Is Medical Ozone Safe when Injected Intra-articularly?

A Comparative Histological Study in Rat

E. ILIAKIS*, I. PETROPOULOS**, A. ILIAKI***, E. AGAPITOS****, G. AGROGIANNIS****

* *Orthopaedic Surgeon Hospital Inspector N.M.Y. Athens; Greece*
** *Orthopaedic Surgeon National Centre for Emergency Aid; Greece*
*** *School of Veterinary Medicine, Aristotle; University of Thessaloniki, Greece*
**** *Laboratory of Anatomy and Pathology; University of Athens, Greece*

Key words: ozone, safety, cartilage

SUMMARY - *The therapeutic use of ozone (O_2-O_3) gas is controversial. Some authors claim the gas mixture is toxic and therefore out of the question, whereas, others hold that it is useful and effective when used following a certain method to treat degenerative diseases, such as knee osteoarthritis, for which the gas has been used empirically. The present work studied the effects of contact of O_2-O_3 with healthy knee tissues of Wistar rats and compared them to intra-articular injection of a substance known to damage the cartilage. Thirty-six Wistar rats were studied in two groups. Mono-iodoacetic acid (MIA) was injected in the first group in a single dose και, whereas O_2-O_3 was injected in the second group in frequent doses three times a week for three weeks. The rats were killed 40 days later and articular cartilage and surrounding tissues were studied histologically. MIA caused degenerative osteoarthritis gradually deteriorating at the knees of the first group whereas no major changes were observed in those of the second group. We conclude that following the methodology of our study medical ozone appears to be safe for use.*

Introduction

Knee osteoarthritis (OA) is a common high cost disease in orthopaedics and decreases the quality of life especially in the elderly. The main characteristic of OA is degeneration of the articular cartilage and the subchondral bone frequently followed by inflammation of the synovial membrane and aggravation of the supporting structure of the knee. The result of all these is pain and dysfunction in the mobility of the knee. Total joint replacement usually constitutes the final suggestion for the rehabilitation of these patients, but it is not free of complications. Thus, when applying this treatment, side-effects, age, weight and general physical condition of the patient must be taken into consideration [1].

Conservative treatment constitutes the first choice and many proposals have been made to alleviate symptoms, expecting at the same time an improvement in the histological features. The usual pharmacological recommendation includes analgesics, non steroidal anti-inflammatory drugs, diacerein, chondroitin sulphate, glucosamine sulphate, magnesium and others. Intra-articular injections also constitute a frequent choice and the injection of corticosteroids in acute pain or hyaluronic acid (when we also seek to improve damage to the cartilage) and have been applied for many years.

Biochemical changes in the levels C6S, C4S, and KS, TN-C [2] chemokine, endothelin and transforming growth factor alpha [3] metalloproteinases (MMP), cytokines such as IL-1 and TNF - alpha [4] and others appear to influence the development of osteoarthritis.

For some years intra-articular injection of medical ozone (O_2-O_3) has been suggested for the treatment of knee osteoarthritis symptoms and positive results have been reported [5]. However, its application appears to be empirical and no histological or biochemical studies have provided evidence for the repercussions of its use.

Ozone's powerful oxidant factor [6] has been reported to be toxic [7,8,9,10] and hence there is scepticism concerning its use for medical purposes. Other studies have shown that if ozone is used rationally it is safe [11] and therapeutic for certain diseases. Medical ozone (O_2-O_3 mix) appears to behave as a bioregulator [12] when it comes into contact with a biological liquid, releasing factors from human endothelial cells [13] and normalizing the cellular redox balance [14,15]. Studies have shown

