

Review Article

Could Ozone Be Used as a Feasible Future Treatment in Osteoarthritis of the Knee?

Marcos Edgar Fernandez-Cuadros[1,2], Olga Susana Perez-Moro[1] and Jose Antonio Mirón-Canelo[3]

[1]Rehabilitation Department, Santa Cristina's University Hospital, Madrid, Spain.

[2]Rehabilitation Department, Santisima Trinidad's Foundation Hospital, Salamanca, Spain.

[3]Department of Preventive Medicine and Public Health, Salamanca's University, Salamanca, Spain.

ABSTRACT

Osteoarthritis (OA) is one of the most disabling and incapacitating diseases on the autonomy of older people, affecting their quality of life. OA produces great impact on pain, function and use of resources, being considered as an important problem of Public Health. OA is a degenerative and progressive disease without treatment nowadays. The goals in OA treatment are to ameliorate symptoms and diminish articular damage. In knee OA, there is destruction of cartilage and subchondral bone, with the consequent narrowing of articular space. Knee OA is multifactorial. Besides the bio mechanic factors, trauma and obesity; it is believed that inflammation plays an important role. Future treatments should act on the regulation of inflammation to diminish progression of OA. There is evidence on several studies and years of experience that state that Ozone acts on the modulation of inflammation. The objective of this paper is to review the main pathophysiological mechanisms involved in knee OA, and to postulate ozone as a promising and ideal conservative treatment, since it could act on several therapeutic targets, besides inflammation.

Introduction

Osteoarthritis (OA) is one of the most disabling and incapacitating diseases on the autonomy of old people, affecting more than one third of the population over 70 years in Spain (Moreno, 2009). OA is the most common arthropathy to an extent that 70% of people over 50 years have radiological signs in some articulation. For that reason, OA becomes an important Public Health problem in old populations, like in Spain (Espallargues, 1996).

OA produces a great impact in suffering, in impaired function and in the use of limited health resources. This fact ameliorates the quality of life in OA patients on their physical, emotional or social aspects (Fernández-Cuadros, 2013).

The normal knee is composed by cartilage, subchondral bone, synovial tissue and articular capsule. In OA of the knee, there is destruction of articular cartilage, narrowing of articular space, sclerosis of subchondral bone, osteophyte formation and subchondral cysts (Batlle 2002; Mishra 2011).

Nowadays, there is no cure for OA. The main goals are to ameliorate the symptoms of the disease such as pain, rigidity, inflammation and to diminish articular damage and joint destruction (Batlle-Gualda 2002; Vaillant, 2013).

OA is related to chronic inflammation (Batlle-Gualda 2002; Valliant 2013). Chronic oxidative damage is involved in the changes and development of knee OA (Borreli 2015). Chronic oxidative stress plays an important role in knee OA, so the future success will be the suppression of oxidative damage without disruption of the antioxidant defense network (Atlas 2005; Weinstein 2013; Borreli 2015).

It is of therapeutic value to act over the modulation and regulation of inflammation to diminish the progression in OA. Besides, the future advances in OA treatment should try to ameliorate the destruction of cartilage and to enhance the cartilage repair. The main goal would be to act over a great number of proinflammatory cytokines located on the affected articulation (Battle 2002).

Several studies and four decades of clinical experience demonstrate that Ozone therapy has proven effects over the modulation of inflammation (Mawsouf 2010).Ozone is a highly reactive molecule that when injected into a joint capsule, it is able to stimulate the fibroblastic joint repairing abilities. It may reduce inflammation and promote new cartilage growth. Ozone is so reactive that it never fails to initiate this reactive activity (Dogan 2014). Different clinical studies support the efficacy of ozone in the reduction of pain and inflammation in OA, recovering mobility (Borrelli 2015).

The objective of this paper is to review the main pathophysiological mechanisms involved in knee OA, and to postulate ozone as a promising and ideal conservative treatment, since it could act on several therapeutic targets.

Osteoarthritis Of The Knee

OA is a degenerative disease that produces pain, diminishes function and affects personal, familiar, social and labor activities. OA has great morbidity and low mortality which makes it a chronic disease especially in older occidental societies (Battle 2002).

The evolution of knee OA is slow and progressive (Battle 2002; Mishra 2011). Once established, OA stabilizes clinical and radiological. Although symptoms can diminish, radiological signs can rarely get better (Dougados, 1992). The radiological worsening occurs in 33-66% of patients. Episodes of pain lasting from days to weeks are a common clinical presentation.

If pain persists for months, collapse of subchondral bone must be suspected. An acute onset of pain could indicate necrosis of femoral bone (Dougados 1992).

OA affects mainly middle aged people. In the earlier age group both sexes are equally affected but later on (50 years and older) females are mainly affected. Obesity, family history, high body mass index (BMI) and repeated trauma are the susceptible precipitating factors to develop OA, but not the only (Mishra 2011). In that context, OA is not only a mechanical problem, but a chronic inflammatory process with cell and biochemical alterations, that justify pharmacological and ozone therapy (De Lucas 2005; Samper-Bernal 2013; Vaillant 2013; Borrelli 2015).

