Clinical Prospective Study on the Use of Subcutaneous Carboxytherapy in the Treatment of Diabetic Foot Ulcer

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ABSTRACT

Diabetic footfoot ulcer (DFU) is a serious complication of diabetes mellitus, and possibly the major morbidity of the diabetic foot. It is the most common foot injury in diabetic patients and can lead to lower-extremity amputation. Management of DFU requires a systematic knowledge of the major risk factors for amputation, frequent routine evaluation, scrupulous preventive maintenance, and correction of peripheral arterial insufficiency.

Carboxytherapy refers to the subcutaneous injection of CO_2 to improve the microcirculation and promote wound-healing by stimulating the microcirculation. Since optimal ulcer-healing requires adequate tissue perfusion, it is considered that carboxytherapy could be useful in the treatment of DFU.

The present prospective clinical study included 40 patients with different sizes and types of chronic DFU. In addition to cleaning of the wound, antibiotics and debridement as necessary, the treatment protocol included blood sugar control, medication, healthy habits, no weight-bearing, and carboxytherapy.

The results showed that this treatment that included carboxytherapy promoted wound-healing and prevented amputation.

These positive effects should be confirmed through a complete study that includes different clinical and instrumental parameters.

INTRODUCTION

Diabetic foot ulcer (DFU) is a major complication of diabetes mellitus. The wound-healing process involves four steps: hemostasis, inflammation, proliferation and remodeling. The proliferation step includes angiogenesis, collagen deposition, granulation, tissue formation and epithelialization. While wound-healing works effectively most of the time, diabetes mellitus delays the normal phases of wound-healing.[1,2] The alteration of the healing cascade in diabetic patients is associated with changes in the extracellular matrix through changes in fibroblasts, which lengthen and decrease in quantity. These changes in fibroblasts, in turn, lead to the reduced production of collagen, elastin and glycosaminoglycan (GAG), and the consequent delay in the healing and contraction of wounds.[3] Diabetic patients also exhibit a reduced ability to generate nitric oxide from L-arginine, which compromises angiogenesis and vasodilatation of the blood vessels.[4]

Uncontrolled diabetic ulcers in the limbs are responsible for more than 25% of hospital admissions among diabetic patients in the U.S. and U.K.[5] Diabetes is the principal cause of nontraumatic lower-limb amputation in the U.S.[6] Everett and Mathioudakis recently reported that "*Mortality rates associated with the development of a DFU are estimated to be 5% in the first 12 months, and 5-year mortality rates have been estimated at 42%.*"[7]

In many countries with low levels of diagnostic technology, the most important measures for avoiding foot complications in diabetic patients are careful inspection and proper care of the diabetic foot. The education of diabetic patients regarding foot hygiene, nail care and proper footwear can help reduce the possibility of an injury that could lead to the development of an ulcer in the extremities.[8,9]

Adequate glycemic control, periodic foot inspection, early recognition and proper medical treatment of these ulcers could prevent up to 85% of amputations,[10,11] and the sooner a diabetic foot ulcer is treated, the better the result.[12]

The risk factors for ulcer formation include diabetic neuropathy, structural foot deformity and peripheral arterial occlusive disease (PAOD). PAOD is four times more predominant in diabetics than in non-diabetics,[13] and arterial occlusion typically involves the tibial and peroneal arteries. Aggravating conditions, like hypertension, hyperlipidemia and smoking, also contribute to the increase in vascular problems in diabetic patients.[14]

Treatment for diabetic wounds includes both general guidelines (no weight-bearing, blood sugar control, and the promotion of healthy habits) and local treatments (e.g., removing pressure from the foot ulcer and using topical and general medications for wound-healing). Surgical procedures, such as skin grafts, debridement to remove dead tissue, and bypass surgery, can be useful to restore circulation.

