REVIEW ARTICLE

An Overview of Ozone Therapy for Treating Foot Ulcers in Patients With Diabetes

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ABSTRACT

Diabetic foot ulcer (DFU) is one of the most common and severe complications of diabetes mellitus, which is becoming increasingly prevalent throughout the world, with high mortality and morbidity. Because of the complex pathophysiological processes involved, DFU is difficult to treat effectively with traditional therapies. Ozone therapy, an emerging method, has been reported as potentially beneficial for closure of DFUs and may gradually move to the forefront of clinical practice. Possible mechanisms of action include antioxidant capacity, pathogen inactivation, vascular and endogenous growth factor modulation, and immune system activation. However, some researchers are skeptical about its safety, and clinical trials are lacking. This article reviews the current research and application of ozone therapy for DFUs.

Key Indexing Terms: Ozone therapy; Diabetic foot ulcer; Mechanism of action; Administration safety. **[Am J Med Sci 2020;■(■):1–8.]**

INTRODUCTION

Diabetes mellitus is a disease characterized by hyperglycemia and glycosuria, caused by impaired insulin function or secretion.[1] Poor long-term management of blood glucose and high plantar pressure are responsible for diabetic foot ulcers (DFUs), one of the quintessential complications of diabetes mellitus, defined as full-thickness wounds that penetrate the dermis (the deep vascular and collagenous inner layer of the skin) and are located below the ankle in diabetic patients.[2] It is conjectured that 125 million people will develop foot ulcer(s) among more than 500 million patients with diabetes by 2025 worldwide.[3] The global prevalence of DFU is 6.3% in the overall population and is expected to rise in the future.[4] Because of repeated infections and pathologic mechanisms such as vascular lesions and neuropathy stemming from diabetes mellitus, conventional medical and surgical treatments of DFUs are not satisfactory. DFUs account for a high proportion of hospital admissions, and gangrene or even amputation can result if the lesion is not treated as soon as possible, leading to nearly 80% of all nontraumatic lower limb amputations.[5,6] Increasing morbidity and mortality have motivated researchers to explore new methods. Ozone, a gas composed of 3 atoms of oxygen with a cyclic structure, was used to treat gangrene during World War I.[7] With the development of pharmaceutical science, ozone therapies have shown positive effects on a variety of acute and chronic ailments such as coronary artery disease, hypersensitive teeth, periodontitis, chronic low back pain, chronic severe hepatitis, and mucosal and cutaneous infections.[8] The use of ozone therapy to treat chronic nonhealing wounds and increasing evidence of its value in treating DFUs have been reported in previous studies. However, it has been approached with suspicion of its potential toxicity, based on its inherently unstable nature and use at inappropriate dosages. This article summarizes the present situation, potential mechanism of action and safety of ozone therapy for DFUs.

PATHOGENESIS, DIAGNOSIS AND CONVENTIONAL THERAPY FOR DFUS

Studies show that independent risk factors such as active smoking, obesity and hyperlipidemia predispose patients with type 2 diabetes to develop DFUs.[9] DFUs associated with neuropathy and angiopathy are characterized by impaired wound healing and prolonged closure time.[10] Diabetic neuropathy results in reduction or loss of sensation and foot dysmorphia, which subjects the foot to increased localized pressure, leading to callus and tissue injuries and eventual ulcer formation.[10] Besides persistent opportunistic infections and mitochondrial destruction in repair cells with growing apoptosis, free radicals increase, and high oxidative stress and antioxidant reduction in the presence of high glucose levels contribute to inadequate tissue perfusion after macrovascular and microvascular damage.[10-13] The intelectin-1 gene is believed to be a protective factor in DFUs with vascular complications, encoding omentin for endothelial vasodilator

and anti-inflammatory activity.[6,14] However, a study demonstrated the relationship between the incidence of DFUs and rs2274907, the allelic variant of the intelectin gene, concluding that the variant is associated with increased prevalence of DFU for men with single-nucleotide variants.[9] Given that tumor necrosis factor-α (TNF-α) is overexpressed in people with DFUs, the TNF-α-308AA and GA genotypes have been reported to increase susceptibility to DFUs.[15] There is a study considering that the ankle-brachial pressure for assessing vascular damage is unavailable in cases of serious limb ischemia.[16] In addition, Tehan et al[17] found that continuous wave Doppler was both more sensitive and more accurate than toe-brachial index or ankle-brachial pressure index for assessing peripheral arterial disease in patients with diabetes mellitus.