In knee OA the main pathological changes are progressive loss of cartilage, meniscus and capsule of the joint (Mishra 2011). Structural changes on knee OA are visible on standard X-ray and include narrowing of the joint space (due to cartilage loss), formation of osteophytes at the joint margins, and bony sclerosis (increased density or thickness of the bone) just underneath the articular cartilage (Goldring 2007). (**Figure 1)**

The cytokines released by chondrocytes implicated in the catabolic process of the extra cellular cartilage matrix are: IL1, IL6, IL8, IL17, LIF, TNF-α, IFN-γ. All of these cytokines produce cartilage destruction (Battle 2002). The proteolytic degradation of extra cellular matrix constitutes an important mechanism of articular degradation. In such a case, the future therapy in OA should produce an inhibition of proteolytic enzymes (MMP or mineral metalloproteinases), nitric oxide synthesis (NOs), proinflammatory cytokines (IL1, IL6, TNF-α), and apoptosis through caspases. On the other hand, OA treatment should favour anti-inflammatory cytokines (IL4, IL10, IL13), and stimulate growth factors (TGF-β, IGF-1) (Battle 2002).

In OA there is augmentation of free radicals of Oxygen. They inhibit collagen and proteoglycans, accelerating the cartilage matrix disintegration, and narrowing the joint space (Yu 2010). IL-1, which depends of the production of ROS (Reactive Oxygen Species), is implicated in the DNA damage to chondrocytes (Davies 2008).

The OA surgical cell therapy goal is to transplant chondrocytes and stem cells in order to recover vascularisation and trophism of bone and cartilage (Battle 2002).

The last option for advanced knee OA is the surgical treatment, in order to replace the damaged articulation. Knee prosthesis is an expensive and invasive surgical procedure not exempt of side effects and complications (Fernández-Cuadros 2013, Borreli 2015).

Ozone Therapy

Ozone is the third strongest oxidant agent, after fluorine and persulphate, a fact that explains its high reactivity (Bocci 2011).

Ozone is the allotropic or unstable form of oxygen (Iliakis 2001, Paolini 2009, Seyman 2012). It is used to treat several infectious, autoimmune, degenerative and orthopaedic diseases (Mawsouf 2011). It is postulated that Ozone has analgesic, anti-inflammatory, immunomodulatory and trophic properties (Paoloni 2009).

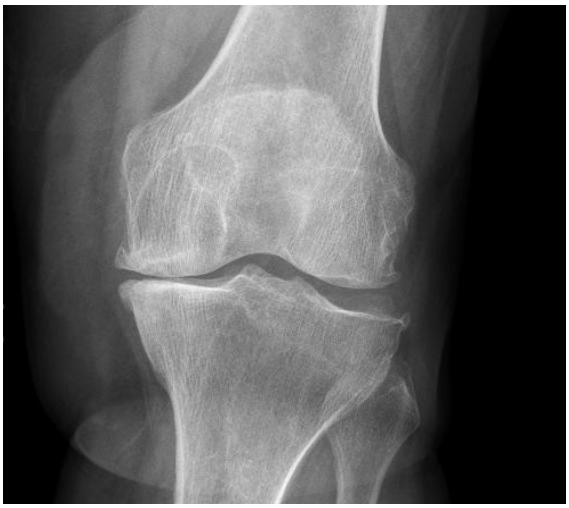

Figure 1: Severe Knee OA and radiological changes.
In this left knee radiography, there is destruction of articular cartilage, narrowing of articular space, sclerosis of subchondral bone, peripheral osteophyte formation and subchondral cysts.

Ozone decomposes spontaneously and therefore it is hardly storable. The ozone concentration is halved at 30ºC within 25 minutes, at 20º within 40 minutes and at minus 50ºC within 3 months (Bocci 2011). Ozone decomposes rapidly, splitting in O_2 and O^- (monatomic O) which is very reactive, and it has anti infectious, antiviral, anti-parasitic, antifungal and antitoxic properties (Madrigal 2007). The Ozone disintegration releases 24,27kcal producing an important peripheral vasodilator effect (Madrigal 2007).

Ozone is not a homeopathic drug. On the contrary, ozone properties have a doses/effect ratio (Bocci 2006; Bocci 2011). Most medical generators deliver ozone concentrations from 1 to up to 70-100ug/ml **(Figure 2)**. The total ozone dose is equivalent to the gas volume (ml) multiplied by the ozone concentration (ug/ml). For different medical applications, the optimal ozone doses must be known (Bocci 2011). However, the therapeutic window is between 10-80 ug/ml (Madrigal 2007).

Ozone dissolved in the plasmatic water reacts immediately with a number of biomolecules and disappears. There are two compounds (reactive oxygen species or ROS and lipid oxidative products or LOPS) which represent the "ozone messengers" and are responsible for the biological and therapeutic effects. ROS are produced immediately, in the early phase (mainly Hydrogen peroxide or H_2O_2) and are responsible for the early biological effects on blood (erythrocytes, leucocytes, platelets). On the contrary, LOS, which are simultaneously produced have a far longer half-life, they reach the vascular system and interact with several organs, where they trigger late effects. Some of these real targets are liver (in chronic hepatitis), vascular system (in vasculopathies), while other organs are probably involved in restoring normal homeostasis (central nervous system, gastrointestinal tract, mucosal associated lymphoid tissue) (Bocci 2006, Mawsouf 2010, Bocci 2011).

