Ozone therapy for diabetic foot

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Abstract

Diabetic foot ulcers (DFU) are a burden to the diabetic community. With increasing medical bills, to unsuccessful treatment, those suffering from DFUs can use alternative therapeutics. First seen in the mid-1800s, ozone (O_3) is thought to be unstable, due to inherent molecular nature. With the help of pharmaceutical science, various O_3 treatments have flourished in the medical community to help those suffering from DFUs. Promising results are seen through numerous studies. Usually, a mixture of both O_2 and O_3 is seen in pressurized machines as administered to the foot ulcer. Foot ulcers, specifically DFUs, need to be assessed, cleaned, and treated as fast as possible for the fastest results. Results such as amputation can be seen if the foot is not attended to as soon as possible. With fast growing clinical trials in O_3 therapy and quick administration of the O_3, O_3 therapy may be on the rise to be at the forefront of treating DFUs. Compelling evidence is seen in clinical trials, but more must be done to fully understand the role of O_3 in DFUs.

Key words: ozone therapy; wound closure; oxygen-ozone; diabetic foot ulcer; diabetes mellitus; Charcot foot; peripheral arterial disease; wound healing

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Introduction

Foot ulcers have an incidence of 4–10% in patients diagnosed with diabetes mellitus and a lifetime incidence of around 25%.[1] Furthermore, it is clear that patients that present diabetic foot ulcers (DFU) are hospitalized more often than without DFU. Amputations can be attributed to ischemia, infection, neuropathy, faulty wound healing, ulceration, gangrene, and initial minor trauma.[2] Recurrent infections in patients suffering from type 2 diabetes mellitus (T2DM) coupled with high plantar pressure are a major determining factor for DFUs.[3] Common treatments of DFU is debridement, offloading areas of friction and other conventional wound managements.[4] If treatment is delayed, the neuropathic and vascular complication of DFU can lead to gangrene or even amputation.[5] Not only is foot problems of diabetic patients seen as a major factor when considering morbidity, there seems to be a cost burden with heavy expenditure associated with treatment for the diabetic foot (DF).[6] The use of ozone (O_3) within the medical community has begun in the mid 19th century.[7] Medicinal application of O_3 is met with great discourse due to its inherently unstable nature. However, it is believed that O_3 can be initiated to pharmaceutical science with great therapeutic benefit to specific biological systems and not just serve as an esoteric approach.[8] O_3 has been seen to be used in many medicinal applications ranging from uses in dentistry to proper sterilization of medical instruments.[7,9] Beneficial effects of O_3 has been seen in orthopedics, mucosal and cutaneous infections.[10] Future effects can show O_3 therapy being used to treat heart failure.[11] Furthermore, there is growing evidence that O_3 can be used to treat DFUs. A study showed that O_3 treatment via rectal insufflation can improve glycemic index along with preventing oxidative stress in diabetic rats.[12] Efficacy can be measured by how well the wound has closed in DFU after O_3 treatment has been administered.[13] Herein we review the evidence for possible use of O_3 therapy on DFU.

Classification of Diabetic Foot Ulcers

There are several different ways to assess the severity of a DFU. The most common classification system surfaced in the 1970s named the Meggit-Wagner classification, summarized in **Table 1**.[14] The Meggit-Wagner classification system has been around the longest, however, some researchers believe it lacks overall substance to describe the ischemia and possible complications. In the early 1990s a more profound classification system, developed by the University of Texas, surfaced and is summarized in **Table 2**.[15] Although these two classification systems are both very much used today, it is believed that the Meggit-Wagner classification is too simple and the University of Texas classification is too complex. That is until Amit Jain proposed a new classification based on infective and non-infective complications as depicted in **Table 3**.[16]

Molecular Genetics of Diabetic Foot Ulcers

The Intelectin 1 (ITLN1) gene encodes a protein known as

Table 1: Meggit-Wagner classification system for diabetic foot ulcer[14]

Grade	Clinical manifestation
0	Pain
1	Superficial ulcers
2	Deep ulcers
3	Ulcers involving bone
4	Forefoot gangrene
5	Full foot gangrene

Table 2: University of Texas classification system for diabetic foot ulcer[15]

	Grade			
Stage	**0**	**1**	**2**	**3**
A	Lesion completely epithelized (pre-/post-ulcerative)	Superficial wound	Wound penetration to tendon or capsule	Wound penetration to bone or joint
B	Infection	Infection	Infection	Infection
C	Ischemia	Ischemia	Ischemia	Ischemia
D	Infection & Ischemia	Infection & Ischemia	Infection & Ischemia	Infection & Ischemia

