Rationalization of the Activity of Medical Ozone on Intervertebral Disc

A Histological and Biochemical Study

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Key words: medical ozone, herniated disc, cytokines

SUMMARY – Ozonetherapy is used for the treatment of immunodeficiency syndromes as well as for the treatment of cardiovascular disease. It is also used for the treatment of low back-pain with promising results although it is not yet well established.

The aim of the current study is the presentation of the effects of ozonetherapy injected intradiscally or paravertebrally.

We present the histological, immunological and biochemical changes in vertebral discs.

Our material consist of human specimens as well as New Zealand rabbits.

Effetti biochimici e istologici dell'ozono sul disco intervertebrale

RIASSUNTO – *L'ossigeno-ozono-terapia è utilizzata per il trattamento delle sindromi da immunodeficenza e così pure in patologia cardio-vascolare. È utile anche nel trattamento delle lombalgie anche se i risultati, che sembrano promettenti, non sono ancora definiti completamente.*

L'obiettivo di questo studio è quello della presentazione degli effetti dell'osigeno-ozono terapia con iniezione intradiscale o paravertebrale.

Presentiamo le modificazioni immunologiche, istologiche e biochimiche, indotte dall'ozono, nel disco intervertebrale.

Il nostro materiale consiste di studi su dischi intervertebrali umani, in vitro, e su conigli neozelandesi.

Introduction

Herniated lumbar disc is accompanied by mechanical[24], and biochemical or immunological[30] alterations of the disc itself as well as its microenvironment, causing low back pain and sciatica.

Surgical discectomy (laminectomy) and the intradiscal injection of substances that cause proteolytic degradation of collagen and proteoglycans are a mechanical way to relieve the symptoms by decompressing the neural structures due to the protrusion of the disc.

Medical treatment on the other hand, is based on the use of analgesics and anti-inflammatory drugs that inhibit the production of various substances responsible for the pain. It has been shown recently that some chemical agents produced by the granulation tissue, which is formed around the

protrusion of the disc, are responsible for the painful symptoms. Interleukin-1α, interleukin-6 and TNF-α are produced by histiocytes, fibroblasts and chondrocytes [9,13,27,28]; many chondrocytes producing cytokines are found at the site of the protrusion of the disc [30]. Cytokines induce the production of prostaglandin E2 by these cells, which causes pain or enhances the sensitivity of the nerve roots to other pain-producing substances, like bradykinin. Substances that inhibit cytokine production, like steroids, are expected to decrease the pain [1,17,18,20,29,32].

Results from various studies have shown that medical ozone (mixture of oxygen/ozone) has various dose-related effects. It may cause alterations of tissue structure [19,35], at high concentration levels, regulation of the immune system (cytokine production) [5,21,22] at medium concentrations, and improvement of the microcirculation at low concentrations [3,8,11,19,34]. The intradiscal or paraspinal injection of medical ozone is at present used for the treatment of lumbar disc syndrome due to disc herniation [7,15,16,33].

The positive clinical results obtained may be due to a combination of the known properties of medical ozone and of the causes responsible for low back and sciatic pain. However, the empirical application of this treatment to patients and the lack of experimental results concerning the effect of medical ozone on the herniated disc weaken the reliability of this method.

This study is an attempt to establish criteria for the proper use of medical ozone in humans. It consists of three parts:

1) The investigation of cytokine production after paraspinal injection of medical ozone in animals, in exactly the same way as applied to humans.

2) The histologic study of the disc structure after intradiscal injection of medical ozone.

3) The study of the *in vitro* effect of medical ozone on human discs and posterolateral ligaments and cartilage from the lateral apophyseal joints after surgical discectomy.

Experimental

Materials

Medical ozone was prepared using the apparatus Multiossigen (I) Model Multitech P.M. 94. The gas was collected with five ml syringes and the needles used for the injections were 26 GX5/8. ELISA reagents for cytokine determination were purchased from Endogen (Cambridge, MA, USA). All chemicals used throughout the study were of the best available grade.

Paraspinal injection of medical ozone in rabbits

Five white New Zealand rabbits were used. One ml of a mixture of oxygen/ ozone in concentrations described below was injected, in one cm depth and one cm laterally from the midline of the spinal processes and two vertebrae above the line joining the higher points of the iliac crest. A total of six injections were applied, twice a week. Three rabbits (P17, P18, P19) received 30 μg of ozone per ml of oxygen, a dose which empirically is used or is regarded as satisfactory for humans [7,15,33]; another rabbit (P20) received a higher concentration of ozone, 55 μg per ml of oxygen, and a fifth rabbit (P21) was used as reference and received only pure oxygen. The animals were sacrificed, two (P17 and P21), four (P18 and P20) and twenty (P21) hours after the sixth injection by intravenous injection of an anaesthetic mixture containing cetamine, methezolame and atropine and three samples from their spinal column were collected. First, the vertebra where the medical ozone was injected together with the surrounding muscle; second, the proximal vertebra with the surrounding muscle and third, a similar section four vertebrae away from the injection point. The samples were stored at –40°C until used for cytokine determination.

