Clinical utility of ozone therapy for musculoskeletal disorders

Omar Seyam[1], Noel L. Smith[2], Inefta Reid[1], Jason Gandhi[1, 3], Wendy Jiang[1], Sardar Ali Khan[1, 4, *]
1 Department of Physiology and Biophysics, Stony Brook University School of Medicine, Stony Brook, NY, USA
2 Foley Plaza Medical, New York, NY, USA
3 Medical Student Research Institute, St. George's University School of Medicine, Grenada, West Indies
4 Department of Urology, Stony Brook University School of Medicine, Stony Brook, NY, USA

*Correspondence to: Sardar Ali Khan, MD, FRCS, FACS, skysalik@gmail.com.
orcid: 0000-0002-4759-530X (Sardar Ali Khan)

Abstract

Oxygen-ozone (O_3) therapy serves as an alternative medical technique that increases the oxygen in the body along with the introduction of O_3. O_3 therapy has finally reached a level where the biological mechanisms of action have been understood, showing that they are in the domain of physiology, biochemistry, and pharmacology. Few clinical applications have been reviewed here as well as exemplifying that O_3 therapy is particularly useful in musculoskeletal disorders. In the therapeutic range, O_3 can be used as a more effective and safe substitute of standard medications. O_3 therapy has been used for many years for its ability to inactivate various viruses, cancer, and acquired immune deficiency syndrome but is now making strides in the treatment of musculoskeletal disorders such as rheumatoid arthritis, lumbar facet joint syndrome, subacromial bursitis, carpal tunnel syndrome, osteoarthritis, hip bursitis, shoulder adhesive capsulitis, herniated disc, and temporomandibular joint disorder.

Key words: oxygen-ozone therapy; osteoarthritis; herniated disc; carpal tunnel syndrome; lumbar facet syndrome; muscle oxygenation, fibromyalgia; cervical disc herniation

doi: 10.4103/2045-9912.241075
How to cite this article: Seyam O, Smith NL, Reid I, Gandhi J, Jiang W, Khan SA. Clinical utility of ozone therapy for musculoskeletal disorders. Med Gas Res. 2018;8(3):103-110.

Introduction

Ozone (O_3) has great oxidizing activity as a soluble gas. When in contact with biological fluids, it forms reactive oxygen species as well as lipid oxidation products.[1] Both of these products react with white blood cells initiating the formation of proteins, cytokines, and red blood cells which increases the tissue oxygen supply. O_3 is used to treat many cases regarding the muscles, tendons, and joints. O_3 therapy raises the pain thresholdas it works based on stimulating antinociceptive apparatus mediated by serotonin and endogenous opioids. Due to neoangiogenesis, O_3 allows for vascularization caused from tissue hyperoxygenation. Therefore, the inhibitory capacity of inflammatory metabolites is improved as well as local tissue trophism. There is a common theme among many literature reports that O_3 reduces local pain which favors the mobility lost during the painful state and recovery of joint function.[2,3] O_3 can be injected by peri-articular, intra-articular, or percutaneous means. It is considered a satisfactory treatment with a low risk of complications and high success rate.

Mechanism of Action

An endogenous cascade is started when beginning the use of O_3 therapy. In response, a stress is induced from the biologically active substrates that are released. Because of O_3's ability to dissolve in the aqueous component of plasma, it can cause this oxidative stress.[4] Hydrogen peroxide and a reactive species (ROS) are formed when O_3 reacts with water and polyunsaturated fatty acids. When inhaled, the O_3 reacts with polyunsaturated fatty acids that are found in the alveolar lining layer. A mixture of lipid ozonation products (LOP) is also formed simultaneously such as malonlydialdehyde, lipperoxyl-radicals, hydroperoxides, isoprostanes, 4-hydroxynonenal, and alkenals.[5] The activation of the transcriptional factor mediating nuclear factor-erythroid 2-related factor 2 (NRF2) is increases with the moderate oxidative stress caused by O_3. The role of NRF2 is to activate the transcription of antioxidant response elements. A variety of antioxidant enzymes attain an increased concentration level upon introduction of antioxidant response elements. Some of the antioxidants include glutathione S-transferase (GST), catalase (CAT), heme oxygenase (HO)-1, superoxide dismutase, glutathione peroxidase, heat shock proteins and quinone-oxidoreductase. most of these enzymes play a role as free radical scavengers in a range of diseases.[5] Depending on the cell's redox status and the amount given, O_3 and other medical gases such as nitric oxide and carbon monoxide have a twofold effect. O_3 overexpresses HO-1 or heat shock proteins (HSPs) of 32 kPa which produces nitric oxide.[6] The expression levels of Hsp70 are upregulated by O_3 which is related to HO-1. Therefore, there may be a developing role in therapy of free radical-based diseases. Heme is enzymatically degraded by HO-1 and can be toxic depending on free iron, amount produced, and bilverdin. Biliverdin is a neutralizer of nitrosative and oxidative stress based on the ability to interact with reactive nitrogen species and NO.[7,8] It was found recently that the heat shock response provided a cytoprotective state during aging, cancer, neurodegenerative disorders and inflammation.[9] Throughout the phylogenetic spectrum, HO isoforms are found to be as regulators of redox homeostasis and dynamic sensors of cellular oxidative stress. Hormesis is a defense mechanism for oxidative insults to

multiple organ systems.[9] O_3 can have a role in hormesis by regulating the proinflammatory and anti-inflammatory effects of prostaglandin formation which is of similar nature to nitric oxide.[10]

How O_3 helps repair musculoskeletal tissues (tissue repair)

When O_3 gets in contact with organic fluids such as plasma, lymph, urine, and saliva, it interacts highly with all tissue components.[11] It can react with antioxidants, glutathione, cysteine, albumin, ribonucleic acid (RNA), and deoxyribonucleic acid (DNA).[12] All these reagents, participate in the ozonation process and formation of lipid oxidation products and ROSs.[11] These two molecules function as biochemical regulators of inflammation and physiological concentrations.[11] ROS are described as being highly unstable, immediate action and a half-life less than one second.[12] It is common that patients report the sensation of well-being during the course of O_3 therapy and this is due to the LOPs which stimulate the central nervous system and endocrine system while also improving hormonal production, neurotransmitter release, and metabolism.[11] It was proved that O_3 was capable of promoting the preservation and increase of endogenous antioxidant systems through a study conducted by Re et al.[13] Another study which dealt with the ozonation of platelet-rich plasma samples results demonstrated the increase of interleukin (IL)-8. The increase of IL-8 allows for the leukocytes to leave the circulation into the tissues to facilitate the phagocytosis of bacteria and necrotic tissue of ulcers.[14] The results also showed an increase in growth factors such as platelet derived growth factor (PDGF), IL, and transforming growth factor beta (TGF-β) 1.[14] Lim et al.[15] that when dermal wounds were exposed to O_3, it increased the activity of factor nuclear kappa B, an important immunomodulatory of inflammation and the expression of TGF-β which is essential for remodeling tissue.

