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Si, proprio così.
A Brescia è nato un Centro Polifunzionale in grado di soddisfare i bisogni di tutta la famiglia. Dove il rapporto umano viene prima di tutto. E dove specialisti e fisioterapisti di alto livello si incontrano con metodi, sistemi e tecnologie avanzatissimi.

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Editorial

Cari Amici e Colleghi

Purtroppo devo aprire questo mio editoriale con una notizia molto triste: da poco è scomparso il nostro Presidente della W.F.O.O.T, Vijay Sheel Kumar, che dopo lunga malattia ci ha lasciati.

Ci lascia un grande uomo che ha vissuto insieme a tutti noi con entusiasmo, passione, impegno, dedizione il cammino dell’ozonoterapia nel Mondo, un maestro di vita, un uomo saggio, un amico, un padre.

Vijay ci mancherai, ci mancherà il tuo sorriso intelligente, il tuo saper essere sempre propositivo e positivo.

Grazie Vijay per tutto quello che hai fatto e che ci hai dato.

Così lo cita il Guardian Health Chronicle

Neurosurgeon turns ozone therapist

Dr Vijay Sheel Kumar is a pioneer in Ozone Therapy, a new treatment in spinal disorders that is non-invasive and causes minimal discomfort to the patients. Technically, Dr Kumar is a neurosurgeon with a deep interest in pain medicine. As a neurosurgeon he always preferred minimal invasive techniques for surgery because of reduced tissue damage that ensured faster recovery. Since 2003 he has been practising ozone therapy full time, as it offers a new way for treating back and leg pain resulting from disc prolapse. Almost three to four hundred patients come to him seeking relief from crippling pain and majority of them are in the age group of 30-50. “However, the youngest patient I treated was 13 years of age and the oldest was 88 year old. The patients that I get come from all over the world and different parts of the country,” shares he.

Ti ricorderemo sempre così.
Vorrei ora, invece, soffermarmi su alcuni aspetti che riguardano il mondo dell’ozonoterapia italiano.

La F.I.O., nata nel 2003 resiste da circa dieci anni con pochissimi fondi a disposizione, facendosi forza sulla necessità di dare credibilità e scientificità a un campo della medicina che viaggia sempre al limite con molti detrattori sempre pronti a crocifiggere il medico ozonoterapeuta.

La F.I.O ha saputo dare ai suoi associati le basi scientifiche per poter lavorare in un range di sicurezza validando scientificamente l’operato dei propri medici, soprattutto grazie alla rivista “International Journal of Ozonetherapy”, oggi unico riferimento sicuro riconosciuto per qualsiasi controversia medico legale, ma anche grazie a Corsi e Congressi seri e credibili.

La F.I.O grazie ai propri legali garantisce anche un ausilio immediato in caso di necessità ai propri associati.

In questi dieci anni siamo intervenuti in difesa dei nostri colleghi in quattordici occasioni, molto spesso gli interventi sono avvenuti in casi pretestuosi “per es.: collega accusato di aver causato una spondilodiscite quando invece trattavasi di un ostecondrite tipo I di Modic etc…”, ma comunque si è dovuto intervenire. Il sottoscritto e il professor Leonardi, che ricordo essere riferimento nazionale AMAMI per casi di controversie in tema di ossigeno-ozono terapia, sono più volte intervenuti a difesa dei colleghi. Cito anche l’importante intervento di Lamberto Re, nostro consigliere presso il Tribunale Sportivo Internazionale nei casi di doping con grande autoemoterapia.

Dopo questa premessa, che spero vi faccia capire quanto sia importante avere una Federazione sempre più forte e compatta (oggi siamo pochissimi medici che hanno rinnovato l’iscrizione alla F.I.O per il 2012), volevo chiedere ringraziando il lavoro e l’impegno da parte di tutti con l’augurio che nel 2012 sempre più colleghi e amici che lavorano con l’ozono vogliano unirsi a noi iscrivendosi alla F.I.O. per far parte di questo gruppo.

Il vostro segretario

Matteo Bonetti
Cari amici e colleghi,

Vi sottopongo questo caso, partendo da quanto venne pubblicato su “Spine dicesi Spine” nel 2007


Fulminating septicemia secondary to oxygen-ozone therapy for lumbar disc herniation: Case report

GAZZERI R, GALARZA M, NERONI M, ESPOSITO S, ALFIERI A.

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Caso Clinico

Paziente: R.A femmina di anni 69, diabetica, afflita da lombalgia acuta complicata da sciatalgia bilaterale, la RM documenta listesi di 1° grado L4 su L5 con protrusione mediana paramediana bilaterale del disco con situazione di conflitto disco radicolare con entrambe le radici L4 (vedi figura 1).

Viene sottoposta a svariate terapie: quali??? Le ometto per portarvi alla riflessione finale.

La paziente viene ricoverato presso l’Istituto Clinico... Ed in due giorni sviluppa un quadro di setticemia...

La RM documenta multipli ascessi nella muscolatura paravertebrale!!! (figura 2) e un’impregnazione patologica della dura madre e delle radici della cauda equina con meningite.

Ora posso concludere facendovi queste domande:

1) La paziente ha fatto ozonoterapia?
Se si, quale approccio: paravertebrale classica causando ascessi paravertebrali e poi sepsi oppure è stato punto il forame o il disco con le conseguenze sovraccitate?

2) Se siamo colpevoli cosa facciamo? (tutti possiamo sbagliare nella vita)

Discussione-Conclusioni

La paziente era diabetica ed è stata sottoposta a dosaggi elevati di steroide per un mese, il dolore è diventata insopportabile e ha assunto anche della morfina, era immunodепressa in maniera grave e non ha mai fatto ozonoterapia.

Questo è lo stesso caso che è costato ad un nostro collega un processo di oltre 5 anni, un esborso economico non indifferente, una perdita di credibilità professionale etc... e per fortuna esisteva la Rivista con gli articoli riconosciuti a livello internazionale e la F.I.O...
Quindi, perdonatemi, ma oggi chi fa ozonoterapia, una terapia dai risultati terapeutici straordinari, deve fare i conti con l’invidia dei colleghi, con il dente avvelenato delle lobbies chirurgiche, sapendo di toccare interessi importanti.

Un altro problema che sta diventando sempre più di attualità è questo: ambulatorio per ozonoterapia? Studio medico per ozonoterapia? Poliambulatori dove si fa ozono?

Cari colleghi anche a questo la F.I.O è pronta a darvi la massima disponibilità per risolvere il vostro problema e aver la possibilità di lavorare in piena tranquillità.

Quindi iscrivetevi alla F.I.O!!!, perché da ora in poi la F.I.O. fornirà la propria consulenza solo ai medici regolarmente iscritti. Devo dirvi che siamo infastiditi da quelli che si iscrivono un anno perché vivono delle difficoltà professionali, e dopo essere stati aiutati dall’Associazione non continuano a sostenerla.

Questo dovevo.

Il vostro segretario

Matteo Bonetti
Inflammatory Mechanisms Involved in the Lumbar Disc Herniation with Hydrated Nucleus Pulposus (Acute Herniated Disc) and the Oxygen-Ozone Therapy
A Different Viewpoint

A.M. Grangeat, M. De Los Ángeles Erario
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Key words: oxygen-ozone therapy, disc herniation, cytokines, interleukins

SUMMARY - The sciatalgy is one of the main reasons for medical consultation worldwide. One of the most common causes of sciatalgy is the intervertebral disc herniation. Since 2002 we have been using the oxygen-ozone therapy to treat the intervertebral disc herniation with hydrated nucleus pulposus. When the herniated disc has a constraining fibrous ring, the response is not always good. We believe that the immune system is the one that reabsorbs the herniated portion of the nucleus pulposus; this mechanism is increased by the action of the oxygen-ozone therapy in therapeutic concentrations, moreover, it aids the scar formation of the fibrous ring. Therefore, at our Institute, we have named herniolisis the procedure we perform at the operating room by administering oxygen-ozone in therapeutic concentrations directly into the intervertebral disc. In this work we have revised the bibliography from the last few years thoroughly to understand the role of the inflammatory response and all the chemical mediators involved in the development of the pathology, to test an explanation on how the oxygen-ozone therapy works in the resolution of the intervertebral disc herniation.

The sciatalgy is a very common cause of pain and disability, which brings about huge expenses to health systems. The studies regarding its prevalence vary significantly and range between 1.2% and 43%, depending on the studied populations. In the USA, the acute lumbar pain generates annually an economic impact of 200 billion dollars and 5.7 million people develop an intervertebral disc related disease. In 40% of the people, the lumbar pain is the result of intervertebral disc pathologies.

In 90% of the cases, the sciatalgy is caused by intervertebral disc herniation, with nerve root compression, but the lumbar stenoses and – though less frequently – the tumors can also be the cause. The treatment of sciatalgy varies considerably, but the guidelines for the diagnosis have been well established throughout the years.

Intervertebral Disc Embryology

The embryological development of the vertebral column is focused on the notochord derived from the mesoderm. In the development of the intervertebral disc, the notochord is important as the center for signaling that mediates the cellular migration, differentiation and survival, and as the physical structure that gives rise to the nucleus pulposus.

The annulus fibrosus and the nucleus pulposus emerge at the same time through different means of development. After 30 days of gestation the sclerotome cells migrate medially from pairs of paraxial somites to condense around the notochord. The condensation adopts a metameric pattern of regions more or less condensed that, later on, will give rise to the fibrous ring and vertebral bodies respectively. The cells in the future region of the fibrous ring adopt fibroblastic morphology. Those cells align and orient themselves to form a matrix deposit support. The organization of the fibrous ring is mediated by actin filaments from the cytoskeleton. Within those cells, the stress fibers form an alignment that forebodes the organization of the mature collagen in the fibrous ring.

Simultaneously with the morphogenesis of the fibrous ring, the notochord contracts within the rudiments of the forming vertebral body, while at the same time it expands inside the intervertebral disc. The notochord is important as the center for signaling that mediates the cellular migration, differentiation and survival, and as the physical structure that gives rise to the nucleus pulposus.

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regions to form the nucleus pulposus. It has been proposed that the biomechanical forces play a part in this transformation.\(^\text{10}\)

The embryogenesis of the intervertebral disc occurs in response to a series of molecular signals that originate from notochordal cells and the neural tube.\(^\text{11}\) The scarce nutritional contribution to the avascular intervertebral disc cells is related to the degenerative pathologies that develop throughout the years. In humans, during the early years in life, the blood vessels that penetrated the fibrous ring and the endplate cartilage since the 35th gestation week start to move away, leaving the disc as a totally avascular structure.\(^\text{14}\) The possible reasons for this to happen are the decrease of nutrient requirements, followed by the initial period of rapid growth or the inability of the circulatory pressure to compete with the great physiological problems surrounding the extracellular matrix.

After the regression, there is evidence that the pathways taken by the blood vessels are never fully remodeled, in the micro-architecture of the surrounding areas, and that they remain as trans-lamellar elements. The idea is consistent with the fact that the disc has a poor remodeling and repairing capacity. The adult disc is almost completely avascular, which is why the resident cells must survive and work in an environment with poor oxygen and nutrients.\(^\text{4}\)

**Normal Intervertebral Disc Anatomy**

The intervertebral discs occupy the space between the vertebral bodies of the column. They are the main joints in the spine and represent the third part of its weight. Their main role is mechanical, since they transmit the loads derived from the body weight and the muscular activity through the vertebral column. The intervertebral discs provide flexibility, allowing flexure and torsion.

They are made of an external fibrous ring of fibrous cartilage that surrounds a central region of a gelatinous structure called the nucleus pulposus. The nucleus pulposus is limited in the superior and the inferior parts by a hyaline cartilage called endplate.\(^\text{15}\)

The central nucleus pulposus contains collagen fibers randomly organized and elastin fibers distributed in a radial way. The fibers are soaked in a gelatinous substance highly hydrated that contains proteoglycans. The main proteoglycan is called aggrecan. Interspersed, there are chondrocyte-like cells that are sometimes located in a capsule inside the matrix. Outside the nucleus pulposus there is the annulus fibrosus formed by a series of concentric rings or lamellae with collagen fibers oriented in parallel with the lamellae. The elastin fibers are among the lamellae.

The cells of the annulus fibrosus in the outer region are fibroblast type, which are elongated, thin and aligned in parallel with the collagen fibers. Towards the inner region cells can be more oval.

The endplate is a very thin structure of hyaline cartilage that lies in the interface between the intervertebral disc and the vertebral bodies. The collagen fibers inside that structure are parallel and horizontal to the vertebrae.\(^\text{15}\)

In healthy adults the intervertebral discs receive almost no blood supply, but they do receive some innervation in the outer lamellae of the annulus fibrosus that can end up in proprioceptors.\(^\text{14}\)

**The Nucleus Pulposus and the Annulus Fibrosus**

The Nucleus Pulposus is the central region of the intervertebral disc. It is a glycoprotein formed by a central protein (hyaluronic acid) attached covalently to glycosaminoglycans. In the case of the aggrecan, the glycosaminoglycans are constituted by chondroitin sulfate and the keratan sulfate. They keep the hydration of the disc through osmotic pressure. Due to the highly negative charge content, they draw water and give a gel-like consistency, occupying a great volume. The water and proteoglycan content is greater in the nucleus pulposus than in the annulus fibrosus.\(^\text{16,19,15}\)

The matrix is a dynamic structure and its molecules are in constant change because of the proteases, matrix metalloproteinases (MMPs) and aggrecanases that are synthesized by the disc cells. The balance among the synthesis, degradation and accumulation of the matrix molecules determine its quality and the performance of the intervertebral disc. The integrity of the matrix is essential to keep the functions of the intervertebral disc, since due to its avascular and non-neural origin it cannot be repaired.\(^\text{20}\)
Disc Herniation with Hydrated Nucleus Pulposus

Regarding the response to treatment with oxygen-ozone therapy, the disc herniation is acute as long as the disruption of the annulus fibrosus and the exposure of the nucleus pulposus to the adjacent tissue remain. In this way, the nucleus pulposus is exposed to the action of the immune system.

The herniated nucleus pulposus resorption is a phenomenon supposedly demonstrated during intervertebral disc herniation. It is important to clarify the molecular events occurring at the nucleus pulposus resorption to understand the pathogenesis and the resolution of the disc herniation. However, it can be frequently observed patients who drag episodic pain crises for many years. And in the consecutive nuclear magnetic resonance images (MRI) we observe images where the morphology and size are modified but never fully resolved. A very frequent case is the different size in the image of the protrusion, but what generally remains is the disruption of the annulus fibrosus. This is appropriate to the criteria of the causes of the inflammation and pain and the persistence of the painful clinical signs throughout the years.

Since 1969 it is known that the pH levels within and surrounding the herniated disc are diminished, which shows an inflammatory reaction in the area surrounding the nerve root causing the sciatalgy. Many studies have contributed to the understanding of the inflammatory process in the intervertebral disc, showing that there is an increase in the production of prostaglandins, leukotrienes, thromboxanes and nitric oxide in the tissues of herniated intervertebral discs. Also, several studies have revealed the greatest expression of pro-inflammatory cytokines in the herniated intervertebral discs, such as tumor necrosis factor alpha (TNFα), interleukine-1 alpha (IL-1α) and/or 1 beta (IL-1β), interferon gamma (IFNγ) and interleukin-6 (IL-6).

Shamji M. et al found increased levels of IFNγ and IL-6 in the herniated intervertebral discs. They also showed that in the herniated discs there is a greater presence of IL-4 and IL-12 compared to the degenerated intervertebral discs. This group of mediators includes products from macrophages that cause the lymphocyte activation and differentiation, as well as the products from lymphocytes that recruit and activate macrophages towards phagocytosis and proteolytic enzyme secretion. All this inflammatory profile – IL-4, IL-6, IL-12 and IFNγ – in the herniated intervertebral discs suggests that the lineage activation of lymphocytes Th1 is associated to the herniated disc pathology. This happens after the herniated material from the nucleus pulposus is exposed to the systemic circulation.

The herniated discs also express ICAM-1 (intercellular adhesion molecule-1), suggesting that products associated with inflammation in the herniated discs can use the molecule to draw effector cells from the immune system. The ICAM-1 is a cell surface glycoprotein which is typically expressed on endothelial cells and cells of the immune system. It functions in the transduction of signals, causing pro-inflammatory effects.