Table 3: Amit Jain's classification system for diabetic foot ulcer[16]

Type	Lesion
Type 1 (infective)	Cellulitis, abscess, necrotizing fasciitis, wet gangrene, osteomyelitis, tinea pedis
Type 2 (non-infective)	Skin and soft tissue: non-healing ulcer, bullae
	Nerve: neuropathies
	Bone/joints: Charcot foot, hammertoes, claw toes
	Vessel: peripheral arterial disease
Type 3 (mixed)	Infective and non-infective complications

omentin. Inherently, omentin has endothelial vasodilator activity and anti-inflammatory actions.[17] For this reason, it is believed to be a protective factor in DFUs since they are vascular complications. Mrozikiewicz-Rakowska et al.[18] sought to evaluate the association of the allelic variant, rs2274907, of the ITLN gene and the occurrence of DF in patients diagnosed with T2DM. A study with 670 individuals was done; 204 individuals had T2DM with DF, 299 had T2DM with no signs of DF, and 167 individuals served as a healthy control. Mrozikiewicz-Rakowska et al.[18] found that neuropathy, ischemic heart disease, retinopathy, obesity, active smoking, and hyperlipidemia were all seen more frequently in patients with T2DM and DF than with T2DM and no DF. Furthermore, allele A of the rs2274907 variant was seen at a more frequent rate in the DF group than the healthy control. His team concluded the variant is associated with increased prevalence of DF.[18] He also found that allele A is sex-specific and is present in men. Therefore, men with single-nucleotide variants (SNV) are at risk for DF. Individuals present with DFU have overexpressed pro-inflammatory cytokines. The cytokines seen present are: tumor necrosis factor α (TNF-α), chemokines (*e.g.*, CCL2), stromal cell-derived factor 1 (SDF-1), and interleukins (IL) 1 and 6.[19] Dhamodharan et al.[20] studied the role and genotyped IL-6 (rs1800795), TNF-α (rs1800629), and SDF-1 (rs361525) SNVs in DFU. This study showed that IL-6-174 CC and GC genotypes protected against diabetes, but not no association with DFU was found. TNF-α-308 AA and GA was correlated with increased susceptibility towards diabetes and DFU. SDF-1-801 AA and GA role was similar to that IL-6-184 CC and GC with protection against DFU as well. This was studied via serum levels of IL-6, TNF-α, and SDF-1 variants, along with other diabetogenic markers.[20]

Diagnosis of Diabetic Foot Ulcers

Patients presented with DFUs are at risk for having peripheral arterial disease (PAD). Furthermore, nearly 50% of patients present with DFU are also present with PAD.[21] A common physical examination for diabetic patients with foot ulcers is the use of ankle brachial pressure-systolic (ABI-s). The use of the ABI-s test is to understand how severe PAD has advanced in patients with DFUs. However, diabetic patients tend to have calcified lower limb arteries, disrupting the systolic pressure in the arteries after the contraction of the heart. ABI-s can be falsely elevated due to the calcified lower limb arteries. A study shows that instead of performing ABI-s one can perform ankle brachial pressure-diastolic (ABI-d). Asbeutah et al.[22] had a size of 51 patients present with DFUs, 26 of which came present with calcified lower limbarteries and 25 of which did not present calcification. Another 25 persons were used as a control. Simply enough, both ABI-s and ABI-d were measured bilaterally *via* a brachial and ankle oscillometric pressure. The use of analysis of variance (ANOVA) showed statistical significance among people with the use of ABI-s and ABI-d leading to the conclusion that ABI-d may be a better tool for patients with DFUs with calcified arteries.[22] More studies have been done to see whether other forms of non-invasive vascular assessment can be performed to asses PAD more significantly. Common device-based diagnostic testing for patients with DFU and possible PAD includes color duplex ultrasonography, MR angiography, radiography, capillaroscopy, phlebography, continuous wave Doppler (CWD) and toe-brachial index (TBI).[23] Some researchers believe that ABI testing is not as effective as others such as CWD or TBI. A study was done where 117 participants were recruited with only 72 present with diabetes and 45 without diabetes. All patients received diagnostic testing *via* ABI, TBI, and CWD from the right lower limb. To properly assess accuracy a color duplex ultrasonography was used. Tehan et al.[24] determined that CWD was both the most sensitive and most specific in determining the presence PAD in patients with diabetes than TBI or ABI. Another study ruled out against ABI because of the significant limitation it places on diabetic patients presented with critical limb ischemia.[25]