Temporomandibular joint disorder (myofascial pain syndrome)

The temporomandibular joints are the joints that connect the jawbone to the skull. Temporomandibular joint disorder also known as myofascial pain syndrome results in pain that limits chewing, talking, and other daily activities. It was found that the use of O_3 therapy has been much more effective than medication therapy in patients with high pain scores which relieves pain and increases the maximum voluntary interincisal mouth opening values. A proposed explanation of why O_3 causes the joint to heal quicker than of the traditional therapy is due to the highly reactive nature of O_3. It is able to stimulate the fibroblastic joint repairing abilities when injected into a joint capsule. It is also able to promote new cartilage growth as well as reducing inflammation. When split into separate oxygen atoms, O_3 is able to react when in contact with a contaminant.[16-18] In fact, it never fails to initiate this reactive activity. A recent study found that 87% patients had either improved or completely recovered from temporomandibular pain.[18] It was concluded that this was a promising new treatment, but the mechanism of action is still being researched. A plausible mechanism can be due to a single oxygen atom oxidizing the contaminants. It is thought that O_3 can stimulate the fibroblastic joint since it is such a reactive molecule. O_3 can react with the contaminant when it splits into single oxygen atoms.[18]

Herniated disc (back pain)

The herniated disc is characterized as a condition affecting the spine in which a tear in the fibrous ring of an intervertebral disc allows the central portion to bulge out.[19] The treatment of lumbar disc herniation is applied with a needle which is placed into the hernia between the inner margin of the facet joint and the lateral nerve root. This has been considered a minimally invasive treatment for nerve root treatment since the O_3 is minimally invasive and the needle is thin. The mechanism of action is as follows: nucleur pulposus is oxidized by proteoglycan which then cause it to denaturize and reduce in volume. The local blood circulation will have reduced when the osmotic pressure is reduced. The doze of O_3 administered is essential and should not pass the capacity of glutathione and antioxidant enzymes to prevent accumulation of hydrogen peroxide and superoxide anion which can possibly degradation of cell membranes.[20] O_3 in a medium of pH = 8 or higher will cause the formation of free radicals. While, in a medium of pH = 7.5 or lower the formation of peroxides occur due to the ozonolysis mechanism. In oxygen-O_3 therapy, O_3 is administered at a nontoxic concentration of 1 to 40 µg of O_3 per milliliter of oxygen. It was found through in studies that consisted of *in vitro* on resected human disk specimens as well as *in vivo* on rabbits that the optimal concentration to administer is 27 µg. O_3 has a direct effect on the disk's nucleus pulposus specifically in proteoglycans at this concentration which results in subsequent cell degradation of the matrix and release of water molecules.[21] The matrix is replaced by fibrous tissues and formation of new blood cells in approximately 5 weeks. A reduction in disk volume is the result of all these events. In a study conducted by Andreula and others,[22] five histologic disk specimens were removed during surgical microdiskecotmy who received intradiscal injects of O_3 at 27 µg/mL. Dehydration of the fibrillary matrix of the nucleus pulposus, signs of regression (fragmentation and vacuole formation), and revealing collagen fibers were all noted in these specimens. Other findings of a herniated disk untreated with medical O_3 are proliferating and signs of new blood cell formation guided by lymphocyte inflammatory tissue and chondrocyte hyperplasia. One of the main therapeutic causes of medical O_3 is a reduction in disk size which can possibly reduce nerve root compression. Furthermore, disk shrinkage can improve local microcirculation and increase the supply of oxygen by reducing venous stasis caused by disk compression of vessels. O_3 therapy also had analgesic and anti-inflammatory effects in treating disk herniation.[23] The action ties into inhibiting release of bradykinin or release of algogenic compounds as well as inhibiting synthesis of proinflammatory prostaglandins. Proinflammatory cytokines such as interleukin can be neutralized by increasing release of antagonists. The results from this study was satisfactory compared to other percutaneous treatments for disk herniation such as enzymatic chemonucleolysis.[24] The two procedures are similar, however oxygen-O_3 therapy is less invasive due to the narrow nature of the needle and less traumatic.[25] Also, there were no anaphylactic or allergic reactions.[25]

Shoulder (Glenohumeral joint)

The shoulder allows for the role of orientating the hand and has a great degree of movement.[26] Specifically, the glenohumeral joint is not a stable ball-and-socket joint, but has high mobility.[26] The rotator cuff muscles allow for joint motility.

These muscles include the infraspinatus, subscapularis, supraspinatus, and teres minor. O_3 therapy for the glenohumeral joint includes injection with the posterior approach under the inferior margin laterally using a 22 G needle. In a case study, Benvenuti[27] reported that a 58-year-old woman experiencing severely limited joint movement and pain underwent a session of O_3 therapy with 10 mL gas mixture at a concentration of 15 µg/mL. Furthermore, it is helpful to puncture the long head of the bicep muscle and subacromial bursa with a 27 G needle in microdoses of 0.5 to 1 mL. After one week, the patient reported a reduction of nocturnal pain.[27] The patient reported a recovery of active shoulder function and total reduction of pain.

Shoulder adhesive capsulitis

Shoulder adhesive capsulitis is a condition where there is a reduction in the arc of active motion.[28] Adhesive capsulitis is characterized with an unknown etiology that has an onset of pain associated with it.[28] The treatment with oxygen-O_3 can help in the reduction of pain as well as inflammation activity. It uses a mixture which is 95–96% oxygen and 4–5% O_3. The reduction and modulation of inflammation activity and a reduction of pain are a few of the many action accomplished with medical O_3. The local injection of O_3 destroys algogenic substances, alters serotonin, and inactivates bradykinin. All of these substances are altered to produce no pain. A release of soluble receptors or anatagonists neutralize proinflammatory cytokines such as interferon-a, tumor necrosis factor-α (TNF-α) and ILs. In addition, the denaturation of cellular proteins such as kallikrein, kininogen, and cyclooxygenase (COX) help form endorphins and modify the pain receptors. Another important activity is given by the muscle relaxant action by direction action on the muscle fibers.[12] Contraindication to this treatment include latent hypoglycemia, hyperthyroidism, favism, pregnancy, and sickle cell anemia. This study has many limitations since it was a case study. It can be a basis for future studies on a better statistical criterion as well as on more patients.