Intradiscal injection of medical ozone to rabbits

Fifteen New Zealand rabbits were used in this experiment divided into three groups, according to the concentration of the ozone applied. Each group of five rabbits to which the same amount of medical ozone was injected for various time periods. The concentration of medical ozone used was 6, 27.5 and 70 μg of ozone per ml of oxygen for each group, respectively, and 0.5 ml was injected intradiscally.

The animals were subjected to general anaesthesia as above, the skin around the area of O4-O5 was cleaned, a cut parallel to the mid line of the spinal processes about one cm from them was made. The intradiscal injection was performed in the nucleus pulposus under radiologic control (figure 1) and the wound was closed by mattress suture; no drainage was necessary. The disc to which the injection was performed was recognized by a mechanical fracture made on the transverse process.

In another rabbit a simple perforation of the O4-O5 disc was made and the disc was removed surgically after five weeks. Another disc from this rabbit was also removed and used as a control.

After 1 and 2 days and 1, 2 and 5 weeks, the animals were sacrificed as described above. The in-

jected discs and the proximal discs were removed with the surrounding soft and hard tissues and preserved in formalin solution for histologic studies.

In another experiment, three discs were removed from a normal rabbit, placed separately in test tubes, covered with Ringer Lactated buffer solution, and 0.5 ml of medical ozone (containing 6, 27.5 and 70 μg of ozone per ml of oxygen, respectively) was injected into each tube through the rubber lid stopper. The tubes were shaken mildly for five min[3], stood at 4°C for one hour and then put in formalin solution for histologic study.

Effect of medical ozone on human discs in vitro

Parts of the facet joints, including cartilage and ligamentous elements of the spinal column were removed surgically as treatment of spinal canol narrowing. Discs from patients after surgical discectomy of herniated discs were also used. The tissues were cut in small pieces, placed in test tubes containing Ringer Lactated buffer solution, and 0.5 ml of medical ozone containing 27.5 μg of ozone per ml of oxygen was injected into each tube several times through the rubber plug. The tubes were shaken mildly for five min and left at 4°C for varions lengths of times as follows: zero, six, twelve, eighteen and twenty four hours, for one, two, three, four and five times, respectively. At the end of the experiment, the discs were transferred to formalin solution and examined for any histologic alterations. Histologic examination was also performed on a disc of a patient who had started ozonotherapy but discontinued it and received surgical discectomy.

Determination of cytokines in tissue extracts

The samples removed from the rabbits which had received paraspinal injections of ozone were transferred to 4°C, the muscles were separated from the vertebrae and cut in small pieces (2-3 cubic mm). Then the tissues were homogenized in a buffer solution (5 ml/g wet tissue) of 0.05 M potassium chloride, 0.05 M sodium acetate pH 6.8 containing the proteinase inhibitors: ε-amino-n-caproic acid (0.1 M), benzamidine hydrochloride (5 mM), phenylmethanesulfonylfluoride (0.4 mM) and N-ethyl-maleimide (10 mM). The metalloproteinase inhibitor sodium EDTA was omitted because it interfered with interleukin determination. Homogenization was performed in a Sorvall homogenizer at 4°C for twenty s at half of the highest speed and then for ten s at the highest speed. The homogenates were then subjected to centrifugation at 4°C for twenty min at 12,000 rpm in a Sorvall centrifuge (rotor SS-24). The supernatants containing the soluble proteins of the tissue were stored at –20°C until use and the precipitates containing mainly myosin were rejected.

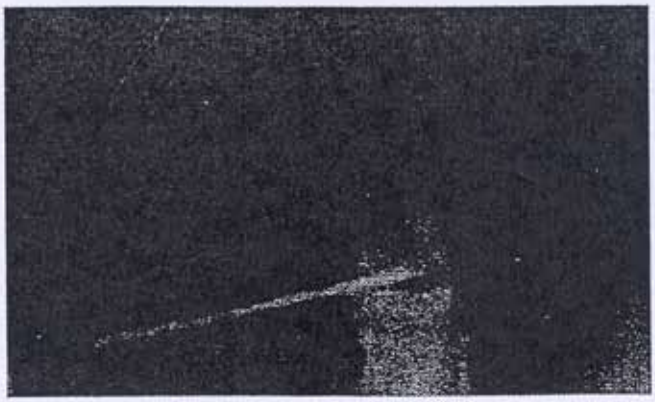

Figure 1 Endodiscal infusion of ozone. The needle is in the center of nucleus pulposus.
Figura 1 Iniezione intradiscale di ozono, nel coniglio. L'ago è al centro del nucleo polposo.