The biological effects of Ozone elicited during exposure to plasma are due to the formation of ROS and LOPs (Ozone

Table 1: Effect of Ozone on target organs and functional modifications (Bocci 2011).

Subtrates	Messengers	Target organs	Functional modificactions
Ozone in plasma	ROS	• Erythrocytes • Leucocytes • Platelets	Improved O_2 delivery. Inmune activation. Release of autacoids and growth factors.
		• Endotelium	Increased reléase of NO^-, generetaion of supergifted erythrocytes. Release of stem cells.
	LOPs	• Bone marrow • Other organs	Upregulation of Oxidative Shock Proteins and antioxidant enzymes (Ozone tolerance).

Figure 2: Ozonotherapy equipment (Ozonosan ® alpha-plus). Dose of 20 µg/ml.

messengers). ROS improves O_2 delivery in erythrocytes. They stimulate leucocytes and immune activation. On platelets Ozone favours the release of autacoids (local hormones) and growth factors. LOPs increase the release of NO^- on the endothelium. They favour the generation of super gifted erythrocytes and release of stem cells on the bone marrow. Finally, LOPs acts over other organs, activating the upregulation of Oxidative Shock Proteins (OSP) and antioxidant enzymes (Superoxide dismutase, catalase and glutathione-peroxidase). (**Table 1)** (Bocci 2006; Bocci 2011).

The antioxidant system has evolved during the last two billion years as an essential defense against Oxygen. Antioxidant system is made up of scavenger components: albumin, Vitamins C and E, uric acid, bilirrubin, cysteine, ubiquinol, alpha lipoic acids and intracellular antioxidants (superoxide dismutase, catalase and glutathione peroxidase), protein (ceruloplasmin, which is able to chelate free iron and copper that otherwise, could favour the formation of hydroxyl radicals). In this context, a small amount of Ozone exposed to blood can undergo a transitory oxidative stress absolutely necessary to activate several biological systems and functions without detrimental or deleterious effects (Bocci 2011).

The purpose of Ozone is to produce acute oxidative stress. This stress must be adequate (otherwise it is a placebo), calculated (neither below nor too much above threshold levels) and transitory (not chronic oxidative stress). It is of paramount importance neither to override the antioxidant system nor to cause any toxicity. The upregulation of the antioxidant system is followed by repeated but small oxidative shocks (Bocci, 2011) **(Table 2).**

Ozone is an effective modulating agent for biological oxidative stress. It stabilizes pro-oxidant and antioxidant systems and also inflammation (Madrigal 2007).

The following situations preclude or limit the use of ozone: a) significant deficit of glucose-6-phosphate dehydrogenase (favism is an haemolytic disease in people lacking that enzyme); b) pregnancy in the early phase (to exclude any mutagenic risk, although unlikely); c) patients treated with angiotensin converting enzyme inhibitors; d) hyperthyroidism, thrombocytopenia, serious cardiovascular instability; and e) allergy to Ozone (Bocci 2011). Except for these situations, Ozone is very safe and it has no side effects, then no formal contraindication is stated by most researchers (Madrigal 2007).

There are several pathologies such as arteriosclerosis, diabetes, ischemia, osteoarthritis, neurodegeneration, nephropathies, chronic viral infections, autoimmune diseases and cancer; where there is an imbalance between oxidants and antioxidants, leading to chronic oxidative burden and death (Bocci 2006, Bocci 2011).

Ozone is a wonder drug because it can produce four extraordinary phenomena: 1) the induction of oxidative shock proteins (OSP); 2) the upregulation of antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase); 3) the reduction and/or normalization of oxidative stress; and 4) the probable release of bone marrow stem cells (Bocci 2006, Madrigal 2007, Bocci 2011).

Ozone mobilizes bone marrow stem cells (BMSC) and produces LOPs, which induce NO synthase (NOs). This produces inhibition of platelet-leukocyte aggregation. Besides, NO produces neovascularisation and neoangiogenesis. NO activates MMP-9 (matrix metalloproteinase 9) indispensable for stem cell mobilization (Bocci 2006, Bocci 2011).

As a resume, Ozone is a molecule able to act simultaneously on several blood components. Ozone produces messengers such as ROS and LOPs; they can act locally or systemically in practically all cells of an organism. Four decades of clinical

Table 2: Objective of Ozone (Bocci 2011).

Ozone produces	**Acute oxidative stress**	Adequate (not placebo) Calculated (neither below nor above threshold) Transitory (not chronic oxidative stress)

experience have shown that ozone can act as a disinfectant, an oxygen donor, an immunomodulatory, a paradoxical inducer of antioxidant enzymes (catalase, glutathione peroxidase, superoxide dismutase), a metabolic enhancer, an inducer of endothelial NO syntheses, and possibly an activator of stem cells with consequent neovascularisation and tissue reconstruction (Bocci 2006, Bocci 2011).