Conventional management of DFUs includes not only diagnosis and classification of the wound, vascular assessment and optimization of medical treatment but also local surgical management including offloading the affected foot, debriding the necrosis quickly and revascularizing the limb when indicated.[18] Annual diabetic screening and preventive care for those at high risk of diabetic foot complications are essential. Emerging surgical techniques such as maggot biological debridement, skin grafting, skin flap transplantation, skin distraction closure to repair soft tissue defects, fat transplantation, platelet-rich plasma for refractory wounds after infection control, lower extremity artery bypass grafting, lower extremity vascular bypass pressure perfusion therapy and tibia lateral moving techniques have been reported.[19] Although conservative remedies are effective for severely infected wounds, prolonged hospital duration is troublesome and the aforementioned emerging methods are not readily available. Although hyperbaric oxygen therapy significantly reduces the occurrence of amputations for diabetic ischemic foot ulcers, there is insufficient evidence and limited availability.[10,20]

MECHANISM OF ACTION FOR OZONE TREATMENT OF DFUS

Antioxidant Capacity

Besides creating hydrogen peroxide (H_2O_2), a reactive oxygen species (ROS) and a mixture of lipid ozonation products, this transient and moderate oxidative stress caused by ozone therapy has increased activation of the transcriptional factor mediating nuclear factor erythroid 2-related factor 2 (Nrf2) simultaneously.[21] The Nrf2's domain is responsible for activating the transcription of antioxidant response elements capable of promoting formation of antioxidant enzymes such as superoxide dismutase, which scavenge free radicals relevant many diseases.[22] Ozone can be used for a longer time to reestablish the balance of the redox system through oxidative preconditioning, stimulating and/or preserving the endogenous antioxidant systems and interfering with the xanthine/xanthine oxidase pathway for ROS production.[23,24] Ozone may have a hormetic role in regulating the anti-inflammatory and pro-inflammatory effects of carbon monoxide, including prostaglandin formation, akin to those of nitric oxide, which has been shown to exert some of its biological actions by modulating prostaglandin endoperoxide synthase activity.[25] Animal models have demonstrated the beneficial effects of prophylactic ozone therapy in controlling the age-related effects of oxidative stress, mediating a mechanism involved in rebalancing the dysregulated redox state in the heart and hippocampus of rats.[26,27] Diabetic patients with hyperglycemia exhibited increased oxidant stress during lipid peroxidation and reduced antioxidant capacity associated with antioxidant depletion via several mechanisms, stimulating auto-oxidative glycosylation, the formation of advanced glycation end products and increasing polyol pathway activity, aggravating destructive processes in the diabetic foot.[28,29] A significant finding of the study was that ozone treatment (1.1 mg/kg with an ozone concentration of 50 μg/mL via rectal insufflation) improved glycemic control and prevented oxidative stress in streptozotocin-induced diabetic rats.[30] Another study concluded that in combination with surgical treatment in patients with diabetic foot, ozone therapies have a positive effect on wound healing by increasing antioxidant capacity, thereby decreasing the duration of hospital stays.[29] A 4-year prospective study hypothesized that an imbalance between ROS and antioxidants is an important pathogenic factor causing insulin resistance, fully supporting a direct link between the presence of H_2O_2 and impaired glucose uptake in adipocytes.[31] Ozone treatment is superior to control treatment in decreasing hyperglycemia, increasing insulin sensitivity and increasing the capacity to maintain cellular redox balance for neuroinfectious diabetic foot associated with hyperglycemia and severe vascular damage.[32] On the other hand, the hypoglycemic effect of ozone treatment is attributed to the inhibition of glycogen depletion and a decrease in the availability of free glucose. However, some toxicologic studies have noted that insulin resistance can be induced by ozone inhalation through muscle c-Jun N-terminal kinase activation in the context of a lean phenotype and no preexisting hyperglycemia, which is opposite to the findings that inhalation exposure to ozone increases insulin sensitivity, probably through a weight loss and leptin sensitization-dependent mechanism, besides stimulating marked local and systemic inflammation in KKAy mice (a model of obese type 2 diabetes characterized by severe hyperglycemia, hyperinsulinemia and insulin resistance in early life).[33,34] The study showed that high doses of ozone increase oxidant enzyme levels and decrease antioxidant enzyme levels, contrary to stimulating the antioxidant defense systems in low doses.[35] Researchers have argued that the total antioxidant status and plasma protein thiol group levels of a blood