Pathologic intervertebral discs show an increase in the expression of IL-17, a product from lymphocyte lineage Th17. This suggests those lymphocytes are involved in the intervertebral disc pathology and maybe they synergize with the INFγ. The disc cells respond to the IL-17 and to the pro-inflammatory co-stimulators TNFα and IFNγ, increasing the production of inflammatory mediators: nitric oxide synthase (NOS), prostaglandin E2 (PGE2), interleukin-6 (IL-6) and ICAM-1 in the cell surface. The pro-inflammatory action of IL-17 depends on its ability to trigger the expression of the inducible nitric oxide synthase (iNOS), which is responsible for the formation of radical nitric oxide (NO). The NO – at low concentrations – can modify intracellular signals that affect the function of immune cells and tissue and organ resident cells; the excessive release of NO results, however, in the inflammatory destruction of tissues.

Equally important to the cellular production of inflammatory cytokines, it is the IL-17 ability to stimulate the expression of ICAM-1 for drawing immune cells. The results show that the intervertebral disc cells respond to the IL-17 and the co-stimulators IFNγ or TNFα, increasing the production of inflammatory mediators and cytokines as well as the chemotactic profile through the regulation of the expression of the ICAM-1. The IL-17 can be an important regulator of the herniated intervertebral disc inflammatory pathology, of the degenerative pathology and/or multiple pathologies.

The Ozone Therapy in Medicine

In the XIX century the ozone was identified as a powerful bactericide and it was used during the First World War to treat soldiers suffering from gas gangrene due to anaerobic infections by Clostridium, at the Queen Alexandra Military Hospital, Germany. In 1935, at the 59th Congress of the German Surgical Society (Berlin), it was introduced as a well-tolerated disinfectant for wounds. Since that moment the application of this therapeutics started to spread. The treatment with oxygen-ozone therapy for back pain has proved very effective, either administering it
directly (intradiscal) or indirectly via intramuscular in the paravertebral muscles. During the last few years over 30,000 patients with disc herniation have been treated successfully in Italy 37.

**Action Mechanisms of Ozone Therapy**

In a physiological environment, the ozone reacts immediately with the antioxidants, polyunsaturated fatty acids, proteins, carbohydrates and, if the concentration is high, with DNA and RNA. Also, it leads to the formation of reactive oxygen species (ROS), lipid oxidation products (LOPs) and oxidized antioxidant compounds 37. In precise doses it is not noxious and it can trigger a great amount of biological responses that manage to take back a chronic oxidative stress situation. In the process the ROS and LOPs are formed. The former work immediately (as early messengers of short action), the latter are distributed to the different tissues through circulation, as late messengers. The hydrogen peroxide (H₂O₂) is the most important ROS that is formed in this process; it spreads quickly from the plasma to the cellular cytoplasm (leukocytes, erythrocytes, platelets) and it triggers stimuli that, depending on the kind of cell, activate different pathways and therefore various biological effects. In the erythrocyte, the pentose phosphate pathway is activated, allowing a greater oxygen supply to the ischemic tissues. This occurs through the displacement of the oxyhaemoglobin dissociation curve towards the right, because of a decrease in the pH (Bhor effect) 40,37.

In the leukocyte cytoplasm, the action of the H₂O₂ oxidizes specific cysteines, activating the tyrosine kinase that phosphorylates the nuclear transcription factor NFκB, allowing the release of a heterodimer that when entering the cellular core activates the synthesis of acute phase reactant proteins and interleukins.

In the case of platelets, the ozone produces a dose-dependent activation, which generates the release of growth factors.

The generated LOPs act as remote messengers and activate endothelial cells, resulting in an increase in the production of nitric oxide (NO). The synthesis of some antioxidant enzymes increase: superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GSH-Rx), glutathione transferase (GSH-Tr) and glucose-6-phosphate dehydrogenase (G6PD) 38. Also, it is induced the action of the hemeoxygenase-1 (HO-1) 39 that generates the production of carbon monoxide (CO), bilirubin and Fe²⁺. The CO cooperates with the NO in the regulation of the vascular tone 40,37.

It has been proved recently that the ozone behavior adjusts to the concept of hormesis, which states that the exposure of a living organism to very low concentrations of an agent, which at high concentrations can be harmful, induces an adaptable and beneficial response. The concept that the ozone will eventually induce an antioxidant response capable of taking back chronic oxidative stress is based on findings about an increase of the antioxidant enzymes levels during the oxygen-ozone therapy. These facts can include the ozone as a hormetin 41.

**The Ozone Therapy in Disc Herniation**

The oxygen-ozone therapy is a minimally invasive treatment for lumbar disc herniation that uses the biochemical properties of the oxygen-ozone mixture. Andreula et al. compared the result of administering oxygen-ozone versus ozone followed by corticosteroid and anesthetics in 600 patients suffering from constrained lumbar disc herniation with nerve root compression. Three hundred patients were administered oxygen-ozone and the other 300 received periradicular corticosteroids and anesthetics plus oxygen-ozone. The progress was measured six months after the treatment and the result they obtained was favorable in both cases, showing that the oxygen-ozone therapy is a useful option to treat lumbar disc herniation when the conservative treatment has failed 42.

In a randomized study, controlled in over 300 patients, Bonetti et al. compared the therapeutic effectiveness of the intraforaminal oxygen-ozone treatment versus periradicular infiltration of steroids in lumbar pain and sciatica. The short-, medium- and long-term results favored oxygen-ozone treatment, besides no adverse reactions were observed. Oxygen-ozone infiltrations not only did reduce the pain, but also acted on its cause, accelerating the natural process of recovery from disc herniation, showing that oxygen-ozone therapy is a first choice treatment, leaving surgery as a second choice 41.

Gallucci et al. compared treatment with intradiscal and intraforaminal injections of steroids versus oxygen-ozone plus steroids, concluding that oxygen-ozone treatment was more effective in a six-month term 40. Furthermore, in 2009, Paoloni et al. performed a multicenter, randomized, double-blind, clinical trial in a cohort of patients with lumbar pain due to disc herniation, assessing the benefits of intramuscular-paravertebral infiltrations of oxygen-ozone, proving to be an effective therapy for the reduction of pain, disability and analgesic drugs intake 41.
**Our View**

Knowing the inflammatory mechanisms and the inflammatory mediators that take part in the intervertebral disc herniation, in addition to the knowledge we have acquired over action mechanisms of the ozone therapy, we are ready to state that the oxygen-ozone therapy modulates oxidative stress that takes place in the disc-radiculal conflict, and it modulates the immune system, which is the one that resolves the disc herniation. As far as we understand, the oxygen-ozone therapy, administered in therapeutic concentrations, would not dehydrate the nucleus pulposus nor diminish its volume. We believe the immune system is modulated by the ozone therapy and thus it phagocytes the herniated portion of the nucleus pulposus, it restores the integrity of the annulus fibrosus and it puts an end to inflammation and radicular pain. We believe the discal disease does not self limit. This is consistent with the idea that the avascular intervertebral disc has a poor or null capacity of remodeling and repairing, and with the fact that the patients progress with pain crises for years and end up developing a genuine nucleus pulposus herniation that imposes a treatment or they develop, through multiple inflammations and scar formations, a chronic fibrosis with calcium deposits that lead to a narrow spinal canal, acquired at that level. As we quoted earlier, the resorption of the herniated nucleus pulposus is a phenomenon supposedly demonstrated during intervertebral disc herniation. The bibliography maintains the idea that eventually the herniated portion of the nucleus pulposus is reabsorbed by the immune system. However, we think that this resolution is partial, that it takes a long time; meanwhile the patient is made to undergo pain, disability and limitations in everyday tasks. Though the whole process can be partially performed by the organism, it is the ozone which helps the immune system to shorten the periods of time and to improve the final quality of this pathology repairing. In other words, the oxygen-ozone therapy manages to shorten the recovery period and improves its final quality. In patients with herniated disc treated with oxygen-ozone therapy, we observe in the routine MRI in T2 a hydrated white nucleus pulposus, while in patients with discal protrusion with constraining ring the ozone therapy does not resolve the protrusion.

**Conclusion**

Because of all these observations and the thorough reviewing of the bibliography, for a few years now, at our health center, we call “herniolisis” the procedure we perform with oxygen-ozone for the treatment of disc herniation with hydrate nucleus pulposus. We believe that the immune system modulated by the ozone therapy phagocytes the herniated portion of the nucleus pulposus, restores the integrity of the annulus fibrosus and puts an end to inflammation and radicular pain.

Moreover, since few years ago it is known the role of the IL-17 in the disc herniation. This interleukin is common in degenerative osteoarthritis process, Chron’s disease and psoriasis, besides disc herniation. We believe this inflammatory mediator opens the door to a new line of investigation, since these are possible diseases to be treated with oxygen-ozone therapy. We need to research which is the effect of ozone over the expression of lymphocytes Th17 and its product IL-17.

**Acknowledgements**

This work has been funded by the Instituto Argentino de Ozonoterapia (IAOT) – Argentine Institute of Ozone Therapy. We are deeply grateful to the Italian, Spanish and Cuban ozone-specialist doctors who have guided us in the last ten years.

We are also grateful to our over thousand patients for their confidence in us.
Low back pain (LBP) is one of the most common and important clinical, social, economic, and public health problems affecting the human population worldwide (1). Around 70% of adults suffer from LBP at some point in their lifetime with various degrees of symptom severity. Additionally, 1.6% to 43% of these patients have LBP associated with sciatic symptoms (2). In the United States, the incidence of chronic low back pain ranges from 15% to 45%, with a prevalence of 30% (1). Most back pain has no recognizable cause...
on imaging studies and is usually attributed to muscle strain or ligament injuries (65%-70%). In 5% to 15% of cases, the source of LBP is related to degenerative joints and disc disease (3). The natural history of disk herniation is favorable; improvement of symptoms is the norm, and most episodes resolve spontaneously or after conservative therapy. However, studies have shown that low back pain is sometimes still present after long periods of time (at least 12 months) in 37% to 54% of patients (1,2).

Besides oral pharmacological and rehabilitation treatments, ozone therapy has emerged as an alternative or additional treatment option for these patients, particularly in Europe (4,5). Despite its widespread use to treat a variety of conditions, ozone therapy remains unknown to most physicians. Ozone (O3) is an allotropic form of oxygen, primarily known for its ecological properties, industrial application and therapeutic effects. Questions persist concerning its potential toxicity as an oxidant agent versus its reported clinical efficacy. Several mechanisms of action have been proposed to explain the efficacy of ozone therapy including analgesic, anti-inflammatory and oxidant action on proteoglycans (e.g., in the nucleus pulposus). Ozone is administered in the form of an oxygen-ozone gas mixture at nontoxic concentrations ranging from 1 to 40 µg of ozone per mL of oxygen, using various percutaneous methods (5).

Percutaneous techniques minimize the invasive nature of surgery, rendering administration more straightforward and faster while sparing healthy tissue and avoiding or minimizing complications such as postsurgical infection (6). Those techniques have been applied as an adjunct treatment for LBP and used in association with ozone injections have yielded good results (4). However, the effectiveness of ozone injections for the treatment of LBP remains a matter of debate. In order to investigate the effectiveness and safety of ozone therapy for this specific purpose, the authors performed a systematic review and meta-analysis of the literature, focusing on observational studies and randomized controlled trials (RCTs) in patients with subacute or chronic LBP.

1.0 Methodology

The methodology utilized in this work follows the systematic review process derived from evidence-based systematic review and meta-analysis of randomized trials (7) and the PRISMA statement (8).

1.1 Inclusion Criteria

1.1.1 Types of Studies

Three review authors screened the abstracts of studies in all languages against the inclusion criteria. They then retrieved all possibly relevant articles in full text for comprehensive assessment of the quality and satisfaction of inclusion criteria.

The review focused on randomized trials, systematic reviews, observational studies, and reports of complications. All studies providing appropriate management with outcome evaluations at 6 months or longer and statistical evaluations were reviewed. Reports without appropriate diagnosis, nonsystematic reviews, book chapters, and case series with fewer than 10 patients were excluded from the initial search in the databases.

1.1.2 Types of Participants

Participants were adults aged at least 18 years with low back pain due to lumbar disc herniation or degenerative disc disease treated by the interventional procedures 2.1.3 below.

1.1.3 Types of Interventions

Interventions were injections of an oxygen-ozone mixture associated or compared with steroids, and local anesthetic applied to intradiscal, intramuscular paraspinous, justaforaminal, periganglionic or epidural, guided by fluoroscopy or tomography.

1.1.4 Types of Outcome Measures

The primary outcome measure was pain relief (short term < 6 months and long-term > 6 months) in accordance with Staal et al (9).

1.2 Review criteria

The search in the databases was performed independently by 3 authors who selected the articles for analysis. Each study was evaluated by 3 physicians for stated criteria and any disagreement was resolved by a fourth physician. The other author was responsible for statistical analysis.

1.3 Adverse Events or Side Effects

Adverse effects and complications were analyzed according to the description of the authors or based on case reports.

1.4 Search Methods for Study Identification

Searches were performed from the following sources:
Ozone Therapy for Low Back Pain

1. PubMed from 1966
2. EMBASE from 1980
   www.embase.com/
3. Cochrane Library
   www.thecochranelibrary.com/view/0/index.html
4. DARE and HTA

Search period included from 1966 through September 2011.

1.4.1 Search Strategy

The search terminology included the terms ozone-therapy, ozone, ozone therapy, chronic low back pain, back pain, pain, failed back surgery syndrome and ozonucleolysis.

At least 3 of the review authors independently, in a standardized manner, performed each search. Accuracy was confirmed by a statistician. All searches were combined to obtain a unified search strategy. Any disagreements between reviewers were resolved by a third author and consensus.

1.4.2 Assessment of Methodological Quality

The methodological quality assessment was performed by 3 reviewers and any discrepancies were evaluated by a fourth reviewer and consensus was reached.

The quality of each individual article included in this analysis was assessed by modified Cochrane review criteria with weighted scores (10) for randomized trials and the Agency for Healthcare Research and Quality (AHRQ) quality criteria for assessment of observational studies (11). Only the observational studies scoring at least 50 on weighted scoring criteria were included for analysis. Methodological quality assessment criteria are described in Tables 1 and 2.

1.5 Data Extraction and Management

Three review authors independently extracted the data from the included studies. Disagreements were resolved by discussion among the 3 review authors; if no agreement could be reached, it was planned a fourth author would decide.

1.6 Measurement of Treatment Effect and Data Synthesis (Meta-analysis)

The authors used a standardized data extraction form for independent inclusion of the study population, intervention, study design, and outcome measures for randomized controlled trials. The meta-analysis was performed using the Review Manager 5.0 (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark) with the random-effect model. Dichotomous data were compared using odds ratio (OR). Respective 95% confidence intervals (CI) were calculated for each estimate and presented in forest plots. The pooled OR, symbolized by a solid diamond at the bottom of the forest plot (the width of which represents the 95% CI), is the best estimate of the true (pooled) outcome. The effect of the treatment was expressed as a ratio of the ozone therapy arm over the control arm.

1.7 Analysis of Evidence

Analysis was conducted using 5 levels of evidence, ranging from Level I to III with 3 subcategories in level II, as illustrated in Table 1(12).

1.8 Recommendations

Grading recommendations were based on the criteria stated by Guyatt et al (13) as illustrated in Table 2.

1.9 Outcomes of the Studies

A study was judged positive if the ozone injections were clinically relevant and effective. Regarding randomized studies, this indicates that the difference in the effect for the primary outcome measure was statistically significant on the conventional 5% level. In a negative study, no difference between the studied group and the controls or no improvement from baseline was reported (9).

Table 1. Levels of evidence based on the quality data available in the literature (USPSTF).

<table>
<thead>
<tr>
<th>Level</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Evidence obtained from multiple properly conducted diagnostic accuracy studies.</td>
</tr>
<tr>
<td>II-1</td>
<td>Evidence obtained from at least one properly conducted diagnostic accuracy study of adequate size.</td>
</tr>
<tr>
<td>II-2</td>
<td>Evidence obtained from at least one properly designed small diagnostic accuracy study.</td>
</tr>
<tr>
<td>II-3</td>
<td>Evidence obtained from diagnostic studies of uncertainty.</td>
</tr>
<tr>
<td>III</td>
<td>Opinions of respected authorities, based on clinical experience descriptive studies Evidence obtained from case reports or reports of expert committees.</td>
</tr>
</tbody>
</table>

Adapted and modified from the U.S. Preventive Services Task Force (USPSTF)(12).
2.0 Results

Our search strategy yielded multiple studies evaluating the effectiveness of ozone injected into the disc and/or periforaminal or at the paravertebral muscles. From the initial search (117 articles) only 35 were reviewed: 30 studies, including 7 randomized trials (14-20) and 23 observational studies, and 5 reports of complications (21-25) (Fig. 1).