Ultimately, optimal healing of diabetic ulcers requires satisfactory tissue perfusion and a healthy, oxygenated wound bed.[15-17] A deficiency of oxygen (hypoxia) in the wound bed slows or stops the normal healing process. Hyperbaric oxygen therapy (HBOT) can be used as an adjunct to comprehensive wound care, however, there is some controversy regarding its effectiveness. Löndahl and co-workers [18] concluded that HBOT promotes wound repair and enhances healing by improving blood circulation, encouraging angiogenesis, and providing more oxygen to tissue in the wound bed; it also helps kill anaerobic bacteria, which cause some of the worst infections in chronic wounds. On the other hand, Fedorko and co-workers [19] wrote that "*HBOT does not offer an additional advantage to comprehensive wound care in reducing the indication for amputation or facilitating wound healing in patients with chronic DFUs (diabetic foot ulcers).*"

Carboxytherapy is an alternative to HBOT that consists of the therapeutic use of carbon dioxide (CO_2) in its gaseous state via either transcutaneous application or subcutaneous injection.

The clinical use of CO_2 is not new. In both Argentina and France, the injection of CO_2 gas has been used to treat peripheral arteriopathies, especially arteriopathy obliterans, in the lower limbs.[20] When CO_2 is injected subcutaneously, it immediately diffuses at the cutaneous and muscular microcirculatory levels, increases microcirculatory vasodilatation and improves flow through a direct action on arteriole smooth muscle cells.[21] Via the Bohr effect, it also increases the tissue PO_2 at the injection site.[22-24]

In addition to these effects, the tissue-stretching during CO_2 injection induces slight inflammation, which triggers tissue repair and regeneration, and increases macrophages, fibroblasts, and

Figure 1. Carboxytherapy device. (a) side view. (b) control panel.