Ozone In Orthopedic Diseases

The first author who had the idea to inject small volumes of ozone in patients affected by tendinitis and myofascial pain was Dr. Alexander Balkany in Zurich in the 1970s. After him Riva Sanseverino (1989), Verga (1989), Siemsen (1995) have begun to treat acute and chronic polyarthritis (OA of the knee, hip, interphalangeal joints, sacroiliac joints, etc.), epicondylitis and carpal tunnel syndrome with intra articular or periarticular insufflation of small volumes of O_2-O_3 or Oxygen-Ozone (5-10 ml in one or three sites with an ozone concentration from 5 to 15ug/ml) with very encouraging results. In Morton's disease (neuroma), up to six infiltrations of gas (4ml each at 20ug/ml) have yielded great pain relief. In a very informative review, Siemsen (1995) reported that application of medical ozone in acute and chronic painful joint diseases is a complementary method of treatment to obtain rapid pain relief, decongestion, disappear of edema, reduction of local temperature and increase mobility. If performed by an expert orthopaedic surgeon, the treatment is not risky and causes only transitory local pain that disappears in 5-10 minutes without any other adverse effect (Siemsen 1995).

The pathophysiology of OA diseases is complex and characterized by the softening and even destruction of articular cartilage, with increased matrix degradation due to collagenases and proteoglycanases. The enzymes may be secreted by activated chondrocytes and monocytes, which by releasing IL-1 and TNF-α amplify the inflammation. Synthesis of PGs increases several fold and there is a natural attempt to maintain a biomechanical adequate matrix. In contrast to Rheumatic Arthritis, pannus does not develop. Joint pain may be aggravated by concomitant synovitis (Bocci 2011).

Drug therapy for OA is symptomatic, aiming to reduce pain and disability. Inhibitors of cyclooxygenase I (COX I) are in wide use, with some side effects, and are being substituted with inhibitors of cyclooxygenase II (COX II), but less successfully. Local injection of glucocorticoids into a given joint can be carried out no more than twice per year (Bocci 2011).

Conventional medicine does not have a cure for OA. Patients search for complementary therapies. By now, innumerable patients have been treated with ozone, and they have experienced great pain relief for a long time. It is surprising that ozone, a potent oxidant, injected into the synovial space does not elicit further inflammation nor degeneration, but it is believed to produce just the opposite paradoxical effect (Bocci, 2011). It is believed that Ozone injected on the synovial fluid generates ROS and LOPs, which are responsible for the positive effects of Ozone therapy: a) possible inactivation-inhibition of release of proteolytic enzymes/proinflammatory cytokines; b) stimulation of proliferation of chondrocytes (probably via H_2O_2-ROS) and fibroblasts, with increased synthesis of matrix and possibly of articular cartilage. The induction of the synthesis of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) are a crucial event in the adaptive response to chronic oxidant state to Ozone. That is the reason why Ozone must be injected at low doses at the beginning; c) probable inhibition of release of bradykinin/synthesis of inflammatory PGs with reabsorption of edema and pain relief; d) an increased release of IL-1 soluble receptor or of some other soluble receptors and antagonists able to neutralize proinflammatory cytokines such as IL-1, IL-8, IL-12, IL-15 and TNF-α; e) conversely, the release of immunosuppressive cytokines (TGF-β1 and IL-10) may inhibit inflammation (Iliakis 2001, Paolini 2009). Among several growth factors, TGF-β1 is interesting because it modulates the expression of integrins and stimulates the synthesis of matrix proteins such as collagen and glycosaminoglycan's (Trippel 1995; Qi and Scully 1997; Grimand 2002, Jani 2012).

In 1988, Verga noted pain relief after infiltrating trigger points in myalgia with O_2-O_3 and proposed to use an indirect technique by injecting the gas into localizable points in the paravertebral muscle (locus dolente) corresponding to the metamere of the herniated disc. Now this is called the indirect approach or chemical acupuncture (named by Bocci since 1988) to clarify that the beneficial results is obtained via the chemical reaction to Ozone (Bocci 2011).

It is hypothesized that the formation of Ozone messengers ROS and LOPs would act in the synovial fluids in two phases. In the first phase Ozone would inhibit inflammation diminishing proinflammatory cytokines (like PGE_2 and leukotrienes via decreasing phospholipase A_2, COX I and COX II inhibition) diminishing kallikrein and bradykinin. It could inhibit the release of serotonin and MPs or metalloproteinases such as collagenase, gelatinase and aggrecanase, avoiding cartilage degradation (Bocci 2011, Buric 2014). On the late phase, Ozone would act over the upregulation of antioxidant enzymes, OSP (HO-1) inhibitory cytokines (IL-4, IL-10, TGF-β), neoangiogenesis, NO syntheses and release of endorphins, ACTH and cortisol. All of these would act in the reparative process in the articular joint by stimulating chondrocytes, fibroblasts and stem cells to synthesize proteoglycans, glycosaminoglycans and collagen (Bocci 2011).

At this moment there are several clinical studies on the efficacy of Ozone in the reduction of pain and inflammation in knee OA or disc herniation (Borreli 2015). In our public hospital (Santa Cristina's University Hospital) we have been treating more than 200 patients with chondromalacia and knee OA in 3 years of follow-up with outstanding results on pain, rigidity,

Table 3: Ozone therapeutic targets in knee OA.