2.1 Randomized Trials

2.1.1 Methodological Quality Assessment

From the 7 randomized trials, 4 (14-17) met the established inclusion criteria. Three of them were excluded from the meta-analysis: one utilized collagenase (19) associated with ozone and steroid; Gautam et al (20) utilized intradiscal radiofrequency with ozone, and the other due to methodological issues that would invalidate the meta-analysis (18). The results of the methodological quality assessment of randomized studies are illustrated in Table 3. The quality assessment criteria ranged from 56 to 84 points for evidence synthesis.

2.1.2 Descriptive results of randomized studies

In the randomized series of 306 patients, Bonetti et al (14) reported that 57.5% of 80 patients in the disc disease group treated with steroid deemed the clinical outcome to be excellent, as did 62.8% of 70 patients in the group with no disc disease after steroid infiltration (Table 4). Whereas in the ozone therapy group, 74.4% of 86 patients with disc disease reported complete remission of pain, as did 75.0% of 70 patients with no disc disease. In this study, differences in favor of O2-O3 treatment were statistically significant in patients with disc disease but not in those without disc disease. In another randomized study, Gallucci et al (16) observed a satisfactory success rate with ozone-therapy combined with intraforaminal and intradiscal steroid and anesthetic injection compared to steroid alone.
Zambello et al (15) randomized 351 patients with low back pain for treatment with either ozone or steroid (epidural) and planned a crossover during the follow-up to the other group in case of failure to respond to treatment after 4 weeks of therapy. The long-term outcome remained excellent or good in 47.3% of 171 patients treated by epidural steroid injections and in 77.1% of 180 patients treated with O2-O3. Eleven patients in the ozone group were subjected to crossover to epidural steroid injections whereas 38 patients in the epidural group were submitted to crossover to the ozone group. Only 36.4% of patients in the crossover group to epidural injection presented excellent/good remission of pain while 70.8% of patients in the epidural group who were submitted to crossover to ozone therapy reported an excellent/good outcome.

Recently, Paoloni et al (17) conducted a multicenter, randomized, double-blind, “simulated therapy”-controlled clinical trial. Thirty-six patients received intramuscular-paravertebral ozone injections whereas 24 received simulated lumbar intramuscular-paravertebral injections. The simulated injection was administered us-
**Table 3. Randomized trials on the efficacy of ozone therapy for low-back pain.**

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Author/Country/Year</th>
<th>Scores for methods criteria</th>
<th>( \text{Author/Country/Year} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Paolini (18)/Italy/2009</td>
<td>Bonetti (15)/Italy/2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>A Homogeneity rep.</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>B Comparability of relevant baseline characteristics</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>C Randomization procedure adequate</td>
<td>4</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>D Drop-outs described for each study group separately</td>
<td>3</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>E (&lt;20% loss to follow-up)</td>
<td>2</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>(&lt;10% loss to follow-up)</td>
<td>2</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>F (\geq50% loss to follow-up) &amp; - &amp; - &amp; 8 &amp; 8 &amp; 8 &amp; 9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G &gt;100 subjects in the smallest group</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Interventions</td>
<td>(\geq100) subjects in the smallest group</td>
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<td>74</td>
</tr>
<tr>
<td>H Pragmatic study</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>I Co-interventions avoided</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>J Placebo-controlled</td>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Effect</td>
<td>K Patients blinded</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>L Outcome measures relevant</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>M Blinded outcome assessments</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>N Follow-up period adequate</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Data-presentation and analysis</td>
<td>O Intention-to-treat analysis</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>P Frequent of most important outcomes presented for each treatment group.</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Total Score</td>
<td>84</td>
<td>70</td>
<td>70</td>
</tr>
</tbody>
</table>


ing a false needle that pricked the skin without piercing it, applied at the lumbar paraspinal level, followed by hand-applied pressure on the same site designed to reproduce the load sensation commonly described after O2-O3 injections. Patients who received ozone had significant lower pain scores (mean visual analog scale [VAS] was 0.66 in the study group and 4.00 in the control group) compared to patients who received simulated therapy. Also, a greater percentage of patients became pain-free (61% versus 33%, \( P < 0.01 \)) in the ozone group. Active ozone therapy was followed by a statistically significant shorter time on nonsteroidal an-
Ozone Therapy for Low Back Pain

### Table 4. Results of randomized studies of ozone therapy for low-back pain

<table>
<thead>
<tr>
<th>Author/Country/Year/Methods/Type of pain</th>
<th>Participants</th>
<th>Design of study/Intervention(s) (guided by CT or fluoroscopy)</th>
<th>Outcome(s)</th>
<th>Result(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bonetti (15), Italy, 2005, RA, DB</td>
<td>306 patients with acute or chronic low back pain and sciatic nerve pain were treated. They were divided into two groups: Group with disc disease (bulging disk, protrusion or extrusion; n=166); group with non-disc vertebral disease (osteoarthritis, spondylosis, facet joint syndrome; n= 140) and received ozone or steroid infiltrations.</td>
<td>The patients were divided into two groups with their subgroups: injections were infiltrated adjacent to neural foramina or facet joint regions guided by CT. G1=Ozone (7mL-25µg/mL): With disc disease: n=86 Non-disc disease: n=70 G2= (Steroid): With disc disease:n=80 Non-disc disease: n=70</td>
<td>Timing: 1 week, 3and 6 months. Outcome measures: (MacNab Score): Excellent: pain free and return to work Good: Pain relief 50% or more Poor: Pain relief 30% or less.</td>
<td>6 months (excellent and good) G1=74.4 % with disc disease and 75.8% in the non-disc disease G2=57.5% with disc disease and 62.8% in the non-disc disease</td>
</tr>
<tr>
<td>Gallucci17, Italy, 2007, RA, DB</td>
<td>159 patients with lumbar disc herniation and radicular pain. All patients complained of pain for at least 8 weeks with poor clinical improvement after conservative treatment.</td>
<td>The patients were divided into two groups: all received intradiscal and intraforaminal injections of a steroid and a local anesthetic or ozone (7mL-28µg/mL). G1(n=82) Steroid / local anesthetic G2(n=77)Steroid / local anesthetic and ozone</td>
<td>Timing: 2 weeks, 3 and 6 months; Outcome measures: Classified as successful if the Oswestry Disability Index was no greater than 20% at follow up and unsuccessful otherwise.</td>
<td>6 months Successful: G1=47% G2=74% Unsuccesful: G1=53% G2=26%</td>
</tr>
<tr>
<td>Paoloni18 , Italy, 2009, RA, DB</td>
<td>60 patients with acute low back pain and/or radiating pain of moderate to severe intensity (VAS ≥ 5) and MRI evidence of disc protrusion with or without disc degeneration in the spinal segments involved in the pain.</td>
<td>The patients were divided into two groups: G1(n=36): Ozone intramuscular paravertebral lumbar infiltrations (3/wk for 5 consecutive weeks) of ozone (20mL - 20µg/mL). G2(n=24): Simulated therapy: injection using a false needle that pricked the skin without piercing it, pressure applied at the lumbar paravertebral level.</td>
<td>Timing: 15 and 30 days, 2 weeks, 3 and 6 months. Outcome measures: (at the end of follow up) Pain free on VAS score ≤ 1, Backill questionnaire, SF-36, Kellner scores.</td>
<td>6 months Pain-free: G1=61% G2=33% Backill score: G1=13.0 G2=+5.6 Kellner and SF-36: No differences between groups MRI findings: Unchanged Drug intake: Decreased</td>
</tr>
<tr>
<td>Zambello16, Italy, 2006, RA, DB</td>
<td>351 patients with chronic irradiating low back pain over sciatic nerve and failure to respond to medical treatment were randomly assigned to one of two groups.</td>
<td>The patients were divided into two groups: G1(n=171): Steroid at intervertebral space G2(n=180): Ozone into the paravertebral muscle, 5mL - 20µg/mL</td>
<td>Timing: 3 weeks and 6 months; Outcome measures: Subjective pain scores (MacNab method Score).</td>
<td>6 months (excellent or good) G1=47.3% and 77.1%</td>
</tr>
</tbody>
</table>

G1 = group 1; G2 = group 2; G3 = group 3; G4 = group 4; WK = week; RA = randomized; P = prospective; O = observational; DB = double blind-ed; B = blinded (patients or evaluator); U = unblended; R = retrospective; CT = tomography; VAS = Visual Analog Scale; MacNab method (excellent and good outcome); MRI/MR (magnetic resonance imaging).
ti-inflammatory drugs, as well as a significant improvement on the disability scale in the patient study group compared to the controls.

The outcome measures of the randomized studies were VAS (17,20), Backill scores (17) and drug intake (17), MacNab’s criteria (15,20), and ODI (16,20).

2.2 Observational Studies

2.2.1 Methodological Quality Assessment

A total of 23 observational studies were considered for inclusion (Fig. 1). Only 8 of these met the methodological quality assessment criteria for inclusion (Table 5) (26-33). The results of the methodological quality assessment showed scores from 50 to 72. Some observational studies met the inclusion criteria, but had an insufficient score in the methodological quality assessment, and thus were only listed in the references (34-46). Furthermore, some studies were excluded for other reasons: one compared ozone therapy with a not well-established treatment (Alarnerf) for low back pain (47); and Baabor et al (48) used another intervention associated with intradiscal ozone.

2.2.1 Descriptive results of observational studies

Among the observational studies, we observed heterogeneous groups of patients, different follow-up periods, and some discrepancy in the computed tomography (CT) or magnetic resonance imaging (MRI) evaluations of morphological criteria (Table 6). Muto et al published 3 studies between 1998 and 2008 (27,28,29) using intradiscal injection of an oxygen-ozone mixture under CT guidance to treat approximately 3,700 patients and reported an 80% success rate at short-term follow-up (6 months) and a 75% success rate at long-term follow-up (18 months), with no major or minor side effects.

Oder et al (26) studied 621 patients to determine associations among the morphology of the disc disease, patient-specific data, and treatment outcomes. Six hundred twenty-one consecutive patients were subjected to CT-guided ozonucleolysis in combination with peri-radicular infiltration by steroids under local anesthesia. Based on the MRI findings of the lumbar spine, the patients were retrospectively divided into 5 diagnostic groups: group I consisted of 205 patients (bulging disc); group II had 185 patients (herniated disc); group III had 66 patients (postoperative patients); group IV had 51 patients (primarily intervertebral osteochondrosis); and group V had 114 patients and included other primary nondiscal changes (intervertebral arthrosis, spinal canal stenosis and pseudoarthrosis). The patients received steroid and an oxygen-ozone mixture into the disc and periganglionic infiltrations by CT guidance. Each patient was monitored for a period of 6 months and documented with the Oswestry Disability Index (ODI) and VAS. Patients younger than 50 years had significantly better values on the VAS and in ODI scores, 6 months after treatment.

Andreula et al (30) reported a 78.3% success rate in patients treated with ozone therapy and periganglionic steroid injection compared with a 70.3% rate in those treated with ozone therapy alone; complications occurred in 2 of 235 patients and consisted of episodes of impaired sensitivity in the lower limb on the treated side, which resolved spontaneously within 2 hours. In a series of 45 patients, Buric et al (31) studied the differences in outcome between intradiscal ozone chemonucleolysis and microdiscectomy in patients with noncontained lumbar disc herniations; they documented that 27 patients (90%) in the chemonucleolysis group showed a statistically significant improvement in pain and function; the same was true in 14 (93.3%) patients in the microdiscectomy group. However, 2 patients dropped out of the ozone chemonucleolysis group because of aggravating symptoms and subsequently underwent surgery.

Das et al (33), in an Indian population cohort study, evaluated 53 consecutive patients with lumbar disc herniation. All presented with clinical signs of lumbar nerve root compression supported by CT and MRI findings. They were treated with a single session of intradiscal ozone therapy. Therapeutic outcome was assessed after 2 years. Pain intensity was significantly reduced following treatment (VAS baseline was 7.58; after 2 years, 2.64). Similar ODI results were seen (P < 0.05). No major complication was observed in this case series.

Xu et al (32) included 187 patients with sciatica and low back pain with positive Lasègue sign and diagnostically verified by CT and MRI exhibited disc protrusion with nerve root or thecal sac compression. They compared the effectiveness rates after one week (103 cases), 2 weeks (61 cases), and 4 weeks (23 cases) treatment sessions of intradiscal ozone therapy. They were evaluated by Macnab criteria at 48 months. The effective rate was 82.02% in all groups. However, there were no significant differences in the total effective rate in the 3 groups (P = 0.280).
Table 5. Methodological assessment of observational studies of ozone therapy

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Weighted Core Points</th>
<th>Oder Austria 2008</th>
<th>Muto Italy 2008</th>
<th>Muto Italy 2004</th>
<th>Andrella Italy 2003</th>
<th>Muto Italy 1998</th>
<th>Buric Austria 2005</th>
<th>Xu China 2009</th>
<th>Das India 2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Study Question</td>
<td>2</td>
<td>2</td>
<td>2</td>
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<td>2</td>
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<tr>
<td>Clearly focused and appropriate question</td>
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<td>2</td>
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<td>2</td>
<td>2</td>
<td>2</td>
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</tr>
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<td>2. Study Population</td>
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<tr>
<td>3. Comparability of subjects</td>
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<td>11</td>
<td>11</td>
<td>14</td>
<td>14</td>
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<tr>
<td>Specific inclusion/exclusion criteria for all groups</td>
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<td>Criteria applied equally to all groups</td>
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<tr>
<td>Comparability of groups at baseline with regard to disease status and prognostic factors</td>
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<td>0</td>
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<tr>
<td>Study groups comparable to non-participants with regard to confounding factors</td>
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<td>0</td>
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<tr>
<td>Use of concurrent controls</td>
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<tr>
<td>Comparability of follow up among groups at each assessment</td>
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<td>Exposure measure equally in all study groups</td>
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<td>5. Outcome measures</td>
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West et al. Rating system to measure the strength of evidence, evidence report, technology assessment No. 47 AHQR Publication No. 02-016 (11).
Table 6. Results of observational studies of ozone therapy for low-back pain

<table>
<thead>
<tr>
<th>Author/Country/year/Methods/Type of pain</th>
<th>Participants</th>
<th>Design of study/intervention (↑) (guided by CT or fluoroscopy)</th>
<th>Outcome(s)</th>
<th>Result(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Das (33), India, 2009 Chronic</td>
<td>53 patients with low back pain due to lumbar disc prolapsed were included in this study</td>
<td>Prospective cohort study</td>
<td>Timing: 2 years Measure outcome: VAS and ODI</td>
<td>_</td>
</tr>
<tr>
<td>Xu (32), China, 2009 Chronic</td>
<td>187 patients with diagnostically confirmed lumbar disc herniation with sciatica and low back pain</td>
<td>Prospective study G1: (103) One week session G2: (61) 2 - week session G3: (23) 4 - week sessions</td>
<td>Timing: 48 mos. Outcome measures: MacNab’s criteria</td>
<td>Not reported</td>
</tr>
<tr>
<td>Muto (29), Italy, 2008 R, O Subacute</td>
<td>In 6 years, 2,900 patients with low back pain and/or sciatica refractory to medical management, lasting at least 2-3 mos. were treated with ozone and selected on the basis of clinical, psychological, neurological and neuroradiological criteria. Patients divided into 4 groups: all procedures were carried out with: ozone (40µg/mL) intradiscal (3 – 4mL) and the foramen (10 mL). G1 (n=2,650 with soft – disc herniation; G2 (n=250) had calcified herniation; G3 (n=350) had multiple herniation and G4 (n=200)had FBSS</td>
<td>Prospective study</td>
<td>Timing: 6 and 12 mos. Outcome measure: VAS (-3 pts), MacNab’s criteria and ODI (-30%)</td>
<td>Not reported</td>
</tr>
<tr>
<td>Oder (26), Austria, 2008 R, O Chronic</td>
<td>621 patients with lumbago or lumboischialgia and degenerative disease of the lumbar spine whose symptoms did not improve after previous conservative procedure They were retrospectively divided into 5 diagnosis groups: G1 (n=205) Bulging disc; G2 (n=185) herniated disc; G3 =66) post-operative patients; G4 (n=51) osteocondrosis and G5 (n114) non-disc disease (spinal canal stenosis, intervertebral arthrosis and pseudoanteriorolisthesis)</td>
<td>Prospective study</td>
<td>Timing:2 and 6 mos. Measure outcome: VAS and ODI</td>
<td>Not reported</td>
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<tr>
<td>Buric (31), Italy, 2005 P.O Subacute</td>
<td>45 patients with acute or sub acute low back pain unresponsive to pharmacological treatment The patients were divided into two groups G1(n=30) ozone inside the disc/30 ml – 30µg/ml G2 (n15) microdiscectomy</td>
<td>Prospective study</td>
<td>Timing: 6, 12 and 18 mos. Outcome measures: VAS, RMDQ, OPRS, MRI scans pre and post- treatment</td>
<td>Not reported</td>
</tr>
<tr>
<td>Muto (28), Italy, 2004 R, O Subacute</td>
<td>2,200 patients with low back pain and/or sciatica refractory to medical management, lasting at least 2-3 mos., subjects were treated with ozone and selected on the basis of clinical, psychological, neurological and neuroradiological criteria. Consecutive patients with degenerative disease, herniated disc, multiple disc herniation, FBSS, calcified disc herniation and disc associated with spinal stenosis received ozone (40µg/mL) intradiscal (3-4 mL) and the foramen (10 mL).</td>
<td>Prospective study</td>
<td>Timing:6 and 18 mos. Outcome measures: Subjective MacNab’s criteria</td>
<td>Not reported</td>
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Ozone Therapy for Low Back Pain