Applying Ozone to Diabetic Foot Ulcers (Table 4)

A study demonstrated that ozonated water, along with ozonated oils, can be used as a disinfectant and healing stimulant.[10,26] This can reap great benefit for individuals suffering from DFUs. Furthermore, O_2–O_3 treatment can improve vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-β), and platelet-derived growth factor (PDGF) levels.[27] The aforementioned growth factors, specifically, TGF-β, seem to increase in patients with DFUs and can heal the localized gangrene and tissue remodeling.[28-30] O_3 causes platelets to aggregate, along with the release of the specific growth factors (*e.g.*, PDGF, TGF-β, and IL-8) which is known to heal wounds rapidly.[28] Furthermore, O_3, when applied to DFUs, eliminates pathogens and the O_2 promotes proliferation of

Table 4: Studies testing ozone for diabetic wound care

Study	Route of administration of O_3	Type of study	Patients information & sample size	Measured parameter	Findings
Wainstein et al.[13]	Ozoter 101 device a noninvasive sealed chamber using O_3-O_2 mixture	Randomized, double-blind, placebo-controlled clinical trial	*n* = 61, 62% male, average age 62.6 with a standard deviation of 9.8 years old	Wound size	Per protocol (PP): Ozone groups *vs.* placebo group: *P* = 0.03 ozone group significantly higher rate of wound closure. Ozone group with wound size < 5 cm *versus* sham group: *P* = 0.006 for total wound closure in ozone group and 50% in sham group
Rosul et al.[40]	Phase 1: 96% O_2 & 4% O_3 at 80 µg/mL 4 times a week for 4 weeks. Phase 2: 98% O_2 & 2% O_3 at 40 µg/mL until week 12	Case study with two groups	*n* = 47	Wound size, level of lipid peroxidation and antioxidant protection indexes, length of hospital stay	Group A *vs*. Group B observations: Significant rate of wound healing, lipid peroxidation, reductions of hospital stay, greater antioxidant protection
Rosul et al.[41]	Group B received traditional therapy. Group A received traditional therapy along with systemic and regional ozone therapy for 12–14 days, one session per day. Local wound treatment by ozone at 4000 µM/L	Caste study, ungrouped	High-level of colonization by microorganisms *n* = 49	Density of microbial colonization	yielded significance in decreased microbial colonization of wounds
Martinez-Sanchez et al.[42]	Group 1 treatment: 20 sessions of ozone *via* rectal insufflation (50 mg/L) and local treatment *via* sealed bag with ozone (60 mg/L) Group 2 treatment: topical and systemic antibiotics	Randomized, grouped, controlled, clinical trial	*n* = 101, group 1: *n* = 52, group 2: *n* = 49	Wound size, glycemic index, endothelial damage *via* oxidative stress	Group 1 *vs*. Group 2 observations: Healing of lesions improve significantly in group 1, fewer amputations seen, no side effects observed, reduced hyperglycemia
Zubarev et al.[43]	Controlled group: underwent operative treatment and standard local treatment along with low-frequency ultrasound. Treatment group: underwent ultrasonic cavitation and ozone therapy of the wound	Grouped case-study	*n* = 120 with a control of *n* = 90	Ablation of wound, microbial contamination, decontamination	Treatment group *vs*. controlled group observation: Decreased microbial contamination, facilitations of growth, prolonging of decontamination
Zhang et al.[27]	Ozone group: non-invasive oxygen-ozone treatment with 52 µg/mL ozone (total volume 20–50 mL) *via* special bag for 30 minutes per day for 20 days *via* ozone generator device + standard treatment Control group standard treatment: debridement once every 2 days and wound dressing	Randomized controlled clinical trial	*n* = 50, control group and ozone group Baseline values not significantly different in wound size	4-Leveled therapeutic effect on wound closure, expression of vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF)	Ozone group had a 92% significant effective rate compared to 64% for the control group (*P* = 0.037) Ozone group had significantly smaller wound size at day 20 (*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)