Cytokine/cell Target	Ozone effect	Article Review
MMPs inhibition	↑	Bocci 2006, Bocci 2011, Buric 2014
NO synthesis	↑	Bocci 2006, Bocci 2011, Borrelli 2015
PGE_2 inhibition	↑	Borrelli 2015
Inhibition of proinflammatory cytokines:		
• IL-1	↑	Bocci 1993, Iliakis 2001, Chang 2005, Jani 2012
• TNF-α	↑	Larini 2001, Madrigal 2007, Borrelli 2015, Chang 2005
• IFN-γ, IFN-β	↑	Bocci y Paulesu 1990, Larini 2001, Bocci 2006, Madrigal 2007, Bocci 2011, Jani 2012, Borrelli 2015
To stimulate anti-inflammatory cytokines:		
• IL4, IL6, IL10, IL13	↑	Bocci 1993, Iliakis 2001, Larini 2001, Chang 2005, Madrigal 2007, Borrelli 2015
Growth Factors:		
• TGF-β, IGF-1	↑	Paulesu 1991, Bocci 1993, Trippel 1995, Qi 1997, Larini 2001, Grimand 2002, Bocci 2005, Madrigal 2007, Fuccio 2009, Bocci 2011, Jani 2012, Borrelli 2015
Cell therapy:		
• Chondrocytes, stem cells	↑	Bocci 2006, Bocci 2011

functioning (measured by WOMAC scale) and recovering of articular joint space (measured by radiographs), and our results will be published soon.

The explanation for that efficacy is that O_2-O_3 acts on several cytokines which are lately responsible for the degradation and the repair of cartilage and bone in OA. Ozone stimulates the production of TGF-β1 which accelerates the cell recovery (Madrigal 2007).Ozone induces the production of cytokines: IFN-γ (Bocci y Paulesu 1990, Madrigal 2007, Bocci 2011), IFN-β, IL-2, IL6, IL-8, TNF-α, TGF-β (Paulesu 1991, Bocci 1998, Larini 2001, Madrigal 2007, Bocci 2011), IL-4 and IL-12 (Madrigal 2007).**(Table 3)**

It would be interesting to examine synovial fluid before and after Ozone therapy, because there are no experimental studies on the mechanism of action of Ozone in knee OA (Borreli 2015, Hashemi 2015).Unfortunately this is not possible because it is difficult to collect patients and most of them are treated privately. However, Chang has recently demonstrated that Ozone in synovial fluid diminishes the production of the proinflammatory cytokines IL-6, IL-1β and TNF-α (Chang 2005). On the other hand, the private industry is no interested in the study of the efficiency of Ozone on pain in knee OA for economic interests. That is the reason why there are only small series of case-reports worldwide. It is of paramount importance to originate and reproduce new and interesting clinical studies that sustain the effectiveness of Ozone, so it could become useful for the benefit of both science and patients.

Ozone Infiltration Technique And Effectiveness In Osteoarthritis Of The Knee

O2-O3 in osteoarticular diseases produces: a) better vascularisation on bones and cartilage, accelerating anabolism and recovery; b) anti-inflammatory effect by ozone activation over PGs (prostaglandins); c) immunomodulatory effect on autoimmune and inflammatory diseases (such as Rheumatoid Arthritis and Osteoarthritis); d) trophic effects on bone and cartilage (Madrigal 2007).

In knee OA there is great variability in terms of side of injection, volume and gas concentration of Ozone therapy (Borreli 2015). The ozone treatment on knee joint could be intra articular, periarticular and subcutaneous. The O_2-O_3 can be applied twice/week for 5-6 weeks. The concentration of O_2-O_3 must be 10ug/ml, the volume of the gas should be 20ml for intra articular injections, 10ml for periarticular and subcutaneous injections **(Figure 3)**. The treatment is painless if the injections are applied slowly (Madrigal 2007).

After the first ozone therapy treatment, some other treatments can be applied afterwards (1-2 per year) for maintenance. It all depends on the effect over the articular physiology of the knee. It is accepted that ozone improves the range of movement (ROM) in Osteoarthritis of the knee (Madrigal 2007).

On knee OA, ozone slows the degenerative process and improves the ROM. Besides, ozone improves the cell permeability and diminishes articular effusion (Madrigal 2007).

The published studies on the use of Ozone in knee OA are limited. Fahmy and Riva –Sanseverino were the first to use Ozone in the treatment of Knee OA and in rheumatoid arthritis (Fahmy 1981, Riva-Sanseverino 1989). Escarpenter reported the treatment of 123 patients with knee OA (Escarpenter 1997). Riva-Sanseverino treated 83 knee OA patients with amelioration of pain (Riva Sanseverino 2002). Milanés in 321

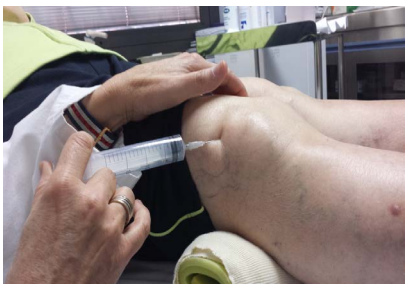

Figure 3: Intra articular infiltration of 20 ml of Ozone.

