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The effect of transcutaneous application of gaseous CO_2 on diabetic chronic wound healing—A double-blind randomized clinical trial

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Abstract
Chronic wounds in diabetics are difficult to treat, therefore, adjuvant therapies have been investigated. Bathing in CO_2-rich water (spa therapy) has been known in Europe for decades for its positive effect on peripheral vascular disorders. Recently, much effort has been invested in developing optimal application methods of CO_2. Uses include subcutaneous injections of CO_2, bathing in CO_2-enriched water, and transcutaneous application of CO_2. To verify the effect of transcutaneous application of gaseous CO_2 on the healing of chronic diabetic wounds, a randomized double-blind clinical research was designed. The research included 30 and 27 wounds in the study and control groups, respectively. In addition to standard treatment, patients in the study group received 20 therapies with medical-grade CO_2 gas and the control group received the same treatment with air. Results showed significantly faster healing in the study group: 20 of the 30 wounds in the study group were healed compared with none in the control group. Mean wound surface and volume in the study group was reduced significantly (surface: 96%, P = .001, volume: 99%, P = .003) compared with a small reduction in the control group (surface: 25%, P = .383, volume: 27%, P = .178). Considering our results, transcutaneous application of gaseous CO_2 is an effective adjuvant therapy in diabetic chronic wound treatment.

KEYWORDS
carboxy therapy, chronic wound treatment, CO2 therapy, diabetic chronic wound, diabetic foot ulcer, transcutaneous CO_2 application

1 | INTRODUCTION

Diabetes mellitus is known for producing long-term complications that almost inevitably occur even when carefully treated so that blood glucose levels mostly remain within the normal range.[1] According to the World Health Organization, the prevalence of diabetes mellitus is highest in Western Europe and the numbers are expected to grow.[2] One of the long-term complications, diabetic foot ulcer, affects approximately 19 to 34% of diabetes patients in their lifetime.[3] Standard therapies for chronic wounds include debridement of necrotic tissue, infection control, and maintenance of the moist wound bed with various dressings. Wound healing progresses from the inflammation phase to proliferation and maturation or remodeling of newly formed tissue, but chronic diabetic wounds usually remain in the inflammatory phase. The results of standard healing methods are often unsatisfactory, therefore, additional and advanced therapeutic options are increasingly being investigated. Advanced therapeutic options include reducing biofilm and infections,[4] development of different scaffolds for delivering growth factors, nanoparticles, antibiotics, and the use of mesenchymal stromal cells.[5-7] Skin substitutes are developed to shorten healing time. Their value is not only in protecting the wound from the risk of infection, but also in delivering added growth factors to enhance healing.[8] However, the mentioned therapeutic procedures cannot be effective without sufficient vascularization.

CO_2-rich water bathing has been known in Europe for decades for its positive effect on the peripheral vascular disorders as spa therapy. Recently, much effort has been made to investigate local and possible systemic effects of CO_2 use and the development of optimal application methods. Described uses of CO_2 include subcutaneous injections of CO_2, bathing in CO_2-enriched water, and transcutaneous application of CO_2.[9-18] Previous studies established a positive influence of CO_2-enriched water or transcutaneous application of CO_2 for different indications, including an improvement of symptoms in Raynaud's phenomenon, intermittent claudication, stage II artheriopathy, and after surgical revascularization procedures.[15-19] To our knowledge, one randomized control trial and one observational trial have been conducted investigating the effect of CO_2-enriched water on chronic wound treatment in humans, one observational study using transcutaneous application of CO_2 in acute and chronic wounds, and one randomized controlled study using subcutaneous CO_2 injections in chronic wound treatment.[13,14,20] All mentioned studies confirmed a positive influence on the healing of chronic wounds. In our opinion, transcutaneous application of gaseous CO_2 has some advantages compared with the other two application methods: it is not invasive like subcutaneous injections of CO_2 and it does not moisturize the wound area like CO_2-enriched water. For these reasons, we decided to use transcutaneous application of gaseous CO_2 as an adjuvant therapy. We designed a clinical study with the aim of evaluating the effect of transcutaneous application of gaseous CO_2 on the healing of diabetic chronic wounds.