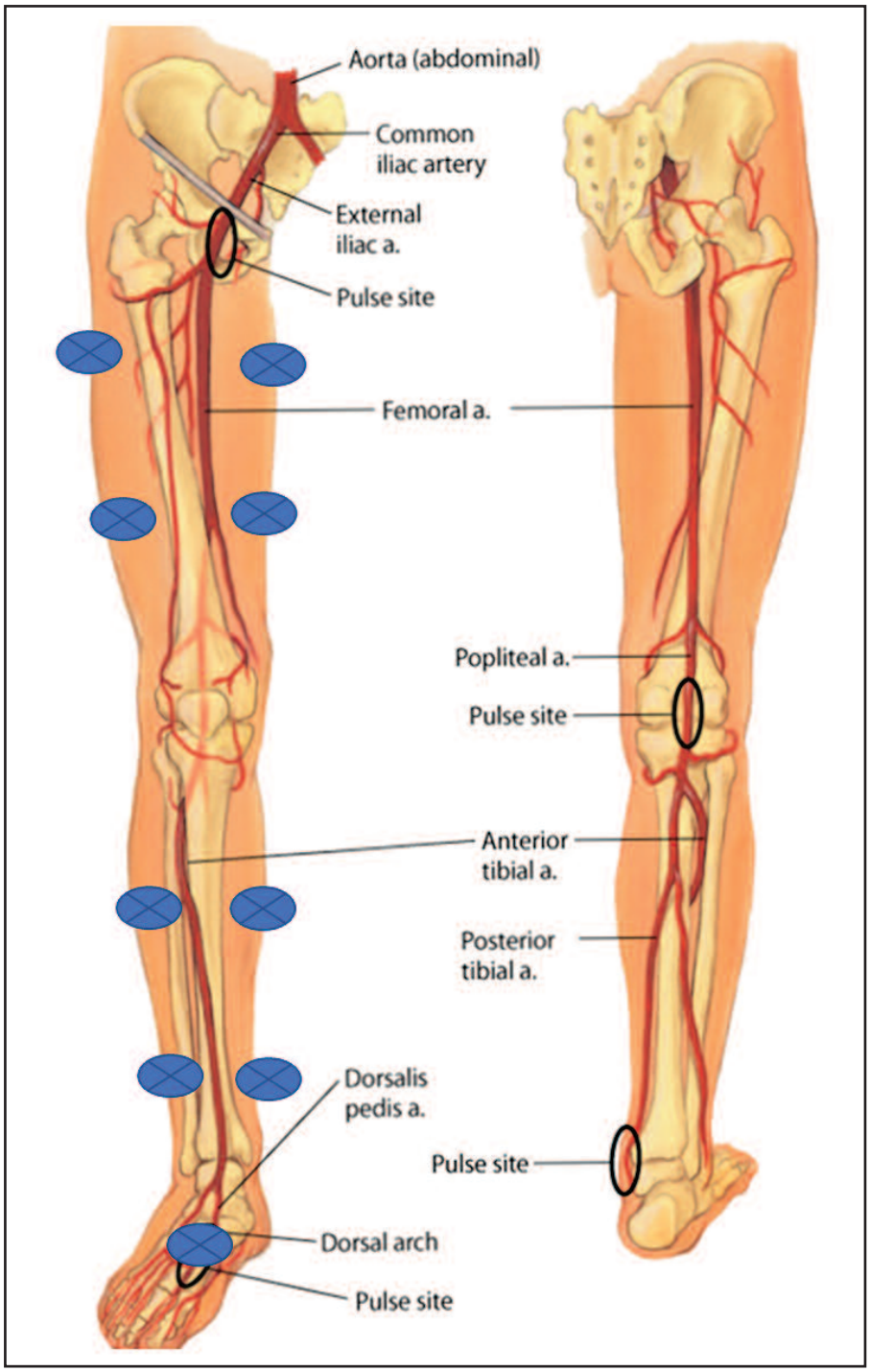

Figure 2. Sites for general CO_2 injection (blue).

endothelial cells, resulting in neo-vascularization and remodeling of the extracellular matrix.[25-30]

Based on these effects of carboxytherapy on the microcirculation of the skin, we decided to start using this technique in diabetic patients with ulcers in their limbs. In this report, we describe our experience with carboxytherapy in 40 patients with diabetic foot ulcers.

MATERIALS AND METHODS

Subjects

This prospective clinical study included 40 patients (13 female/27 male) with different sizes and types of chronic diabetic foot ulcers who were treated between January 2015 and January 2017.

The treatment protocol included blood sugar control, medication, promotion of healthy habits, no weight-bearing, and carboxytherapy. All of the patients were volunteers from the community from different regions of Algeria. They all provided their written informed consent for the treatment.

Of the total 40 patients, 6 patients had previously received hyperbaric oxygen therapy and 3 had received LED therapy. The remaining 31 patients had been treated only with local dressings, which was the only treatment available in that part of Algeria. The average duration of carboxytherapy was 7 months (Case 6 was treated with CO_2 for only three weeks).

Healing was evaluated according to the following criteria: a change in the odor of the dressing, the presence of angiogenesis, new networks of blood vessels, changes in the exudate, a change in local wound color, re-epithelialization from the edges of the wound, and improvement in the general condition of the patient (reduction of fever and better glycemic balance).

Carboxytherapy

The CO_2 device (Carboxy-pen®, Anti-Aging Medical Systems, Montrodat, France) consists of a handpiece and a control panel (Fig. 1). The electronic control allows the user to select different parameters, including the flow velocity, the gas volume and the dose. The CO_2 itself must be sterile and suitable for medical use.

CO_2 is injected both systemically (at the blue sites in Fig. 2) and locally at the wound site.

Figure 3 shows an example of where CO_2 was injected in and around a wound.

RESULTS

Clinical cases

Our experiences with 6 of the 40 cases are summarized below. All of the carboxytherapy patients were treated with metronidazole (500 mg, 2 tablets/day) to prevent anaerobic infection. In all cases, treatment was as follows:

75 cc or less (depending on tolerability by the patient) of CO_2 was injected at each injection site (Fig. 2) on the internal and external sides of the right and left legs along the route of the large vessels at a depth of 4mm (intradermic).

The same dose of CO_2 was then injected in and around the wound.

CASE 1

A 56-year-old diabetic male under insulin treatment presented with 2nd and 3rd degree burns on both feet with no healing over the previous 6 months.