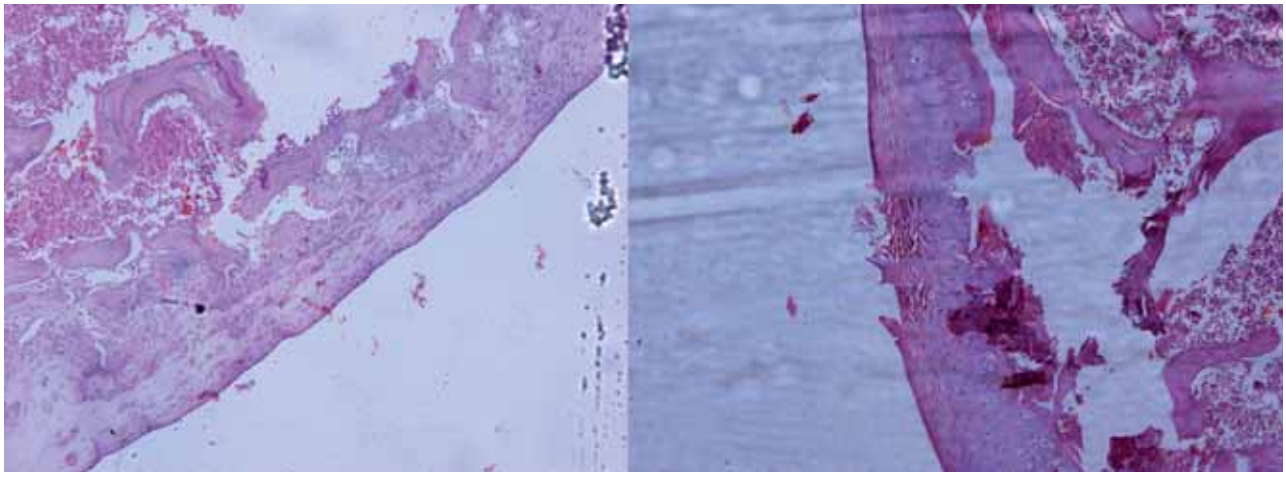

Figure 1 Sacrifice at 10 days. Initial degeneration of the cartilage with modification of the natural tissue to fibrosis, hyperaemia of the subchondral bone. H.E.×45.

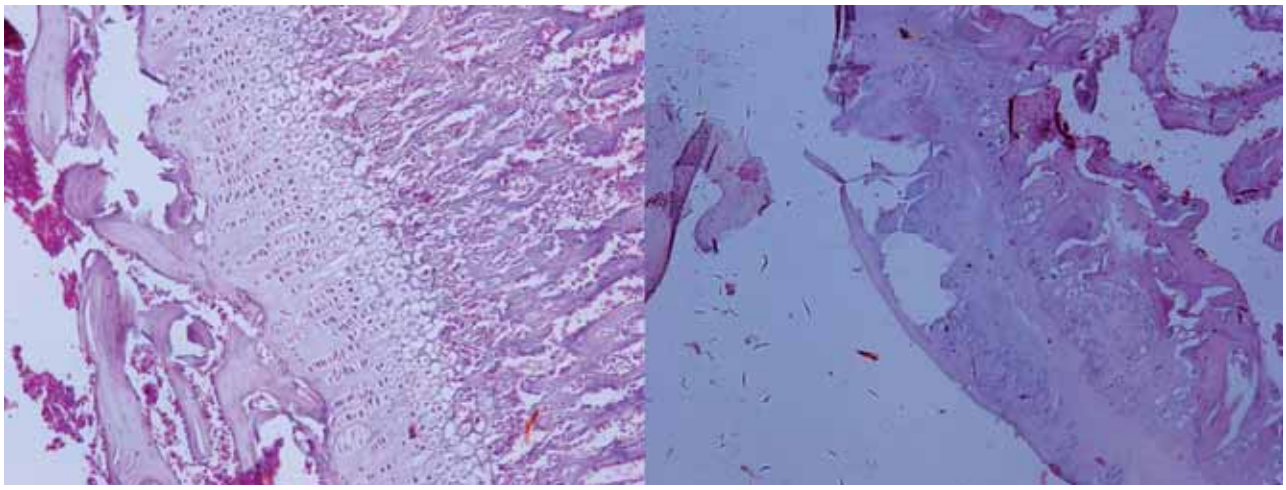

Figure 2 Sacrifice at 40 days. Total destruction of the architecture of the articular cartilage. H.E.×45.

its capacity to alter the levels the cytokines 16 such as TNF-alpha [17], IL [18] and platelet-derived growth factor (PDGF), transforming growth factor beta1 (TGF-beta1), interleukin-8 (IL-8) and other [19,20] indications of a likely effect of this gas on the articular cartilage when it is injected intra-articularly. Provided that ozone is indicated for use as a therapeutic means, due to its well-known antiseptic property [21,22 23,24], it would also succeed in reducing the potential dangers of septic arthritis after intra-articular injection of the gas mixture.