sample indicate the precise doses of ozone needed to optimize treatment. An individual treatment would achieve the correct dosage on a day-by-day and case-by-case basis by developing more accurate antioxidant status indicators.[36]

PATHOGEN INACTIVATION

Bactericidal effects of ozone *in vitro* have been confirmed by a wide variety of studies. As a potent oxidant, ozone destroys bacterial cell walls by oxidizing phospholipids and lipoproteins on the cytosolic membrane and thereby destroying its integrity. As this occurs, accompanied by changes in the bacterial envelope's permeability, ozone infiltrates the microorganism to oxidize glycoproteins and glycolipids and damage enzymatic function, causing cell death and lysis.[21] Specifically, a previous study has demonstrated that ozone therapy as an adjunct to vancomycin increases the ability to eliminate methicillin-resistant *Staphylococcus aureus* mediastinitis in animal models.[37]

The precise antimicrobial mechanisms *in vivo* by which ozone is used for patients with DFUs are not clearly known. A trial was carried out in which ozone-oxygen treatments in conjunction with conventional treatment conferred a significantly higher rate of complete wound closure than sham treatment among patients with DFUs who completed the study protocol, especially when foot ulcers with a surface area <5 cm^2.[10] And superiority of zone treatment in this trial might be implicated in bactericidal capabilities, controlling the wound infections and increased fluid drainage induced negative pressure by the ozone generation device.[10] A case study has also called attention to the role of ozone in the treatment of Buruli ulcer (*Mycobacterium ulcerans*) applied by insufflation with a sealed bag; the ulcer was eventually eradicated, perhaps via the same mechanism mentioned above.[38] Marked bactericidal effects in wound cleansing, which improves wound healing and decreases the frequency, expenditure, and complications of antibiotic therapy, have been reported in a case series study that demonstrated successful treatments in 87% (46% of them complete healing) of patients with chronic nonhealing wounds, among whom 50 (79%) had diabetic foot problems treated with ozone.[20] Rosul and Patskan[39] observed decreased microbial colonization of wounds in patients with high levels of colonization by microorganisms after traditional therapy along with systemic and regional ozone treatment. Ozone can be used as a disinfectant and healing stimulant, fully supporting the study that reported reduced microbial colonization, enhanced growth and prolonged decontamination were shown in a group treated with ultrasonic cavitation and ozone therapy.[40] These effects may offer insight into the role of ozone treatment in diabetic foot bacterial infections. Nevertheless, whether ozone's antimicrobial action is influenced by different environments *ex vivo* and by germicidal spectrum remains to be confirmed for the treatment of common drug-resistant infections in DFUs.