Initial classification

- ◆Wagner Grade 2
- ◆University of Texas Grade 2 Stage B

History

- ◆Medical history = diabetes type 2
- ◆Family history = diabetes type 2 and high blood pressure
- ◆Basic Metabolic Panel = anemia
- ◆Doppler ultrasonography of the lower extremities = plaque with weakened flow
- ◆Radiology = no osteitis
- ◆Evolving diabetic neuropathy
- ◆Cytobacteriological study of secretion + antibiogram = Streptococcus B sensitive to gentamicin

Treatment

- ◆Lesions were cleaned daily with 0.9% normal saline and sterile compresses.
- ◆Antibiotics were prescribed for 6 weeks (Metronidazole 500 mg 2 tablets/day and gentamicin 80 mg 1 injection IM/day).
- ◆Carboxytherapy was administered on a schedule of 1 session every 15 days for 20 sessions, for a total duration of 10 months.

Figures 4 and 5 show the left foot before and after treatment (Fig. 5 (a) 1, (b) 2, (c) 4 and (d) 6 months), respectively.

Figures 6 and 7 show the right foot before and after treatment (Fig. 7 (a) 1, (b) 2, and (c) 4 months), respectively.

Figure 8 shows both feet after 9 months of treatment.

Final classification

- ◆Wagner Grade 0
- ◆University of Texas Grade 0 Stage A

Figure 3. Example of sites for the local injection of CO2 in and around a wound.

Figure 4. Photograph of Case 1 (left foot) before treatment.

Figure 5. Case 1 (left foot) after treatment for (a) 1, (b) 2, (c) 4, and (d) 6 months of treatment.

Figure 6. Case 1 (right foot) before treatment.

Figure 7. Case 1 (right foot) after treatment for (a) 1, (b) 2, and (c) 4 months of treatment.

Figure 8. Case 1 after treatment for 9 months.

CASE 2

A 62-year-old diabetic male under insulin treatment presented with amputation of the left great toe with poor wound-healing over the previous 6 months.

Initial classification

- ◆Wagner Grade 3
- ◆University of Texas Grade 3 Stage B

History

- ◆Medical history = diabetes type 2
- ◆Surgical history = amputation of the left great toe
- ◆Family history = diabetes type 2 and high blood pressure
- ◆Basic Metabolic Panel = anemia
- ◆Doppler ultrasonography of the lower extremities = plaque with weakened flow
- ◆Radiology = osteitis
- ◆Evolving diabetic neuropathy
- ◆Cytobacteriological study of secretion+ antibiogram = Proteus sensitive to ciprofloxacin

Treatment

- ◆The lesion was cleaned daily with 0.9% normal saline and sterile compresses with medical honey.
- ◆Antibiotics were administered for 8 weeks (Metronidazole 500 mg 2 tablets/day and ciprofloxacin 500 mg 2 tablets/day)
- ◆Carboxytherapy was administered on a schedule of 1 session every 7 days until the lesion started to improve (at 2 months), and then 1 session every 15 days, for a total of 23 sessions over a duration of 10 months.

Figure 9 shows a photograph at the initiation of treatment. In the photograph at 2 weeks (Fig. 10), osteitis is visible.

Figures 11-15 show the results after treatment for 3, 3, 5, 6 and 8 months, respectively.

Final classification

- ◆Wagner Grade 1
- ◆University of Texas Grade 0 Stage A

Figure 9. Photograph of Case 2 before treatment

Figure 10. Case 2 after treatment for 2 weeks. Osteitis is visible.

Figure 11. Case 2 after treatment for 3 months.

Figure 12. Case 2 after treatment for 3 months.

Figure 13. Case 2 after treatment for 5 months.

Figure 14. Case 2 after treatment for 6 months.

Figure 15. Case 2 after treatment for 8 months. Final result with hyperkeratosis.

CASE 3

A 54-year-old diabetic male under insulin treatment presented with altered wound healing at 9 months after amputation of the left fourth toe with many fibrin filaments and plantar infection.