patients diminished pain and improved articular function (81% of success) (Milanés 2009). Baeza-Noci has reported 80% success in knee and hip OA, while de Lucas published 74% in Knee OA (de Lucas 2005, Baeza-Noci 2011). More recently, Cabot found 82-87% success in 57 knee OA/chondromalacia patients after ozone treatment (Cabot 2012). **(Table 4)**

Intra articular Ozone injections are an effective and costless procedure that controls pain in knee OA (Muto 2004, Paolini 2009). Many authors state that Ozone diminishes pain and improves function in knee OA (Benvenuti 2006, Moretti 2010, Mishra 2011, Calunga 2012, Li 2013, Samper 2013, Camelia 2014, Hashemi 2015). Ozone is also capable to reduce edema and inflammation (Siemsen 1995, Benvenuti 2006, Madrigal 2007, Yu 2010, Valliant 2013). **(Table 4)**

Severe OA is not a contraindication to use Ozone; these patients improve almost the same as low grade OA, but the time free of symptoms is significantly shorter (Baeza-Noci 2011).

The experience of the previous authors and the current literature demonstrate the efficacy of ozone against joint pain, inflammation and swelling. Patients are capable of recovering function in walking, going up and down stairs, washing, dressing and feeding (Benvenuti 2012).

Ozone is a good treatment with high success rate, minor complications and easy execution, reproducibility and sustainable outcome (Benvenuti 2006). It is mandatory to be well trained to perform these approaches (Alexandre 2014). Ozone is so safety and cheap, that the cost/effectiveness approach could prove that Ozone is even better than systemic drug treatments (Alexandre 2014).

Conclusions

Knee OA is a progressive condition with no cure nowadays. The treatment is focused on amelioration of symptoms. Knee OA is related to genetic, biological and biomechanical factors and recently to chronic inflammation. There is a long time evidence (four decades of clinical use) of the use of Ozone on many diseases and especially on treating musculoskeletal disorders such as knee OA. Ozone is able to inhibit inflammatory cytokines, MMP (mineral metalloproteinases), NO (nitric oxide), PGs and to stimulate anti-inflammatory cytokines, growing factors, chondrocytes and stem cells. Ozone diminishes inflammation and favours the trophism, vascularisation and repair of articular cartilage and subchondral bone. The action of Ozone over different targets on knee OA postulates it as a promising and wonderful therapeutic weapon capable to diminish pain, articular destruction and recover function and quality of life. Because of all the expected potentials attributed to Ozone therapy in different medical specialties (including knee OA), there is the hope that Ozone therapy will be part of the official medicine in all public Hospitals as it was in ours.

Table 4: Ozone clinical effect in knee OA.

Clinical variable	Ozone effect	Article Review
Pain	↓	Fahmy 1981, Riva-Sanseverino 1989, Siemsen 1995, Escarpenter 1997, Riva Sanseverino 2002, Muto 2004, Benvenuti 2006, Milanes 2009, Paolini 2009, Moretti 2010, Mishra 2011, Cabot 2012, Calunga 2012, Li 2013, Samper 2013, Hasehmi 2015
Function	↑	Benvenuti 2006, Milanes 2009, Yu 2010, Mishra 2011, Cabot 2012, Li 2013, Samper 2013, Hasehmi 2015
Rigidity	↓	Samper 2013, Camelia 2014,
Inflammation	↓	Siemsen 1995, Benvenuti 2006, Madrigal 2007, Yu 2010, Valliant 2013,

Conclusions

To Saturnino Díaz Trujillo, librarian at Santa Cristina's University hospital.

REFERENCES

1. Alexandre A, Baeza J, Kumar V.S (2014). Ozone in Non-Rheumatic Locomotor System Pathologies.ISCO3 - International Scientific Committee of Ozone Therapy. Madrid.
2. Atlas SJ, Keller RB, Wu YA, Deyo RA, Singer DE (2005). Long-term outcomes of surgical and nonsurgical management of sciatica secondary to a lumbar disc herniation: ten years results from the main lumbar spine study. Spine 2005;30;927-35.
3. Baeza-Noci, J (2011). Ozonoterapia en la gonartrosis y coxartrosis. Ozonetherapy in osteoarthritis. Río de Janeiro, Brazil. 2011.
4. Battle-Gualda E, Benito-Ruiz P, Blanco-García F, Martin-Mola E (2002). Manual SER de la Artrosis. Sociedad Español de Reumatología. España.
5. Benvenuti, P (2006). Oxygen-ozone treatment of the knee, shoulder and hip. A personal experience. Rivista italiana di ossigeno-ozonoterapia, 2006; 5: 135-44.
6. Bocci V.A, Paulesu L (1990). Studies on the biological effects of Ozone. Induction of ITF-γ on human leucocytes. Haematologica; 75: 510-15.
7. Bocci V.A, Valacci G, Corradeschi F, Fanetti G (1998). Studies on the biological effects of Ozone. 8: Effects on the total antioxidant status and on the interleukin-8 production. Mediat.Inflamm; 7: 313-17.
8. Bocci V.A, Valacci G, Corradeschi F, Aldinucci C, Silvestri S, et al.(1998). Studies on the biological effects of Ozone. 7: Generation of reactive oxygen species (ROS) after exposure on human blood to ozone. J. Biol. Regulat. Homeost. Agent; 12: 67-75.
9. Bocci, V. A (2006). Scientific and medical aspects of ozone therapy. State of the art. *Archives of medical research*, *37*(4), 425-435.
10. Bocci V (2011). Ozone: a new medical drug. 2nd Edition., Springer Verlag. Dordrecht, the Nederlands, Germany.