Hip bursitis (inflammation)

Hip bursitis can be characterized as the swelling of bursae. The bursae are fluid-filled sacs that cushion muscles and tendons.[29] The hip is similar to the shoulder since it is also a ball-and-socket joint. However, the hip has much more flexibility in movement as compared to the shoulder where it is attached to the trunk. It is common that patients are not aware they have a hip inflammation since they might complain about the pain in the anterior knee and thigh rather than the hip itself. O_3 therapy can be used to alleviate hip pain due to functional overload, trochanteric bursitis, pain caused by initial and late coxarthrosis, and hip tendonitis. It is also helpful to associate oxygen-O_3 therapy along with prescribed exercises and a period of rehabilitation. In a case report,[27] a 54-year-old man was treated by a cycle of 5 therapy session of O_3. The dosage consisted at a concentration of 25 µg/mL at 55 mL oxygen) through weekly intervals. It was injected at a lateral approach where the method of infiltration was called peritrochanteric. The patient referred difficulty walking with deambulation for approximately 50 meters and nocturnal hip pain.[27] The joint pain subsided after the first treatment session with an improvement in trochanteric swelling. At the end of the treatment cycle daytime and nighttime pain had disappeared. By the subsequent follow-up visit which was approximately 5 weeks later, there were no signs of bursitis. Signs of limping stopped and the patient was able to take the stairs without any difficulty. This study demonstrates the versatility and efficacy of O_3 therapy when administered in small doses through weekly intervals.

Osteoarthritis (OA) (knee)

OA is a degenerative disease that affects function and produces pain.[30] O_3 infiltration accelerates anabolism and produces better vascularization on cartilage and bone. Regarding knee osterarticular disease, there is a great variability in terms of gas concentration of O_3 as well as the side of injection. On the knee joint, it could be either periarticular, intra articular, and subcutaneous. Currently, there is no cure for knee OA. Because of this there is a focus on the amelioration of the symptoms caused by knee OA. O_3 improves the range of motion (ROM) and slows down the degenerative process. It also is able to inhibit chondrocytes, stem cells, inflammatory cytokines, nitric oxide, and mineral metalloproteinases. The published studies on O_3 regarding the knee are limited. Riva Sanseverino[31] were the first people to use O_3 to treat the knee OA. Over the many years, intra articular O_3 injections have found to be a costless procedure that is effective. Patients who have severe OA will improve at about the same rate as those with low grade OA. The pathophysiology of OA is characterized by the destruction and softening of articular cartilage along with increased matrix degradation due to proteoglycanases and collagenases. Activated chondrocytes and monocytes release enzymes which by releasing TNF-α and IL-1 multiply the inflammation. The synthesis of prostaglandins increases and there is an attempt to maintain biomechanical matrix. It is believed that the synthesis of O_3 Messengers LOPs and ROS would act in two phases in the synovial fluids. O_3 would inhibit the proinflammatory cytokines such as prostaglandin E2 (PGE2) during the first phase. During the second phase, O_3 will act over inhibitory cytokines such as IL-10, TGF-β, and IL-4, antioxidant enzymes, and neoangiogenesis. These are all working together in the process of repairing the articular joint by stimulating fibroblasts, chondrocytes, and stem cells.

Carpal tunnel syndrome

Carpal tunnel syndrome is caused by a compressed nerve in a narrow passageway on the palm side of the wrist.[32] Secondary forms are associated with a variety of diseases such as hypothyroidism, diabetes mellitus, oestrogen therapy, rheumatoid arthritis, amyloid disease.[33] The mechanism of oxygen O_3 treatment is based on three mechanisms that are shared by the treatment of herniated disc in the spine. By increasing trans-tissue and intra-oxygenation along with reduced lymphatic stasis and hypoxia, there is an indirect vessel-mediated decompression of the nerve roots. Second, by inhibiting the release of polymorphonucleates through increasing immunosuppressive cytokines and proteinase through macrophages.[34] Third, inhibiting the release of prostaglandins and pro-inflammatory bradykinins, action on the cell-mediated inflammatory response would take place.[35] Oxygen-O_3 therapy

seems to guarantee improvements of symptoms after one year compared to steroid injections. A study conducted by Zambello et al.,[36] showed that 90% of patients had a significant improvement after O_3 injection. 17% had a good control of symptoms and 70% of patients no longer had symptoms after a one year follow up.[36] However, further studies should be conducted to see if symptoms remain or not after two years or more to evaluate the long-term effects.

Partial tear of the supraspinatus tendon

The supraspinatus tendon is part of the rotator cuff.[37] Oxygen-O_3 therapy is one of the many methods that can be used to relieve the symptoms. In a study,[38] which consisted of 40 patients, specifically 26 males and 14 females whom had shoulder pain for approximately six months. In order for patients to be included in the study, the size of the tear of the supraspinatus had to be less than 1.5 cm. Ultrasound guidance is recommended since the blind infiltration does not guarantee to get properly in the shoulder joint.[38] It was concluded that the ultrasound-guided infiltration of oxygen-O_3 therapy proved to be an effective treatment method in partial tears of the supraspinatus tendon. However, it is necessary to do further research requiring a large sample size.

Subacromial bursitis

First degree spondylolisthesis and spondylolysis-spondylosis is a defect of part of the vertebral arch between the superior and inferior spinal processes. Spondylolisthesis is if the defect results in a forward shift of one of the vertebral body on one another.[39] Eighty-three percent of the 18 patients in this had a complete recovery from pain immediately.[40] The twofold action of O_3 in the perganglionic region and in the lysis points of the neural arch or pars interarticularis region innervated by Luschka's recurrent nerve is an explanation for such fast pain.[21] The use of the gas mixture directly next to the lysis points on Luschka's nerve by exploiting the well-known analgesic and anti-inflammatory effects of the oxygen-ozone mixture. The spine is innervated by Luschka's recurrent nerve or posterior primary branch and vertebral plexus.[41] The plexus innervates the end plates, longitudinal ligament, vertebral bodes, and relative peridural tissues in the pars interarticularis region or the neural arch.[41] The anti-inflammatory and analgesic effects of the oxygen-O_3 mixture infiltrates directly proximal to the lysis point on Luschka's nerve.[42] Prostaglandin levels and cytokine levels are normalized with a reduction of reactive oxidant species and an increase in superoxide dismutase production. A eutrophizing effect occurs when subsequent infiltration into the periganglionic region takes place both adjacent to the nerve root compressed at the level of muscle spasm and injured by accompanying disc protrusion.[43,44] A good final outcome is accounted due to the combined action.

