Treatment of Chronic Nonhealing Leg Ulceration with Gaseous Nitric Oxide: A Case Study

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Abstract

Background: Despite the best clinical practice, chronic nonhealing ulcers of the lower extremities present a significant challenge. Nitric oxide (NO) has been shown to play a significant role in biological functions including wound healing and as an antimicrobial agent in nonspecific immune response.

Objective: Our goal was to study the effect of gaseous NO (gNO) administered directly to a two-year-old nonhealing chronic venous ulcer in a 55-year-old male presenting with a 30-year history of severe venous disease.

Methods: gNO (200 ppm) was applied to the lower extremity using a delivery system connected to a "single patient use" plastic boot, at 1.0 L/min.

Results: The patient received an average of 8.1-h treatments for 14 consecutive nights. On day 0 the wound was malodorous and covered by bacterial biofilm with little healthy granulation tissue present. Following 3 days of gNO treatment, healthy granulation tissue was noted with absence of malodorous odor. At day 14, the ulcer was significantly reduced in size ($p = 0.014$) and almost completely reepithelialized. Day 10 post-treatment did not reveal any deterioration in healing. Six weeks later, the wound was 90% healed. At 26 weeks post gNO discontinuation, the ulcer was completely healed.

Conclusions: This single case study demonstrated that gNO as a topical agent was well tolerated by the patient without any report of discomfort or side effect. The result of wound healing was very promising and warrants future exploration.

Sommaire

Antécédents: Malgré les meilleures pratiques cliniques, les ulcères chroniques non guérissables des membres inférieurs représèntent un défi de taille. Il a été démontré que le monoxyde d'azote (NO) joue un rôle important dans les fonctions biologiques, notamment la cicatrisation des plaies, et agit comme agent antimicrobien dans les réponses immunitaires aspécifiques.

Objectif: Administrer du NO gazeux directement sur un ulcère veineux chronique non guérissable de deux ans, chez un patient âgé de 55 ans souffrant de maladies veineuses graves depuis 30 ans.

Methodes: 200 ppm de NO gazeux ont été appliqués sur les membres inférieurs au moyen d'un système de livraison connecté à des bottes en plastique à usage unique, à 1lpm

Résultats: Le traitement de 14 nuits consécutives a nécessité en moyenne 8,1 heures. Au jour 0, la plaie était malodorante et couverte de mucilage bactérien, avec peu de tissu de granulation sain. Après trois jours de traitement au NO gazeux, on a noté la présence de tissu de granulation sain avec une absence de mauvaise odeur. Au jour 14, la taille de 1'ulcère a été réduite considérablement ($p = 0{,}014$) et une réépithélialisation presque

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complète a eu lieu. Au jour 10 après la fin du traitement, aucun signe de détérioration n'a été détecté. Six semaines plus tard, la plaie était guérie à 90 %. Á la 26e semaine suivant la fin du traitement, l'ulcére était complétement guéri.

Conclusion: Ce cas unique montre que le NO gazeux comme médicament topique a été bien toléré par le patient sans mention de sensations gênantes ni d'effets secondaires. Les résultats de la guérison sont très prometteurs et méritent une exploration plus poussée.

Chronic ulcers of the lower extremities are a significant public health problem. Besides the large financial burden placed on the health care system for their treatment, they cause a heavy toll in human suffering. As our population ages and with the current obesity crisis in North America, venous, diabetic, and pressure ulcers are likely to become ever more common. Approximately 4 million (1% of population) people in the United States develop chronic lower leg ulcers, the majority classified as diabetic or venous leg ulcers, and this number can climb to 4%–5% in older (>80 years of age) patients.[1]