Key Message

- a randomized double-blind clinical study confirmed the effect of transcutaneous application of gaseous CO_2 on chronic wound healing in diabetic patients. After 20 CO_2 therapies in four weeks, 20 of 30 wounds healed completely, while in the control group receiving 20 placebo therapies with air, none of the 27 wounds healed completely. According to our results, transcutaneous application of gaseous CO_2 is an effective adjuvant therapy for treatment of chronic wounds in diabetic patients.

2 | MATERIALS AND METHODS

2.1 | Patients

The research was designed as a prospective randomized double-blind study, performed at the University Medical Center Ljubljana between 1 November 1 2018, and 1 November 2019. All patients included in our study had one or more chronic wounds that did not heal despite standard treatment. Standard treatment protocol before inclusion into the study included debridement of the necrotic tissue, maintenance of the moist wound bed, and control of the infection. Patients were randomly distributed into study and control groups and did not know if they were receiving CO_2 therapy or placebo treatment. The observed parameters were wound area and wound volume (measured with the use of a 3D scanner for area and volume evaluation, non-contact 3D laser scanner DAVID SLS-2 (David Vision Systems GmbH, Koblenz, Germany) and evaluation of the Falanga wound bed score which was performed by a third party blind to the group to which the patient belonged.[21]

The research initially included 47 diabetic patients with 61 chronic wounds. Before inclusion, the patients were familiarized with the study protocol and received all the relevant information on the study from medical staff. The patients were then asked to give written informed consent. The study was approved by the National Medical Ethics Committee of the Republic of Slovenia (approval ID: KME 84/02/16).

The patients were randomized into study and control groups using random number generator. During the treatment, four patients from the control group were excluded: (a) two patients required amputation before the end of the placebo treatment, (b) one patient exhibited a significant increase in wound size during the placebo treatment (952% increase in volume and 294% increase in area), and (c) one patient exhibited severe hypertrophy of the wound bed.

All patients included in the study continued with the standard treatment protocol. CO_2 or placebo therapies were used as adjuvant therapy in addition to standard treatment.

The basic characteristics of the remaining patients are shown in Table 1.

2.2 | CO_2 therapy

After an initial assessment, the patients in the study group underwent treatment with CO_2 therapy using the Peripheral vascular rehabilitation system (PVR system; Derma Art, Brežice, Slovenia). The PVR system is a device that enables non-invasive transcutaneous application of therapeutic concentration 99.9% gaseous CO_2 to the body, prevents unwanted inhalation of CO_2 by patients and continuously monitors the quality of the ambient air. Lower extremities of the subjects were isolated in a therapeutic wrap (single use, low-density, made from biocompatible polyethylene), sealed at the waist and connected to the PVR system. After this, air was first pumped out of the therapeutic wrap, then the wrap was filled with 99.9% CO_2 gas. The therapy lasted for 45 minutes.

Each patient from the study group underwent CO_2 therapies until the wound was healed or for a maximum of four weeks (meaning 20 CO_2 therapies that were performed on workdays only), whichever came first. After the completion of CO_2 therapies, the unhealed wounds were again evaluated, given a wound bed appearance score, and scanned for area and volume evaluation.

2.3 | Placebo therapy

Patients in the control group underwent the same procedure with the PVR System, except that the therapeutic wrap was filled with air.

2.4 | Statistical analysis

For statistical analysis, a paired T-test or chi-square test was performed to compare the variables prior to and after treatment and between the groups. The mean differences and 95% confidence intervals (95% CI) were calculated with two-sided probability (P) values. The level of significance was set at $P < .05$. Statistical analysis was performed using IBM SPSS Statistics, v. 24 (IBM Corp, Armonk, New York).

3 | RESULTS

Table 2 shows wound area, volume, and wound bed appearance according to the Falanga score prior to and after CO_2 therapy or placebo treatment.