Lumbar facet joint syndrome

Lumbar facet joint syndrome can be described as pain at the joint between two vertebrae in your spine. It is a condition which affects about 80% of people who have lower back pain. The main mechanism of action may be considered as follows: there is an immediate oxidation by which the proteoglycan in the nucleus pulposus could be oxidized immediately and the osmotic pressure is reduced.[45] The volume of the nucleus pulposus would decrease as well as necrotize, denaturize, and atrophy. The local blood circulation would also be changed when the osmotic pressure is reduced. The symptoms may be improved when increasing the oxygen supply. Regarding the anti-inflammatory effect, as the vein, lymphoid tissue, and nerve root were compressed by the annulus fibrosus and herniated nucleus pulposus, the lymphatic backflow and venous was obstructed which was accompanied by exudation and nerve edema. To induce an immune response, antigenic substances such as B-lipoprotein and glycoprotein could be released that would result in aseptic inflammation and adhesion. The pain of disc herniation could be caused by these factors. Lastly regarding the analgesic effects, enzyme products and inflammatory mediators stimulate the nerve endings near ligament and on the disc surface that causes the pain that is associated with disc herniation. In order to attain pain relief, the strong oxidative activity of O_3 can inactivate the above inflammatory mediators. O_3 injected into the middle of the disc through the conventional posteriorlateral route had produced the desired result for small or medium size disc herniations.[22] Regarding the large disc herniation, the symptoms were not eliminated quickly and the efficacy was limited due to the hernia compressing the nerve root. The efficacy of O_3 therapy is poor with patients who have large lumbar disc herniation and greatly significant with those who have a small or minimal disc herniation. Since the annulus fibrosus was either completely or partially ruptured, it was found that the O_3 could diffuse through the tissues that were torn surround the vertebral or spinal disc. This would cause the hernia and nucleus pulposus to not be fully oxidized. In a study conducted by Lu et al.,[46] the treatment of large lumbar disc herniation with percutaneous O_3 injection was greatly effective. An improvement of local ablation and eliminating local aseptic inflammation was achieved through O_3 injection in or around the hernia areas. Therefore, it was concluded through using percutaneous O_3 injection with the inner margin of facet *via* the posterior-lateral was an effective method. It is important to note that the concentration of O_3 should be acceptable in quantity and the rupture of the annulus fibrosus by high intradiscal pressure should be avoided in order to ensure efficacy. Low pressure of repeated injection of O_3 was suggested for large disc herniation. This can be accomplished by repeatedly pushing and pulling the syringe to allow the O_3 to completely oxidize and contact the nucleus pulposus. New O_3 is then injected and the residual O_3 is abandoned to avoid rupture of the annulus fibrosus.

Cervical disc herniation

Cervical disc herniation is wear and tear of a disc in the neck. It is suggested that the material from the nucleus pulposus may act as a chemical or immunologic irritant the nerve and these mechanisms may produce an inflammatory effect.[47] Studies have hypothesized that the injection of O_3 induces overexpression of antioxidant enzymes which then neutralizes excessive ROS formation. In the degenerated nucleus pulposus, the intradiscal injection of O_3 can accelerate the degradation of proteoglycans.[14] The biochemical modification of the medium in the extraduralspace is one of the important aspects of it.[48] The cause of radicular pain is A2 phopholipase independent of a direct inflammatory process or immunological response.[47] A2 phospholipase is responsible for the prostaglandins and arachidonic acid liberation. It has been shown that there are

high levels of A2 phospholipase in herniated discs. A powerful stimulus to the activation of antioxidant defense is the result of O_3 injected in the peridural space of the conjugation foramen and disc. Even in cases that had extruded cervical disc pathology, injections were still performed and had great results. This is most likely due to the fact that normal tissues and the isolate fragment are separated.[48]

Rheumatoid arthritis

Rheumatoid arthritis is characterized as an autoimmune disease where the body's immune system attacks the joints instead of bacteria and viruses.[49] The syndromes include hyperplasia of synovial cells, excess synovial fluid, and forming pannus which can damage joint deformities and articular cartilage.[50,51] The etiology for rheumatoid arthritis is still not understood. The common treatments are immunologic purging, advanced surgical treatment, and drug therapy.[52] However, O_3 therapy is a new treatment in treating rheumatoid arthritis can overcome these limitations at a certain level. The therapeutic mechanism of O_3 still remains unclear. Previous research has showed that O_3 can reduce the activity of TNF-α in the inflammatory tissues and suppress synovial hyperplasia and joint swell in rheumatoid arthritis in rats.[53,54] Therefore, a study was conducted to treat bovine collagen II-induced RA in rats with intraarticular injection of O_3 at various concentrations.[55] Rats were injected with complete Fruend's adjuvant bovine collagen II that successful established a rat model of rheumatoid arthritis.[55] In the O_3 treated groups, the optimal concentration for treating rheumatoid arthritis was 40 μg/mL.[55] They also had higher TNF-receptor (TNF-R)1 indicating that O_3 can reduce synovium injury in rats with RA and lower synovial TNF-α and TNF-R2 levels.[55] A plausible mechanism is the reduction in TNF-R1 and rheumatoid arthritis TNF-a levels and increase the level of TNF-R1 which increase synovial cell apoptosis and preventing synovial cell proliferation.[55]

Systemic sclerosis

Systemic sclerosis is a chronic tissue disease characterized by vascular abnormalities in the joints, internal organs, and fibrosis.[56] The etiology and clinical manifestations of scleroderma are still not understood; this is why it is difficult to treat systemic sclerosis.[56] The inflammatory processes can be limited by O_3's potential in reducing the proliferation of neutrophils and mastocytes, increasing concentration of prostacyclin 6-keto-prostaglandin F1α (6-keto-PGF1α), impeding the release of acute phase proteins, and decreasing the concentration of prostacyclin (PGF)2α resulting from the oxygen radicals on arachidonic acid.[57] The results from this study have proved to slow down the progression of the illness by limiting the activation of the immune system. It had also increased the movability of interphalanx joints and decreased the thickness of the skin.[57] O_3 is able to penetrate the epidermis water-fat barrier and also has a good solubility in serum which is why it has a good effect on the skin.[57] The vasodilating effect of O_3 allows for the decrease of skin score index, decrease of arterial blood pressure, increase in angle of interphalangeal joints all through the synthesis of nitric oxide synthase.[57]