Despite recent advances in chronic wound care, many ulcers of the lower extremity do not heal. A variety of factors can potentially influence wound healing, including presence of infection, necrotic tissue, poor tissue handling, and impaired tissue perfusion. Wound healing is also influenced by other clinical conditions such as advanced age, diabetes, and steroid administration.[2] By definition, such ulcers show no evidence of healing after 6–12 weeks of best clinical practice.[3] One of the major factors affecting nonhealing chronic wounds is the presence of significant bacterial burden interfering with the normal process of healing. Recently, it has been recognized that the wound bacterial burden may be composed of a bacterial "biofilm," which is a complex, organized network of bacteria and tenacious film that is nearly impossible to eradicate with conventional antibiotics.[4–6] The persistence of these biofilms disrupts the normal wound-healing process.[7] Strategies to deal with these biofilms usually consist of physical removal by curettage and the use of topical antimicrobial agents. These expensive methods and products are often ineffective with frequent and relatively rapid reoccurrence; clinical research is needed to formulate new approaches.

One such potential approach may be the use of exogenous gaseous nitric oxide (gNO), identical to the endogenously produced molecule, which plays a critical role in various bodily functions. These included the vasodilation, neurotransmission, regulation of wound healing, and regulation of immune response to infection. Nitric oxide is also cytotoxic toward various organisms such as bacteria.[8,9] Its specific role in wound healing has been demonstrated to be important and related to vasodilation, angiogenesis, anti-inflammation, and antimicrobial actions.

Preliminary dose-ranging investigations have shown the antibacterial effects of direct application of nitric oxide to microorganisms and that the optimal exposure dose of 200 parts per million (0.02%) gNO had the predicted effect on common bacterial strains contributing to wound infections in both *in vitro* and *in vivo* animal models,[19] without any indication of systemic adverse effects.[20] Parallel *in vitro* studies on human dermal fibroblasts exposed to 48 h of continuous 200 ppm gNO showed no sign of reduced viability or loss of cellular function.[21] These encouraging results supported further exploration for the use of gNO in a clinical application. To our knowledge, direct application of gNO to a nonhealing wound has not been reported in the literature. The purpose of this pilot case study was to evaluate the feasibility and potential role of short-term gNO in reducing the wound biofilm and thus improving wound-healing status in a two-year-old nonhealing chronic ulcer that had not previously responded to conventional therapies.

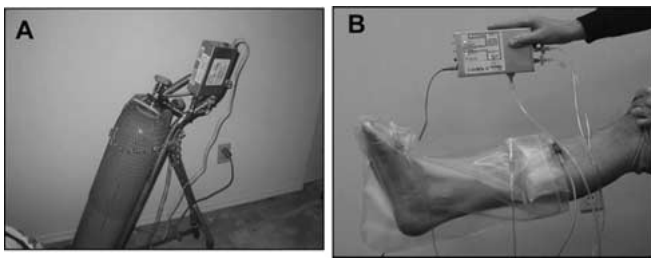
Materials and Methods

Gaseous Nitric Oxide Delivery (Fig. 1)

Nitric oxide gas (ViaNOx-H, VIASYS Healthcare, Allentown, PA, USA) was applied to the lower extremity with use of a gas-diluting delivery system (CidaNOx Delivery System) designed specifically for the study (PulmoNOx Medical Inc., Edmonton, Alberta, Canada). This CidaNOx delivery system contains an internal air pump for dilution of the gNO and a flow control circuit to dilute the 800 parts per million (ppm) in the NO source cylinder down to the therapeutic level of 200 ppm. The total flow from the system was 1.0 L/min and included one-quarter of a liter per minute (250 ml/min) flow of gNO. Several internal pressure sensors assure the dilution flow is operational and monitor the system. The flow of nitric oxide was limited to 250 ml/min by a mechanically set pressure regulator and a mechanical flowmeter that have no external controls that could be changed by the patient. The concentration of nitric oxide delivered was assured by measurement of the CidaNOx output with a calibrated nitric oxide analyzer (AeroNOx, Pulmonox Medical Inc.) that is approved for monitoring inhaled NO in human patients.