11. Borrelli E, Alexandre Al, Iliakis E, Alexandre An, Bocci V (2015). Disc herniation and knee arthritis as chronic oxidative stress diseases: the therapeutic role of oxygen ozone therapy. J Arthritis; 4:161. doi:10.4172/2167-7921.1000161.

12. Buric J, Rigobello L, Hooper D (2014). Five and ten years follow-up on intradiscal zone injections for disc herniation. Int J Spine Surg, 8:17.

13. Cabot J (2012). Técnica combinada de Ozonoterapia y factores de crecimiento en la artrosis de rodilla. Revista Espanola de Ozonoterapia; 2(1):35.

14. Calunga J L, Menendez S, Leon R, Chang S, Guanche D, et al. (2012). Application of ozone therapy in patients with knee osteoarthritis. Ozone: Science & Engineering; 34(6): 469-475.

15. Camelia C, Madalina I, Tatiana M, Marilena P, Oana A (2014). The role of ozone therapy in maintaining the articular function and in relieving the pain for patients with knee osteoarthritis. ARS Medica Tomitana; 20(1):25-29.

16. Chang JDS, LU HS, Chang YF, Wang D (2005). Ameliorate effect of Ozone on cytokine production on mice infected with human rheumatid arthritis sinovial fibroblast cells. Rheumatol Int; 26:141-151.

17. De Lucas JC (2005). Peripheral infiltrations with ozone. Indications, techniques and clinical experience. Rev Soc Esp Dolor; 12 NºExtra.II:3-9.

18. Davies CM, Guilak F, Weinberg JB, Femor B (2008). Reactive nitrogen and oxygen speies in interleukin-1-mediated DNA damage associated with osteoarthritis. Osteoarthritis Cartilage; 16:624-30.

19. Dogan M, Ozdemir Dogan D, Duger C, Ozdemir Kol I, Akpmar A, et al. (2014). Effects of High-Frequency Bio-Oxidative Ozone Therapy in Temporomandibular Disorder-Related Pain. *Medical Principles and Practice*, *23*(6), 507-510.

20. Dougados M, Gregen A, Nguyen M, Thiesce A, Listrat V et al. (1992). Longitudinal radiologic evaluation of osteoarthritis of the knee. J Rheumatol, 19:378-83.

21. Escarpanter Bulies J. C, Valdes Diaz O, Sanchez Rauder R, Lopez Valdes Y, and Lopez Garcia C (1997). Resultados terapéuticos en la osteoartritis de la rodilla con infiltraciones de ozono. Revista Cubana de Investigaciones Biomédicas; 16(2): 124-132.

22. Espallargues M, Alonso J, Ruigomez A, Anto J M (1996). Trastornos osteoarticulares en el anciano: aproximación a su impacto poblacional. Med Clin (Barc); 100:601-6.

23. Fahmy Z (1981). Ozon-sauerstofftherapie in der rheumatologie. In Proceedings (Vol. 5).Ozone-weltcongress. Wasser Berlin.

24. Fernandez-Cuadros M.E (2013). Analisis de la calidad de vida en pacientes con prótesis total de rodilla. Tesis Doctoral. Universidad de Salamanca. Espana.

25. Goldring MB, Goldring SR (2007). Osteoarthritis. J Cell Physiol; 213:626-34.

26. Grimand E, Heymann D and Redin F (2002). Recent advances in TGF-β in cartilage disorders. Cytokine Growth Factor Rev; 13: 241-57.

27. Hashemi M, Jalili P, Mennati S, Koosha A, Rohanifar R, et al (2015). The effect of Prolotherapy with hypertonic dextrose versus Prolozone (Intraarticular Ozone) in patients with knee Osteaoarthritis. Anesth Pain Med; 5(5): e27585.

28. Iliakis E, Valadakis V, Vynios D H, Tsiganos C. P, and Agapitos E. (2001). Rationalization of the Activity of Medical Ozone on Intervertebral Disc A Histological and Biochemical Study. Rivista di Neuroradiologia; 14(1 suppl): 23-30.

29. Jani P, Patel G, Yadar P, Sant L, Jain H (2012). Ozone therapy: the alternative medicine of the future. Int J Pharm Bio Sci; 2(4):196-203.

30. Larini A, Aldinucci C and Bocci V (2001). Ozone as a modulator of the immune system. In proccedings of the 15th Ozon World Congress, London. UK, 11th-15th September 2001, Medical Therapy Conference (IOA 2001, Ed.), Speedprint MacMedia Ltd, Ealing, London, UK, pp.1-10.