Fibromyalgia

Fibromyalgia is seen as a rheumatic disease which means that it causes myofascial pain or soft tissue pain.[58] The mechanism of O_3 is as follows. After the O_3comes into contact with the blood, it immediately reacts with various reducing molecules such as antioxidizing agents, unsaturated fatty acids containing double bonds to produce reactive oxygen species. Both lipid peroxidation products and hydrogen peroxide are generated, when O_3 reacts with polyunsaturated fatty acid (PUFA).[59] Enzymatic antioxidant systems such as aldehyde dehydrogenase and glutathione-transferase neutralize the toxicity of both molecules (LP and hydrogen peroxide). They act as secondary messengers which stimulate further generation of antioxidant enzymes.[60] This can be done if O_3 is administered in quantities that are able to achieve a therapeutic effect which can protect against radicals and is nontoxic. A case which a 45-year-old woman was administered oxygen-O_3 therapy biweekly sessions equating to a total of 12 sessions. Due to a lowering of painful symptoms, the patients experience a sense of well-being. An improvement in the asthenia was seen due to a greater oxygenation of tissues because of O_3. There were no side effects seen in this case. The limitation from this study is the small number of patients in which it was conducted. It was found that O_3 glycolysis was sped up through the activation of the mitochondrial respiratory chain.[61,62] The O_3 mixture will cause an increase in oxygenation level due to the increased efficiency of the antioxidant enzyme system, enhance serotonin production, and microcirculation. The production of endorphins was enhanced by the motor plate. So patients who had fibromyalgia had improved their daily activities by 40% and sleeping disorders by 6%.[63]

Muscle oxygenation

The role of O_3 therapy was observed for hypoxic tissues, those in which tissues were below-normal level of an adequate oxygen supply.[64] A study has demonstrated that O_3 therapy can change the level of oxygenation in resting muscles by measuring directly the pressure of oxygen.[64] Through autohemotransfusion, O_3 therapy avoids lung toxicity from oxidative stress. The effects of O_3 are mediated by rapid oxidation of blood substances. Hydrogen peroxide and peroxidated lipoproteins both are reactive oxygen species that can activate the hexose monophosphate shunt. Charge modification is done by the increase of malonyl aldehyde and lipid peroxidation as well as an improvement of blood rheology and flexibility of erythrocyte membrane. The collaboration of nitric oxide, adenosine, and prostaglandins can decrease vascular resistance. It is hypothesized that this will lead to blood flow redistribution. This is supported by the data collected in the study which shows the correlation between the change in pressure of oxygen post-ozone therapy and the initial oxygenation. Another possible mechanism to explain the results of this experiment, is the increase in production of 2,3-diphosphoglycerate in erythrocytes and lipid peroxidation of red blood cell membranes can be achieved with the activation of the hexose monophosphate shunt.[65] These would both cause a shift to the right in the oxyhemoglobin dissociation curve ultimately leading to an increase of release of oxygen to the tissues.[65]

Spinal muscle disorder (horse)

The harmonious movement of the spine and balance are the

result of the muscles running along the spine to the dock of the tail. Muscle fatigue is caused by soft tissue spinal lesions. Impaired performance in athletic horses is mainly caused by changes in the thoracolumbar spine. One of the many pathologic changes affecting the thoracolumbar spine is soft tissue spinal lesions. The muscles may suffer varying degrees of inflammation after intense muscular stress. In the study conducted, all four horses had a positive response to O_3 therapy.[66] They were all able to increase their trotting speed due to the stiffness that was relieved and increase in posterior muscle chain thrust. The mechanism of action of O_3 is described as the O_3 coming in contact with the blood on different targets. Since O_3 is very active it reacts when it comes in to contact with blood or any biological fluid. O_3 first reacts with polyunsaturated fatty acids then with proteins, antioxidants (ascorbic acid and glutathione). When O_3 reacts with bio molecules, it produces a molecule of ROS which is hydrogen peroxide and two molecules of lipid oxidation products. The ROS activates the pentose phosphate pathway. The lipid oxidation products that are produced as 4-hydroxynonenal and malonaldehyde.[67] Since they are toxic, they undergo a dilution in the circulation and get metabolized in the blood circulation. Overall, the beneficial effects seen by O_3 therapy is the increase in availability and delivery of oxygen, adenosine triphosphate (ATP), and glucose within ischemic tissues, enhances implantation of bone marrow stem cells at the site of lesion which can provide neovascularization, tissue regeneration, and angiogenesis. Ballardini[66] did not notice any short or long-term effects when administering different treatment cycles to the same horse; he always noticed a positive response from the horse.

Spinal pain

About 80% of the world's population has a symptom of low back pain.[68] In general, the pain one suffers from a herniated disc is caused from inflammation and not compression. The use of O_3 therapy is recommended to treat back pain before doing any surgical procedures. There are two techniques one can attempt: direct approach and the indirect approach. O_3 acting as a chemical reactant by needle insertion refers to the indirect approach. Whereas, the direct approach is done by direct insufflation of the oxygen-O_3 mixture of a concentration at approximately 30 μg/mL and preceded by needle insertion in the pathologic intersomatic space. Eighty percent of 63,000 patients have shown good outcomes confirmed by magnetic resonance imaging (MRI) controls and computed tomography.[48,69] In the future, it is essential to conduct more studies to assess the role of variables such as place of needle, needle type, O_3 concentration, and oxygen amount. The mechanism of action underlying O_3 therapy for the direct method is: O_3 reacts with biomolecules when dissolved in interstitial water, which then results in the formation of reactive oxygen species such as hydrogen peroxide and hydroxyl radicals.[70,71] The matrix degenerates with disappearance of the herniated material, when ROS reacts with proteoglycans of the nucleus pulposus,[72] thusleading to a lower of sensitivity of axons. Alternatively, nociceptors can be stimulated when algesic endogenous substances released during perineural ischemia. The indirect approach consists of one to four injection of 5–10 mL of O_3. The disappearance of pain because of the complex series and chemical and neurological reactions have defined it as a chemical acupuncture. The O_3 concentration must be between 18–25 μg/mL.[73] If it is higher than 20 μg/mL, it can be too painful, and if it is too low, then it won't be effective. Therefore, it is essential to maintain the right balance since it can cause risky vasovagal reflex and lipothymia during initial treatments. Contrarily, the pain threshold rises after five to seven treatments therefore, the concentration of O_3 increases, but must not exceed 30 μg/mL. The infiltration of O_3 therapy uses the paravertebral muscles as a route. Regarding the indirect method, the mechanism of action underlying O_3 therapy is described as O_3 reacting with PUFA, LOPs, and anti-oxidants. The final therapeutic effect is achieved with these compounds stimulating local C-nociceptors. Altogether, it is concluded that injection of O_3 either into the paravertebral muscles or intradiscally has indicated long-term pain relief.[16,74]

Lumbar spinal stenosis (LSS)