Controlled degeneration of the articular cartilage for experimental purposes can be caused by mechanical techniques [25] or by injection of chemical substances into the knee joints [26]. Monoiodoacetic acid (MIA) is a material that could cause osteoarthritis in the knees of Wistar rats similar to those of humans [27,28]. Rodents are the most widely employed species for these studies, because their night and day activity has been studied extensively and several models of osteoarthritis were developed using mice, hamsters and Wistars. The present study constitutes the first stage of comparison of histological changes and recommended methodology for the proof of action of intra-articular injection of O_2-O_3 mix and MIA in knee tissues.

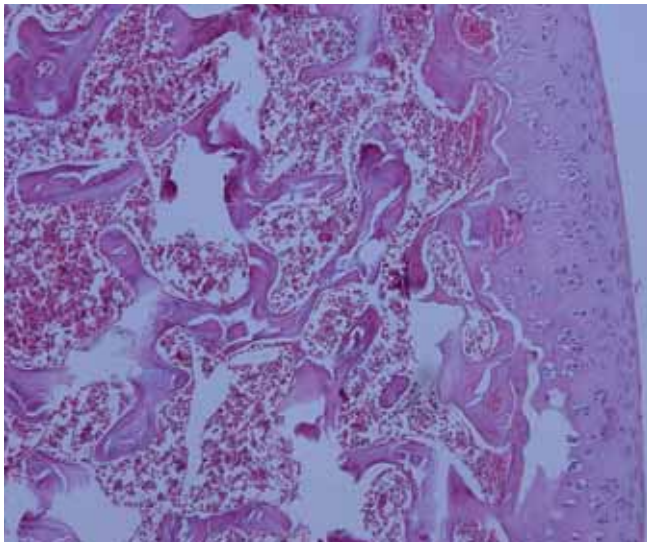

Figure 3 Normal articular cartilage (water, collagen and proteoglycans the surface layer thicker than the deeper layers). Tissue sections of the normal articular surface where the cells of the main membrane of histocell fibroblasts fat cells and mast cells are evident.

Figure 4 The tissues in and around the knee (apart from the cartilage) did not show any major alterations in the long run.

Material and Method

Thirty-six (36) male wistar rats weighing 150-180g were employed in our study. A variable volume (5 to 50 µL) automatic pipette was required. An ozone generator (Multiossigen mod. PM 93, Italy). Biochemical reactors (Iodoacetic acid I 8136 amp 5×500 mg, Sigma-Aldrich). The animals were divided into two groups. After anesthesia and disinfection a pre-set quantity was administered intra-articularly:

1. First group (18 Wistars): MIA was injected into the posterior joint of the right knee while the posterior joint of the left knee (placebo) was injected with NaCl solution.
2. Second group (18 Wistars): O_2-O_3 mix was injected into the posterior joint of the right knee while in the left knee joint (placebo) was injected with O_2.

The animals remained in plastic cages throughout the experiment. They were exposed to light every twelve hours (6-18) and the temperature was maintained at 20°C. The subjects received a short anesthesia with the use of ether, long enough for the action, and after the treatment they were returned to their cages. The rats were killed by ether overdose.

