ Place of birth _____

Home address _____

Zip code _____ Town _____ Province _____ Country _____

Phone _____ Fax _____ e-mail _____

WORK ADDRESS:
Service/Division/Institute _____

Company/University _____

Address _____

Zip code _____ Town _____ Province _____ Country _____

Phone _____ Fax _____ e-mail _____

PAYMENT:

	Before March 15th	After March 15th
Regular fee:	❏ 250 €	❏ 350 €
F.I.O. Members:	❏ 100 €	❏ 200 €
W.F.O.O.T Members:	❏ 100 €	❏ 200 €

THE PAYMENT HAS BEEN MADE BY:

❏ Bank cheque number _____

of Bank _____ in favour of Koinè eventi snc

The cheque must be sent, possibly by registered mail, to the organizing Secretariat Koinè eventi snc Via Fontane, 24 - 25133 Brescia, together with the registration form.

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UBI - Banco di Brescia F.le n. 27 - Via Ambaraga n. 126 (BS) c/c 19000 - CIN: R- ABI 3500 - CAB 11290
IBAN: IT40R0350011290000000019000 ❏ BIC/SWIFT : BCAIT21090

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Name _____

Address _____

Fiscal Code (Italian) _____

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