LSS can be characterized as the narrowing of the spinal canal of the lumbar area.[75] The three main symptoms that LSS gives rise to are radicular pain or discomfort, low back pain, and neurological intermittent claudication.O_3 therapy blocks phospholipase A2 which is the same enzyme that epidural steroid injections target.[76-78] Therefore, O_3 can serve as a better substitute steroid since it has the same mechanism of action while being a much safer drug. The neurological pain in LSS can be improved by the microcirculation that O_3 induces.[79] In a study conducted by Baeza-Noci,[76] patients with spinal stenosis underwent O_3 therapy for 10 biweekly sessions along with 5 weekly sessions. Each injection of O_3 (10 mL) had a concentration of 20 μg/mL. After 1 year, from the baseline, 74% of patients improved with excellent results.[76]

Complications of O_3 therapy in musculoskeletal disorders

The reactivity of O_3 gives rise to a cascade of reactions such as the lipid ozonation products acting as signal transducer molecules, peroxidation of lipids leading to changes in membrane permeability.[80] Endogenous mediators of inflammation are released by the activation of lipases through which LOP activates the lipases.[81,82] It is the O_3 which reacts with unsaturated fatty acids such as in the pulmonary cell bilayers and lung lining fluid. Enzyme inactivation occurs when there is a loss of functional groups in enzymes. Cell death or cell injury may occur from these reactions. Hazardous effects on lung alveoli can occur with the combination of nitrogen dioxide (NO_2) and O_3 that are in photochemical smog. These effects can be prevented by free radical scavengers such as vitamin E, C or dietary antioxidants. In an *in vitro* study, peroxides were found to be formed by the presence of O_3 and oxidized arachidonic acid.[83] The activity of prostaglandin endoperoxides are comprimable to that of arachidonic acid peroxides. The aggregation of human platelets in platelet-rich plasma was seen with arachidonic acid peroxides. While, the presence of vitamin E and indomethacin, presented no signs of aggregation of human platelets in platelet-rich plasma.[83] Therefore, this suggests that they can treat O_3 toxicity.

Conclusion

O_3 therapy is becoming an effective treatment option for musculoskeletal disorders as it promotes tissue hyperoxygenation as well as treating painful syndromes affecting muscles,

tendons, and joints. Though O_3 has indicated great success in most indications mentioned in this review, there still needs to be further research conducted to determine its activity for treatment of plantar fasciitis, costochondritis, and myofascial syndrome. In order to prevent the common side effects that O_3 therapy causes, it is essential to continue researching the utility of O_3 therapy in all indications.

Acknowledgments
The authors are thankful to Drs. Kelly Warren, Todd Miller, and Peter Brink (Department of Physiology and Biophysics, Stony Brook University School of Medicine, Stony Brook, NY, USA) for departmental support, as well as Mrs. Wendy Isser and Ms. Grace Garey (Northport VA Medical Center Library, Northport, NY, USA) for literature retrieval.

Author contributions
OS designed, organized, and wrote the review article; designed the outline; solved queries related to scientific publications from the journals. NLS performed Medline searches, aided in writing the review article and critiqued the literature. IR revised the article to add logical reasoning and corrected the literature. JG critiqued and applied logical reasoning to the literature. WJ critiqued and applied logical reasoning to the literature. SAK formulated clinical concepts, reviewed the article, and corrected the reference. All authors have read and approved the manuscript provided.

Conflicts of interest
The authors have no conflicts of interests to declare.

Financial support
None.

Plagiarism check
Checked twice by iThenticate.

Peer review
Externally peer reviewed.

Open peer reviewer
Nemoto Edwin, University of New Mexico Health Sciences Center, USA.

References

1. Cardoso CC, Carvalho JC, Ovando EC, Macedo SB, Dall'Aglio R, Ferreira LR. Action of ozonized water in preclinical inflammatory models. *Pharmacol Res*. 2000;42:51-54.
2. Lopes de Jesus CC, Dos Santos FC, de Jesus LMOB, Monteiro I, Sant'Ana MSSC, Trevisani VFM. Comparison between intra-articular ozone and placebo in the treatment of knee osteoarthritis: A randomized, double-blinded, placebo-controlled study. *PLoS One*. 2017;12:e0179185.
3. Cardelli R, de Santis F, Dall'Olio M, Leonardi M. Osteoarthritis of the hip treated by intra-articular infiltration of oxygen-ozone and hyaluronic acid (Hyalubrix®). Preliminary results. *Int J Ozone Ther*. 2008;7:66-69.