Initial classification

- ◆Wagner Grade 2
- ◆University of Texas Grade 2 Stage B

History

- ◆Medical = diabetes type 2 and high blood pressure.
- ◆Surgical = amputation
- ◆Family = diabetes type 2 and high blood pressure

Exam results

- ◆Basic Metabolic Panel = normal
- ◆Doppler ultrasonography of the lower extremities = plaque with weakened flow
- ◆Radiology = No osteitis
- ◆Evolving diabetic neuropathy
- ◆ECB pus + antibiogram = Haemophilus influenza sensitive to Ciprolon

Treatment

- ◆Lesions were cleaned daily with 0.9% normal saline and sterile compresses.
- ◆Antibiotics were prescribed for 8 weeks (Metronidazole 500 mg 2 tablets/day and Ciprolon 500 mg 2 tablets/day)
- ◆Carboxytherapy was administered on a schedule of 1 session every 15 days for 10 sessions, for a total duration of 5 months.

Figures 16-22 show photographs at the initiation of treatment, and after treatment for 1, 2, 3, 3.5, 4 and 48 months, respectively.

Final classification

- ◆Wagner Grade 0
- ◆University of Texas Grade 0 Stage A

Figure 16. Case 3 before treatment.

Figure 17. Case 3 after treatment for 1 month.

Figure 18. Case 3 after treatment for 2 months.

Figure 19. Case 3 after treatment for 3 months.

Figure 20. Case 3 after treatment for 3.5 months.

Figure 21. Case 3 after treatment for 4 months.

Figure 22. Case 3 final control photo one year after the last session of carboxytherapy.

CASE 4

A 70-year-old diabetic male under insulin treatment presented with a lesion of the right foot that had not healed for 3 years and showed many fibrin filaments.

Initial classification

- ◆Wagner Grade 2
- ◆University of Texas Grade 2 Stage B

History

- ◆Medical = diabetes type 2 and high blood pressure.
- ◆Surgical = none
- ◆Family = diabetes type 2 and high blood pressure

Exam results

- ◆Basic Metabolic Panel = anemia
- ◆Doppler ultrasonography of the lower extremities = plaque with weakened flow and pedis stenosis
- ◆Radiology = no osteitis
- ◆Evolving diabetic neuropathy
- ◆ECB pus+ antibiogram = Staphylococcus aureus sensitive to pristinamycine

Treatment

- ◆Lesions were cleaned daily with 0.9% normal saline and sterile compresses.
- ◆Antibiotics were prescribed for 6 weeks (Metronidazole 500 mg 2 tablets/day and Pristinamycine 500 mg 2 tablets/day)
- ◆Carboxytherapy was administered on a schedule of 1 session every 15 days for 16 sessions, for a total duration of 8 months.

Figures 23-27 show photographs at the initiation of treatment, and after treatment for 1, 2, 3, and 6 months, respectively.

Final classification

- ◆Wagner Grade 0
- ◆University of Texas Grade 0 Stage A

Figure 23. Case 4 before treatment.

Figure 24. Case 4 after treatment for 1 month.

Figure 25. Case 4 after treatment for 2 months.

Figure 26. Case 4 after treatment for 3 months.

Figure 27. Case 4 after treatment for 6 months.

CASE 5

A 53-year-old diabetic male under insulin treatment presented with a wound in the left foot that had not healed for 8 months and which showed localized necrosis.

Initial classification

- ◆Wagner Grade 4
- ◆University of Texas Grade 3 Stage D

History

- ◆Medical = diabetes type 2 and high blood pressure.
- ◆Surgical = none
- ◆Family = diabetes type 2 and high blood pressure

Exam results

- ◆Basic Metabolic Panel = anemia
- ◆Doppler ultrasonography of the lower extremities = plaque with weakened flow
- ◆Radiology = osteitis
- ◆Evolving diabetic neuropathy
- ◆ECB pus+ antibiogram = Klebsiella sensitive to ciprofloxacin

Treatment

- ◆The lesion was cleaned daily with 0.9% normal saline and sterile compresses with medical honey.
- ◆Antibiotics were administered for 8 weeks (Metronidazole 500 mg 2 tablets/day and ciprofloxacin 500 mg 2 tablets/day)
- ◆Carboxytherapy was administered on a schedule of 1 session every 15 days, for a total of 15 sessions over a duration of 7.5 months.

Figures 28-34 show photographs at the initiation of treatment, and after treatment for 1, 2, 3, 4, 7 and 48 months, respectively.