The 200 ppm gNO from the CidaNOx Delivery System flowed out to a "single patient use" plastic boot that covered the patient's lower extremity. The boot had an inflatable cuff near the top that provided a low-pressure seal. A secondary air outlet from the CidaNOx unit managed the inflation of the cuff. The patient connected

FIGURE 1 (**A**) The gaseous nitric oxide delivery device consisting of a source cylinder of 800 parts per million (ppm) nitric oxide (ViaNOx-H, Viasys Healthcare). (**B**) A gas diluting system (CidaNOx, PulmoNOx Medical Inc.) providing 200 ppm nitric oxide gas balanced air at 1.0 L/min to a sealed plastic "boot."



the pump outlet to the cuff connector until it was inflated and then the connector was sealed closed with the provided clamp. The gNO flow was then connected to the inlet connector near the toe of the boot and the return line to the connector near the top of the boot. The return line passed through the CidaNOx unit and then out through a scavenger consisting of charcoal and potassium permanganate that absorbs the nitrogen oxides. The CidaNOx Delivery System had two toggle positions, one for delivery of gNO and the other for delivery of air only. At the end of the treatment period, the patient switched the delivery flow to air only so as to clear the boot of remaining gNO before taking the boot off.

Case Study

This case study involved a 55-year-old man with a 30 year history of severe venous disease, both deep and superficial, related to deep vein thrombophlebitis. Initially, while in his twenties, the patient developed bilateral nonhealing venous leg ulcers that were surgically treated. The surgical sites healed but the ulcers continued to recur. Approximately 26 months ago, the patient presented with a small ulcer located just below the medial malleolus of the left ankle. Although not increasing in size, this ulcer did not completely heal with two years of standard of care therapy.

Most of the time the wound base was covered with a biofilm- a tenacious, yellow-colored, gel-like material. Edema control was maintained by using graduated compression stockings. Antimicrobial dressings were tried including Manuka Honey, a starch iodine preparation (lodosorb, Smith & Nephew, Largo, FL, USA), and colloidal silver (Aquacel AG, ConvaTec, Princeton, NJ, USA). His wound was frequently debrided in order to physically remove the biofilm. This was generally ineffective as the biofilm was frequently noted to be present again at the next visit. Twenty percent benzyol peroxide lotion was applied every few days in order to trigger the development of granulation tissue; however, this was ineffective as well. At times there would be improvement as the ulcer would appear to become covered with new skin only to break down weeks later. This poor progress to complete closure was noted despite wound care that addressed proper moisture balance, wound bed preparation, and treatment of the underlying disease.

This failure of his wound to close had a significant impact on quality of life for this patient. He made clinic office visits at least once a month for the entire two years. The cost of the treatment, including the surgeon's time and treatment materials (several thousand dollars), put pressure on the health care system as well as on the patient, with him having to travel several hours each visit for treatment. As previous treatments proved ineffective, the patient was invited to participate in this experimental study. Following a discussion of the experimental therapy and potential risks, an informed consent was obtained.

The patient was seen at the clinic where the wound was assessed and photographed (Fig. 2). The treatment regimen was explained and the use of the CidaNOx Delivery System and boot was demonstrated. Arrangements were made to meet at the patient's home the following day to set up the equipment and for him to have a repeat training on the use of the treatment system. Training included use of the system as well as safety information on using the gas equipment.

The patient was instructed to continue wearing supportive stockings and to use a hydrofiber dressing (Aquacel, Convatec) on the wound when not receiving gNO treatment. During the gNO treatment, he removed the supportive stocking and replaced the Aquacel dressing with a porous, low adherence dressing (ETE, Molnlycke Health Care, Sweden), which had previously been shown to allow the diffusion of gNO through it (data not shown).