Table 6 (cont.). Results of observational studies of ozone therapy for low-back pain

<table>
<thead>
<tr>
<th>Author/Country/year/Methods/Type of pain</th>
<th>Participants</th>
<th>Design of study/intervention (guided by CT or fluoroscopy)</th>
<th>Outcome(s)</th>
<th>Result(s)</th>
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<tbody>
<tr>
<td><strong>Andreula (30), Italy, 1998</strong></td>
<td>600 patients with chronic low back pain resistant to conservative treatment, with positive sings of nerve root involvement, with or without hypoaesthesia or paraesthesia, with appropriate dermatome distribution and CT or MRI findings in live with the patient’s clinical Picture</td>
<td>The patients were divided in two groups: G1 (n = 211) ozone Intradiscal/4ml and periganglionic/8ml-30µg/ml G2 (n = 235) ozone + steroid</td>
<td>Timing 6 mos. Outcomes measures: Subjective MacNab’s criteria</td>
<td>6 mos. (excellent and good) G1 = 70.3% G2 = 78.3%</td>
</tr>
<tr>
<td><strong>Muto (27), Italy, 1998</strong></td>
<td>93 patients with low back and/or sciatica, lasting two or more mos., were treated with ozone</td>
<td>The patients were divided into two groups and all received ozone = 15ml; 30µl/mL Intradiscal and Intraforaminal G1 (n = 58) with neurological deficit G2 (n = 58) Without neurological deficit</td>
<td>Timing: 6 mos. Measure outcome MacNab’s criteria</td>
<td>6 mos. (good or excellent) G1 = failure in all patients G2 = success in 77.58% of patients</td>
</tr>
</tbody>
</table>

G1 = group 1; G2 = group 2; G3 = group 3; G4 = group 4; RA = randomized; P = prospective; O = observational; DB = double blinded; B = blinded (patients or evaluator); R = retrospective; FBSS = failed back surgery syndrome; CT = tomography; VAS = Visual Analog Scale; ODI = Oswestry Disability Index; McGill = McGill questionnaire of pain; MacNab method (excellent and good outcome); RMDQ = Roland-Morris Disability Questionnaire; OPRS = Overall Patient Rating Scale; MRI/MR (magnetic resonance imaging); EMG (electroneuromyography).

2.3 Effectiveness

Overall, the observational studies revealed positive results for short- and long-term relief of pain. From the randomized studies, intervention was found superior to the control, with OR 2.66 (95% CI, 1.94 to 3.63), and P < 0.00001 as shown in Fig. 2.

These studies evaluated ozone applied at the para-vertebral muscle and juxtaforaminal at the herniated disc level. Three of them compared ozone injections utilizing an active control group (steroid injections) (14,15,16). Paoloni et al (17) utilized a sham control group with a simulated injection that was administered using a false needle that pricked the skin without piercing it, applied at the lumbar paraspinale level, on the same site designed to reproduce the load sensation commonly described after O2-O3 injections.

243 Level of Evidence

The indicated level of evidence is II-3 for ozone therapy applied intradiscally and II-1 for ozone therapy applied paravertebrally on long-term relief in low back pain secondary to disc herniation (12).

2.5 Recommendations

Based on Guyatt et al (13), grading the strength of recommendations and quality of evidence in clinical guidelines, the recommendation is 1C for ozone therapy applied intradiscally and 1B for ozone applied at the paravertebral muscles or periforaminally.

2.6 Complications

Complications secondary to ozone therapy are rarely documented in the literature. In this review, regarding ozone therapy for low back pain, we encountered predominantly case reports of 5 different types of complications. Giudice et al (22) reported bilateral vitreo-retinal hemorrhages following ozone therapy for lumbar disc herniation. Furthermore, one case of thunderclap headache after oxygen-ozone therapy...
related to pneumoencephalus as a consequence of inadvertent intrathecal puncture was recently published (24). Ginanneschi et al (23) reported a case of a patient who experienced paresthesias along the anterolateral compartment of the left leg and hypoesthesia over the dorsum of the left foot, suggesting spinal nerve injury occurring a few minutes after percutaneous intradiscal infiltration of ozone for L4-L5 disc herniation. In 2004, Corea et al (21) published a report of vertebrobasilar stroke during ozone therapy. In 2 of 235 patients, Andreula et al (30) reported episodes of impaired sensitivity in the lower limb on the treated side, which resolved spontaneously within 2 hours. Fabris et al (34) reported a subcutaneous hematoma at the puncture site.

3.0 Discussion

The present review has added methodological improvements compared to previous review articles; the search database was wider and covered all languages, focusing on articles that used ozone alone in at least one group of patients. Final evidence was separated by the route of ozone administration. In addition, the authors performed a rigorous selection of RCTs that made possible a meta-analysis. Steppan et al (29) published a review in which data was extracted mainly from observational series; one was an unpublished study and one was a randomized trial on intradiscal ozone injections for the treatment of pain related to herniated discs. Although the authors have made a meticulous computation of data and wrote similar conclusions about the effectiveness and safety of this method, it probably would not be considered a meta-analysis if it had been submitted to the present review board.

Regarding the observational studies, 23 were initially selected according to the inclusion criteria, but only 8 could be included according to the rigorous methodological assessment criteria (11). Most studies lost points in the characterization of the study population because they did not specify the diagnosis. Probably, in future studies the authors should add diagnostic criteria and if needed, diagnostic procedures. The excluded studies also lack outcome measures and some of them had poor statistical analysis (which was absent in some of them). Furthermore, excluded studies contained heterogeneous populations of patients with low back pain, including patients with lumbar disc herniation, degenerative disease, acute pain, chronic pain, and patients with and without a history of operations. In addition, regarding the analysis of results, in some studies it was not clear what primary and secondary outcomes were expected; functional scales were diverse and in most cases not comparable. Most comparative studies used statistical analysis to aid the conclusions, but some of them have unacceptable confusion between normal and non-normal data distribution, resulting in the inappropriate choice of statistical tests. Furthermore, these studies often do not describe bias and limitations. Some studies include a large number of patients, a long period of follow-up and a careful surgical technique, but do not have appropriate design or statistical analysis (39). Another study did not compare with a method established in the literature, so it was excluded (46).

Among the selected RCTs, 3 of them compared ozone treatment with an active control group (steroid or steroid with local anestheti) and one study compared ozone injection with a sham procedure. No placebo-control study was found among the articles included in this review. This seems to be a tendency when treatment-resistant pain is the issue. Currently,
Ozone Therapy for Low Back Pain

ethics committees seem to favor studies based in an active controls comparison group. In addition, this makes patient recruitment faster because patients have a better acceptance when there is no placebo involved. Although this is not a consensus, it seems logical that an established treatment is probably better than the placebo effect. So, if any new treatment is to be tested, it could be perfectly compared to an active control group and in this way the placebo effect would also be overcome. On the other hand, patients tend to think that new treatments are more efficacious than the established ones because of their novelty. This makes us think that the novelty usually carries a strong placebo effect. This controversy still keeps placebo-controlled trials as the gold standard methodology. However, in the near future this methodological recommendation will probably be reviewed because practical issues point to more progressive methodology for active control studies in pain literature.

Some of the studies have evaluated the morphology of the disc by MRI or CT scan during follow-up. Buric et al (31) evaluated the clinical and morphological results of patients with disc disease and observed that 15 of the 30 patients showed clinical improvement, performing post operative MRI imaging. Eight of these patients had a substantial reduction of over 50% in herniation volume. Two patients had a volume reduction of less than 50%, whereas 5 patients had no substantial variation in herniation volume. Muto et al (27) observed a reduction in the size of the herniated disc in only 8 cases out of the 45 patients who had improved. In 2004, Muto et al (28) documented a reduction in herniated disc size in 63% of cases, confirming persistent satisfactory outcome. Thus, these authors stated that the equation large herniation = major symptoms, small herniation = minor symptoms, does not always hold true. It seems quite natural to assume that clinical signs and symptoms of disc herniation are not caused only by mechanical compression but that biochemical factors play an important role in inflammatory sensitization and immune response in the epidural environment of the nerve roots and ganglia. Based on the same reasoning, it seems logical to presume that mechanical removal of herniated tissue may not always be needed and that reducing the inflammatory process could essentially be sufficient to treat the symptoms. This hypothesis was partially confirmed by the cited study (49,50). On the other hand, patients who were clear candidates for surgery had no improvement after ozone therapy. Muto et al (27) observed treatment failure in all 35 patients previously selected for surgery who presented a herniated or protruded disc with radicular pain associated with neurological deficit. In the work of Buric (50), 2 patients dropped out of the ozone therapy group because of aggravating symptoms and were subsequently operated on. In another observational study (30), among patients treated with ozone and whose treatment had failed, outcomes were poor in 25% and poor with recourse to surgery in 4.7%. Among the patients in the steroid group and anesthetic injection group, 50 (16.7%) had poor results and 15 (5%) were referred for surgery.

The majority of the studies reviewed included patients with discogenic disease at one or more levels between L3 and S1(14-16,26,27,29-31). However, other series included heterogeneous groups of patients with other primary nondisc diseases such as canal stenosis, postsurgical fibrosis (failed back surgery syndrome), disc protrusion with vertebral instability, facet arthropathy, calcified herniation, intervertebral osteochondrosis, and pseudoanterolisthesis. In the first group, positive results were achieved in 75-80% of treated patients. In patients with a nondisc disease, the rate of sustained improvement ranged between 44 to 70% in all groups, independent of the morphological classification of the spinal disease (26,28,29). This suggests that ozone therapy may have an important role in low back pain relief, independent of the source of disease.

Ozone is a strong oxidizing agent that quickly reacts and oxidizes the proteoglycans in the nucleus pulposus, which results in a small reduction of disc volume and subsequently contributes pain relief. The suggested premise is that a small volume reduction results in a significant decrease in pressure. In addition, it has been shown to have anti-inflammatory/analgesic and natural antibacterial effects (5,52). Additional discussion of ozone’s mechanisms of action can be found elsewhere (51).

Ozone therapy for lumbar disc herniation is a procedure that is considered generally risk-free or as low as 0.1% (48) and has low or no adverse effects at concentrations used for therapeutic application (10-40 µg/mL). However, in the present review, 6 reports of side effects related to ozone infusion were found. Similar descriptions of transitory paresthesia suggested transient root dysfunction, although the mechanisms underlining the reported sensations are still not clear. Assuming the presence of microfractures of the annulus fibrosus, one possibility is that an abrupt, transient pressure spike in the region of the spinal canal and cerebrospinal fluid (CSF) pressure after disc infiltration could be related to...
the transient paraesthetic symptoms. A similar mechanism was postulated as the cause of acute bilateral intraocular hemorrhages after injection of the O2-O3 mixture (22). Concerning the pathophysiology of the lesion, it could be hypothesized that an abrupt and transient increase of CSF pressure causes focal damage by means of mechanical transmission of pressure in the CSF, manifesting in the form of direct root trauma. The occurrence of retinal hemorrhages immediately after rapid injection of air into the subarachnoid space during myelography or after epidural injection of corticosteroids has also been previously described (52,53).

Infection secondary to oxygen-ozone injection therapy is extremely rare. Recently, Gazzeri et al (25) reported a case of fatal septicemia secondary to Escherichia coli infection after ozone therapy for lumbar disc herniation, in which a pyogenic lumbar muscle involvement and septic pulmonary embolism were present. The most likely pathophysiologic mechanism in these cases was probably iatrogenic; that is, the direct inoculation of the bacteria by injections due to an inadequate asepsis procedure as has occurred in other percutaneous spinal procedures (25,30).

4.0 Conclusion

This systematic review and meta-analysis of ozone therapy for low back pain secondary to herniated disc indicated the level of evidence is II-3 for ozone therapy applied intradiscally and II-1 for ozone therapy applied at the paravertebral muscle and periforaminally for long-term pain relief based on USPSTF criteria (12). The available evidence yielded a 1C strength of recommendation (13) for ozone therapy applied into the disc and 1B for ozone applied at the paravertebral muscles or periforaminally. The evidence was derived from randomized control trials within this meta-analysis and observational studies. In addition, the low costs of ozone therapy may account for its wider use in the percutaneous treatment of herniated lumbar discs (54) and other causes of back pain. Injections can be repeated if necessary and complications or side effects are rare. Therefore, this method may be considered an option to treat lumbar disc herniation-related low back pain that has failed to respond to conservative treatment, representing an alternative to surgery. However, future studies are necessary to demonstrate whether ozone therapy effects persist over time.

References

Ozone Therapy for Low Back Pain

References continue below

Ozone Therapy as a Treatment for Low Back Pain Secondary to Herniated Disc...


Effectiveness of Oxygen-Ozone and Hyaluronic Acid Injections in De Quervain's Syndrome

M. MORETTI
Poliambulatorio Oberdan; Brescia, Italy

Key words: oxygen-ozone, hyaluronic acid, De Quervain's syndrome

SUMMARY - De Quervain's syndrome is a tenosynovitis of the short extensor tendon and of the long abductor tendons of the first finger of the hand frequently found in our clinical experience. The effectiveness of traditional physical therapies proposed and cortisone injections must be to verify and even surgery on a long time does not always adequate results. The injection with oxygen / ozone and hyaluronic acid are effective in other forms of overload tendinopathy. We want to assess whether the SDQ can be effectively treated with this therapy.

The De Quervain's Syndrome

The De Quervain's syndrome (SDQ), is a tenosynovitis that affects the tendons long abductor (LAT) and short extensor (SET) of the first finger of the hand that slide together in a single sheath within the channel of the extensors. The disease often occurs in people who perform violent or highly repetitive movements with the first finger of the hand. For this is common among musicians, the tailors, who use a lot the mouse or computer keyboard and mothers, especially in the last feeding period, when the child becomes heavier; so that at the time of the description of the disease by De Quervain, this was known as a disease of the nannies and embroiderers. The common cause of SDQ is the edema that surrounds the tendons which are located in the wrist on the side of thumb. The irritation causes swelling of tissues around the tendon thus causing a change volume of the area or causing a thickening of tendons, and this means that they cannot slide as they should. The SDQ is not uncommon to find associated with carpal tunnel syndrome, trigger finger or the rhizarthrosis.

The musculoskeletal ultrasound can be used to show the presence of excess synovial fluid that distends the synovial sheath (figure 1).

Therapy

The most used remedies are the classical physical therapies (laser, ultrasound and iontophoresis) that not have proved effectiveness and cortisone injections, whose effectiveness has not been demonstrated unambiguously and that can cause rupture and infection of the treated tendon. The oxygen-ozone therapy is widely used as a remedy for the SDQ and it is a treatment that was effective in our experience. Hyaluronic acid is an effective treatment for other overload tendinopathies but there is no scientific literature on the subject.

Aim of the study

We want to assess if injections with O2/O3 and HA, already tested for other tendinopathies are a good way to heal the SDQ.