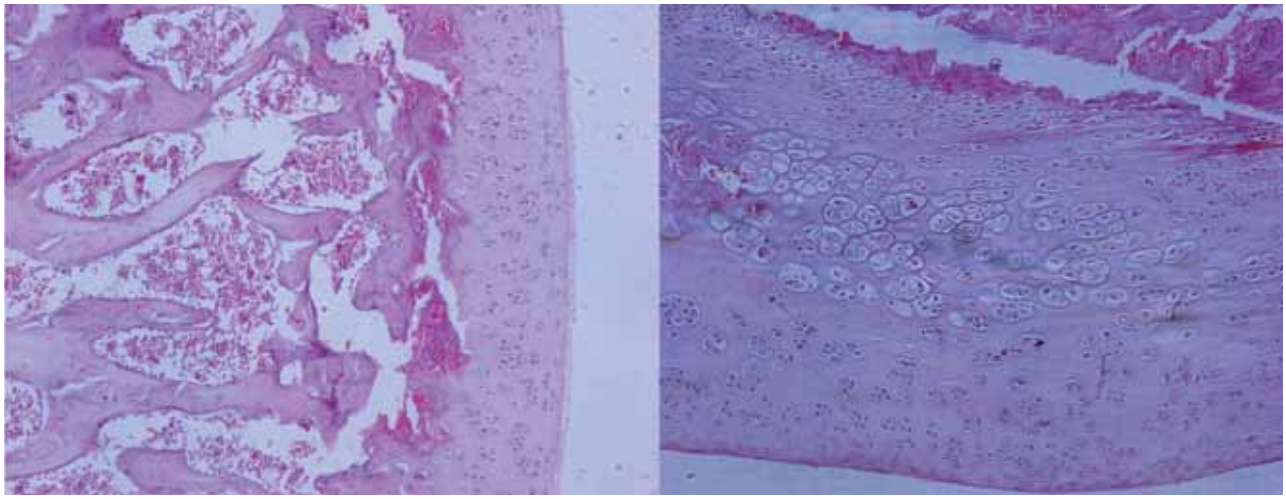

Figure 5 A microscopic image of the right knee joint treated with infusion of medical ozone (O_2-O_3 mix) H.E.×45. Note that the natural architecture is maintained. Inflammatory evidence on the soft tissues of the joint was absent.

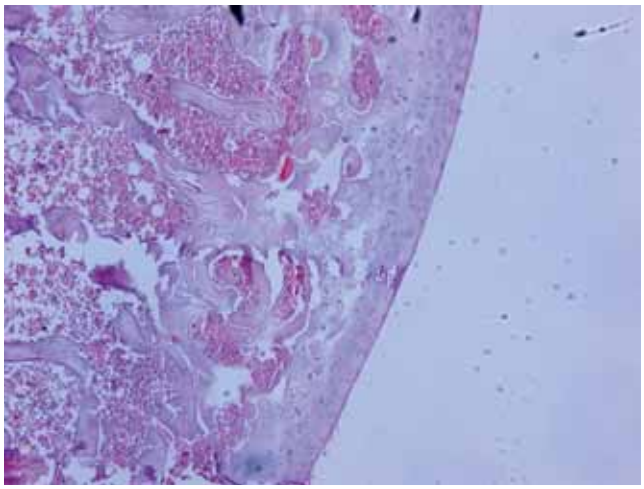

Figure 6 Microscopic images of left knees infused with oxygen (O_2). Note that the natural architecture of the tissues is maintained and articular inflammation is absent. H.E.×45.

The Wistars in the first group (MIA) were killed at two different times. Nine (9) rats ten days later, and nine rats 40 days later. The quantity of MIA had been set at 0.3 mg (corresponding to 0.05 ml of substance) per knee.

For the Wistars in the second group (O_2-O_3 mix) the O_2 and the O_2-O_3 mix injections were made three times a week for nine sessions in total. The administered quantity had been set at 0.05 ml in concentrations of 20 μg O_3/ml O_2 concentration empirically used for the treatment of osteoarthritis in humans.

The cartilage samples and the synovial membrane were studied histologically after being put in test tubes containing formalin 1/10.

Results

MIA

A) Macroscopically, the tissues in and around the knee (except for the cartilage) did not show major alterations.
B) Macroscopically and microscopically, the articular cartilage presented considerable degenerative alterations which gradually worsened in two phases with characteristics of non-specific arthritis (figures 1 and 2).
C) Knee joints infused with 0.9% NaCl (placebo) (figure 3).
D) During the study there were no side effects

or animal losses except for the reduction of the mobility of the right knee in the animals of the first group (MIA).

O_2-O_3 mix

A) During the histological testing, no significant change occurred in the cartilage or synovial membrane of the healthy knee animals, either after ozone administration (figures 4 and 5) at the predefined concentration for our experiment, or after the oxygen (figure 6).
B) No undesired event or animal loss.
C) On the contrary, a shorter recovery period was observed after the anesthesia and an increased mobility of the animals.

Discussion

Ozone in the form of an O_2-O_3 gas mix has been recommended for many years as a treatment for many diseases [23,29,30]. Initially, its application was limited to the positive nutritional effects on tissues [31] due to the improvement the gas induces on the circulation [32,33,34,35,36,37,38,39,40]. Today, the knowledge and wide use of medical ozone as a treatment in the form of major AHT and/or AHTs constitutes a challenging, safe and effective method if applied properly [23, 29].

Today's widest use of O_2-O_3 is mainly with the treatment of herniated disc and its symptoms. Its administration in the form of a gas mixture is direct (intradiscal or intraforaminal) [41,42] or indirect by intramuscular injection into the paravertebral muscles [43].