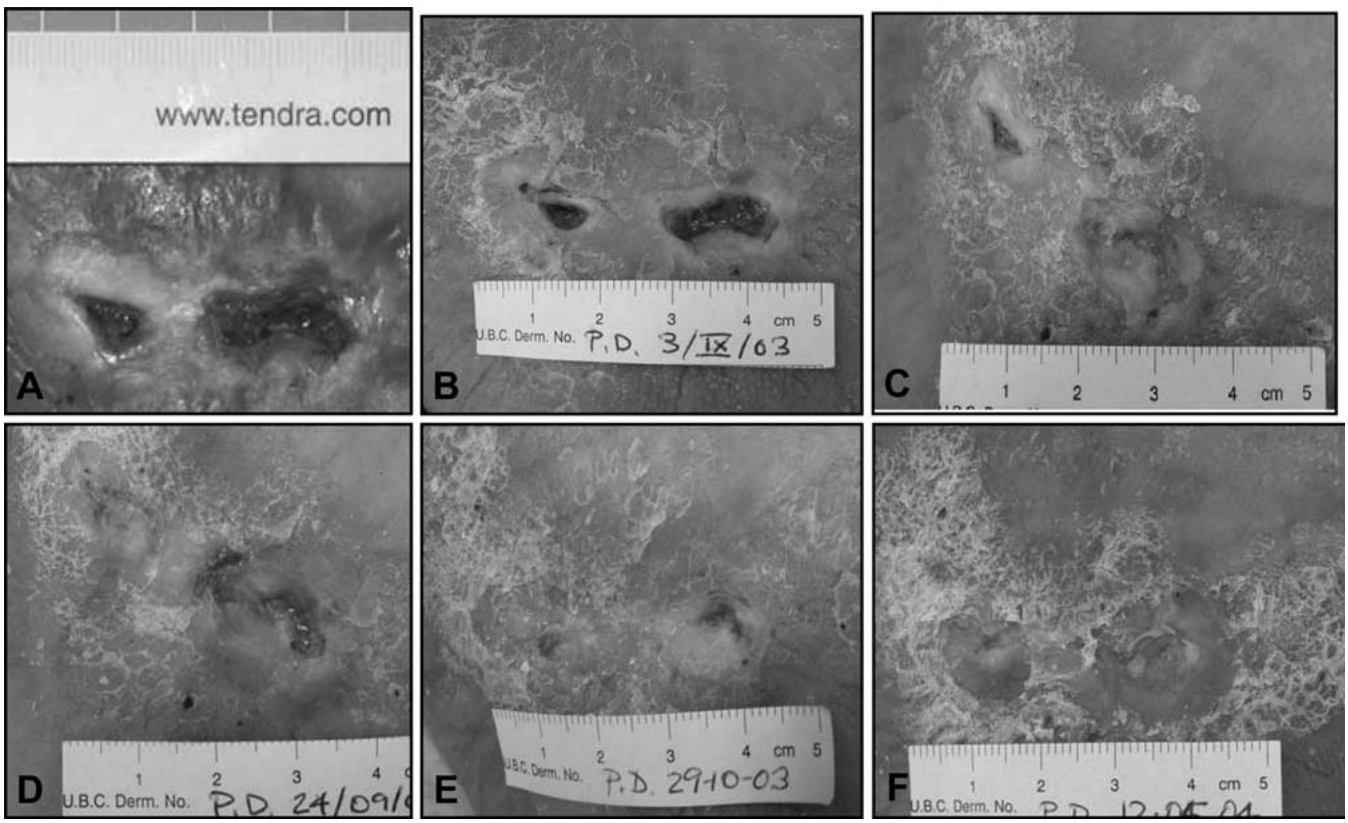
The *in vitro* experience with various microorganisms suggested that it would take at least 8 h to attain the cidal action of the gNO. However, since the limited animal experience suggested that 72 h did not completely sterilize the wound site and we wanted to explore the potential for wound bed preparation and accelerated wound healing from prolonged use, we chose to extend the treatment beyond three days. It was decided to stop at 14 days to evaluate the short-term effects and explore the possibility that the short-term effects would improve the longer-term outcome. The patient was encouraged to wear the gNO boot as often as possible during each 24-h period. As the patient worked during the day, it was decided that it would be most practical to wear the boot and receive the gNO treatments only while in bed at night.

The patient recorded the date, time, and duration of each treatment period on a data sheet, and any significant observations related to the wound, treatment, or equipment. The wound size (cm^2) was measured using digital photography and densitometry technique (Scion Image β-4.02, Scion Corp., Frederick MD, USA).