The quantitative determination of the cytokines, interleukin-1β (IL-1β), interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α) was performed by the ELISA assay purchased by Endogen, which can be applied to human and rabbit cytokines.

Preparation of samples for histology

The tissues fixed in formalin solution were embedded in paraffin sliced with a microtome, stained with hematoxylin-eosin and subjected to light microscopy, and thick 3 mm to examine collagen fibrils and cell integrity.

Results

Effect of ozone on cytokine production (by rabbit muscles)

The effect of ozone on the production of the three cytokines, interleukin-1β, interferon-γ and tumour necrosis factor-α by rabbit muscles was examined for two different concentrations of medical ozone, 30 and 55 μg, and the results were compared to those obtained from the control experiment, where pure oxygen was injected (table 1).

The injection of 30 μg of ozone per ml of oxygen seemed to increase the amount of TNF-α in the area of the injection. The increase became higher four hours after the injection and remained high after twenty hours. On the other hand, in the proximal area, a rapid increase in TNF-α concentration was observed (maximum value at two hours), followed by a decrease which was maintained up to

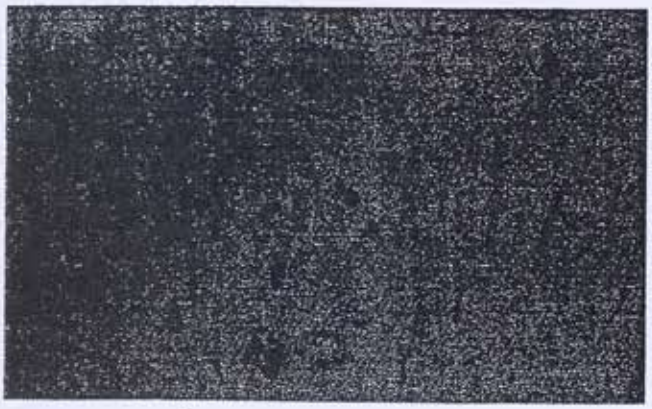

Figure 2 Histologic examination of intravertebral disc with intense oedeme (H.E. x 40).
Figura 2 Preparato istologico da disco intervertebrale: edema intenso (HE 40 x).

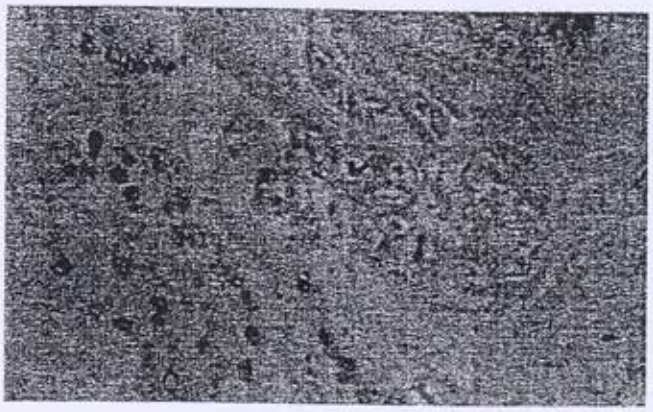

Figure 3 Intermediate fibrosis of the disc with degeneration of the cells (H.E. x 40).
Figura 3 Grado intermedio di fibrosi discale con degenerazione cellulare (HE 40x).

Figure 4 Full degeneration of the disc.
Figura 4 Completa degenerazione discale.

twenty hours. The injection of 55 μg of ozone per ml of oxygen seemed not to affect TNF-α concentration in the area of the injection. It seemed that this amount of medical ozone had a concomitant cytotoxic effect and thus, no difference in TNF-α concentration was measured.

The effect of medical ozone on IL-1β was somewhat different. The injection of 30 μg acted by increasing the concentration of IL-1β initially (first two hours). Four hours after the injection, a decrease was observed, which was maintained in the proximal area up to twenty hours, while in the area of the injection and the distal area even more increased concentrations of IL-1β were measured. The injection of 55 μg of ozone per ml of oxygen produced a dramatic decrease of IL-1β in the area of the injection and proximally to it, but an increase in the distal area, possibly by maintaining the cells in an activated status because of the high amounts of medical ozone injected, which diffused continuously to that area.

The injection of 30 μg of medical ozone had a slightly positive effect on IFN-γ production two hours after it, but after four hours a considerable decrease was observed. IFN-γ production was restored after twenty hours, only in the proximal area of the injection, while in the area of the injection it continued to be decreased, possibly because medical ozone was diffused from the vertebra of the injection. In the distal area, a small increase was measured after four hours and a small decrease after twenty hours, but these differences seemed not to be significant. The injection of 55 μg of medical ozone seemed to act similarly. It decreased strongly the amount of IFN-γ in the area of the injection and proximally to it, but increased it in the distal area.