Results show that before the treatment, the mean area of wounds in subjects included in the study and control groups were comparable ($P_{area}{}^{ac}$ = .625), but the wound volume in the study group was almost significantly larger ($P_{volume}{}^{ac}$ = .059).

After the CO_2 or placebo treatment, there was a significant difference in the area and volume of the wounds between the study and control groups ($P_{area}{}^{bd}$ = .001; $P_{volume}{}^{bd}$ < .001).

In the study group, 20 of 30 (67%) wounds were healed following CO_2 therapies. Moreover, the mean area and volume values of the remaining wounds were reduced significantly (Figures 1 and 2).

In the control group, none of the wounds were healed after the end of the placebo treatment, but the mean volume and area decreased in comparison with the mean values before the placebo treatment, although the differences were not significant (Figures 1 and 2).

The Falanga wound bed score prior to the treatment was comparable in the study and control groups

TABLE 1 Basic characteristics of the study and the control groups

	Study group	Control group	P
No. of wounds	30	27	
No. of patients	21 (17 M, 4F)	22 (18 M, 4F)	.872
Average age of patients (years ± SD)	64.6 ± 11.6	65.7 ± 10.7	.720
BMI (mean kg/m^2 ± SD)	30.5 ± 4.3	28.9 ± 5.0	.233
Diabetes mellitus type	2 (type 1), 19 (type 2)	1 (type 1), 21 (type 2)	.617
Wound age (average months ± SD)	8.8 ± 14.3	6.7 ± 8.2	0.518
Etiology	ischemia: 1 neuropathy: 13 ischemia + neuropathy: 16	ischemia: 0 neuropathy: 14 ischemia + neuropathy: 13	.551

Abbreviations: BMI, body mass index; F, females; M, males.

TABLE 2 Wound parameters before and after treatment in the study and control groups

	Study group (30 wounds)		Control group (27 wounds)	
	Before CO_2 therapies[a]	After CO_2 therapies[b]	Before placebo treatment[c]	After placebo treatment[d]
Average wound area (mm^2 ± SD)	486 ± 707	35 ± 109	407 ± 453	305 ± 390
Average wound volume (mm^3 ± SD)	1746 ± 3011	35 ± 126	613 ± 518	438 ± 417
Wound bed appearance (Falanga score)	A:1	A:10	A:2	A:10
	B:20	B:0	B:22	B:15
	C:8	C:0	C:7	C:3
	$P_{Falanga}{}^{ab} = .001$		$P_{Falanga}{}^{cd} = .024$	

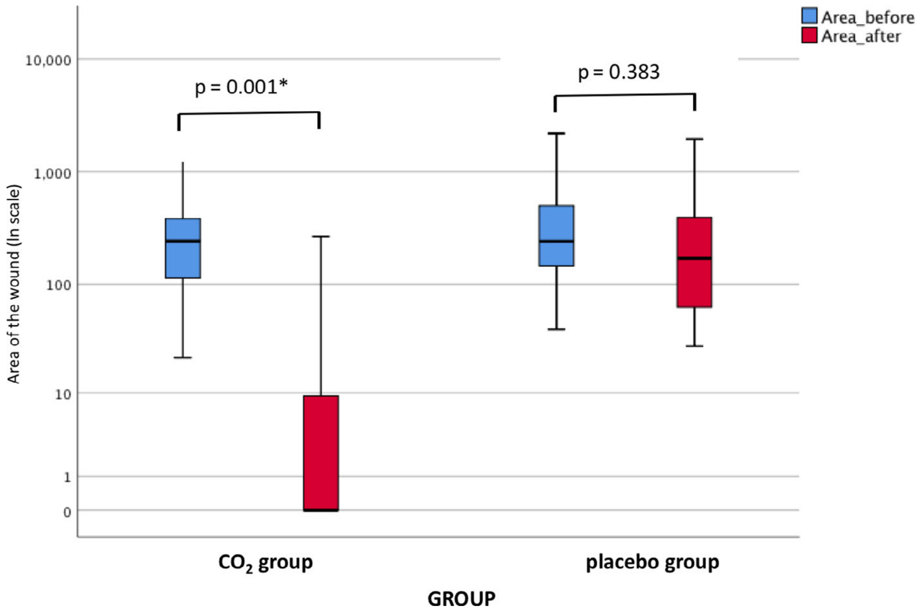

FIGURE 1 Area of the wound before and after treatment in the study and control groups