Results

The patient self-administered the treatment for 14 consecutive nights. The nocturnal treatment duration varied

FIGURE 2 (**A**) Pretreatment image (day 0) shows the initial presentation of the ulcer prior to use of the nitric oxide gas. The wound base is covered by a biofilm and there is little healthy granulation tissue present and no evidence of new skin growth from the edges. The wound was malodorous. (**B**) Following 24 h of nitric oxide gas at 200 ppm for 8 h a night over 3 days there is healthy granulation tissue noted in the ulcer base. There is also early evidence of new skin growth from the edges. Concomitantly, there is less biofilm present. (**C**) Following 14 days of treatment, the ulcer clearly is diminished in size. The ulcer is almost completely epithelialized. Nitric oxide treatment was discontinued at this time. (**D**) Ten days after 14 days of treatment there does not appear to be any deterioration of the wound. (**E**) Six weeks after 14 days of treatment the wound is judged to be about 90% healed. No deterioration is noted and the ulcer is much healthier than on day 0. (**F**) Twenty-six weeks after 14 days of treatment the wound is completely closed with healthy granulation tissue.

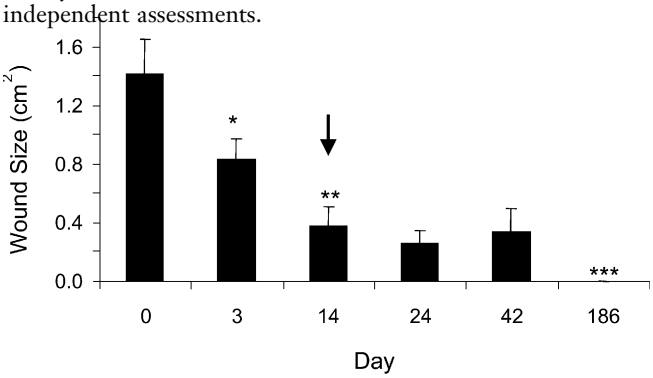


from 6.5 to 9.75 h per treatment. The cumulative wound exposure to 200 ppm gNO during the 14 treatment periods was 105.25 h. The wound was assessed and photographed on day 0 (Fig. 2A, pretreatment), day 3 (Fig. 2B, following accumulative 24 h of gNO exposure), and day 14 (Fig. 2C). The wound was also assessed and photographed ten days following the completion of the 14-day treatment (Fig. 2D) and in the 6 and 26th week following the completion of the treatment (Fig. 2E and F, respectively).

During the active treatment period, the subject was assessed with respect to the use of the CidaNOx system. The subject found the system easy to use in a fixed location, found the application of the bag comfortable, and never reported any pain associated with its use. He noted that the upper part of the bag sometimes slipped off and suggested that it could be better sealed. He suffered no bleeding episodes. Figure 2A shows the initial presentation of the ulcer prior to use of the gNO. The wound base was covered by a biofilm and there was little healthy granulation tissue present and there was no evidence of new skin growth from the edges. The wound was malodorous.

After 24 h of NO exposure (3 days at 8 h/day), for the first time there was healthy granulation tissue noted in the ulcer base. There was also early evidence of new skin growth from the edges observed. The malodorous odor was also absent. Concomitantly, there was less biofilm present (Fig. 2B). At 14 days of therapy (Fig. 2C) the ulcer clearly had diminished in size. By then it had almost completely epithelialized. Significant wound size reduction was observed as early as day 3 of gNO treatment ($p = 0.014$), with approximately 75% reduction in wound area by the end of gNO therapy at day 14 (Fig. 3). The wound was further assessed 10 days after cessation of gNO treatment (Fig. 2D). There did not appear to be any deterioration of the wound during this time, although the ulcer was judged to be incompletely healed. No significant deterioration in wound size was observed compared to the last day of gNO treatment (Fig. 3). Six weeks later the wound was judged to be about 90% healed with no deterioration in wound size or epithelialization (Fig. 2E and Fig. 3). At 26 weeks post NO discontinuation, the ulcer was noted to be completely healed and reepithelialized (Fig. 2F). Over the entire post-treatment time,