Ozone is a disputed gas. According to some authors, it is a powerful oxidant harmful to the human body. Scepticism concerning its administration and doubts over its therapeutic indications in any of its forms constituted a conviction for a considerable number of doctors. Ozone application, which was a personal choice of the consulting physician. Although the gas was administered empirically, physicians ascertained daily remarkable therapeutic results, absence of side-effects and their patients' appreciation for their method. Its acknowledgement and acceptance in the broader medical discipline has changed since Bocci's research became known and a set of rules and conditions under which O_3, once considered potentially toxic, can be therapeutically useful, was introduced. Today we know why and how the result is achieved in a series of diseases [23,29,30] and how this low-cost and manageable element can be used.

Studies on orthopaedic diseases are mentioned in clinical findings, dealing mainly with disc herniation. The satisfactory outcome has been ascribed to the activation of the immunogenic system by the ozone, which has an anti-inflammatory action due to lipid peroxidation products with a consequent inhibition of cyclooxygenase-2 [44,45].

A crucial factor, which accompanied the monitoring of results, was the strengthened constancy of the studied gas with the construction of ozone generators which continuously monitor the concentration of ozone in real time by a photometer calibrating system.

An initial histological and biochemical study examined: *a*) histologically, discs from patients operated while treated with O_2-O_3, and *b*) histologically and bio-chemically, discs and their soft tissues on animals (rabbits) after intradiscal or paravertebral infusion. Findings showed the close connection between ozone concentration in the change of the volume of the δ□σκου and the levels of cytokines (IL,IF,TNF) [46,47]. Ozone concentration within the O_2-O_3 mixture injected is the most crucial factor because it is what controls the movement of blood vessels and other elements of the blood. The concentration and quantity of O_2-O_3 chosen in this study was the one which has long been used empirically, adjusted to the weight of the animal.

This is the first of a series of studies designed to show whether the medical ozone, empirically used for the treatment of knee osteoarthritis, is experimentally safe, and whether it promotes the re-establishment of the damaged cartilage. The methodology followed was the same for both groups, with the only modification being the route of administration. Multiple infusions of medical ozone were chosen (three times a week for three weeks) so that the results will go to extremes, if they are destructive. The O_2-O_3 concentration selected was the one which has been used empirically by man for patients suffering from knee osteoarthritis: 20 μg O_3/ml O_2. The schedule of the histological and clinical effects is also described in this study. Based on this data concerning the animals (Wistar rats their general clinical condition before, during and after MIA and O_2-O_3 infusion was simultaneously monitored. Special emphasis was placed to the mobility of the knees front and rear (treatable). It was deemed possible that for a further comparison to demonstrate the heterosided knee of the study 0.9% NaCl be administered for the MIA and O_2 for the O_2-O_3.

The histological samples were O_2-O_3 by a specialized histopathologist and the Mankin [48,49] (table 1) method was employed for its evaluation (tables 2-7). The intra-articular infusion of O_2-O_3 did not cause apparent changes in the mobility of the knees and has not caused histological damage to the articular cartilage or the synovial membrane

Table 1 Histological and histochemical grading system for evaluation of articular cartilage degeneration (Mankin et Al).

Category	*Grade*
I. Structure	
Normal	0
Surface irregularities	1
Pannus and surface irregularities	2
Clefts to transitional zone	3
Clefts to radial zone	4
Clefts to calcified zone	5
Complete disorganization	6
II. Cells	
Normal	0
Diffuse hypercellularity	1
Cloning	2
Hypocellularity	3
III. Safranin-O staining	
Normal	0
Slightly reduced	1
Moderately reduced	2
Severely reduced	3
No dye noted	4
IV. Tidemark integrity	
Intact	0
Crossed by blood vessels	1

Table 2

O_2-O_3 Dx 40 Days	*Score*
Dx1	1
Dx2	3
Dx3	1
Dx4	0
Dx5	2
Dx6	3
Dx7	2
Dx8	3
Dx9	1
Dx10	2
Dx11	0
Dx12	2
Dx13	3
Dx14	4
Dx15	2
Dx16	2
Dx17	1
Dx18	0

(tables 2 and 3). The clinical condition that mainly concern the mobility of the studied knees is comparable to the one where the knees were infused with pure oxygen or saline solution

Monoiodoacetic acid (MIA) and the degenerative findings it caused proved the correctness (tables 4-7) of the employed technique and the fact that it always constitutes a sufficient method for causing experimental osteoarthritis and comparison of therapeutic material when needed. All the materials used were bearable for all the animals judging from the observation of the behaviour of our samples (fatality, aggressiveness, claudication, cordial disorder).