Final classification

- ◆Wagner Grade 0
- ◆University of Texas Grade 0 Stage A

Figure 28 Case 5 before treatment.

Figure 29. Case 5 after treatment for 1 month.

Figure 30. Case 5 after treatment for 2 months.

Figure 31. Case 5 after treatment for 3 months.

Figure 32. Case 5 after treatment for 4 months.

Figure 33. Case 5 after treatment for 7 months.

Figure 34. Case 5 final control photo one year after the last session of carboxytherapy (November 2017).

CASE 6

A 54-year-old diabetic female under oral antidiabetic treatment presented with a lesion on her left foot that had not healed for 6 months.

Initial classification
- ◆Wagner Grade 1
- ◆University of Texas Grade 1 Stage B

History
- ◆Medical = diabetes type 2
- ◆Surgical = appendectomy
- ◆Family = diabetes type 2 and high blood pressure

Exam results
- ◆Basic Metabolic Panel =normal
- ◆Doppler ultrasonography of the lower extremities = plaque with weakened flow
- ◆Radiology = no osteitis
- ◆Evolving diabetic neuropathy
- ◆Cytobacteriological study of secretion + antibiogram = Staphylococcus aureus sensitive to gentamicin

Treatment
- ◆The lesion was cleaned daily with 0.9% normal saline and sterile compresses.
- ◆Antibiotics were administered for 3 weeks (Metronidazole 500 mg 2 tablets/day and gentamicin 80mg 1 injection IM/day)
- ◆Carboxytherapy was administered on a schedule of 1 session every 7 days for 2 sessions.

Figures 35-37 show photographs at the initiation of treatment, and after treatment for 1 and 3 weeks, respectively.

Final classification
- ◆Wagner Grade 0
- ◆University of Texas Grade 0 Stage A

Figure 35. Case 6 before treatment.

Figure 36. Case 6 after treatment for 1 week.

Figure 37. Case 6 after treatment for 3 weeks.

DISCUSSION

Delayed healing and chronic wounds are some of the basic characteristics of diabetic patients. Wounds in the extremities of diabetic patients that require long-term treatment affect all areas of the patient's life and lead to a reduced quality of life. For example, diabetic foot ulceration is a major precondition to amputation; 85% of diabetic patients undergoing amputation have had a prior foot ulceration.[31]

Microcirculation in diabetic ulcers in the limbs is significantly different from that in healthy skin, and can lead to local hypoxia, edema, microaneurysms and microhemorrhages, as observed by video capillaroscopy. Revascularization is the key to treatment for ischemic diabetic lesions.[32] Various medical, physiotherapy and surgical techniques have been developed to improve the microcirculation and thus avoid negative effects on tissue tropism. In this clinical study, in addition to basic treatment of the lesion (cleaning, antibiotic treatment, correction of support), patients with diabetic foot ulcers were treated by the subcutaneous injection of CO_2. At the vascular level, CO_2 increases vascular tone and produces active microcirculatory vasodilatation. CO_2-induced vasodilatation results from its direct activity on arteriole smooth muscle cells.[21]

In addition, CO_2 promotes Bohr's effect, a mechanism that allows the transfer of tissue CO_2 to the lungs and

the transfer of lung O_2 to tissues through the oxy-hemoglobin dissociation curve. When administered through an external route, CO_2 promotes this mechanism, resulting in a higher tissue oxygenation and neoangiogenesis.

Of the 40 patients in this study, none required amputation. Furthermore, 31 showed complete recovery and 9 showed partial recovery.

CONCLUSION

Based on the present observations, carboxytherapy appears to be a valid treatment option in diabetic patients with microcirculatory disturbances that produce trophic injury. While further studies will be needed to compare the results with carboxytherapy to those with other techniques, none of the 40 patients we treated required amputation and all made a full functional recovery.

The results of this prospective clinical study may help to improve the treatment of diabetic patients with impaired wound-healing in their extremities and consequently improve their quality of life. STI

AUTHORS' DISCLOSURES

The authors have no conflicts of interest to disclose.

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