Materials and methods

To assess whether injections with O2/O3 and Al are a good way to heal the SDQ 100 patients were selected. Patients had to have pain for at least
Effectiveness of Oxygen-Ozone and Hyaluronic Acid Injections in De Quervain’s Syndrome

M. Moretti

Results

The 100 patients selected were for 68% women, mean age of 53.3 years and for 32% men with a mean age of 53.4 years. The initial pain, expressed with the VAS scale, is a result of 7.9 and the end of 1.9 as shown in figure 2. The patient satisfaction is showed in the table 1 and in figure 3.

Conclusions

The SDQ is a very frequent tenosynovitis in patients who perform works that involve the continuous use of the first finger of the hand and the traditional therapies have not proven unequivocal-
largely effective. The O$_3$O$_2$ and AI injections have been shown in our first assessment an effective method to heal the SDQ but an evaluation is required on a larger sample to propose this treatment on a larger scale as an alternative to conventional physical therapies and cortisone injections.

Table 1 Satisfaction of the treated patients.

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<thead>
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<th>Satisfaction</th>
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<td>Total</td>
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References

Ossigeno Ozono Terapia
Il razzionale scientifico e l'utilizzo pratico
Fondazione di Verbania - Sabato 22/09/2012
Modulo di iscrizione

EVENTO ACCREDITATO ECM
Referente: 100 medici

Programma

8.30 Accoglienza e iscrizioni
9.00 Apertura dei lavori
9.20 Prima sessione mattutina
11.00 Coffee break
11.30 Seconda sessione mattutina
13.30 Lunch
14.30 Sessione pomeridiana
16.30 Compilazione ECM
16.45 Chiusura dei lavori

Comunicazioni

Presentazione del corso
Domano Delbarba
9.00 - 9.30

Saluto delle autorità
Matteo Bonetti
9.30 - 9.50

Intervenuto
Matteo Bonetti, Alessio Zambello, Annetta Pacico, Alberto Uderzo, Umberto Cattol, Alessandro Parravincini
13.30 - 15.30

Workshop
Infiltrazioni eco-guidate
Viganò, Boraso, Pasquali, Vaccini, altri
15.30 - 16.30

Relatori e moderatori:

Matteo Bonetti
Alessio Zambello
Annetta Pacico, Alberto Uderzo
Umberto Cattol, Alessandro Parravincini
Viganò, Boraso, Pasquali, Vaccini, altri

Miglioramento gestionale e di formazione nell'ambito della comprensione e dell'applicazione pratica dell'ossigeno ozonoterapia.

Sabato
22 settembre 2012
Tecnoparco del Lago Maggiore
Fondazione di Verbania
Can Oxygen-Ozone and Hyaluronic Acid Injections Be a Valid Alternative to Cortisone?

M. MORETTI
Poliambulatorio Oberdan; Brescia, Italy

Key words: oxygen-ozone, hyaluronic acid, cortisone

SUMMARY - Lateral epicondylitis (LE) is a frequent condition of the common extensor tendon of the wrist, hand and fingers. The existing studies about the corticosteroid injections, the acupuncture and physical therapies for the LE don't show evidence of effectiveness but these remedies are frequently used. The \( O_2-O_3 \) injections \((O_2O_3)\) are a valid treatment for the LE as well as the injections of hyaluronic acid (HA) in our experience and according with scientific literature. We want to asses if \( O_2O_3 \) and HA injections together are a valid alternative to cortisone, acupuncture and physical therapies for the treatment of LE.

Lateral epicondylitis (LE) is a common condition of the common extensor tendon of the wrist, hand and fingers, but relatively little is known about its etiology and associated risk factors. The diagnosis is quite simple since the patient reports pain on the epicondylus when efforts are made or by compressing the point reported as painful. Ultrasound can be useful to assess the LE magnitude and to evaluate the presence of vascular signal and of ossealcific metaplasias in severe cases. The existing studies about corticosteroid injections for the LE are not conclusive even this remedy is frequently used. Many trials were conducted but corticosteroid injections appear to be relatively safe and seem to be effective only in the short term (2-6 weeks). The Acupuncture and physical therapies don’t give good results in the treatment of the LE too. The \( O_2-O_3 \) injections \((O_2O_3)\) are a valid treatment for the LE as well as the infiltration of hyaluronic acid (HA).

Aim of the study

We believe that the use of cortisone injections is a methodology to be abandoned, and so we want to evaluate whether the use of the \( O_2O_3 \) and HA may be an effective remedy for epicondylitis.

Material and Methods

50 patients were selected for evaluating the effectiveness of injections of \( O_2O_3 \) and HA to heal the LE. All the patients had already been subjected to rest, use of orthopedic brace, traditional physical therapies and cortisone injections with no benefit. All patients were subjected to ultrasound exam before being included in the study. In figure 1 it is possible to observe an example of a ultrasound exam that shows a common extensor tendon with vascular signal, indicative of overload syndrome. Before starting treatment was done to complete a questionnaire which was given the intensity of pain according to VAS scale. The treatment was repeated three times and consisted of a injection first with the \( O_2O_3 \) (5 ml at 14-16 µg / ml) and then with HA (16 mg in 4 ml). The pain was then assessed by VAS scale again two weeks after stopping treatment.

Results

The 50 patients included in the study were 30 men and 20 women. The average age of the total sample was 48.7 (±6.1), for the men was 48.7 (±9.3) and 46.4 (±8.1) for women. The pain before the treatment of the total sample was 7.6 (±0.9), for the men 7.6 (±1.2) and 7.5 (±1) for the women. The pain two weeks after the treatment was 2.1 (±0.7) for the total sample, 2.2 (±0.9) for the men and 1.9 (±0.7) for the women. All the results are shown in the table 1 and in the graphics 1 and 2.

Conclusions

The injections with \( O_2O_3 \) and HA are a valid method in the treatment of EL with no collateral
Can Oxygen-Ozone and Hyaluronic Acid Injections Be a Valid Alternative to Cortisone? M. Moretti

analysis that demonstrates unequivocally the most effective treatment with O₂O₃ and HA compared with cortisone.

Table 1  Result of the study

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Woman</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>48.7 (±9.3)</td>
<td>46.4 (±8.1)</td>
<td>48 (±6.1)</td>
</tr>
<tr>
<td>Pain Before treatment</td>
<td>7.6 (±1.2)</td>
<td>7.5 (±1)</td>
<td>7.6 (±0.9)</td>
</tr>
<tr>
<td>Pain After treatment</td>
<td>2.2 (±0.9)</td>
<td>1.9 (±0.7)</td>
<td>2.1(±0.7)</td>
</tr>
</tbody>
</table>

effects and may represent a valid alternative to the cortisone in the short term. However it must be conduct a study on a larger sample with statistical

Graph 1  Pain before and after the treatment in the total sample.

Graph 2  Pain before and after the treatment in the men and in the women.

Figure 1  Example of ultrasound exam of the common extensor tendon of one of the patients involved in the study.
References


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A Rational Solution for the Problem of the Ozonated Saline Infusion

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Key words: ozonetherapy, albumin, hydrogen peroxide, alkenals, antioxidant response

SUMMARY - While the biochemical and molecular mechanisms of the ozonated autohemotherapy are well defined, the use of ozonated saline infusion should be proscribed because it is unreliable.

Introduction

In 1974, Wolff\(^1\) proposed a new medical approach consisting in mixing in a sterile glass, 500 ml bottle about 150-200 ml of human blood with an equal volume of a gas mixture composed of about 95% oxygen and 5% ozone. After a few minutes of mixing the two phases, the ozonated blood is infused into the donor patient. This approach remained empirical for almost three decades because it remained unclear how ozone acted in blood. However now, not only we have ozone generators able to produce precise ozone concentrations in real time but we know exactly all the mechanisms of actions of ozone in blood.\(^{12}\) Ozone is very soluble in the water of plasma and reacts immediately with some of the hydrosoluble antioxidants and with the polyunsaturated fatty acids present in the albumin molecules. Within a few minutes ozone is exhausted but generates two fundamental messengers: hydrogen peroxide and 4-hydroxy-nonenal (4-HNE) among the alkenals. The first has a free access into all blood cells and activates a number of important biochemical pathways and the second binds to either the Cys34 of albumin or to GSH forming a fairly stable adduct. After a few minute of mixing blood with the gas phase, the ozonated blood is infused into the donor patient and the alkenal-adducts can enter into a great variety of the body cells. This second small oxidative stress is very important because, by activating the Nrf2-ARE pathway, leads to the upregulated synthesis of several antioxidant proteins, phase-2 enzymes and heme-oxygenase-1.\(^3\) In conclusion today by using minute and precise dosages of ozone ranging from 10-80 µg/ml ozone per ml of blood (or 0.21-1.68 mM ozone) it is possible to reverse the chronic oxidative stress typical of cardiovascular and degenerative diseases.\(^4\) This therapy is able to block the progression of chronic diseases and all the pharmacological parameters are now well defined without any risk of toxic effects. Actually most of the patients, after a few treatments, report a feeling of wellness. Administration of antioxidants is insufficient because it does not actively change the cell response.

Owing to the number of patients and scarce resources, it is most unfortunate that Russian physicians have adopted the cheap and quick procedures of infusing ozonated saline. This approach is unreliable because imprecise and not pharmacologically acceptable by Western Health Authorities. Saline is bubbled with 2-3 µg/ml ozone, that is safe, but most uncertain regarding the dosage and with an effect similar to a placebo. This procedure is becoming fashionable among European unscrupulous physicians with uncertain but likely toxic ozone dosages. Obviously this empirical approach deeply discredit the aim and validity of scientifically sounded ozonetherapy.

This negative situation can be corrected by the following method: a 250 ml glass bottle is filled with 50 ml of physiological saline and 100 ml of pure human albumin (4% concentration). To this “artificial serum” containing 26.6 mg/ml of albumin, 1.0 ml of a diluted solution of Ascorbic Acid (AA) is
added as obtained from a commercial ampoule of AA (0.1 g/ml) by taking 0.5 ml of it and diluting and mixing with 10 ml sterile water. The final AA concentration in 150 ml is of 33 µg/ml Finally 100 ml of O₂-O₃ gas mixture (Ozone dose: 3 mg) are added and, after one minutes of gentle mixing, the 150 ml solution (final O₃ concentration: 20 µg/ml) are infused with a G22 needle. This procedure allows the generation of the ozone messengers thus closely resembling the classical ozonetherapy: this method has proved to be very beneficial and atoxic in “dry” macular degeneration women with poor venous accesses⁴. While therapeutic albumin is safe, even the best screened human serum cannot be used.

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Rectal Administration and its Application in Ozone therapy

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Key words: rectal insufflations, ozone, ozone therapy, autohemotherapy, rectal way

SUMMARY - The rectal administration of ozone is one of the oldest systemic and local forms of application. The biological effects of the Rectal Insufflations of Ozone (RIO) has been demonstrated extensively either experimentally or clinically. Furthermore, preclinical studies demonstrated its low toxicity. RIO has been now extended to treat many diseases and is increasingly being used as a systemic therapeutic form. RIO is already being viewed as an alternative to Mayor autohemotherapy (MAH). Using standardized clinical protocols a therapeutic success can be reached with RIO. Handling the advantage and disadvantage of RIO, not as alternative to MAH but used properly (e.g. pediatric, geriatric, when MAH cannot be performed because i.v. is difficult due to unfavorable vein conditions, etc.), this method is a valid route of O/O2 administration.

Introduction

Rectal administration of drugs has been used since ancient times to produce local effects. In addition, the rectal route may be used for systemic administration of drugs.1 The rectal administration of ozone is one of the oldest systemic and local forms of application. Rectal insufflations of ozone (RIO) was first proposed by Aubourg (1936) for treating chronic colitis and fistulae. Actually, the biological effect of RIO has been demonstrated extensively either experimentally or clinically. Furthermore, preclinical studies demonstrated its low toxicity. That’s why the application of RIO has been now extended to treat many diseases.

Based on animal investigations and a comprehensive proctological study, rectal insufflation with an O2/O2 gas mixture is increasingly being used as a systemic therapeutic form, and is already being viewed as an alternative to Mayor autohemotherapy (MAH). In addition, it is the method of choice in pediatrics. The main disadvantage of RIO are connected with: 1) The variation in doses because of possible flatulence, the presence of a more or less abundant luminal content and the neutralization of O2/O2 by faecal material produce erratic absorption. 2) Composition, viscosity, pH and surface tension of rectal fluids have great effects on drug bioavailability. 3) It’s hypnotized that the O2/O2 concentration used is too high and during prolonged use may be mutagenic. 4) Not well accepted because cultural patients’ attitudes to rectal drug administration.

However, preclinical and clinical studies demonstrated that using standardized clinical protocols a therapeutic success can be reached using RIO. Handling the advantage and disadvantage of RIO, not as alternative to MAH but used properly (e.g. pediatric, geriatric, when MAH cannot be performed because i.v. is difficult due to unfavorable vein conditions, etc.), this method is a valid route of O2/O2 administration.

Consideration of the rectum and anus anatomy and physiology

The large bowel is a closed receptacle, 1-7 m long, with an ileoocaecal valve at its cephalad end, which prevents reflux, and the dentate line of the anus at the caudal end.7 While the transverse colon always has a mesentery, the ascending colon has a mesentery in only 12% of people and the descending colon has one in 22%. The sigmoid colon also has a mesentery and is sometimes unusual long (dolicolon) a feature which facilitates torsion or volvulus. The rectum, totally sheathed in longitudinal muscle fibres, is continuous with the anal canal, where the external sphincter of voluntary muscle provides an additional sheath. The levator ani sling muscle
acutely angles (at 60°-105° in normal subjects) the rectoanal junctions forwards, its nerve supply running on its upper aspect and thereby being liable to damage by inordinate stretching of the muscle, for example, during childbirth (Figure 1).18

The colorectum is lined with columnar epithelium as far as the dentate line in the middle of the anal canal, where sensitive squamous epithelium in continuity with that of the perineum takes over. Submucous anal glands may extend deeply into the sphincter. The anal canal has a high pressure zone resulting from tonic contraction of the internal and external sphincters, which is responsible for continence. Voluntary contraction can, however, double this pressure (squeeze pressure). Anorectal sensation permits discrimination of solids from gas 18. The pressure induced by rectal insufflation of O₂ during therapy also stimulates continence. Patients should be advised of this fact and will be invited to control this sensation at least for 5 min.

The rectum is normally empty, and when people awaken and eat breakfast, enhancing left colonic motility, faeces enter the rectum, and the person is called to stool. Sitting on the toilet helps to straighten out the anorectal angle and faeces enter the anal canal, to be passed if the passage is not voluntarily stopped. Further faeces from as far cephalad as the splenic flexure may be passed, the average daily volume being 150 mL. It is possible to delay expulsion: the rectum can accommodate passively adistension of up to 400 mL, maintaining a low rectal pressure, and faeces may even be propelled back into the sigmoid colon 18,19.

During RIO₂, once O₂ enter into the rectum the substrate of reaction will be feces, flatus and mucus. The main characteristics of those components are:

**Mucus Secretions.** The mucosa of the large intestine, like that of the small intestine, has many crypts of Lieberkühn; however, unlike the small intestine, there are no villi. The epithelial cells contain almost no enzymes. Instead, they consist mainly of mucous cells that secrete only mucus. The great preponderance of secretion in the large intestine is mucus. This mucus contains moderate amounts of bicarbonate ions secreted by a few non–mucus-secreting epithelial cells. The rate of secretion of mucus is regulated principally by direct, tactile stimulation of the epithelial cells lining the large intestine and by local nervous reflexes to the mucous cells in the crypts of Lieberkühn 18.

Mucus in the large intestine protects the intestinal wall against excoriation, but in addition, it provides an adherent medium for holding fecal matter together. Furthermore, it protects the intestinal wall from the great amount of bacterial activity that takes place inside the feces, and, finally, the

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**Figure 1** Anatomical aspect of the large bowel, and schematic representation of the rectal ozone application (adapted from Irving and Catchpole) 18.

**Figure 2** A schematic figure showing the thicknesses of the → 2 mucus gel layers in vivo in different region of the rat gastrointestinal tract (adapted from Atuma et. al.) 36.
mucus plus the alkalinity of the secretion (pH of 8.0 caused by large amounts of sodium bicarbonate) provides a barrier to keep acids formed in the feces from attacking the intestinal wall.

The mucus of the small intestine has only one layer, whereas the large intestine has a two-layered mucus where the inner, attached layer has a protective function for the intestine, as it is impermeable to the luminal bacteria. The thicknesses of the 2 mucus gel layers are particularly reinforced in the colon (Figure 2).