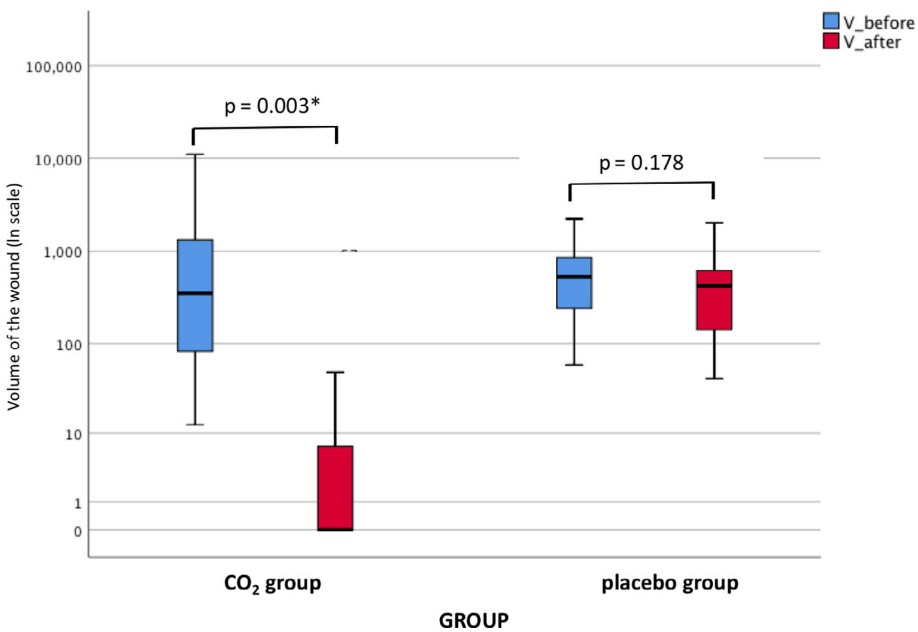

FIGURE 2 Volume of the wound before and after treatment in the study and control groups