4. Bocci V, Larini A, Micheli V. Restoration of normoxia by ozone therapy may control neoplastic growth: a review and a working hypothesis. *J Altern Complement Med*. 2005;11:257-265.
5. Inal M, Dokumacioglu A, Özcelik E, Ucar O. The effects of ozone therapy and coenzyme Q_{10} combination on oxidative stress markers in healthy subjects. *Ir J Med Sci*. 2011;180:703-707.
6. Bocci V, Aldinucci C, Mosci F, Carraro F, Valacchi G. Ozonation of human blood induces a remarkable upregulation of heme oxygenase-1 and heat stress protein-70. *Mediators Inflamm*. 2007;2007:26785.
7. Mancuso C, Capone C, Ranieri SC, et al. Bilirubin as an endogenous modulator of neurotrophin redox signaling. *J Neurosci Res*. 2008;86:2235-2249.
8. Barone E, Trombino S, Cassano R, et al. Characterization of the S-denitrosylating activity of bilirubin. *J Cell Mol Med*. 2009;13:2365-2375.
9. Dattilo S, Mancuso C, Koverech G, et al. Heat shock proteins and hormesis in the diagnosis and treatment of neurodegenerative diseases. *Immun Ageing*. 2015;12:20.
10. Mancuso C, Pistritto G, Tringali G, Grossman A, Preziosi P, Navarra P. Evidence that carbon monoxide stimulates prostaglandin endoperoxide synthase activity in rat hypothalamic explants and in primary cultures of rat hypothalamic astrocytes.*Brain Res Mol Brain Res*. 1997;45:294-300.
11. Bocci V. Ozone as Janus: this controversial gas can be either toxic or medically useful. *Mediators Inflamm*. 2004;13:3-11.
12. Bocci VA. Scientific and medical aspects of ozone therapy. State of the art. *Arch Med Res*. 2006;37:425-435.
13. Re L, Mawsouf MN, Menendez S, Leon OS, Sanchez GM, Hernandez F. Ozone therapy: clinical and basic evidence of its therapeutic potential. *Arch Med Res*. 2008;39:17-26.
14. Bocci V. Biological and clinical effects of ozone. Has ozone therapy a future in medicine? *Br J Biomed Sci*. 1999;56:270-279.
15. Lim Y, Phung AD, Corbacho AM, et al. Modulation of cutaneous wound healing by ozone: differences between young and aged mice. *Toxicol Lett*. 2006;160:127-134.
16. Rahimi-Movaghar V, Eslami V. The major efficient mechanisms of ozone therapy are obtained in intradiscal procedures. *Pain Physician*. 2012;15:E1007-E1008.
17. Bocci V, Zanardi I, Travagli V. Has oxygen-ozonetherapy a future in medicine.*J Exp Integr Med*. 2011;1:5-11.
18. Daif E.Role of intra-articular ozone gas injection in the management of internal derangement of the temporomandibular joint. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2012;113:e10-e14.
19. Jordan J, Konstantinou K, O'Dowd J. Herniated lumbar disc. *BMJ Clin Evid*. 2009;2009:1118.
20. Bellomo G, Mirabelli F, Salis A, et al. Oxidative stress-induced plasma membrane blebbing and cytoskeletal alterations in normal and cancer cells. *Ann N Y Acad Sci*. 1988;551:128-130.
21. Iliakis E, Valadakis V, Vynios DH, Tsiganos CP, Agapitos E. Rationalization of the activity of medical ozone on intervertebral disc a histological and biochemical study. *Riv Neuroradiol*. 2001;14:S23-30.
22. Andreula CF, Simonetti L, De Santis F, Agati R, Ricci R, Leonardi M. Minimally invasive oxygen-ozone therapy for lumbar disk herniation. *AJNR Am J Neuroradiol*. 2003;24:996-1000.
23. Bocci V, Luzzi E, Corradeschi F, Paulesu L, Di Stefano A. Studies on the biological effects of ozone: 3. An attempt to define conditions for optimal induction of cytokines. *Lymphokine Cytokine Res*. 1993;12:121-126.
24. Matsui H, Terahata N, Tsuji H, Hirano N, Naruse Y. Familial predisposition and clustering for juvenile lumbar disc herniation. *Spine (Phila Pa 1976)*. 1992;17:1323-1328.
25. Leonardi M. Disc puncture under fluoroscopic guidance. *Riv Ital Ossigeno-Ozonoterapia*. 2002;1:73-78.
26. Terry GC, Chopp TM. Functional anatomy of the shoulder. *J Athl Train*. 2000;35:248-255.
27. Benvenuti P. Oxygen-ozone treatment of the knee, shoulder and hip: A personal experience. *Rivista Italiana di Ossigeno-Ozonoterapia*. 2006;5:135-144.
28. Neviaser AS, Neviaser RJ. Adhesive capsulitis of the shoulder. *J Am Acad Orthop Surg*. 2011;19:536-542.
29. Hirji Z, Hunjun JS, Choudur HN. Imaging of the bursae. *J Clin Imaging Sci*. 2011;1:22.
30. Sinusas K. Osteoarthritis: diagnosis and treatment. *Am Fam Physician*. 2012;85:49-56.
31. Riva Sanseverino E. Intensive medical physical treatment of osteoporosis with the AID of oxygen-ozone therapy. *Europa Medico Physica. 1989*;25:163-170.
32. Ibrahim I, Khan WS, Goddard N, Smitham P. Carpal tunnel syndrome: a review of the recent literature. *Open Orthop J*. 2012;6:69-76.
33. Katz JN, Simmons BP. Clinical practice. Carpal tunnel syndrome. *N Engl J Med*. 2002;346:1807-1812.