From the above results, it may be concluded that medical ozone affected the production of the cytokines analyzed. TNF-α increased, IFN-γ decreased and IL-1β increased rapidly and then decreased.

Histologic examination of discs after intradiscal injection of medical ozone

The smaller concentration of medical ozone examined (six μg), produced initially a mild oedema of disc resulting finally, in the fifth week, in some focal degenerative alterations of collagen fibrils without affecting the cells. At concentrations of 27.5 μg, an interstitial oedema was initially observed, together with eosinophilic degeneration of cytosol and shrinkage of cell nucleus. The alterations were mild on the first day (figure 2), where only the oedema could be observed and became more intense on the second day. Multiple alter-

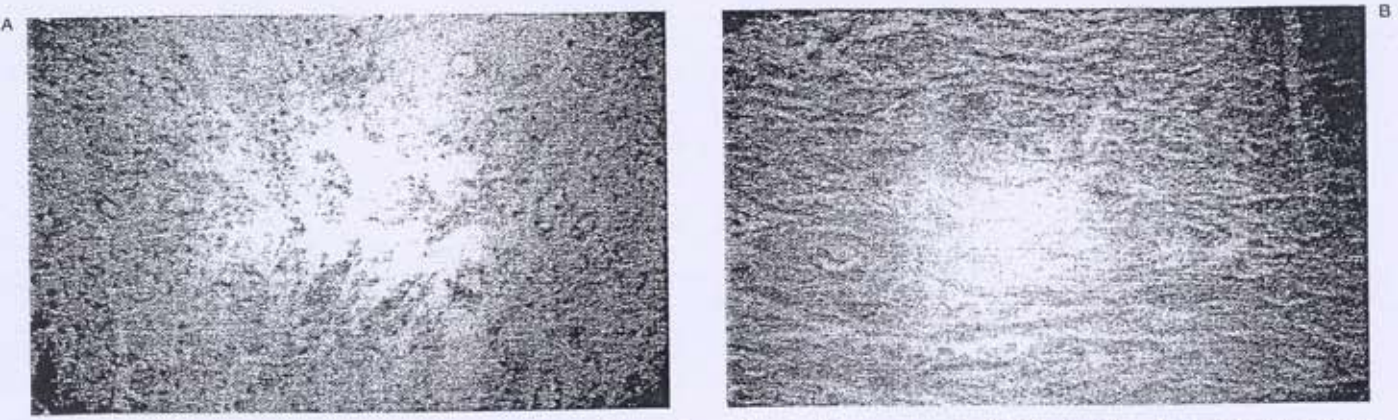

Figure 5 A) Inflammation of the synovia is apparent. B) Synovia are intact after exposure to O_2/O_3 for 6h and concentration 30 µg 0.3/ml O_2.
Figura 5 A) Infiammazione sinoviale. B) Sinovia intatta dopo esposizione all'ozono, concentrazione di 30 µg in 0,3 ml di ossigeno, per sei ore.

ations, with shrinkage of cell nucleus and cellular degeneration, were observed in the first week. In the second week (figure 3), the oedema decreased, the cell degeneration continued and the disc volume decreased. In the fifth week (figure 4), the disc became fully degenerated, free of cells, and the matrix was replaced by fibrous connective tissue. The concentration of seventy µg of medical ozone resulted in rapid alterations of the disc. The interstitial oedema, the cytoplasmic degeneration and shrinkage of the cell nucleus became apparent from the first and second days after the intradiscal injection. These findings were more intense in the disc of the animal sacrificed one week after the injection. Then the structural alterations and the oedema subsided and in the fifth week the oedema was observed only in the peripheral part of the anulus fibrosus; the nucleus pulposus appeared more hypoplastic.

In the case of rabbit with perforation of the disc without the injection of medical ozone, no degenerative alterations were observed but only a mild interstitial oedema. Lamellar bone thinner than mature bone was observed peripherally, with smaller gaps compared to normal. Osteoblastic activity and bleeding, the latter possibly due to a contact of the needle with bone, were also observed.

No osteoblasts were observed in normal disc, while a mild osteoclastic activity existed. The fibrous tissue protecting the vertebrae was rich in blood vessels. No excitement or inflammation was observed in any of the animals.