The animals whose knees were infused with O_2-O_3 recovered faster after the completion of the treatment. This is possibly due to the development of some kind of metabolic acceleration mechanism of the narcotic substance (ether) or directly due to oxygen-ozone which could have entered the circulation during the filtering through the synovial membrane.

The increase in life expectancy means a greater frequency of knee osteoarthritis of the elderly which in conjunction with negative working practices that cause cartilage disorders even at younger ages make new methods for the treatment and alleviation of the symptoms even more imperative. Medical ozone is already used empirically by many physicians as a supplementary or single therapy. If the histological results confirm these findings this will give a crucial boost to this method for treatment knee disease.

Leaving aside the ozone production machine, the cost of the materials and the gas mix is minimal. The already extensive bibliography on ozone behaviour in the body and especially in the immune system and herniated disc, and its local antiseptic activity, gave a starting point and provided scientific grounds for this therapeutic method.

Table 3

O_2 Sn 40 Days	*Score*
Sn1	4
Sn2	3
Sn3	4
Sn4	2
Sn5	1
Sn6	2
Sn7	2
Sn8	1
Sn9	1
Sn10	3
Sn11	1
Sn12	2
Sn13	4
Sn14	1
Sn15	2
Sn16	3
Sn17	3
Sn18	2

Table 4

MIA Dx 10 days	*Score*
Dx 1	10
Dx 2	9
Dx 3	8
Dx 4	10
Dx 5	12
Dx 6	12
Dx 7	9
Dx 8	11
Dx 9	9

Table 5

0.9% NaCl Sn 10 days	*Score*
Sn1	2
Sn2	4
Sn3	1
Sn4	2
Sn5	1
Sn6	4
Sn7	3
Sn8	1
Sn9	3

Table 6

MIA Dx 40 Days	*Score*
Dx1	14
Dx2	12
Dx3	13
Dx4	13
Dx5	14
Dx6	14
Dx7	12
Dx8	13
Dx9	14

Table 7

0.9% NaCl Sn 40 Days	*Score*
Sn1	4
Sn2	2
Sn3	3
Sn4	3
Sn5	4
Sn6	2
Sn7	2
Sn8	3
Sn9	1

It is imperative that this be proven experimentally before any extensive use. The present study shows us that there was no histological damage and the next study will investigate the therapeutic properties of O_2-O_3.

Conclusion

This research shows that the intra-articular infusion of MIA caused osteoarthritis in the animals very soon after injection. This step is considered

necessary for the study of treatment techniques for knee maintenance or operation, initially on animals and later on humans.

The intra-articular infusion of medical ozone to the healthy tissues of the knees of Wistar rats does not cause local damage or any undesired systematic or local events. On the contrary, the shorter recovery period after the anesthesia and more rigorous activity of the Wistars was observed compared to the rats treated with MIA. These findings could constitute the grounds for a study of pathological knees and the first step towards the dictum "primum non nuocere" for a potential therapeutic method.