fibroblasts. This helps rebuild the intercellular matrix, healing the area around the DFU.[30] Bulynin et al.[31] demonstrated that O_3 has antibacterial properties and developed a new method for treatment *via* hydropressive treatment of wounds. A stream of ozonized fluids is generated under a pressure of 350 ATM and an "OZh-2" apparatus is used. The wound is cleansed rapidly, reduces chance of infection, and period of treatment.[31] One study showed that the use of hyperbaric oxygen therapy can ameliorate dermal wound healing.[32] Travagli et al.[30] believe that the accelerated trend of wound closure in a young population may be due to O_2 tension by O_3 in the surrounding wound area that acts as an antibacterial substance to decrease bacterial infection. In the late 20th century, a team of German scientists used O_3 on skin ulcers caused by diabetes. They used a polythene-bag for 25 minutes on average with a concentration ranging from 10 to 80 µg/mL. The different concentrations aforementioned were used with how severe the ulcers were. As the patients' ulcers healed, a lower concentration of O_3 was used.[33] Záhumenský et al.[34,35] stated that offloading and debridement of the area around the DFU is a key step in beginning therapy for neuropathic ulceration. Furthermore, the healing process can be accelerated by O_3, however, one should wear proper footwear to alleviate unnecessary pressure to the foot. In non-healing wounds, a combination of O_2-O_3 therapy might

be of benefit. A study showed that it can heal and reduce the pain due to the inherent disinfectant properties and scavenging properties of endogenous oxygen free radicals.[36] O_2-O_3 is recognized as a disinfectant because it is known to inactivate bacteria by destroying their envelope through oxidation of specific proteins and lipids. Moreover, interferon, TNF, and IL-2 activate the immune system.[7,36,37] This can explain why a reduction in infection is seen when O_3 therapy is used for DFUs. A few studies showed that when patients come present with orthopedic wounds, O_3 therapy can be applied after initial conventional treatment.[38,39]

Safety of Ozone Therapy

The utility and safety of intralesional O_3 injection in the treatment of chronic wounds has not yet been reliably assessed.[44] Though O_3 therapy is typically safe without adverse reactions,[45] it may be toxic if administered outside its therapeutic window.[46] Because intralesional O_3 injection has not yet been used in any of the studies involving DFUs, its safety has not yet been ascertained. These injections may inadvertently drive the superficial infection into the deeper tissue.[44] Moreover, O_3 therapy is not recommended for deep, heavily infected or necrotic wounds.[35]

Uzun et al.[44] described a case of a diabetic patient who developed severe foot necrosis and infection after receiving intralesional O_3 injections for a non-healing wound. Major complications that have been linked to O_3 injections for lumbar disc herniation[45] include vitreo-retinal hemorrhages,[47] ventral and dorsal root injury,[48] vertebrobasilar stroke,[49] pyogenic discitis, and ventral epidural abscess,[50] and fulminant septicemia and death.[51] Skin irritation may follow topical O_3 application while respiratory irritation may occur due to gas emitted from the generator.[35] In O_2-O_3therapy *via* autohemotransfusion for psoriasis, Marchetti et al.[52] described a fatal gas embolism.

When tested in pregnant rats, teratogenic or embriotoxic effects (*i.e.*, fetal malformations) of O_3 administered *via* rectal insufflation have not been identified.[53] In the end, to prove the inocuity of O_3, more controlled clinical trials are warranted.

Preventive Care

Common measures individual can take to prevent DFUs is to get an annual DF screening along with care intervention, especially those at high risk of DF complications.[54] Another measure, limited to individuals who have experienced ulceration, is nerve decompression surgery. It can prevent further ulceration along with amputation.[55] PAD is a major complication of DF and can determine the prognosis of healing. According to the Eurodiale study group, a neuropathic ulcer shift to neuroischemic ulcer is quite often. Assessing PAD *via* imaging and revascularization can dramatically reduce health risks if treated appropriately and on time.[56] Diabetic neuropathic ulcers can be managed by removing calluses, controlling infection, and reducing the amount of weight placed on the afflicted foot.[57] Many physicians agree that one of the greatest ways to decrease rates of mortality and morbidity among those who suffer from DF complications is early recognition.[58] Holistic care is available across the world *via* Multi-Disciplinary Foot Clinics.[59] Callus formation in DF patients serves a high level of predictability of ulcer formation. One can remove such calluses *via* urea-based preparations.[60] It behooves patients to consider preventative measures because a study has showed individuals with DFUs have a poor health-related quality of life.[61]

Conclusion

The purpose of this review is to provide the scientific community with up-to-date clinical trials, peer-reviewed studies, and compelling evidence on O_3 therapy in the treatment of DFUs. Further studies with a larger sample size would corroborate existing evidence.

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Author contributions

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

Conflicts of interest

The authors have no conflicts of interest.

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