Composition of feces and flatus. The feces normally are about three-fourths water and one-fourth solid matter that itself is composed of about 30% dead bacteria, 10% to 20% fat, 10% to 20% inorganic matter, 2% to 3% protein, and 30% undigested roughage from the food and dried constituents of digestive juices, such as bile pigment and sloughed epithelial cells. The brown color of feces is caused by stercobilin and urobilin, derivatives of bilirubin. Short chain fatty acids (acetate, propionate, butyrate) are metabolic products of anaerobic bacterial fermentation of dietary fiber and resistant starch, are also present in the colon luminal content.

The odor is caused principally by products of bacterial action; these products vary from one person to another, depending on each person’s colonic bacterial flora and on the type of food eaten. The actual odoriferous products include indole, skatole, mercaptans, and hydrogen sulfide. Gases produced intraluminally (H₂, CO₂, and CH₄) comprised approximately 74% of flatus, and rapid CO₂ and H₂ productions were responsible for high passage rates. A positive correlation between flatus H₂ and CO₂ suggested that CO₂, like H₂, mainly was a bacterial product. Whereas methanogens and H₂S-producing bacteria usually are mutually exclusive in feces, CH₄ and H₂S did not negatively correlate, indicating coexistence of both organisms in the colon.

The dose of O₃ applied directly in the rectum during RIO₃, is evidently reduced in different proportions because it’s reaction with the luminal content (flatus, feces and mucus). To by past this effect, the German School of ozonotherapy assume empirically a tree fold increment in the rectal dose compared to the same dose administered by the MAH. For example, for a dose of 1.5 mg (30 µg/mL / 50 mL) by autohemotherapy, the corresponding rectal dose will be 4.5 mg (22.5 µg/mL / 200 mL).

Blood vessels from the lower part of the rectum connect with the inferior vena cava instead of merging into the portal vein. The rectal tissues are drained by the inferior, middle and superior haemorrhoidal veins, but only the superior vein connects with the hepatic-portal system (Figure...
Table 1 Some preclinical studies using rectal administration of ozone.

<table>
<thead>
<tr>
<th>Animal model of</th>
<th>Animal Specie</th>
<th>Dose mg/kg</th>
<th>O₃ concentration (µg)/[volume (mL)] / (No. Sessions)</th>
<th>Results (in brief)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepato cellular damage</td>
<td>Rats</td>
<td>1.0</td>
<td>50 [4.4-5] (15)</td>
<td>Hepato protection</td>
<td>León F. et al. 1998 [21].</td>
</tr>
<tr>
<td>Renal ischaemia</td>
<td>Rats</td>
<td>0.5</td>
<td>50 [2.5-2.6] (15)</td>
<td>Reduce renal damage</td>
<td>Barber E. et al. 1999 [22].</td>
</tr>
<tr>
<td>Hepatic ischaemia-reperfusion</td>
<td>Rats</td>
<td>1.0</td>
<td>50 [4.4-5] (10)</td>
<td>Protective effect</td>
<td>Peralta C. et al. 1999 [23].</td>
</tr>
<tr>
<td>STZ-induced diabetes</td>
<td>Rats</td>
<td>1.1</td>
<td>50 [5.5-5] (10)</td>
<td>Reduces markers of oxidative and endothelial damage</td>
<td>Al-Dalain S.M. et al. 2001 [24].</td>
</tr>
<tr>
<td>Hepatocellular damage</td>
<td>Rats</td>
<td>1.0</td>
<td>50 [4.4-5] (15)</td>
<td>Prevent anaerobic glycolysis and oxidative stress induced by CCL₂</td>
<td>Candelario-Jalil E. et al. 2001 [25].</td>
</tr>
<tr>
<td>Hepatic ischaemia-reperfusion</td>
<td>Rats</td>
<td>1.0</td>
<td>50 [5.5-5] (15)</td>
<td>Similar mechanisms of protection of ischaemic and ozone oxidative Preconditionings</td>
<td>Ajamieh H.H. et al. 2002 [26].</td>
</tr>
<tr>
<td>Hepatic ischaemia-reperfusion</td>
<td>Rats</td>
<td>1.0</td>
<td>50 [5.5-5] (15)</td>
<td>Hepato protection</td>
<td>Ajamieh H.H. et al. 2004 [27].</td>
</tr>
<tr>
<td>Cisplatin-nephrotoxicity</td>
<td>Rats</td>
<td>0.36/0.72/1.1/1.8/2.5</td>
<td>20/30/50/70 <a href="15">9</a></td>
<td>Renal protection</td>
<td>Borrego A. et al. 2004 [29].</td>
</tr>
<tr>
<td>Hepatic ischaemia-reperfusion</td>
<td>Rats</td>
<td>1.0</td>
<td>50 [5.5-5] (15)</td>
<td>Protein synthesis is involved in the protective mechanisms</td>
<td>Ajamieh H.H. et al. 2005 [30].</td>
</tr>
<tr>
<td>STZ-induced diabetes</td>
<td>Rats</td>
<td>1.1</td>
<td>50 [5.5-5] (10)</td>
<td>Preserved b-cells functions and reduced hyperglycemia</td>
<td>Martínez-Sánchez et al. 2005 [31].</td>
</tr>
<tr>
<td>Chronic renal failure</td>
<td>Rats</td>
<td>0.5</td>
<td>50 [2.5-2.6] (15)</td>
<td>Renal protection</td>
<td>Calunga et al. 2005 [32].</td>
</tr>
<tr>
<td>Cisplatin-nephrotoxicity</td>
<td>Rats</td>
<td>1.1</td>
<td>50 [2.5-2.6] (15)</td>
<td>Renal protection</td>
<td>Borrego A. et al. 2006 [33].</td>
</tr>
<tr>
<td>Hepato cellular damage</td>
<td>Dogs</td>
<td>1.9-2.4</td>
<td>20 [97-121] [(15)*]</td>
<td>Hepato protection</td>
<td>Li-Jie L. et al. 2007 [34].</td>
</tr>
<tr>
<td>Parkinson</td>
<td>Rats</td>
<td>0.7</td>
<td>25 [5] (20)</td>
<td>Neuro protection</td>
<td>Re L. et al. 2008 [35].</td>
</tr>
<tr>
<td>Red blood cell rheology</td>
<td>Rabbits</td>
<td>1.5-0.94/ 1.1/0.74</td>
<td>20 [150] (15/21/36)</td>
<td>Improvement</td>
<td>Artis et al. 2010 [36].</td>
</tr>
<tr>
<td>Arthritis</td>
<td>Rats</td>
<td>0.5/0.7/1.0</td>
<td>40-60 [5-6] (15)</td>
<td>Improvement</td>
<td>Mawsouf N. et al. 2011 [38].</td>
</tr>
<tr>
<td>Renal ischaemia-reperfusion</td>
<td>Rats</td>
<td>0.5</td>
<td>ND [ND] (10)*</td>
<td>Protective effect in preserving renal function and morphology</td>
<td>Fernández Iglesias A, et al. 2011 [39].</td>
</tr>
<tr>
<td>Endotoxic shock</td>
<td>Mice</td>
<td>0.2-0.4</td>
<td>(5)</td>
<td>Inhibits TNF-alpha production</td>
<td>Zamora Z.B. et al. 2004 [40].</td>
</tr>
</tbody>
</table>

Legend: “Tree groups with different doses; † tree groups with the same dose but different follow-up times (tree times schedules); ‡ one group with progressive dose increment; † post ischemia reperfusion treatment; ƞ one treatment every other day for 30 days. ND, non-defined.”
Evidence of the effectiveness and toxicity of rectal insufflation of ozone

Most of the preclinical model used to study the pharmacological effects of ozone therapy used the rectal way because it’s applicability in experimental conditions. Selected examples are shown in Table 1. Dose ranges from 0.2 to 79 mg/kg b.w. were used. All case referred a positive pharmacological effect without side effects. The O₃ concentration was in general between 10 µg – 50 µg, with exception of highest dose in one experiment. Tissue protections by a mechanism mediated by the synthesis of proteins (essentially antioxidant enzymes) was the main pharmacological effect finding in animal models.

Clinical trials using RIO, demonstrate its therapeutic effectiveness in different pathological conditions (Table 2). In all case it was reported no side effects, even in one of the longer clinical trials (patients was followed by 20 years) 24 no side effects was founded. Preclinical or clinical studies that compare the effect of RIO₃ to other administration ways, found controversial results, for example:

1. Medicaments absorbed in the lower part of the rectum are delivered directly into the systemic circulation, thus avoiding any first-pass metabolism.

Table 2 Selection of some clinical studies using rectal administration of ozone.

<table>
<thead>
<tr>
<th>Diseases</th>
<th>No. Sample</th>
<th>Dose (mg)</th>
<th>O₃ concentration (µg) / [volume (mL)] / (No. Sessions)</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIDS diarrhea</td>
<td>5</td>
<td>2.7-30</td>
<td>ND (21-28)</td>
<td>Effective</td>
<td>Carpendale M.T. et al. 1993³⁶</td>
</tr>
<tr>
<td>Arteriosclerosis obliterans</td>
<td>18</td>
<td>ND</td>
<td>ND</td>
<td>Improvement</td>
<td>Romero Valdés A. 1993³⁶</td>
</tr>
<tr>
<td>Asthma</td>
<td>37</td>
<td>10</td>
<td>50 [ 200 ] (20)</td>
<td>Improvement</td>
<td>Hernández Rosales F.A. et al. 2005⁵</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>52</td>
<td>10</td>
<td>50 [ 200 ] (20)</td>
<td>Improvement</td>
<td>Martínez-Sánchez et al. 2005⁵</td>
</tr>
<tr>
<td>Hypertensive Pregnant Women</td>
<td>15</td>
<td>3-12</td>
<td>20–40 [ 150-300 ] (21)</td>
<td>Improve the umbilical flow indices and reduce anti hypertensive therapy</td>
<td>Tanbouli T. et al. 2009²⁴</td>
</tr>
<tr>
<td>Coronary Artery Disease</td>
<td>40</td>
<td>10</td>
<td>50 [ 200 ] (20)</td>
<td>Improvement</td>
<td>Delgado-Roche et al. 2011²³</td>
</tr>
<tr>
<td>Retinitis Pigmentosa</td>
<td>56</td>
<td>8</td>
<td>40 [ 200 ] (20)</td>
<td>Improvement and increasing their quality of life.</td>
<td>Copello M. and Menendez S. 2011²⁴</td>
</tr>
<tr>
<td>Cerebral disorders</td>
<td>43</td>
<td>ND</td>
<td>15-25 [ 15-120 ] (20)</td>
<td>Improvement</td>
<td>Diaz E. et al. 2011²⁴</td>
</tr>
<tr>
<td>Pulmonary emphysema</td>
<td>20</td>
<td>6</td>
<td>30 [ 200 ] (20)</td>
<td>Improvement</td>
<td>Calunga F.J.L. et al. 2011²⁴</td>
</tr>
<tr>
<td>Portal vein oxygenation in liver cirrhosis</td>
<td>15</td>
<td>12</td>
<td>40 [ 300 ] 12</td>
<td>Improve portal vein oxygenation</td>
<td>Zaky S. et al. 2011²⁴</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>26</td>
<td>8</td>
<td>40 [ 200 ] (20)</td>
<td>Improved prothrombin time, without modify bleeding time</td>
<td>Martínez-Sánchez et al. 2012²⁵</td>
</tr>
</tbody>
</table>

Legend: *treated twice a year during 20 years. †treated every 3 months for one year, ozone volume depend of the age (1 year 15-20 mL; 1-3 years, 20-35 mL; 4-10 years, 40-75 mL; 11-15 years, 75-120 mL). ††treated every 3 months for six months. ND, non-defined.
the best response was obtained in the order; MAH at 8 mg better than MAH at 4 mg better than RIO, at 10 mg.

In a clinical trial in non-diabetic patients with obliterator atherosclerosis, stadium II, (intermittent claudication) there was a significant improvement in comparison to the control group (conventional medical treatment). The improvement was independent of the administration routes (RIO₂, MAH, i.m.)

Probably a correspondence between the dose used in MAH and RIO, will be reached using standard protocol during RIO₂, that minimised the reduction in the real O₂ dose as a result of it reaction of with the luminal content. We should take into consideration that the dose used by MAH is also subjected to modification subject by subject. This happen as a result of the different content of antioxidant levels in serum.

In most of the preclinical assays there were not observed adverse effect during rectal application of ozone. Not even in a study that use 2600 µg (dose 79 mg/kg) repeated in 90 sessions was found any damage associated to the treatment. The in vivo genotoxic effect of O₂/O₃ was studied in leukocytes and exfoliated colorectal cells of rats using the Comet assay (single cell gel electrophoresis assay, SCGE). O₂ final dose 42 mg/kg b.w. (525 µg) was applied during 4 days by RIO₂ simulating human RIO₂. The genotoxic effect of O₂ was measured in exfoliated colorectal cells at 24, 48 and 72 h and in leukocytes at 0, 2, 6, 24, 48 and 72 h after the last exposure to O₂. As a result, a significant increase of the primary DNA damage was observed in exfoliated colorectal cells as well as in the peripheral blood leukocytes. The highest values of DNA damage were observed at 48 h and 24 h after the last exposure to O₂/O₃ mix in exfoliated colorectal cells and in leukocytes respectively. However, after 72 h of the last exposure a significant decrease of DNA damage was observed in both cell types, indicating an evident recovery of the DNA primary damage induced by the treatment.

There are several reactive intermediaries of O₂ that could cause primary DNA damage to leukocytes; some of them are H₂O₂, aldehydes and other inorganic and organic peroxides. These reactive intermediaries have different diffusion rates according their liposolubility and molecular dimensions. Stated that H₂O₂ is the reactive oxygen specie that more easily cross cell membranes. This ability could make H₂O₂ the most probable candidate of the observed early effect of O₂ in lymphocytes. Another group of O₂ intermediaries that could be related to this are long chain aldehydes, such as hexanal, heptanal and nonenal. Their diffusion rates are slower than that of H₂O₂, precisely because they have to overcome the energetic barrier, imposed by their liposolubility, in order to leave membranes and diffuse into the cytosolic environment. Little is known about the diffusion properties of the other O₂ intermediaries (ozonides, liperoxides) in biological systems, but their reactivity and liposolubility might determine a diffusion rate slower. The decrease of Comet lengths 48-72 h O₂ after treatment could be a result of the tissue recovery by cell death, cell turnover and DNA repair. It has been reported that the repair of single DNA strand breaks caused by oxidative damage, occurs in a few minutes, while repair of double DNA strand breaks may take up to 1 h.

When leukocytes and colorectal cells DNA damage is compared, it is observed that DNA damage is higher in leukocytes than in colorectal cells. This unexpected result can be due to the fact that colorectal cells are directly exposed to O₂ and might, therefore, display higher levels of DNA damage. The most probable explanation is that colorectal mucous epithelium, particularly the goblet cells, produces mucin providing a defence mechanism against toxic bio-products of metabolism, pathogenic micro-organisms and xenobiotics.

RIO₂ is used for the treatment of colitis. However, a study that examine the effect of ozonized water (20 µg/mL) enema on normal and inflamed rat colonic mucosa shown that O₂ therapy caused no macroscopic damage. Nevertheless, O₂ therapy induced microscopic colitis, which lasted for at least a week and was accompanied by increase in segmental weight, myeloperoxidase and nitric oxide activity, and prostaglandin E2 generation. In addition, O₂ therapy had no protective effect on inflamed mucosa. This study suggesting that ozone water therapy had a deleterious effect on normal colonic mucosa. In contrast, a study in rabbits, using Ozone 2600 µg (dose 79 mg/kg) repeated in 90 sessions was found that O₂ did not cause adverse effects and did not show significant changes relative to tissue damages and they increased enzymes activities belonging to the first line antioxidant defenses. These results demonstrate that ozone/oxygen mixture administered by rectal insufflation is innocuous and it is able to increase the antioxidant defense of the organism. In addition, most of the long term clinical studied did not found any collateral effects after RIO₂ application.

**Procedure for rectal insufflation of ozone**

RIO₂ is a method of ozone therapy second only to MAH. RIO₂ should be done following right steps in order to guaranty the maximal efficacy of the procedure.
Preparation

Before administering rectal medicine, the door to the room should be closed to assure patient privacy. The patient should be encouraged to empty his or her bladder and bowels before the procedure. After removing lower garments and underwear, the patient should be positioned in bed on his or her left side, with the top knee bent and pulled slightly upward, lifting the upper buttocks will enable visualization of his or her rectal opening. A waterproof pad should be placed under the patient’s hips to protect the bedding, and a sheet should be draped over the patient to cover all of his or her body except the buttocks.