31. Li J. H, Zhou L. X, Li G. Y, and Cheng B (2013). [Treatment of middle-aged and aged patients with knee osteoarthritis of yang-deficiency induced cold-damp syndrome by ozone combined Chinese materia medica: a clinical research]. Zhongguo Zhong xi yi jie he za zhi Zhongguo Zhongxiyi jiehe zazhi= Chinese journal of integrated traditional and Western medicine/Zhongguo Zhong xi yi jie he xue hui, Zhongguo Zhong yi yan jiu yuan zhu ban; 33(4), 471-475.

32. Madrigal C (2007). Tratado de Ozonoterapia. Editorial la Salud Naturalmente. Madrid, Espana.

33. Mawsouf N, El-Sawalhi M M, Shaheen A. A, Darwish H. A, Martinez-Sanchez, et al. (2011). Effect of ozone therapy on redox status in experimentally induced arthritis. Revista Espanola de Ozonoterapia, 1(1), 32-43.

34. Milanes J. R, Acosta W, and Trinchet E (2009). La ozonoterapia/resultados del tratamiento en pacientes con artrosis degenerativa sintomática de rodilla. Revista Mexicana de algologia; 6:18-23.

35. Mishra S. K, Pramanik R, Das P, Das P. P, Palit A. K, et al. (2011). Role of intra-articular ozone in osteo-arthritis of knee for functional and symptomatic improvement. Ind J Phys Med Rehabilit, 22(2), 65-9.

36. Moreno Palacios JA, Catedra Valles E, Plazas Andreu N, Sancho Loras R, Manjon-Cabezas Subirats J et al. (2009). Comparación de los resultados en función de la edad de pacientes ancianos con artroplastia total de reemplazo. Rev Esp Geriatr Gerontol; 44(3):120-23.

37. Moretti M (2010). Effectiveness of Treatment with Oxygen-Ozone and Hyaluronic Acid in Osteoarthritis of the Knee. International Journal of Ozone Therapy; 9(1):25-29.

38. Muto M, Andreula C, Leonardini M (2005). Treatment of herniated lumbar disc by intradiscal and intraforaminal oxygen-ozone O_2-O_3 injection. J Neuroradiol; 31:183-9.

39. Paoloni M, Di Sante L, Cacchio A, Apuzzo D, Marotta S,

et al (2009). Intramuscular oxygen-ozone therapy in the treatment of acute back pain with lumbar disc herniation: a multicenter, randomized, double-blind, clinical trial of active and simulated lumbar paravertebral injection. *Spine*; *4*(13):1337-1344.

40. Paulesu L, Luzzi E and Bocci V (1998). Studies on the biological effects of Ozone: 2. Induction of tumor necrosis factor (TNF-α) on humans leucocytes. Lymphokine Cytokine Res; 7:313-17.

41. Qi W.N, Scully S.P (1997). Extracellular collagen modulates the regulation of chondrocytes by transforming growth factors-β1. J. Orthopaed, Res 15:483-490.

42. Riva Sanseverino E (1989). Knee-joint disorders treated by oxygen-ozone therapy. Europa Medicophysica; 25(3):163-170.

43. Riva Sanseverino E (2002). Trastornos de las articulaciones de las rodillas tratadas con oxígeno-ozono. Instituto de Fisiología Humana. Universidad de Bolonia.

44. Samper-Bernal D, Rovira-Duplaa G, Monerris-Tabasco M, Gonzalez-Palomares M, Mazo-Sanchez V (2013). Tratamiento de la gonalgia por gonartrosis con ozono intraarticular. Rev Soc Esp Dolor; 20(3):107-112.

45. Seyman D, Ozen N. S, Inan D, Ongut G, and Ogunc D (2012). Pseudomona aeruginosa septic arthritis of knee after intra-articular ozone injection. New Microbiologica; 35(3):345-348.

46. Siemsen C (1995). Ozon-anwendung bei akuten und chronischen Gelenkeikrankungen, in Ozon-Handbuch. Grundlagen. Prävention. Therapie (E.G. Beck, and R. Viebahn-Hänsler, Eds.), Ecomed, Landsberg, pp.V-9.21-V-9.2 14. 1995.

47. Trippel S.B (2009). Growth factor actions on articular cartilage. J. Rheumatol; 43:129-132.

48. Vaillant JD, Fraga A, Diaz MT, Mallok A, Viebahn-Hansler R, et al (2013). Ozone oxidative postconditioning ameliorates joint damage and decreases pro-inflammatory cytokine levels and oxidative stress in PG/PS-induced arthritis in rats. European Journal of Pharmacology; 714:318-24.

49. Weinstein AM, Rome BN, Reichmann WM, Collins JE, Burbine SA et al. (2013) Estimating the burden of total knee replacement in the United States. J Bone Joint Surg AM; 95:385-392.

50. Yu B, Lin Q. R, Wang B. W, Zhu Q, and He X. F (2010). Effect of Intra-Articular Ozone Injection on Degenerative Knee Cartilage in Rats. *Int J Ozone Ther*, *9*(1), 14-20.

ADDRESS FOR CORRESPONDENCE

Cristian Rodriguez-Aguayo[2,4] , [2]Department of Experimental Therapeutics, [4]Center for RNA Interference and Non-Coding RNA, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030, USA. E-mail: CRodriguez2@mdanderson.org