HEMORHEOLOGY AND ENDOGENOUS GROWTH FACTOR MODULATION

Given the more efficient mitochondrial respiratory chain in the presence of ozone therapy, oxygen levels inside the cell increase as a result of transmembrane flow of oxygen.[41] By facilitating the glycolytic rate, ozone autohemotransfusion increases adenosine triphosphate and 2,3-diphosphoglycerate in the cell. For this reason and because of the Bohr effect, the oxygen-bound hemoglobin is unloaded into ischemic tissues more easily, a direct consequence of rightward shift of the oxygen-hemoglobin dissociation curve.[21] Many studies have provided evidence that reducing chronic oxidative stress by ozone therapy promotes erythroblast differentiation, contributing to a progressive increase in erythrocytes and oxygen carrying capacity.[21] It has been found that prostacyclin, a known vasodilator, can be induced when ozone is applied.[42] Additionally, some researchers have speculated that an increase in endothelial production of nitric oxide is associated with ozone's inducement of antioxidant enzymes. Although the activity of nitric oxide is disrupted by endothelial generation of superoxide, ozone triggers the enzymes to ameliorate the negative effects of ROS responsible for deleterious vasoconstriction.[43] Ozone may also induce a hypocoagulative state with prolonged thrombin time and partial thromboplastin time and increased tissue plasminogen activator.[44] Therefore, these complex mechanisms improve blood circulation and oxygen delivery to affected ischemic tissues. Other studies of ozone's effects have shown that it reduces the blood viscosity of patients with peripheral arterial disease and increases pain-free walking distance.[10,45]

DFU is characterized by decreased collagen deposition related to reduced expressions of endogenous growth factors, which include angiogenic and leukocyte chemotactic factors such as vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-β), and platelet-derived growth factor (PDGF), which are vitally important for formation of granulation tissue, ulcer epithelialization and wound closure.[46,47] Increasing the expression or level of growth factors with ozone treatment has been confirmed to be useful in the wound healing of DFUs, as demonstrated by Kim et al,[48,49] who proposed that the therapeutic mechanism of ozonated olive oil on acute cutaneous wound healing is attributable to increased expression of PDGF, TGF-β and VEGF. A study found that the oxygen-ozone treatment via bagging significantly increased the rate of wound healing in association with higher expressions of VEGF, TGF-β and PDGF in the ozone group than in the control group at the early stage of DFUs.[47] Although adequately powered, high-quality randomized controlled trials with longer follow-up durations are lacking, the effects of ozone

treatment on hemorheology in patients with DFUs should be considered.

IMMUNE SYSTEM ACTIVATION

The development of inflammatory responses in patients with DFUs is related to infections due to immunologically mediated acute phase reactions manifested by increased levels of amyloid A and C-reactive protein. Studies devoted to the influence of immune changes in the course of long-term diabetes showed significantly reduced chemotaxis of neutrophils and impaired phagocytic activity associated with reduced monocyte adhesion to vascular endothelium.[50] In addition, it has been reported that elevated levels of circulating and pathogenic immune complexes and their deposition in the endothelium lead to an inflammatory response by activating the complement cascade. Complements C1, C2, C3 and C4 were significantly higher in type 2 diabetic patients than in healthy controls, inducing the formation of microvascular and macrovascular disease.[51]