Table 1 **Concentration of cytokines, ng/g wet muscle tissue**

		TNF-α	IL-β	IF-γ
30 µg O_3				
17-D	2h	7.3	4.0	22.0
17-P	2h	10.1	6.3	29.0
17-O3	2h	4.8	4.0	25.0
18-D	4h	8.8	1.6	37.6
18-P	4h	6.1	2.0	7.6
18-O3	4h	10.0	1.0	1.5
19-D	20h	10.8	3.5	17.5
19-P	20h	2.4	1.7	4.5
19-O3	20h	8.4	4.9	23
50 µg O_3				
20-D	4h	16.2	10.9	74.5
20-P	4h	4.6	2.0	1.3
20-O3	4h	2.6	0.5	3.7
O_2				
21-D	2h	4.9	2.3	30.7
21-P	2h	6.2	3.0	10.4
21-O2	2h	3.6	2.0	20.0

D = distal, P = proximal, O3 = point of injection

Histologic examination of human discs after in vitro incubation with medical ozone

The histopathologic study of the human discs treated in vitro with medical ozone has shown that the inflammation of the posterior joints was reduced (figure 5), in accordance with the relationship between degeneration and cytokine pres-

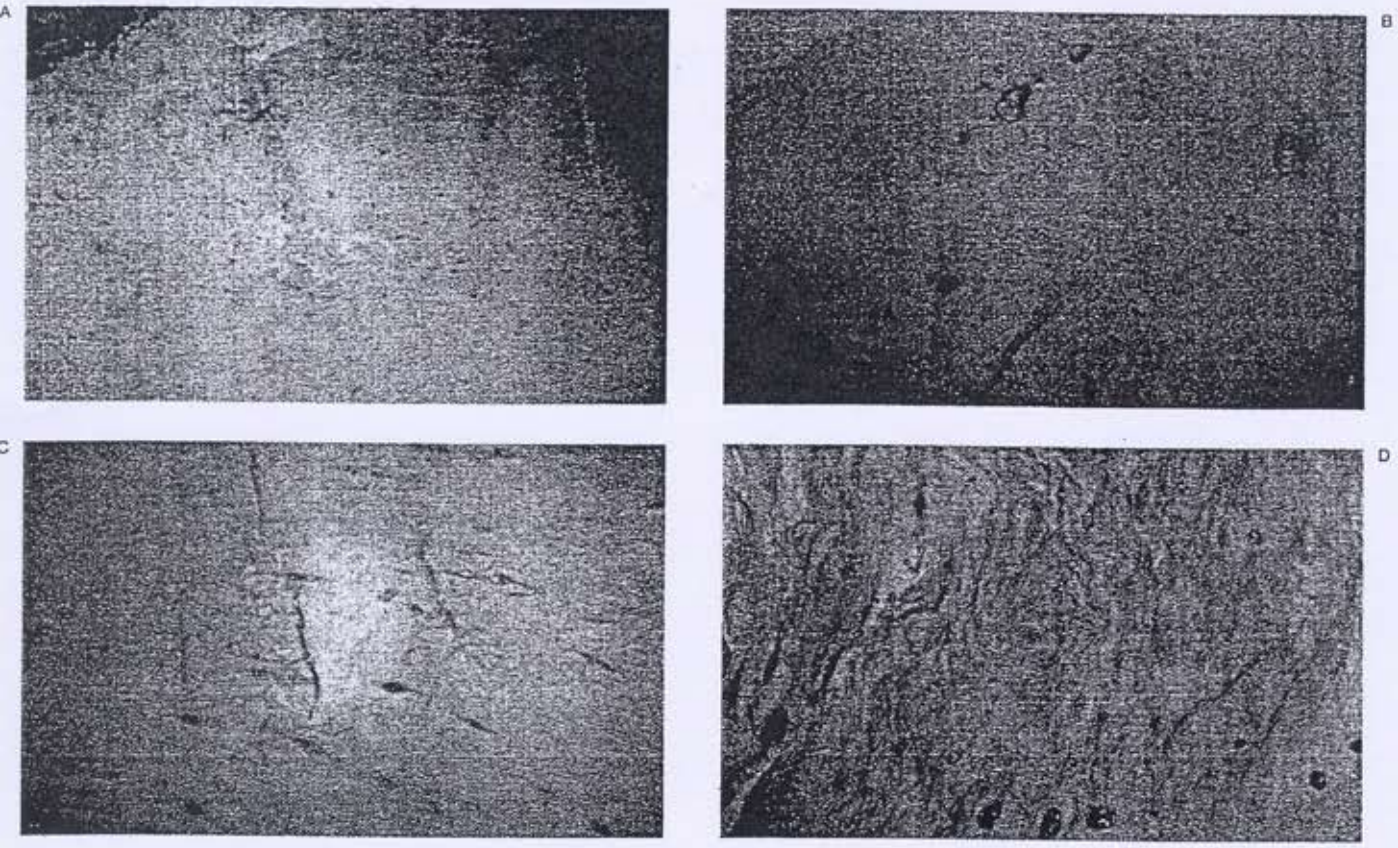

Figure 6 A) After the first addition of ozone; the signs of degeneration are obvious (H.E. x 125). B) After 3 consecutive additions of ozone; the signs of degeneration. C) After 4 additions of ozone; intense degeneration. D) After 5 additions of ozone; degeneration of collagen and appearance of oedema.
Figura 6 A) Chiari segni di degenerazione dopo una prima iniezione di ozono (HE 125x). B) Segni di degenerazione dopo tre consecutive iniezioni. C) Degenerazione intensa dopo quattro iniezioni. D) Alla quinta iniezione, degenerazione del collagene e edema.

ence [12,22]. In addition, degeneration of the disc was observed (figure 6) which was apparent on cells and collagen fibrils. The histologic examination of the disc from the patient who interrupted the medical ozone treatment showed degeneration of the disc and the presence of newly formed blood vessels (figure 7).