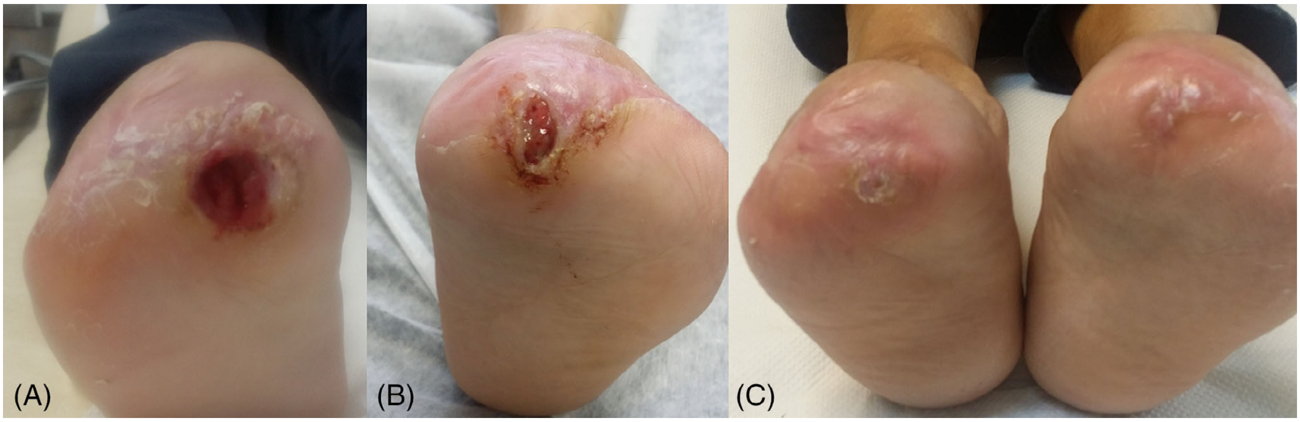

FIGURE 3 A 62-year-old male with diabetes and peripheral artery disease (PAD), that had persisting wounds for 8 months (A and B). Both wounds were completely in 4 weeks with transcutaneous CO_2 application (C)

FIGURE 4 A 66-year-old male with diabetes, PAD and Charcot arthropathy had persisting wound for 6 months (A). The wound was completely healed in 4 weeks with transcutaneous application of CO_2 (B)

($P_{\text{Falanga}}{}^{ac}$ = .343). The wound bed score according to Falanga significantly improved in both the study and control groups, although the difference in the study group was far more significant (see $P_{\text{Falanga}}{}^{ab}$ and $P_{\text{Falanga}}{}^{cd}$). After the treatment, the Falanga score was significantly better in the study group compared with the control group ($P_{\text{Falanga}}{}^{bd}$ = .001).

Figures 3 and 4 show wounds prior to and after CO_2 therapies.

4 | DISCUSSION

The results of our research confirmed that transcutaneous application of gaseous CO_2 significantly improved the healing of chronic wounds in diabetic patients. Wounds in study group patients that received CO_2 therapy healed significantly faster compared with the control group (Table 2). After CO_2 therapy, 66% of the wounds healed completely compared with 0% in the placebo group.

Wollina et al. presented the results of transcutaneous application of gaseous CO_2 in 86 patients with chronic wounds.[13] Similar to our research, these patients received the same transcutaneous CO_2 therapy once daily, but the exposure time is not specified. They performed the treatment for 6 to 14 days—until they observed granulation tissue formation and improved microcirculation. At that stage, they covered the wounds with mesh grafts.

Our results are similar to Wollina's. Wollina et al. noted improved granulation and microcirculation, much like we did in our research. Moreover, the Falanga score improved significantly (Table 2) in our research in both the study and control groups, although the improvement in the study group was far more significant.

The decision whether to prolong CO_2 therapy to complete the healing or to cover the wound when the wound bed enables mesh graft coverage (as in Wollina's case) depends on the wound size and the decision of the patient and physician.

Wollina did not specify the duration of a single therapy, but we decided for 45 minutes. The reason is based on the findings of Finzgar's research where the effect of transcutaneous application of gaseous CO_2 on the microcirculation in healthy men was investigated.[22] The temperature of the skin in the exposed leg reached a plateau in 30 minutes. Since Finzgar et al. included healthy men and we were treating patients with diabetes who have impaired circulation, an additional 15 minutes of exposure time were added to ensure the maximum effect.

Shalan et al. performed an observational study on 22 diabetic patients with chronic wounds.[20] There was no control group. They immersed their patients' feet into CO_2_enriched water once daily for 30 minutes, for 15 days. During this time, they noticed improved blood flow (confirmed by Doppler flowmeter) and improvement in wound color. There was no significant improvement in wound area reduction. The authors rightfully assumed that CO_2 treatment would probably improve wound healing if the treatment lasted longer.

Three years later, Abdulhamza et al. conducted a study using the same device as Shalan.[14] They increased the number of therapies three times compared with Shalan et al. and indeed confirmed significant improvement in wound healing compared with standard treatment methods.

Abdulhamza et al. performed a study similar to ours except theirs was not double blind.[14] They included 100 diabetic patients with chronic wounds divided into study and control groups. The study group received both standard treatment and CO_2 therapy. CO_2 therapy was not administered as gas as in our study but as CO_2-rich water. The patients immersed their feet in CO_2-enriched water for 30 minutes, three times per week for three months. After the treatment, a significant improvement was seen in the study group in Doppler imaging values of the large leg arteries and veins and a decrease in the wound size was noted. The control group showed no differences in the observed parameters.