Ozone reacts with polyunsaturated fatty acid *in vivo*, forming various peroxidation compounds and H_2O_2, which has been shown to play a regulatory role in the process of signal transduction, promoting a plethora of immune responses such as increases in tumor necrosis factor (TNF) interferon, and interleukin (IL)-2.[21,52] The increases in IL-2 are correlated with initiation of immune response mechanisms. Additionally, H_2O_2 mediates the activity of nuclear factor kappa B (NF-κB) and TGF-β by facilitating the action of tyrosine kinases that will phosphorylate I-kappa B, a subunit of the transcription factor NF-κB.[53] These 2 factors are known to increase immunoactive cytokine release and upregulation of tissue remodeling. A previous study called attention to the link between a sufficient rise in H_2O_2 levels and notable increase in IL-8, which also activate NF-κB, allowing production of ROS scavengers. Low doses of ozone have been confirmed to inhibit prostaglandin synthesis and increase secretions of macrophages and leukocytes. These effects may offer insight into why a reduction in infection is seen when ozone therapy is used for DFUs. They found that erythrocyte sedimentation rate was a strong diagnostic factor of osteomyelitis and severe infection, and high C-reactive protein levels could be a prognostic risk factor for foot amputation in DFU.[18] Their study demonstrates that average healing time is significantly lower than the median healing time measured in the control group, and some previous studies found that systemic use of ozone therapy reduced C-reactive protein and erythrocyte sedimentation rate to less than those of the control group.[18] However, some epidemiological studies suggest that diabetic patients may be more vulnerable to the adverse health effects of exposure to high ambient concentrations of ozone. It has been well established that macrophages play a central role in these inflammatory responses. New research in KKAy mice provides important findings that ozone inhalation induces marked systemic inflammation, as evidenced by increases in plasma TNF-α levels, messenger ribonucleic acid expression of pro-inflammatory cytokines, and pro-inflammatory macrophage infiltration in epididymal adipose tissues and lung, but does not significantly change the profile of T cells isolated from pulmonary lymph nodes, suggesting that the role of T cells in ozone-induced inflammation may be trivial.[34] The intraperitoneal administration of ozonation isotonic sodium chloride solution in conditions of chronic inflammatory processes in diabetic old rats with alloxan-modeled diabetes did not have protective effects on the hemostasis system and proteolysis of blood plasma.[54] The influence of ozone treatment on the immune system during ulcer healing is associated with administration dosage and complex physiological mechanisms that are only partly understood.

ADMINISTRATION SAFETY OF OZONE THERAPY

Offloading and debridement of the area around the DFU are important in initial therapy for neuropathic ulcerations, and proper footwear is essential to alleviate unnecessary pressure to the foot. Indications for ozone therapy for diabetic wounds are listed in Table 1. There are 2 forms of ozone therapy for wounds: topical treatment and systemic treatment. Topical treatment includes bagging, tenting, compresses, local injection and ozonized olive oil. Systemic treatment includes rectal insufflation and autohemotherapy.[20] Researchers believe that the toxicity of ozone depends on the dosage, and controlling the dosage and choosing the administration method carefully are essential.[55] Topical ozone treatment is appropriate for wide areas such as the trunk or buttocks. A study evaluating the role of ozone therapy in managing diabetic foot problems found that the ozone-oxygen mixture via ozone bagging is initially applied at higher concentrations (70-80 μg/mL) to exert its oxidative effect on necrotic tissue, is decreased to lower concentrations (60-40 μg/mL) for microbicidal effects, and can be reduced further (≤25 μg/mL) for the metabolically stimulating and immunomodulatory effects of ozone as the healing process continues.[20] The frequency of bagging is gradually decreased over the prolonging of treatment time. Ozone tent administration procedures, dosages and frequency are exactly the same as those for bagging.[20] A burning sensation caused by topical intolerance to ozone can occur. Skin irritation and even dermatitis may follow topical application at high ozone concentrations, and respiratory irritation may result from the escape of ozone from the generator. Ozone therapy significantly increases airway resistance without changing the compliance or elastic characteristics of the lung, but this pulmonary toxicity depends on critical parameters such as the alveolar surface area, minimal volume of the alveolar surface lining (about 30 mL), minimal antioxidant capacity (many times less than that of plasma), and

TABLE 1. Diabetic wounds indications for ozone therapy.