Discussion

The present study was undertaken to examine the applicability of medical ozone for the treatment of herniated disc, and generally low back pain and sciatica. Medical ozone has been studied until now in the cases of posttraumatic or degenerative knee-joints [25], osteoporosis [26] and systemic arthropathy [10]. Medical ozone has also been used for the treatment of orthopaedic diseases or low back pain [7,15,16,33], without any references on how it acts.

In our study, we decided to apply intradiscally medical ozone at three different concentrations for the following reasons: 1) Six μg of ozone per ml of oxygen is used in cases of venous insufficiency, lipodystrophy and it is believed to induce TNF-α production in vitro, and to be harmless to any tissue. 2) Twenty-seven to thirty-two μg of ozone per ml of oxygen is empirically preferred for paraspinal injections in herniated disc cases. 3) Fifty five μg of ozone per ml of oxygen (in case of paravertebral injection) is in the range of concentrations which can affect the immune system according to Bocci [2-6]. 4) Seventy μg of ozone per ml of oxygen can produce oedema and alterations in tissue structure, and is believed to act similarly to chymopapain [19,35].

The results of the histologic study suggested that the optimum concentration of medical ozone for positive clinical results was 27.5 μg of ozone per ml of oxygen. At this concentration, a mild oedema appeared initially, together with simple histologic alterations and degeneration of the cells and the collagen fibrils. The thinning of the anulus fibrosus, the presence of proliferative zones, the

onset of cartilage calcification and the decrease of the total volume of the disc together with its transformation to fibrous connective tissue would contribute to the decompression of the neural roots. Newly formed blood vessels were noted which were also observed in the disc of the surgically treated patient. On the other hand, the concentration of 6 μg of ozone per ml of oxygen seemed not to be sufficient to produce any alterations in the intervertebral disc and therefore it is regarded as ineffective. The concentration of 70 μg of ozone per ml of oxygen produced strong oedema, which continued for many days. This is an undesirable effect for herniated discs, because of the increase in neural compression, while its final results seemed not to be better than those obtained with 27.5 μg of ozone.

The *in vitro* exposure of the discs to medical ozone produced similar results with those obtained from the *in vivo* experiments with comparable concentrations, where the animals were sacrificed the first postoperational day. The fact that disc degeneration occured *in vitro* after multiple injections of medical ozone, while *in vivo* one injection was enough, (see intradiscal infusion) suggested that medical ozone had some immediate biologic activity (figure 8). This might be the result of the release of free radicles due to the reduction of ozone to oxygen, which could affect either the matrix of the disc directly or after activation of cellular proteolytic enzymes via dose-dependent alterations in cytokine production. The best histological results were evident five weeks postoperatively suggesting that the expected positive clinical results appear after some time.

The above findings were also supported by the determination of cytokine levels, locally and distally, after paraspinal or intramuscular injection of medical ozone in rabbits, where these results were comparable to those obtained after autohaemotransfusion.

Our results are encouraging because they show that: 1) The local injection of medical ozone at a concentration of 30 μg of ozone per ml of oxygen increases the concentration of TNF-α around the disc and decreases pain and inflammation. 2) IL-1β, which increases matrix degradation and consequently the pain due to disc herniation, is greatly decreased when a concentration of medical ozone of 55 μg per ml of oxygen was used. 3) IFN-γ is highly decreased locally and increased distally. All these findings suggest that medical ozone contact with the disc causes degeneration of the extracellular matrix, resulting in shrinkage and decompression of the neurons around it. This might proceed probably together with the improvement of blood circulation (oxygenation of neurons)[31], the decrease in lactic acid and inflammatory cytokines, resulting in the decrease of low back pain and sciatica.