References

1 McAlindon TE, La Valey MP, Guilin JP et Al: Glucosamine and chondroitin for treatment of osteoarthritis: a systematic quality assessment and meta analysis. JAMA 283: 1469-75, 2000.
2 Hasegawa M, Nakoshi Y, Tsujii M et Al: Changes in biochemical markers and prediction of effectiveness of intra-articular hyaluronan in patients with knee osteoarthritis 19: 2007.
3 Appleton T, McErlain DD, Henry JL et AL: Molecular and histological analysis of a new rat model of experimental knee osteoarthritis. Ann N Y Acad Sci 1117: 165-74, 2007.
4 Fernandes JC, Martel-Pelletier J, Pelletier JP: The role of cytokines in osteoarthritis pathophysiology. Biorheology 39: 237-46, 2002.
5 Riva Sanseverino E: Knee-joint disorders treated by oxygen-ozone therapy. Eur Med-phys 25, 163, 1989.
6 Bocci V: Ozone as Janus: this controversial gas can be either toxic or medically useful. Mediators Inflamm 13: 3–11, 2004.
7 Goldstein BD, Balchum OJ: Effect of ozone on lipid peroxidation in the red blood cell. Proc Soc Exp Biol Med 126: 356-8, 1967.
8 Freeman BA, Miller BE, Mudd JB: Reaction of ozone with human erythrocytes. In: Lee SD, Mudd JB eds. Assessing Toxic Effects of Environmental Pollutants, Ann Arbor, MI: Ann Arbor Science Publishers 1979: 151-171.
9 Uppu RM, Cueto R, Squadrito GL et Al: What does ozone react with at the air/lung interface? Model studies using human red blood cell membranes. Arch Biochem Biophys 10: 257-66, 1995.
10 Górnicki A, Gutsze A: In vitro effects of ozone on human erythrocyte membranes: an EPR study. Acta Biochim Pol 47: 963-71, 2000.
11 Bocci V: Is it true that ozone is always toxic? The end of a dogma. Toxicol Appl Pharmacol 216: 493-504, 2006.
12 Bocci V: Ozone as a bioregulator. Pharmacology and toxicology of ozonetherapy today. J Biol Regul Homeost Agents 10: 29, 1996.
13 Valacchi G, Bocci V: Studies on the biological effects of ozone: 11. Release of factors from human endothelial cells. Mediators Inflamm 9: 271-6, 2000.
14 Bocci V: Does Ozonetherapy normalize a cellular redox Balance? Med Hypotheses 46: 150-4. 1996.
15 Leon OS, Menendes S, Merino N et Al: Ozon oxidant preconditioning: a protection against cellular damage by free radicals. Mediators Inflamm 7: 289-94, 1998.
16 Bocci V, Luzzi E, Corradeschi F et Al: Studies on the biological effects of ozone: 3. An attempt to define conditions for optimal induction of cytokines. Lymphokine Cytokine Res 12: 121-6, 1993.
17 Paulesu L, Luzzi E, Bocci V: Studies on the biological effects of ozone: 2. Induction of tumor necrosis factor (TNF-alpha) on human leucocytes. Lymphokine Cytokine Res 10: 409-12, 1991.
18 Bocci V, Valacci G, Gorradeschi F et Al: Studies on the biological effects of ozone. Effects of the total antioxidant status and on interlukin-8 production. Mediat Inflamm 7: 313-7, 1998.
19 Valacchi G, Bocci V: Studies on the biological effects of ozone: 10. Release of factors from ozonated human platelets. Mediators Inflamm 8: 205-9, 1999.
20 Bocci V, Luzzi E, Corradeschi F et Al: Studies on the biological effects of ozone: 6. Production of transforming growth factor 1 by human blood after ozone treatment. J Biol Regul Homeost Agents 8: 108-12, 1994.
21 Dyas A, Boughton B et Al: Ozone killing action against bacterial and fungal species, microbiological testing of a domestic ozone generator. J Cl Pathol 36: 1102-1104, 1983.
22 Bocci V: Biological and clinical effect of ozone. Has ozone therapy future in medicine? Br J Biomed Sci 56: 270-9, 1999.
23 Bocci V: A new medical drug . Dordecht, The Netherlands: Springer 2005.
24 Thanomsub B, Anupumpisit V, Chanphetch S et Al: Effects of ozone treatment on cell growth and ultrastructural changes in Bacteria. J Gen Appl Microbiol 48: 193-9, 2002.
25. Azures B. Blanckaert A Thiechart M: Experimental post-contusive osteoarthritis of the knee: quantitative microscopic study of the patella and the femoral condyles. J Rheumatol 14: 119-121, 1987.
26 Bendele AM: Animal models of osteoarthritis. J Muscoloskel Neuron Interact 1: 363-376, 2001.
27 Guingamp C, Gegout-Pottie, Philippe L et Al: Mono-iodoacetate-induced experimental osteoarthritis: a dose-response study of loss of mobility, morphology and biochemistry. Arthritis Rheum 40: 1670-1679, 1997.
28 Williams J M, Brandt KD: Triamcinolone hexacetonide protects against fibrillation and osteophyte formation following chemically induced articular cartilage damage. Arthritis Rheum 28: 1267-1274, 1985.
29 Bpcci VA: Scientific and medical aspects of ozone therapy. State of the art. Arch Med Res 37: 425-35, 2006.
30 Re L, Mawsouf MN, Menéndez S, et Al: Ozone therapy: clinical and basic evidence of its therapeutic potential. Arch Med Res 39: 17-26, 2008.
31 Rokitansky O: Klinik und Biochemie der Ozontherapie. Hospitalis 52: 1572-1576, 1982.
32 Barber, Menendez, Leon et Al: Prevention of renal injury after induction of ozone tolerance in rats submitted to warm ischaemia, Mediat Inflamm 8: 37-41, 1999.
33 Coppolla L, Verrazzo G, Giunta R et Al: Oxygen ozone therapy and haemoreological parameters in peripheral chronic arterial occlusive disease. Trombosi e Aterosclerosi 3: 83, 1992.
34 Clavo B, Catala L, Perez Jl et Al: Effect of ozone therapy on cerebral blood flow: a preliminary report. Based Complement Altern Med 1: 315-9, 2004.
35 De Monde A, Van Der Zee H, Bocci V: Major ozonated autohemotherapy in chronic limb ischemia with ulcerations. J Altern Complement Med 11: 363-7, 2005.
36 Giunda R, Coppola A, Luongo C et Al: Ozonized autohemotransfuzion improves hemorheological parameters and oxygen delivery to tissues in patients with peripheral occlusive arterial disease, Ann Hematol 80: 745-8, 2001.
37 Larini A, Bocci V: Effects of ozone on isolated peripheral Blood mononuclear Cells. Toxicol In Vitro 19: 55-61, 2005.