Study	Type of study & sample size	Concentration and route of ozone administration	Measured parameters	Findings & side effects	Mechanism of action
Wainstein et al[10]	Double-blind, randomized, placebo-controlled clinical trial. n = 61 (ozone group: n = 32;placebo group: n = 29)	Ozone group: ozoter 101 device a noninvasive sealed chamber using ozone-oxygen mixture in 2 phases. Phase 1 delivered 96% oxygen and 4% ozone (80 μg/mL) for up to 4 times a week for 4 weeks. Phase II delivered 98% O_2 and 2% O_3 (40 μg/mL) until the 12th week. Placebo group: sham treatment along with usual diabetic foot ulcer care.	Wound closure	Of the patients completing per protocol, wound closure was significantly greater than controls ($P = 0.03$), expressly in patients with small ulcers initially (≤5 cm^2). Side effects: control group (n = 2) ozone group (n = 5, including osteomyelitis, fever, wound infection and pulmonary congestion)	Bactericidal capabilities and a Reduction of blood Viscosity improves Perfusion; Induced negative pressure by the device may enhance fluid removal and increase perfusion;
Izadi et al[18]	Randomized, grouped single-blind randomized clinical trial. n = 200 (group1: n = 100; group 2: n = 100)	Group 1: ozone therapy: local (bagging and ozonized olive oil and solution) and systemic ozone through rectal or intravenous (minor and major ozone therapy) twice a week (at an interval of at least 24 h) until wound closure besides the standard DFU treatment. Group2: only the standard DFU treatment	Wound size, wound grade, healing time, FBS, ESR, and CRP before and after treatment. Baseline values in average surface area of ulcers and FBS, ESR, CRP are not significantly different	All complete wound closure in the ozone group. ozone group has significantly lower mean healing time (p: 0.012) and lower amputation rate after treatment, FBS is increased while CRP, ESR are decreased in control group, three variables in ozone group are decreased and these values are less than control group .No side effects	Ozone therapy reduces the FBS level significantly due to systemic using. The antioxidant effect; reduction of infections.
Myroslav et al[29]	Case study with 2 groups (n = 47) Subgroup A: n = 23 Subgroup B: n = 24 Baseline values not significantly different in measured parameters	Subgroup A: traditional treatment along with systemic therapy via physiologic saline ozonized using intravenously + regional therapy via ozonized 0.9% NaCl solution and ozonized sea buckthorn oil for 12-14 days, one session per day. Subgroup B: traditional treatment sugar correction, anticoagulants and daily dressing with antiseptics	Wound healing time; cytological examination from wounds; level of malondialdehyde, ceruloplasmin and catalase, length of hospital stay	Significantly faster in wound healing, regenerative type of cellular reaction, malondialdehyde lower and ceruloplasmin, catalase higher after therapy ($P < 0.02$); reductions of hospital stay in subgroup A; degenerative type of cellular reaction; aforementioned parameters no sizeable changes after treatment ($P > 0.05$) in subgroup B, No side effects	Improvement of lipid peroxidation, greater antioxidant protection; regenerative cellular reaction

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TABLE 1. (*continued*)

Study	Type of study & sample size	Concentration and route of ozone administration	Measured parameters	Findings & side effects	Mechanism of action
Martinez-Sanchez et al[32]	Randomized grouped Controlled clinical trial n = 101 (group 1: n = 52; group 2: n = 49)	Group 1 treatment: local treatment via sealed bag with ozone (60 mg/L) and ozonized sunflower oil + 20 sessions of ozone via rectal insufflation (50 mg/L) Group 2 treatment: topical and systemic antibiotics	Glycemic index; lesion size; biochemical markers of oxidative stress; endothelial damage	Group 1 vs. Group 2 observations: significantly lower blood glucose and more reduction in wound size, fructolysine, advanced oxidation protein products, malondialdehyde, peroxidation, potential, and total peroxides .More increase in nitric oxide, reduced glutathione, glutathione peroxidase. No side effects	Activation of SOD; preventing oxidative stress; increasing insulin sensitivity; maintain the cellular redox balance
Zhang et al.[47]	Randomized controlled clinical trial. n = 50 (ozone group: n = 25; control group: n = 25)	Ozone group: noninvasive oxygen-ozone treatment in a special bag for 30 min per day for 20 days plus standard treatment. Control group: only standard treatment including debridement once every two days and wound dressings	0-3 grades therapeutic effect on wound closure; wound size at baseline and day 20; Tissue biopsies at baseline and day 11; expressions of vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-β), and platelet-derived growth factor (PDGF) proteins	Ozone group has significantly higher effective rate than that of control group (92% vs. 64%, $P < 0.05$). More reduction of wound size ($P < 0.001$), more collagen fibers ($P = 0.012$), VEGF, and PDGF levels significantly higher ($P < 0.05$), significantly higher TGF-β, at day 11 ($P < 0.05$) in ozone group. No side effects	Partially due to potential induction of VEGF, TGF-β, and PDGF at early stage of the treatment