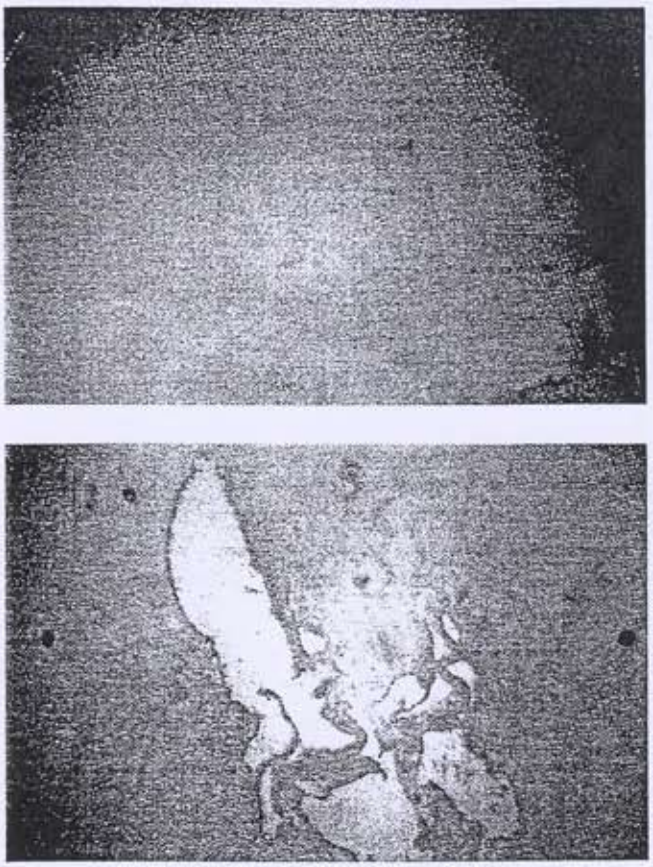

Figure 7 Appearance of new blood vesels with red blood cells inside them.
Figura 7 Angioneogenesi, vedi testo.

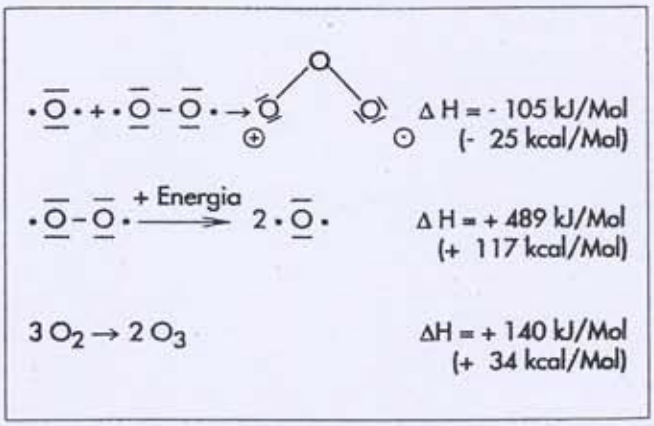

Figure 8 The liberation of energy during the decomposition of ozone (Rilling S, Viebahn R: The use of Ozone in Medicine. Hang KF (eds). II Edition, Heidelberg 1987).
Figura 8 Liberazione dell'energia nel corso della scomposizione dell'ozono.

Conclusions

Paraspinal injections of medical ozone are a painless medical treatment for herniated disc and its symptoms at low cost. This method might have positive results even in serious cases, where the patients cannot or do not want to be subjected in surgery. The optimium concentration of medical ozone seems to be between 30 and 55 μg ozone per ml of oxygen. This treatment could be applied to humans simply in the form of intradermal injections under radiologic control.

The result, concerning the disc, seems to be the same, independently of whether the injection is intradiscal (only one injection) or paraspinal (multiple injections). In the latter case, the improvement of the physical state of the patients is more rapid.