FIGURE 3 Wound size reduction is presented following nitric oxide gas treatment. Significant decrease in area was observed following 3 and 14 days of gNO application to the wound (*p = 0.019 vs. day 0; ** p = 0.014 vs. day 3). Wound status did not deteriorate after removal of treatment (arrow, day 14). Wound was completely healed following 26 weeks (186 days; ***$p < 0.01$ vs. day 3). Values are means and standard deviations from three independent assessments.



there were no changes to the dressing regimen and no other antimicrobials or antibiotics were used.

Discussion

Successful wound healing involves numerous physiological responses, including the inflammatory response, angiogenesis, the development of fibrous tissue, and re-epithelialization. NO is both directly and indirectly involved in each of these physiological processes. Many wound-resident cells, such as macrophages, neutrophils, endothelial cells, vascular smooth muscle cells, keratinocytes, lymphocytes, and fibroblasts, have the ability to synthesize or affect the synthesis of NO.[10] Several studies have suggested a role for NO in wound healing by showing that, in some situations, impaired wound healing results from the absence of NO.[22–25] Specifically, some studies attempted to reverse impaired wound healing by administering dietary arginine or adenoviral-mediated expression of human iNOS (iNOS is a designation for "inducible" NO synthase enzyme; here, the enzyme activity may be increased by the presence of inflammatory substances or bacterial lipopolysaccharides) in NOS Knockout mice, with both showing some degree of success.[23–25]

NO acts by way of multiple mechanisms. Some are due to its chemical reaction with oxygen which forms free radicals, whereas others are due to its affinity with enzymes containing heme or Fe (iron). Also, NO may regulate expression of various genes whose products are important in the healing process. The multiple sites of action of NO partially explain its potential ability to enhance wound healing. Wound macrophages have been shown to be a major component of iNOS expression in the early inflammatory phase of repair. Fibroblasts express both eNOS and iNOS, and the NO that they produce participates in the regulation of collagen synthesis by these cells. (eNOS is the designation for "endothelial" NOS. This enzyme differs from iNOS in that it is not "inducible"; rather, its activity depends on intracellular calcium ion levels.) Keratinocytes at wound margins strongly express iNOS.[26]

The effect of NO on keratinocyte function appears to be dose-dependent. At low levels increased proliferation is observed, whereas at high levels cellular proliferation is inhibited and differentiation occurs.[13,27] A role for NO in keratinocyte function is suggested as iNOS inhibition results in significant impairment of reepithelialization, whereas NO production is associated with the acquisition of a locomotory phenotype (migration of cells toward center of the wound).[28] NO has also been shown to participate in the formation of new blood vessels, as pharmacological inhibition of NOS prevents capillary organization *in vitro*.[29] The effects of NO on angiogenesis may be mediated via pro-angiogenic cytokines, such as vascular epithelial growth factor (VEGF), whose activity is in part NO-dependent.[30]

The average time for ulcers that result from venous stasis disease to heal under optimal care ranges from 12 to 16 weeks. Our patient, who had a nonresponsive ulcer for more than two years, exhibited a positive response to a brief exposure to gaseous nitric oxide. His wound decreased in size, a granular base was established, and the malodorous smell was eradicated during this two-week period. Further studies and randomized controlled trials will be able to answer whether a longer exposure or a different concentration, once the biofilm was eliminated, would have made a difference in the closure of the lesions.

Conclusion

In the single case study we have described here, it was shown that 200 ppm gNO as a topical agent was well tolerated by the patient and augmented wound healing. Gaseous NO could be safely delivered nocturnally at home, with good compliance. There were no adverse side effects associated with the treatment or its delivery. The result achieved in this single patient is very promising but warrants additional investigation. Currently there is no available or effective treatment for many types of chronic wounds. If results such as these continue to be favorable, gNO may become a valuable adjunct to the wound-healing process.

Acknowledgments

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