After placing a bedpan within quick access, the nurse should explain the procedure to the patient. This explanation should include the importance of breathing slowly through the mouth to enhance relaxation of the rectal sphincter and to avoid oppositional pressure. The patient should be made aware that there may be an urge to push the medicine out, but that he or she should try to hold it for at least 10 - 15 min after instillation, as most rectal medications need time to be absorbed.

The nurse should wash his or her hands and put on gloves. The foil wrap should be removed from the rectal catheter. External lotions, ointments or creams can be applied directly, using a gloved finger or a 4×4 gauze pad. Prior to administering the tip of the catheter, or applicator should be lubricated with a water-soluble lubricant. To insert a rectal catheter, the lubricated, tapered end of the catheter should be placed at the rectal opening and gently pushed into the rectum. The catheter should be pushed continually toward the umbilicus until the full length of the nurse’s gloved index finger has been inserted into the rectal opening (i.e., about 3 inches, or 7.5 cm, for an adult patient). When inserting a rectal catheter into children, the catheter should be pushed about 1 inch (2.5 cm) beyond the rectal opening, or up to the first knuckle of the nurse’s index finger. When inserting a rectal catheter into infants, the little finger should be inserted one-half inch (1.25 cm) beyond the rectal opening. The buttocks should be released and the finger removed.

Volume and Concentration

A good starting point for most first time users is 100 mL (assuming the concentration is between 10 – 20 µg/mL). Volume and concentration will be adjusted progressively depend of the redox status and the particular pathology of the patient. However, concentration superior to 40 µg/mL and volumes higher than 300 mL are not recommended. The concentration usually chosen for therapeutic effect from rectal insufflation is between 10 – 30 µg/mL. This concentration may be higher (up to 60 µg/mL) for treating bleeding or acute colitis, bacterial, or parasitic infections.

The volume of gas used is extremely important as well. Performing rectal insufflation is somewhat like blowing up a balloon. Too much gas could cause damage to the intestinal tract. Furthermore, increasing, or decreasing the volume of gas used will change the overall dose of ozone. For example, if the concentration used then is 20 µg/mL, and volume of gas 100 mL the dose correspond to 2 mg of O₃. For detail about the recommended dose see the Madrid Declaration on ozone therapy.

If the concentration used causes irritation or discomfort consider lowering the concentration used or discontinuing treatment until irritation subsides. In most case a cycle of 15-20 RIO₃ was practice with a rest time of 3 months, or adjusted depends on the pathology.

In summary, RIO₃ is a valid therapeutic choice in ozone therapy. Pre-clinical and clinical studies demonstrated that using standardized clinical protocols a therapeutic success can be reached.


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Abstracts

May 25-27, 2012 - Shenyang City - China
Analysis of the Effect of different types of lumbar disc herniation treated by medical ozone.

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Abstract:

Objective: To compare the effects of ruptured lumbar disc protrusion and unruptured lumbar disc protrusion treated by ozone, to access its different mechanisms and provide evidences for clinical application.

Methods: To collect 1000 patients diagnosed with Lumbar intervertebral disc lesions from Jan, 2009 to Dec, 2011. Among these patients, male: 562, female: 438, age: 19-75 years old, the median pain time were 22 weeks. According to MRI and DSA in disc implanted by ozone, these lesions were divided into four subtypes: 1. 488 patients (48.8% in total) with anular disruption of non-prominent type, 2. 303 patients (30.3% in total) with anular disruption prominent type, 3. 122 patients (12.2% in total) with fibrous ring of non-ruptured non-prominent type, 4. 87 patients (8.7% in total) with fibrous ring of non-ruptured prominent type. There were no statistical differences in the age, sex and the median pain time. The operative method taken in patients with contralateral supine, ipsilateral midline adjacent to open at the puncture site in 7-9cm, to wear in the disc lesion with the 21G needle, injecting 5-10ml the O2 mixed gas with concentration for 40ug/ml. To retreat needle to the intervertebral foramen and then injected with 40ug/ml concentration of O2, mixed gas 10-15ml and anti-inflammatory analgesic solution (with Debaosong 7mg, 2% lidocaine 5ml and 500ug Vitamin B12). The follow-up time was 1 week, 3 and 6 months after the operation. The clinical effect was evaluated with MacNab; evaluation of the pain relief is according to criterion of ODI, various types of effects using the X2 test.

Result: After 1 week, 3 and 6 months follow-up MacNab group, the efficacy rate were: the anular disruption of non-prominent type: 68.15%, 82.80%, 85.98%; anular disruption prominent type: 65.13%, 79.49%, 81.54%; fibrous ring of non-ruptured non-prominent type: 60.28%, 75.61%, 79.44%; fibrous ring of non-ruptured prominent type: 47.55%, 60.78%, 64.22%. There were obvious differences between anular rupture types and fibrous ring of ruptured types (P<0.05), the evaluation of ODI of lumbar intervertebral disc protrusion before and after the treatment with medical ozone, the follow-up before and 6 months after the treatment (P<0.01), the results are: anular disruption of non-prominent type: 68.37%, 20.47% (85.98%); anular disruption prominent type: 67.25%, 24.74% (81.54%); fibrous ring of non-ruptured non-prominent type: 65.47%, 27.37% (79.44%); fibrous ring of non-ruptured prominent type: 69.78%, 35.65% (64.22%). Six months after the curative effect of follow-up between men and women (P>0.05), fiber ring to the rupture outstanding charm 81.69% of men, 86.15% of women; Fiber ring broken outstanding type 78.72% of men, 82.37% of female. Fibrous ring of non-ruptured non-prominent type 76.28% of men, 80.26% of female; Fibrous ring of non-ruptured prominent type 65.22% of men, 61.46% of female.
Conclusion:

Treatment of disc herniation with medical ozone had curative effect, the anular disruption of non–prominent type had the best efficacy, the result of anular disruption prominent type is good, and the fibrous ring of non–ruptured non–prominent type is better, but fibrous ring of non–ruptured prominent type had had effect. There had no significant difference between male and female. Its mechanism is effective to reduce the aseptic inflammation and edema response of nerve root and spinal dural, relieve clinical symptom, but the effect on reducing disc hernia is limited.

Keywords: ozone treatment, disc disease, anular disruption, fibrous ring of non–ruptured, clinical evaluation

Clinical evaluation of sequential medical ozone therapy for the Primary liver cancer patients after trans–arterial chemoembolization

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ABSTRACT

[Background]

Primary liver cancer including intrahepatic bile duct cells and hepatocellular carcinoma was the most common malignant tumor in the clinic. It's characteristic was the high degree of malignancy, the it can occur at any age, but more common in 40–50 years. The ratio of male to female ratio is 2–5:1. Most already advanced patients when surgical resection is low, and high recurrence rate. Therefore, the majority of hepatocellular carcinoma require effective and non–surgical approaches for treatment.

Interventional treatment has been used as the preferred treatment to Unresectable advanced hepatocellular carcinoma patients. At present, the interventional treatment of primary liver cancer has grown from a single technology and methods into a variety of methods simultaneously, treating the symptoms of a more complete system and integrated treatment. In paper, transcatheter hepatic arterial chemoembolization is one of the most common methods in the interventional treatment of liver cancer. Majority It will occur in patients with liver dysfunction mainly in the decreased serum albumin, serum transaminases and alkaline phosphatase levels of the compensatory mechanisms of the normal liver tissue, who with hepatocellular carcinoma after interventional therapy; a improved, But restored to the preoperative level fier 5–7 days. Some experts believe that part of the liver cancer chemoembolization caused by acute and chronic liver injury is difficult to restore.

Some patients repeatedly involved in the treatment can lead to cirrhosis of liver function damage. Generally in our country patients with hepatocellular carcinoma accompanied by hepatitis and cirrhosis of the liver, The damage is not easy to restore after the interventional treatment, and can even lead to the occurrence of hepatic encephalopathy and liver failure; intervention in patients found in clinical diagnosis and treatment of liver cancer after death from many is not the cancer itself, but due to
depletion of liver function; Due to multiple factors of the tumor, surgery, psychology, quality of life in patients with tumor survival, autoimmunity and regulatory function of the lower; the treatment of patients with liver function failure is limited, there is no effective reversal measures. often too much medication to poor liver itself functions to withstand the pressure of drug metabolism increase, but may further damage the risk of liver function. How to slow liver cancer involved in the damage of liver function, relieve postoperative clinical symptoms, improve patients' quality of life is still an important clinical issue.

In the 1980s, ozone blood therapy has been the beginning as an auxiliary method of treatment of malignant tumors, studies have shown that medical ozone can increase the body's immune function, enhance chemotherapy efficacy and reduce toxicity of chemotherapy drugs in the prevention and treatment of viral hepatitis. Some scholars use the method of blood transfusion and rectal gas injection of medical ozone related clinical and basic research. medical ozone have a positive effect in preventing liver function damage, enhancing and regulating the immune delay liver fibrosis. Based on the medical use of ozone has been a clinical research, the liver disfunction, the organism immune damage, reducing the quality of life, and the decline of many of the problems after the interventions for liver cancer, and explore the place of medical ozone application to intervene in the development of liver cancer after surgery, the treatment for observation of their chemotherapy reduce postoperative pulmonary embolism response, protecting the liver functions, to improve the quality of life in terms of their impact, and prove its ability to intervene in the treatment of liver cancer.

[Objective]

Observed ozone treatment of primary liver cancer interventional postoperative clinical efficacy; explore the advantages and mechanisms of the efficacy of ozone treatment of the disease; provide new ideas for ozone clinical diagnosis and treatment of liver cancer.

[Material and Method]

1. The selected cases were from the South Hospital from May 2011 to October 2011, a total of 40 cases of primary liver cancer patients. 35 males and 5 females. The average age of 54. 18 cases of liver function in patients with child classification of a level, 22 cases of b level; tumor markers (afp) were abnormal; blood had no obvious abnormalities.

2. Experimental groups and treatment processing: 40 cases of liver cancer patients were randomly divided into experimental and control groups, the experimental group after interventional therapy plus medical ozone rectal infusion therapy; the control group only received conventional tumor intervention surgery. Medical ozone prepared through three oxygen generator, medical oxygen experimental ozone concentration of 40μg/ml. Perfusion methods as: medical ozone gas 150ml slowly through the anus into the rectum with thin conductive air hose, not exhausting to retain 10min. Once a day, 5–7 days in a row.

3. Statistical analysis: liver cancer patients met the diagnostic criteria for a total of 40 cases, the use of 1:1 by age, gender balanced random into groups, using the double entry method entered into the database, patients with surgery, postoperative liver function indicators, indicators of tumor markers (afp) patients before and after points of quality of life, physical condition scores were recorded and used for statistical analysis sas 9.1.3. Before and after the measurement data: repeated measure analysis of variance; disorder classified information: the chi–square test; orderly classification data: Wilcoxon test was used; selected test standard of a = 0.05; p <0.05 indicated a significant difference, p> 0.05 indicates no significant difference.
[Result]

Two groups of patients in the group stage, age, gender, disease treatment before former KPS points, no significant differences between the quality of life (P>0.05). According to the evaluation of clinical effect of standards, through repeated measures analysis of variance, physical condition comparison of scoring points, no significant differences between the experimental and control groups (P=0.1478), before and after treatment of a significant difference between two points in time (P<0.0001), there are interactions between two factors (P<0.0001), analysis of the separate effects of the group, Experimental group of KPS integral average rise 7.55 after treatment than before treatment, control group KPS points after treatment than before treatment with an average rise of 0.3, notes treated in KPS group effect on the index than the control group. Experimental group improved physical status score comparison 1 (5%), stability of 19 cases (95%), controlled stability of 20 cases, and there is no improvement and deterioration; the rank sum test, =1,P=0.3173, can't believe that there are differences between the two treatments. Quality of life scores effective 5 cases of clinical experimental group (25%), stable 15 cases (75%), the control group effective 1 (5%), stability of 19 cases (95%), the rank sum test, =3.0588,P=0.0803, no significant differences between the two groups. Life quality scoring integral comparison, experimental group and control group Zhijian differences no significantly sexual (P=0.1499), treatment before and after two time points Zhijian differences has significantly sexual (P<0.0001), two factors Zhijian exists interactive role (P=0.0064), should analysis all group of separate effect, experimental group scoring treatment Hou than treatment Qian average rise 4.6, control group treatment Hou than treatment Qian average rise 1.5, description experimental group in index Shang effect better than control group. AST, ALT, GCT three indicators no significant differences between the control group and the experimental group (P>0.05), and there are differences between two points in time before and after treatment (p <0.05), two factors zhijian exists interactive role (P<0.05), should analysis all group of separate effect, experimental group three a index in treatment hou reduced degree average level are than control group, description experimental group in this three a index shang effect better than control group. TB, and DB, and AFP three a index experimental group and control group zhijian no significantly sexual differences (P>0.05), treatment before and after and two time points Zhijian has differences (P<0.05), two factors zhijian no interactive role (P>0.05), Description the experimental group and the control group in these three indicators are on curative effect and no significant difference between the effect. ALB, ALP, the two indicators no significant differences between the control group and the experimental group (P>0.05), before and after treatment with no significant differences between two points in time (P>0.05), no interaction between the two factors (P>0.05), in the experimental group and the control group the two indicators have no significant effect on.

[Conclusion]

Primary liver cancer after intervention combined with medical ozone treatment can improve patients' physical condition and quality of life in the efficacy score, and be more effective in reducing the content of aspartate and alanine and valley GCT, and protect liver function; This shows that medical ozone combined interventional treatment of primary liver cancer, which can play a sum of synergy, and have a good effect to attenuate efficiency ,protect the liver function and improve the body's quality of life. So further confirmed the usefulness of medical ozone is objective and rational, The treatment is worthy of further clinical application and promotion.

Key words: primary liver cancer; intervention; medical ozone; liver function; quality of life;
Prevention of RI

No reperfusion injury in hibernating mammals
G. Wasser

1) No reperfusion injury in hibernating mammals

2) Why is it so important to talk about reperfusion injury? Main stream medicine did not find any remedy despite intensive research. The amount of damage induced by RI is documented in the fact that we are losing 25% of liver transplants by the radical formation induced by reperfusion of oxygen enriched blood. Another field is the percutaneous coronary intervention. Here we lose 10% of the patients over the next days and another 25% over the time of five years by the same mechanism.  

3) This nice black bear is just emerging out of hibernation. After staying in his den for six to seven month without feeding, urinating, or defecating the animal wakes up and starts life in springtime.  

4) Why are we talking then about hibernation in connection with RI? We do so because the animals do not encounter RI after emergence in springtime. One reason might be the still partially disabled metabolism down to 53% of BMR (Basal Metabolic Rate) during several weeks after emergence.

5) Small animals hibernate interrupted by periods of arousal. These periods which consume 80% of the spent energy during hibernation protect the neuronal system from damage induced by long-term hypo-metabolism. In autumn a falling level of growth hormone level triggers fat storage and a rise of the hormone in the late denning time prepares the animals for spring time.

6) Here we see some data of the physiology of hibernating like duration, reduction of metabolism, body temperature, heart beat rate, oxygen consumption in bears, and the Comparism with small mammals like ground squirrels.

7) Data from 6 hibernating bears transmitting the same metabolic changes. Exception is a pregnant bear exhibiting higher body temperature.

8) Comparism of ECG in summer time and hibernation and reduction of breathing frequency.

9) Hibernation is induced by active inhibition of metabolism. The production of ATP is reduced, the glycolysis is restricted to low levels, and the overall metabolism relays on lipid utilization (b-oxidation). Even other energy consuming processes like gene expression, transcription and translation are also impaired.

10) Damage of the hypothalamic area or ventromedial nucleus in brains of ground squirrels disrupted the cause of hibernation and led to early death after emergence.

11) Body temperature levels below 30°C endanger the neuronal system inducing damage to the neuronal structure and the development of neuronal deficits. This might be the reason why bears do not show signs of arousal as in the case of squirrels with a body temperature minimum of 2.9°C.

12) Here we see a Comparism of the BMR April to October, during hibernation, and after emergence. Very
important is the hampered metabolism after emergence.

13) If we compare the body temperature of bears and small mammals like squirrels we see a dramatic difference. The reason might be the better insulation of bears in comparison with squirrels.

14) If we compare the body temperature with the BMR we see a good relationship. In bears the reduction of body temperature to 30.4°C induces a reduced metabolism to about 25%.