References

1 Beautier B, Krochin N et Al: Control of cachectin (tumor necrosis factor) synthesis. Mechanisms of endotoxin resistance. Science 232: 977-980, 1986.
2 Bocci V: Ozonization of blood for the therapy of viral diseases and immunodeficiencies. A hypothesis. Med Hypotheses 39: 30-34, 1992.
3 Bocci V: Autohaemotherapy after treatment of blood with ozone. A reappraisal. J Int Med Res 22: 131-144, 1994.
4 Bocci V, Paulesu L: Studies on the biological effects of ozone. 1) Induction of interferon gamma on human leucocytes. Haematologica 75: 510-515, 1990.
5 Bocci V, Luzzi E et Al: Studies on the biological effects of ozone. 5) Evaluation of immunological parameters and tolerability in normal volunteers receiving ambulatory Autohaemotherapy. Biotherapy Biophys Acta 1271: 67-64. 1995.
6 Bocci V, Lucci E et Al: Studies on the biological effects of ozone. 5) Evaluation of immunological parameters and tolerability in normal volunteers receiving ambulatory autohaemotherapy. Biotherapy 7: 83-90, 1994.
7 Bonetti M, Cotticelli B et Al: Oxygen-ozone therapy vs epidural steroids injections. Rivista di Neuroradiologia 13: 203-206, 2000.
8 Coppola L et Al: Oxygen-ozone therapy and peripheral chronic arterial occlusive disease. Thombosi & Aterosclerosi 3(2): 83-89, 1992.
9 Dayer JM, Breard J et Al: Participation of monocyte-macrophages and lymphocytes in the production of a factor that stimulates collagenase and prostaglandin release by rheumatoid synovial cells. J Clin Invest 64: 1386-1392, 1979.
10 Fahmy Z: Ozone therapy in rheumatic disease. Kongrelberientht 10 A. Ozon therapie bei Erkrankungen der rheumatischen Formenkreises. Ozo Nanhrichten 4: 79, 1985.
11 Franzini M et Al: Subctaneous oxygen-ozone therapy in inductive hypodermatitis and in localised lipodystrophies a Clinical Study of Efficacy and Tolerability. Acta Toxicologica et Therapeutica, 1994.
12 Goldring S, Goldring M: Cytokines and Skeletal Physiology. Clin Orthop and rel resh 324: 13-23. 1996.
13 Guerne PA, Carson DA, Lotz M: IL-6 production by human articular chondrocytes. Modulation of its synthesis by cytokines, growth factors, and hormones in vitro. J Immunol 144: 499-505, 1990.
14 Halliwell B: Free radicals and antioxidants a personal view. Nutr Rev 52: 253-265, 1994.
15 Iliakis E: Ozone treatment in low back pain. Orthopaedics 1: 29-33, 1995.
16 Iliakis E: Utilizzo dell'ossigeno-ozonoterapia nella pratica ortopedica. Acta Toxicol Ther XVII(2-3): 249-253, 1996.
17 Kawakami M, Ikeda Y et Al: Stydies of conditions and agents that stimulate and inhibit production of cachectin by macrophages. In: Patton W, Mitchel J, Turner P (eds): IUPHAR 9th International Congress of Pharmacology. Macmillan Press, London 1984: 377-384.
18 Luedke CE, Cerami A: Interferon-γ overcomes glucocorticoid suppression of cachectin tumor necrosis factor biosynthesis by murine macrophages. J Clin Invest 86: 1234-1240, 1990.
19 Mattashi R: Ozonetherapia. Organizzazione Editoriale Medico Farmaceutica, Milano 1985: 1-179.
20 Miyasaka N, Sato K et Al: Augmented interleukin-1 production and HLA-DR expression in the synovium of rheumatoid arthritis patients. Arthritis Rheum 31: 480-486, 1988.
21 Paulescu L, Luzzi E, Bocci V: Studies on the biological effects of ozone. 2) Induction of tumor necrosis factor (TNF-α) on human leukocytes. Lymphokine Cytokine Res 10: 409-412, 1991.
22 Paulescu L et Al: Studies on the biological effects of ozone. 2) Induction of tumor necrosis factor (TNF-α) on human leukocytes. Lymphocaine e Cytokaine Research 10(5): 409-412, 1991.
23 Pelletier JP: Pathological partwass of osteoarthritis. Int Congr and Symp. Series No 208. Royal Society of Medicine Press 1994.
24 Resnick D, Niwayama G: Diagnosis of bone and join disorders. W.B. Saunderes, Philadelphia PA 1988.
25 Riva Sanseverino E: Knee-joint disorders treated by oxygen-ozone therapy. Eur Medicophysica 25: 163-170, 1989.
26 Riva Sanseverino E: Intensive medical physical treatment of osteoporosis with the aid of oxygen-ozone therapy. Europa Medicophysica 25(3): 163-170, 1989.
27 Shinmei M, Masuda K, Kikutsi T: The role of cytokines in chondrocyte mediated cartilage degeneration. J Reumatol 18 (Suppl 16): 32-34, 1989.
28 Shinmei M, Masuda K et Al: Production of cytokines by chondrocytes and its role in proteoglycan degradation. J Reumatol 18 (27 Suppl): 89-91, 1991.
29 Snyder DS, Umanne ER: Corticosteroids inhibit murine macrophase la expression and interleukin 1 production. J Immunol 129: 1803-1805, 1982.
30 Takahashi H et Al: Inflammatory cytokines in the herniated disc of the lumbar spine. Spine 21(2): 218-224, 1996.
31 Utito J. Prokop DJ: Synthesis and secretion of underhydroxylated procollagen at various temperatures by cells subjects to temporally anoxia. Biochem Biophys Commun 60: 414. 1974.
32 Vacca A, Martinotti S et Al: Transcriptional regulation of the interleukin 2 gene by glcocorticoid hormones. J Biol Chem. 8075-8080, 1990.
33 Verga C: Nuovo approccio terapeutico alle ernie e protrusioni discali. Rivista di Neuroradiologia 2 (suppl), 1989.
34 Verrazzo G, Coppola L et Al: Hyperbaric oxygen,oxygen-ozone therapy and rheologic parameters of blood in patients with peripheral occlusive arterial disease. Undersea Hyperbar Med 2: 17-22, 1995.
35 Viebahn R: The use of ozone in medicine. 2nd ed. Karl F. Hang Publishers, Heidelderg 1994: 1-178.

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