CO_2 therapy has two kinds of effects: immediate and delayed. The immediate effects are vasodilatation and the Bohr effect.

As noted by many authors, CO_2 therapy has a vasodilatory effect probably influenced by a NO-dependent pathway.[13,14,17,23-26] Savin as well as Sato confirmed vasodilatation and increased blood flow after a single exposure to CO_2 therapy compared with a placebo in humans.[17,26] Minamiyama and Yamamoto used intravital microscopy to confirm subcutaneous vasodilatation after CO_2 therapy in rats.[27]

Another mechanism that is involved immediately after application is the Bohr effect, which was confirmed by Sakai.[12] The Bohr effect means that in increased pCO_2 changes the $Hb\text{-}O_2$ dissociation curve (shifts to the right) so the Hb releases more O_2 and the tissue gets more oxygenized. Hartmann already showed improved tissue oxygenation after CO_2 immersion of the feet for 20 minutes.[19] Sakai additionally verified this effect in vivo in seven healthy volunteers and confirmed the change between oxygenated and deoxygenated Hb during single transcutaneous application of dry CO_2 therapy.[12]

A single treatment with CO_2 therefore exerts vasodilatation and elevates oxygen release from Hb via the Bohr effect.[12,17,24,26] In order to enhance wound healing, CO_2 application must be repeated to maintain tissue supply with oxygen and to induce neoangiogenesis, a delayed effect of CO_2 therapy. The importance of repetition of CO_2 therapy can be observed from comparison of Shalan's and Abdulhamzah's studies.[14,20] To the best of our knowledge, no research on humans exists investigating the effect of repetition and duration of transcutaneous CO_2 application on angiogenesis. But there are several well-designed studies on rats.

Oda et al. used 40 rats which were divided into four groups after an induced femur fracture.[28] The lower limbs of the rats were shaved and CO_2 therapy was applied on the lower part of the body. The first group had their bags filled with air (control), the second with CO_2 for one week (1w group), the third for two weeks (2w group), and the fourth for three weeks (3w group), each for 20 minutes daily. After three weeks, the animals were sacrificed and evaluated. Results showed a fracture union with callus formation in 90% of the 3w group, 60% in the 2w group, 30% in the 1w group and 20% in the control group. The fracture union rate was significantly higher in the 3w group vs the control. In the 3w group, angiogenesis and the vascular endothelial growth factor were significantly enhanced compared with the control group.

Ueha et al. measured running distance in rats which trained on an activity wheel daily.[29] One group received a 10-minute gaseous CO_2 therapy daily using a sealed plastic bag covering the lower part of the body. The other training group with no additional treatment served as the control. Running distance was compared daily. After 10 days, no difference in the running distance was observed, but after 25 days, the CO_2-treated rats ran significantly longer distances compared with the controls, with the differences getting more significant up to the final day on the fourth completed week. Histology confirmed significantly increased capillary density and mitochondrial number in the CO_2-treated rats compared with the non-treated controls.

The strength of our study is its methodology, because it was designed as a controlled double-blind randomized study, and that it was performed on patients representing the intended treated population. To our knowledge, our study and Wollina's are the only ones investigating transcutaneous application of gaseous CO_2 on diabetic wound healing, but Wollina had no control group. Limitations of our study are the differences in the wound size and volume as well as differences in demographic data of the patients. However, the limitations were minimized since the demographic data of patients and initial wound evaluation were comparable between both groups.

Considering our results and effects of CO_2 therapy described in the literature, transcutaneous application of gaseous CO_2 seems to be an effective adjuvant therapy in the treatment of chronic wounds. Chronic wounds are sometimes very difficult to treat; therefore, it is important to know what kind of adjuvant therapy is available and confirmed as effective. Since the mode of action of CO_2 therapy is improved vascularization, it can be combined with previously described advanced therapeutic approaches in order to treat even the most persistent wounds.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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