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; FBS, fasting blood sugar.

the cumulative effect of inhaling ozone over months. Ozonized water compresses are ideal for recent superficial wounds or after a course of bagging, and they soften the tissue to prepare a safe and clean field for debridement of necrotic wounds.[20] Local subcutaneous ozone injection is not routine and is used only when indicated. Note that intralesional ozone injection has not yet been applied in any of the studies involving DFUs, and the utility and safety of this regimen in the treatment of chronic wounds have not been reliably ascertained. Some studies recommend administering injections only with daily monitoring of the injected wound, by an operator with good experience and training, and ozone therapy is not recommended for deep, heavily infected or necrotic wounds.[20] One study reported the case of a diabetic patient who developed severe foot necrosis and infection after receiving intralesional ozone injections for a nonhealing wound because the superficial infection was driven into deeper tissue.[56] With the exception of the venipuncture and an occasional minor extravasation of blood, clinical improvement in edema and pain was seen with ozone autohemotherapy, and further progress was slow but steady and promoted complete healing of ulcerations in diabetic patients afflicted with intractable leg ulcers. In a study of a patient with diabetic foot gangrene, Wagner classification 4 has shown satisfactory wound healing after daily bagging with daily rectal insufflation, followed by treatment with ozonated olive oil until healing is complete.[20] Although rectal insufflation is convenient and practically free of risks or adverse reactions if dosages are well controlled, teratogenic effects of this application method in pregnant rats have been seen but are not understood.[6] Although the side effects of ozone therapy have markedly decreased because of the potent antioxidant capacity of human plasma and ozone concentrations precisely measured in real time within the precise therapeutic range ascribed to modern ozone generators, knowing its toxicity and orthodox application according to well-defined and safe protocols is important. Given their low antioxidant capabilities, direct contact with the eyes and lungs by ozone, a potent oxidant, should be avoided. One study identified and reviewed 3 relevant clinical trials (212 participants total) by searching the medical literature up to March 3, 2015, that investigated ozone therapy for DFUs, and the researchers were unable to draw any conclusions about the effectiveness of ozone therapy for treating DFUs, because the studies were too small to reliably represent clinically meaningful differences, with methodological flaws and poor quality.[8] Although ozone therapy might reduce length of hospital stay and wound size in the short term compared with antibiotics, there is no evidence suggesting it promotes overall healing or reduces the number of adverse events.[8,32] When applied together with standard therapy, ozone may not reduce ulcer size compared with standard therapy alone. There was no evidence of an increase in adverse events with ozone therapy, and the intention-to-treat analyses did not demonstrate a significant benefit of ozone therapy over standard therapy (41% versus 33%, $P = 0.34$).[8,10]

CONCLUSIONS

The mechanisms of action of ozone have not been elucidated completely, and the therapeutic window for ozone dosage has not been standardized to minimize the risk of toxicity. Adjunctive therapies should be applied with caution after conventional management strategies fail. More controlled clinical trials are warranted to demonstrate the comprehensive effects on DFUs.

AUTHOR CONTRIBUTIONS

QW and QC conceived the concept of the review. QW wrote the manuscript. QC designed and formatted the table. QW and QC read, edited and reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

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