38 Shinriki, Suzuki, Takama et Al: Susceptibilities of plasma antioxidants and erythrocyte constituents to low levels of ozone. Haematologia 29: 229-239, 1998.
39 Tylicki L, Biedunkiewicz B, Nieveglowski T et Al: Ozonated autohemotherapy in patients on maintance hemodialysis: influence on lipid profile and entotelium. Artif Organs 28: 234-7, 2004.
40 Verrazzo G, Coppola L, Luogo C et Al: Hyperbaric oxygen, oxygen-ozone therapy, and rheological parameters of blood in patients with peripheral occlusive arterial disease. Undersea & Hyperbaric Medicine 22: 17-22, 1995.
41 Andreula C, Simonetti L et Al: Minimally Invasive Oxygen-Ozone Therapy for Lumbar Disk Herniation. Am. J. Neuroradiol 24: 996-1000, 2003.
42 Muto M, Andreula C, Leonardi M: Treatmentof herniated lumbar disc by intradiscal and intraforaminal oxygen–ozone injection. J Neuradiol 31: 183-9, 2004.
43 Torri, Della Grazia, Casadei: Clinical experience in the treatment of lumbar disk disease, with a cycle of lumbar muscle injections of an oxygen-ozone mixture. Int J Med Biol 1999.
44 Bochov VN, Leitinger N: Antiinflammatory properties of lipid oxidation products. J Mol Med 81: 613-26, 2003.
45 Tamoto K, Yamasaki A, Nochi H et Al: Ozonidesa of olive oil and methyl oleat inhibit the expression of kB/nF k B pathway in lipopolysaccaharide-stimulated macrophage-like THP1 cells In: Proceedings of the 17th World Ozone Association Congress Strasburg France 2005: 37.
46 Iliakis E, Frangakis E, Agapitos E: Histological changes of intervertebral disks in rabbits after intradiscal injection of ozone. EFORT. Barcelona 1997.
47 Iliakis E, Valadakis V, Vynios DH et Al: rationalization of the activity of medical ozone on intervertebral disc: a histological and biochemical study. Riv Neuroradiol 14: 23-30, 2001.
48 Mankin H, Dorfman H, Lippiello L et Al: Biochemical and metabolic abnormalities in articular cartilage from osteoarthritic human hips. II. Correlation of morphology with biochemical and metabolic data. J Bone Joint Surgical Am 53: 523-37, 1971.
49 Kleemann Krocker D, Cedrano A, Tuischer J et Al: Altered cartilage mechanics and histology in knee osteoartitis: relation to clinical assessment (ICRS GRADE) osteoartritis and cartilage 13: 958-963, 2005.

Emmanuil Iliakis, MD
Orthopaedic Surgeon
Efroniou 8 Pagrati
15634 Athens - Greece
Tel.: 00392107228340
E-mail: driliakis@hotmail.com