34. Bocci V.Ozone as a bioregulator. Pharmacology and toxicology of ozonetherapy today. *J Biol Regul Homeost Agents*.1996;10:31-53.
35. Simonetti L, Raffi L, Cenni P, Agati R, Leonardi M. Pharmacological mechanisms underlying oxygen-ozone therapy for herniated disc. *Rivista Italiana Di Ossigeno*. 2003;16:S201-204.
36. Zambello A, Fumagalli L, Fara B, Bianchi MM. Oxygen-ozone treatment of carpal tunnel syndrome. Retrospective study and literature review of conservative and surgical techniques. *Int J Ozone Ther*. 2008;7:45-48.
37. Volk AG, Vangsness CT Jr. An anatomic study of the supraspinatus muscle and tendon. *Clin Orthop Relat Res*. 2001:280-285.
38. Moretti M. Effect of treatment with O_2-O_3 and hyaluronic acid in partial tear of the supraspinatus tendon. *Int J Ozone Ther*. 2012;11:98-100.
39. Donnally IC, Dulebohn SC. Lumbar Spondylolysis and Spondylolisthesis. *Treasure Island: StatPearls*. 2018.
40. Bonetti M, Fontana A, Albertini F. CT-guided oxygen-ozone treatment for first degree spondylolisthesis and spondylolysis. *Acta Neurochir Suppl*. 2005;92:87-92.
41. R. Jinkins J. The pathoanatomic basis of somatic, autonomic and neurogenic syndromes originating in the lumbosacral spine. *Rivista di Neuroradiologia*. 1995;8:S35-51.
42. Bocci V, Luzzi E, Corradeschi F, et al. Studies on the biological effects of ozone: 4. Cytokine production and glutathione levels in human erythrocytes. *J Biol Regul Homeost Agents*. 1993;7:133-138.
43. Bocci V. Does ozone therapy normalize the cellular redox balance? Implications for therapy of human immunodeficiency virus infection and several other diseases. *Med Hypotheses.* 1996;46:150-154.
44. Bocci V, Luzzi E, Corradeschi F, Silvestri S. Studies on the biological effects of ozone: 6. Production of transforming growth factor 1 by human blood after ozone treatment. *J Biol Regul Homeost Agents.* 1994;8:108-112.
45. Muto M, Andreula C, Leonardi M. Treatment of herniated lumbar disc by intradiscal and intraforaminal oxygen-ozone (O_2-O_3) injection. *J Neuroradiol*. 2004;31:183-189.
46. Lu W, Li YH, He XF. Treatment of large lumbar disc herniation with percutaneous ozone injection via the posterior-lateral route and inner margin of the facet joint. *World J Radiol*. 2010;2:109-112.
47. Saal JA, Saal JS, Herzog RJ. The natural history of lumbar intervertebral disc extrusions treated nonoperatively. *Spine (Phila Pa 1976)*. 1990;15:683-686.
48. Alexandre A, Coro L, Azuelos A, et al. Intradiscal injection of oxygen-ozone gas mixture for the treatment of cervical disc herniations. *Acta Neurochir Suppl*. 2005;92:79-82.
49. Ogrendik M. Rheumatoid arthritis is an autoimmune disease caused by periodontal pathogens. *Int J Gen Med*. 2013;6:383-386.
50. Rhee DK, Marcelino J, Baker M, et al. The secreted glycoprotein lubricin protects cartilage surfaces and inhibits synovial cell overgrowth. *J Clin Invest.* 2005;115:622-631.
51. Cassim B, Shaw OM, Mazur M, et al. Kallikreins, kininogens and kinin receptors on circulating and synovial fluid neutrophils: role in kinin generation in rheumatoid arthritis. *Rheumatology (Oxford)*. 2009;48:490-496.
52. Ma MH, Kingsley GH, Scott DL. A systematic comparison of combination DMARD therapy and tumour necrosis inhibitor therapy with methotrexate in patients with early rheumatoid arthritis. *Rheumatology (Oxford)*. 2010;49:91-98.
53. Cho HY, Morgan DL, Bauer AK, Kleeberger SR. Signal transduction pathways of tumor necrosis factor--mediated lung injury induced by ozone in mice. *Am J Respir Crit Care Med*. 2007;175:829-839.
54. Fakhrzadeh L, Laskin JD, Laskin DL. Ozone-induced production of nitric oxide and TNF-alpha and tissue injury are dependent on NF-kappaB p50. *Am J Physiol Lung Cell Mol Physiol.* 2004;287:L279-285.
55. Chen H, Yu B, Lu C, Lin Q. The effect of intra-articular injection of different concentrations of ozone on the level of TNF-alpha, TNF-R1, and TNF-R2 in rats with rheumatoid arthritis. *Rheumatol Int*. 2013;33:1223-1227.
56. Hasan O, Jessar M, Ashar M, Noordin S, Ahmad T. Systemic sclerosis: Clinical manifestations, anesthetic and orthopedic considerations in a patient. *Int J Surg Case Rep.* 2018;42:24-28.
57. Nowicka D. Thermography improves clinical assessment in patients with systemic sclerosis treated with ozone therapy. *BioMed Research International*. 2017;2017:7.
58. Buskila D. Fibromyalgia, chronic fatigue syndrome, and myofascial pain syndrome. *Curr Opin Rheumatol*. 1999;11:119-126.
59. Stone JR, Yang S. Hydrogen peroxide: a signaling messenger. *Antioxid Redox Signal*. 2006;8:243-270.
60. Bocci V, Borrelli E, Travagli V, Zanardi I. The ozone paradox: ozone is a strong oxidant as well as a medical drug. *Med Res Rev*. 2009;29:646-682.
61. Bocci V. *Ozone: A New Medical Drug*. Dordrecht, The Netherlands: Springer. 2005.
62. Bocci V. *Oxygen-Ozone Therapy: A Critical Evaluation*. Springer Science & Business Media. 2013.
63. Vélez BPL. Ozone therapy, a supplement for patients with fibromyalgia. *Revista Española de Ozonoterapia.* 2014;4:39-49.
64. Clavo B, Perez JL, Lopez L, et al. Effect of ozone therapy on muscle oxygenation. *J Altern Complement Med*. 2003;9:251-256.
65. Giunta R, Coppola A, Luongo C, et al. Ozonized autohemotransfusion improves hemorheological parameters and oxygen delivery to tissues in patients with peripheral occlusive arterial disease. *Ann Hematol.* 2001;80:745-748.
66. Ballardini E. *Oxygen-ozone therapy for spinal muscle disorders in the horse.* Vol 42005.
67. Bhatt J, Bhat A, Dhama K, Amarpal A. An overview of ozone therapy in equine: an emerging healthcare solution. *J Exp Biol Agric Sci*. 2016;4:S203-210.
68. Freburger JK, Holmes GM, Agans RP, et al. The rising prevalence of chronic low back pain. *Arch Intern Med*. 2009;169:251-258.
69. Alexandre A, Buric J, Paradiso R. Intradiscal injection of O2-O3 to treat lumbar disc herniations: Results at five years. *Rivista Italiana di Ossigeno-Ozonoterapia*. 2002;1:165-169.
70. Ueno I, Hoshino M, Miura T, Shinriki N. Ozone exposure generates free radicals in the blood samples in vitro. Detection by the ESR spin-trapping technique. *Free Radic Res*.1998;29:127-135.
71. Borrelli E. Mechanism of action of oxygen ozone therapy in the treatment of disc herniation and low back pain. *Acta Neurochir Suppl*. 2011;108:123-125.
72. Bocci VPR, Pogni R, Corradeschi F, et al. Oxygen-ozone in orthopaedics: EPR detection of hydroxyl free radicals in ozone-treated "nucleus pulposus" material. *Neuroradiol J*. doi:10.1177/197140090101400106.
73. Torri G, Grazia AD, Casadei C. Clinical experience in the treatment of lumbar disk disease, with a cycle of lumbar muscle injection of an oxygen + ozone mixture. http://www.biaccabi.com/edocs/um_torri.html. Accessed at 2018-09-03.
74. Magalhaes FN, Dotta L, Sasse A, Teixera MJ, Fonoff ET.Ozone therapy as a treatment for low back pain secondary to herniated disc: a systematic review and meta-analysis of randomized controlled trials. *Pain Physician*. 2012;15:E115-129.
75. Lee SY, Kim TH, Oh JK, Lee SJ, Park MS. Lumbar stenosis: a recent update by review of literature. *Asian Spine J*. 2015;9:818-828.
76. Baeza-Noci J. Spinal ozone therapy in lumbar spinal stenosis. *Int J Ozone Ther.* 2007;6:17-24.
77. Rosenberg SK, Grabinsky A, Kooser C, Boswell MV.Effectiveness of transforaminal epidural steroid injections in low back pain: a one year experience. *Pain Physician*. 2002;5:266-270.
78. Abdi S, Datta S, F Lucas L. Role of epidural steroids in the management of chronic spinal pain: a systematic review of effectiveness and complications. *Pain Physician*. 2005;8:127-143.
79. Bocci V, Paulesu L. Studies on the biological effects of ozone 1. Induction of interferon gamma on human leucocytes. *Haematologica*. 1990;75:510-515.
80. Di Filippo C, Cervone C, Rossi C, et al. Antiarrhythmic effect of acute oxygen-ozone administration to rats. *Eur J Pharmacol.* 2010;629:89-95.
81. Pryor WA, Squadrito GL, Friedman M. A new mechanism for the toxicity of ozone. *Toxicol Lett*. 1995;82:287-293.
82. Pryor WA, Squadrito GL, Friedman M. The cascade mechanism to explain ozone toxicity: the role of lipid ozonation products. *Free Radic Biol Med.* 1995;19:935-941.
83. Mustafa MG. Biochemical basis of ozone toxicity. *Free Radic Biol Med*. 1990;9:245-265.

Received: 2018-05-30
Accepted: 2018-08-09

C-Editor: Yang LJ, Zhao M; S-Editor: Yu J; L-Editor: Wang L; T-Editor: Jia Y