15) In line with these observations is the reduction of oxygen consumption. Despite the higher body temperature of bears the oxygen consumption is in line with the data in small mammals.

16) Now we can compare hibernation and its reduction of BMR with ischemia and reperfusion. After reduction of metabolism close to full cessation in ischemia reperfusion will enhance the respiratory change to full working speed releasing a maximum of radicals. Afterwards it comes to a decline due to the damage of ROS. The 50% is an estimated level.

17) Therefore the proactive, intermediate step of BMR adaption to about 50% is missing in ischemia and reperfusion leading to excessive ROS production and consequently following tissue damage.

18) In long term hibernators the ATP content and PC (Phosphocreatine) content is decreased to low levels. In this time the ATP/PC ratio is greatest with 5/1 picturing the reduced PC production. Simultaneously the glycolysis is reduced in the cardiac muscle by 36%, in skeletal muscle by 3%, and in the liver by 9%. ⁹

19) This foil demonstrates the glycolysis reduction.

20) We know by experiments that ATP locally delivered will reduce the oxygen demand by 25%. With other words, we diminish the production of ROS by a reasonable extend causing less or now damage by RI.

21) In experiments harvested cardiac muscle cells release ATP. Note: this is in experiments under un-physiological conditions.

22) Cells after 10 min of asystoly and harvesting do not release any amount of ATP.

23) This experiment shows that dead cells do not release ATP. In other words, necrotic or death cells contain virtually no ATP. The briefly exposed cells to ischemia or depolarization undergo apoptosis with still functioning complex IV of the respiratory chain producing basic ATP amounts.

24) Investigations into neutrophil migration cannot be performed in the vial since in situ there is an environment containing ATP at an average level of 1.0 mmo–1 blood.

25) Isolated rabbit hearts perfused with oxygen enriched Ringer solution will consume 25% less oxygen after addition of nmolar ATP levels. ⁹

26) This is not only the case in hearts under experimental (i.e., outside the body) conditions with no nervous input, steady temperature etc., but even in experiments with human volunteers. But precaution: in rats the ATPase activity of the endothelium is four fold higher than in human endothelium.

27) Nmolar ATP infused into the coronary system will not change the heart beat rate, the cardiac output, and the wedge pressure. But this procedure will increase the coronary blood flow to more than 160% and will reduce the oxygen demand and consumption by minus 27%. ⁹

28) If we calculate the absolute amount of oxygen consumption in hibernation and ischemia we might come to the following amounts.
29) In animal trials the ATP content in the MCA cortex area and Penumbra exhibited a totally different picture. 7

30) We have to talk about ATP under physiological conditions. Normally most of the ATP in plasma originates out of RBCs and not out of the endothelium or other blood cells. Extracellular administered ATP enhances ATP pools in liver tissue and RBCs. 8

31) The mean value of ATP in peripheral blood is 1.0 mmol*ml^-1 blood. Two valuable publications are listed on this page. 9

32) In case of induction of ischemia ATP is readily released into the coronary system causing expansion of the vessels. This ATP escapes outward directed the ATPases of cellular membranes.

33) The ATP release of hypoxic and oxygenated cells shows this foil.

34) If we have a look on other effects of ATP delivery we have to take into account the thrombus generation after ischemia. The main players are the endothelia cells in influencing platelets. They show a very different pattern of ATP and AMP degradation in contrast to smooth muscle cells.

35) Endothelial cells produce adenosine after lag time, but metabolize ATP fast and extracellular adenosine is taken up readily by RBCs. Smooth muscle cells produce adenosine rapidly and consume it slowly.

36) If we look at other cells: Lymphocytes release ATP and nucleotides in order to regulate the immune system. High ATP concentrations stimulate in vivo DNA synthesis in bone marrow and in thymocytes, and they inhibit the synthesis in spleen, lymph nodes, and peripheral blood lymphocytes. Note: Ecto-ATPase is to be distinguished from Na^+ and K^+ -stimulated ATPase residing in the plasma membrane, too. 10

37) Interestingly in chronic lymphatic leukemia ATP opens cation channels increasing the Na^+ influx by 12 fold in bone marrow and by 3, 5 fold in the periphery.

38) All B-cells express ecto-5'-NT. This enzyme breaks AMP to P, and adenosine. The B-cell lymphoma cells do not exhibit this enzyme. They are prone to ATPase induced cellular stress and death by overload of Na^+ ions after ATP delivery.

39) Here we see the overload of Na^+ ions inducing depolarization and death.

40) In contrast RBCs in rats and humans (unpublished) do not degrade ATP.

41) The only explanation for the inability of red blood cells to degrade ATP is that there is a lack of ecto-ATPases and ecto-5'-nucleosidases.

42) RBCs do not degrade ATP by a good reason: by their sheer numbers the degradation of ATP would erase the signal function of shrinking ATP levels (climbing adenosine levels) as the indicator for disturbance (inflammation).

43) Leukocytes detect lack of ATP if the receptors are taken by adenosine. This adenosine is not necessary the one originating out of ATP released by themselves. As long as there is the normal range of ATP they travel unexcited.

44) This picture explaining the activation of neutrophils has a weak point. It does not take into account that there is always ATP in the serum (1.0 mmol/1 blood) and inflammation initiates higher amounts of adenosine. Besides that, RBCs secrete ATP, too. 11

45) Corrected information. In the graph before there was no ATP located in serum.

46) Even in a publication as late as 2006 the existence of an ATP transporter was denied. Citation: 'since ATP is a polar molecule it cannot pass the lipid bilayer of the cell membrane'.

58
47) A short description of the ATP transporter. RBCs lack the 5'-Nucleosidase.

48) Another look on the ATP transporter. 13

49) It is therefore prudent to assume that RBCs exhibit ATP transporters while lacking ecto-5'-nucleosidases.

50) Not only mammalian cells secrete ATP into the extracellular space but cells behave in a similar way. Diminishing the extracellular ATP content rendered the plants to death by fumonisin B1 (FB1). 16

51) In human lymphoblasts the activity of ecto-5'NT is lower than that of ecto-ATPases or ecto-ADPases. T cells in contrast to B cells exhibit nor or only low ability to degrade ATP. As mentioned before human and rat red blood cells are not able to degrade extracellular ATP.

52) ATP is degraded by peripheral white blood cells to AMP accumulating in the extracellular space. Inosine and hypoxanthine are formed in the intracellular department.

53) Leukocytes detect lack of ATP if the receptors are occupied by adenosine. This adenosine is not necessary the one originating out of ATP released by themselves.

54) Extracellular ATP inhibits neutrophil-mediated cytotoxicity, similar to ADP and AMP. Intracellular degradation of ATP occurs mainly through deamination producing no adenosine. Extracellular degradation in contrast follows AMP dephosphorylation building up reasonable amounts of adenosine.

55) In the case of platelets ADP is pro-aggregatory and makes the platelet aggregation autocatalytic. ATP enhances platelet cAMP content and opposes platelet aggregation.

56) Adenosine is cleared from the bloodstream by white blood cells and is simultaneously taken up and metabolized to inosine and hypoxanthine. In contrast to this behavior RBCs take adenosine up and produce ATP.

57) Ozone therapy mimics the emergence in the end of hibernation and the time after. ROS development is hampered and damage from radicals prevented.

58) Difference between hibernation and hypothermia: in hibernation is the thermo regulation down sized (respiratory chain running low level), lower temperature reached by conductance. The central down regulation prevents reactive shivering. In hypothermia the down regulation of the temperature is reached by cooling in ice water or by external cooling device. Shivering is suppressed by pharmaceuticals.

59) In short: hibernation shows down sized metabolism, hypothermia down sized temperature. In hibernation after emergence slowly up regulated metabolism, low oxygen consumption, diminished ROS formation. In hypothermia we find downsized temperature. Warming induces full oxygen demand and ROS production.

60) Under ozone treatment the dangerous period of maximal oxygen demand is bypassed allowing reduction of inflammation and recovery.

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Ozone treatment for post-operative cerebral edema

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The ozone has been used in medicine for hundreds of years, in recent years, emphasis and attention has been focused on the use of medical ozone in neurosurgery, especially for the treatment of cerebral edema. Now, 12000 cases has received ozone treatment with significant improvement of cerebral edema in our department since August, 2010. Herein, some cases have been proposed as the following.

1. Treatment for cerebral edema after resection of hemisphere tumor

Zhang xx, male, 56-year-old, ID: 505471, was admitted to us on July 12, 2010. He was diagnosed as left thalamencephal glioma. Surgical removal of tumor was performed on July 14, then he received ozone treatment since July 16, twice per day. Repeated CT showed the significant improvement of the edema, without any shift of midline. The following course was uneventful, he was on the mannitol routinely, twice per day.

2. Occlusion of middle cerebral artery

Feng xx, male, 74-year-old, ID: 410260, he suffered from an occlusion of left middle cerebral artery and was admitted on July 12, 2010. Ozone treatment started on July 17, twice per day, edema was significantly improved as demonstrated by CT scan on July 21. Mannitol treatment did not continued in the following course as a result of improved states.

3. Treatment for cerebral infarction after MCA aneurysms surgery

Guo xx, ID: 504316, male, 38-year-old, on July 23, 2010, was hospitalized with sudden headache for one day. He lost consciousness with dilated pupilla, diagnosed as left temple hemorrhage, cerebral hernia as a result of rupture of aneurysms. Surgery of clipping aneurysms and hemorrhage removal was immediately carried out. However, the cerebral infarction occurred on 4 days after operation, the bone was removal to decompress the brain on July 23, 2010. The ozone treatment was following on July 16 (one, per day). CT showed significant improvement of the cerebral edema. Following courses was stable, he fully recovered from illness when he was discharged.

Discussion

Ozone, also known as activated oxygen or trivalent oxygen, is an extremely reactive oxidizing agent. Ozone facilitates an increase in the partial oxygen pressure of arterial blood through the oxygen saturation of both plasma and erythrocyte hemoglobin, which can induce a decrease in both general and local tissue hypoxia, activation of gas metabolism in the zone of ischemia. Ozone is able to stimulate glucose metabolism in the erythrocytes, formation of 2,3-diphosphoglycerate – substance contributing to the more complete oxygen release by oxyhemoglobin and shift of oxyhemoglobin dissociation curve to the right. Thus, more oxygen is released to the tissues, particularly affected by ischemia. Owing to the interaction with corpuscle membrane lipids, ozone increases the deformability of the erythrocytes and decreases their aggregation and thus improves fluidity of blood in micro flow bed. Ozone can decrease platelet aggregation and activat of fibrinolysis, ozone is able to prevent thrombosis at the areas of decelerated blood
flow and facilitates the lysis of produced small thromboses thus improving the rheological properties of blood. Ozone can facilitate a decrease in arteriolar spasm and opening of non–functional capillaries thus activating microcirculation in the ischemized tissues.

Vasogenic edema occurs after brain operation which is the most common type of brain edema, it is characterized by the influx of fluid and solutes into the brain through an incompetent blood–brain–barrier (BBB), which has been demonstrated by the increased EB content in the animal test.

Pathophysiology of cerebral edema is complex. Damaged cells swell, injured blood vessels leak. Cellular and blood vessel damage follows activation of an injury cascade. The cascade begins with glutamate release into the extracellular space. Calcium and sodium entry channels on cell membranes are opened by glutamate stimulation. Finally, hypoxia depletes the cells' energy stores and decrease ATPase activity and reducing the deformability of the erythrocytes, increase the risk of arteriolar spasm, resulting in the microcirculation disturbance, these injury cascade can be target of Ozone as mentioned above, it is also proved to be effective by our clinical date. Our date has suggested that ET play a key role in the pathophysiology of cerebral edema. However, the relationship of Ozone and ET are still not well understand, it will be explored in our further study.
Clinical function scores and motor evoked potentials of patients with acute cerebral infarction before and after major ozonated autohemotherapy: A control study

The general military hospital of Guangzhou Wu Xiaona Peng Kairun

Abstract

Objective: To explore the effect of major ozonated autohemotherapy (MOAH) on the clinical motor function recovery of patients with acute cerebral infarction (ACI) according to U.S. National Institutes of Health Stroke Score (NIHSS), Modified Rankin Scale (mRS), transcranial magnetic stimulation motor evoked potential (MEP). Methods: Eighty-six patients with ACI were randomized into two equal groups (n=43). All the patients in the two groups were administered 450mg XueShuantong injection (a kind of traditional Chinese patent medicine, The main component is arasaponin.) and 0.1g Aspirin, or 50–75mg Clopidogrel bisulfate tablets, once every night. Meanwhile, in ozone group, the patients were treated with MOAH, once a day and (10 ± 3) days as a course. Recovery of neurological function was evaluated by NIHSS, mRS Index, MEP through transcranial magnetic motor stimulation. Results: Before treatment, the NIHSS, mRS index and the cortical potential amplitude of MEP extraction rate, CMCT and amplitude had no significant difference between the two groups (P>0.05), but after treatment, all were significantly different from that before the treatments (P<0.05). The total effective rate in ozone group was higher than that in control group (P<0.05). The cortical potential raised rate (70.7%) of upper limbs in ozone group was higher than that in control group(68.3%) (P<0.05), but the MEP amplitudes of both upper and lower limbs were not higher than that in control group (P>0.05). The CMCT of upper limbs in experimental group (8.0 ± 2.44) ms shortened obviously than that in control group (9.1 ± 2.77) ms (P<0.05). The MEP amplitudes of upper limbs [3.30(0.75,4.79)] mv in experimental group was significantly higher than upper MEP amplitude in the control group [2.40 (0.64,4.09)] mv (P<0.05). NIHSS was positive to CMCT (r = 0.782) (P<0.05) and MEP amplitudes of the upper limbs (r = 0.847) (P<0.05). Conclusion: MOAH has certain positive effect on the recovery of motor function of patients with ACI, and the MEP index including CMCT and amplitude of upper limbs could efficiently evaluate the motor function.

Key words: Major Ozonated Autohemotherapy, Acute Cerebral Infarction, Motion Evoked Potential, NIHSS, mRS
Observation on Analgesic Effect of A Rotary Probe (Decompressor System) Combined with Ozone injection on Lumbar Intervertebral Disc Protrusion

ABSTRACT

Objective: To explore the clinical effect and the analgesic effect of a rotary probe (decompressor system) combined with Ozone injection on Lumbar Intervertebral Disc Protrusion. Methods 60 cases of Lumbar Intervertebral Disc Protrusion were divided randomly into a treatment group (group A) and a control group (group B) with random number table, 30 cases in each group. The study involved 32 males and 28 females with an average age of 48.7 years (range, 34 to 62 years). After treatment, the visual analogue scale (VAS) was adopted to examine and compare the main pain score, and the clinical were compared between two groups from the symptoms and signs in accordance with efficacy criteria. Result There were significant differences in the main pain score between the two groups (P < 0.05), and there were significant differences between the two groups in the therapeutic (P < 0.01). Conclusion A rotary probe (decompressor system) combined with Ozone injection is a convenient and highly effective therapy for Lumbar Intervertebral Disc Protrusion, and this method can effectively improve the pain of the patients, so it should be to promote the use of clinical.

KEY WORDS Percutaneous, automated percutaneous lumbar diskeectomy; Lumbar Intervertebral Disc Protrusion; Ozone injection;

Clinical study of ozone injection combined with manual release by extradural anesthesia for lumbar disc herniation. CHEN Dong-yu, LIU Zhi-hui, SHI Ying, WANG Xiang, ZHANG Ming-cai, CHEN Yuan-chuan, ZHAN Hong-sheng Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai 200021, China

Abstract Objective To observe the curative effect of ozone injection combined with manual release by extradural anesthesia for lumbar disc herniation. Methods A total of 112 patients with the pain of lumbar and lower limbs, Lasègue sign positive, and confirm by CT or MRI scan that lumbar disc herniation. All the patients were treated with percutaneous oxygen-ozone injection into lumbar intervertebral disc and around the nerve roots under X-ray guidance, and then manual release the conglutination of nerve roots by extradural anesthesia. Results 112 cases were invalid on follow-up from 1 through 10 months, the pain and about 105 cases (94.12%) have improve in the pain and dysfunction. All of patients were all successfully treated without any complications. Conclusion The ozone injection combined with manual release by extradural anesthesia for lumbar disc herniation is a safe, minimally invasive and effective method for these patients with pain of lumbar and lower limbs and dysfunction.

Key Words: Ozone injection; Lumbar Disk Herniation, manual release, extradural anesthesia
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