The Effectiveness of Negative Pressure Therapy in Diabetic Foot Ulcers with Elevated Protease Activity: A Case Series

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Objective: Despite several works have described the usefulness of negative pressure therapy (NPT) in the treatment of diabetic foot ulcers (DFUs), no studies have reported its ability in the proteases modulation in DFUs. The aim of this work was to evaluate the role of NPT as a protease-modulating treatment in DFUs.

Approach: We conducted a prospective study of a series of diabetic patients affected by chronic DFUs. Each ulcer was assessed for matrix metalloproteinases (MMPs) activity with a protease status diagnostic test at the baseline and after 2 weeks of NPT.

Results: Four patients were included. All patients had type 2 diabetes with a disease duration of ≈ 20 years. A1c was 79.5 ± 15.3 mmol/mol. Ulcer area was $>5 \text{ cm}^2$ in all cases. All wounds showed elevated protease activity (EPA) at the baseline. After 2 weeks, all patients showed a normalization of MMPs activity.

Innovation: NPT showed its effectiveness in the reduction of EPA in chronic DFUs.

Conclusion: This study confirms the role of NPT in the positive modulation of protease activity also in chronic DFUs.

Keywords: foot ulcers, negative pressure, chronic wounds

INTRODUCTION

PHYSIOLOGICAL WOUND HEALING requires a complex series of overlapping phases: (1) hemostasis, (2) inflammation, (3) proliferation and (4) remodeling.¹ Each alteration of this process leads to impaired wound healing.

Although acute wounds follow an orchestrated progress through all the healing phases, the inflammatory phase appears prolonged in chronic wounds, leading to alteration of the balance between promoting healing factors and nonhealing factors.²

Proteases have a main role in this process. They are enzymes involved

in the removal of damaged tissue, especially the extracellular matrix (ECM); they break down the capillaries basement membrane to allow the migration of vascular endothelial cells; they facilitate cellular movement through the collagen matrix, by cutting the attachment of the cell membrane to the matrix. Moreover, proteases are needed for contraction and final ECM remodeling.³

Elevated levels of matrix metalloproteinases (MMPs) and reduced levels of their endogenous tissue inhibitors of metalloproteinases (TIMPs) have been shown in chronic wounds.³⁻⁶ It has been hypothesized that increased

activity of these enzymes could cause excessive degradation of ECM proteins, interfering in a key passage of wound healing.

Many studies focused on dressings able to reduce levels of MMPs.^{7–14}

Among these, negative pressure therapy (NPT) plays an interesting role. NPT is a noninvasive therapy system that applies controlled negative pressure using a vacuum-assisted closure device.¹⁵ Its role in the management of the wound bed environment has been widely described.^{16–21} Some authors reported a reduction in expression of MMPs in wounds of various etiologies.^{22–25}

CLINICAL PROBLEM ADDRESSED

The aim of our study is to evaluate the efficacy of NPT to reduce MMPs activity in diabetic foot ulcers (DFUs) with elevated protease activity (EPA).

MATERIALS AND METHODS

We conducted a prospective study of a series of diabetic patients presenting at the Diabetic Foot Unit of the University of Rome Tor Vergata with an extensive foot tissue loss caused by previous infection or gangrene. The study follows the principles outlined in the Declaration of Helsinki. Verbal informed consent was obtained from all subjects involved in the study.

Inclusion criteria were as follows:

- Chronic wounds (relative wound area reduction at 4 weeks <50%).²⁶
- A2 foot ulcer as defined by the University of Texas Diabetic Wound Classification (no ischemia/no infection/no bone exposure).

- Absence of clinical signs of infection (purulent secretion, pain, edema, perilesional cellulitis, and malodorous wound).
- Adequate blood flow established by transcutaneous oximetry (TcPO₂) >30 mmHg.²⁷
- Absence of necrotic tissues.
- Adequate off loading.
- Any treatment with local or systemic antibiotics.
- Any local or systemic steroids therapy.

Each patient was assessed for sex, age, type, and duration of diabetes, glycated hemoglobin (A1c) levels, serum creatinine levels, nutritional values, and previous lower limb revascularization. Local blood perfusion and peripheral neuropathy were evaluated by TcPO₂ and vibration perception threshold, respectively.

MMPs activity of each ulcer was evaluated by a protease test at the baseline and after 2 weeks of NPT Fig. 1.

Protease test

To identify EPA wounds, we used the WOUND-CHEK™ Protease Status diagnostic test. It is an immunochromatographic test for the qualitative assessment of human neutrophil-derived inflammatory protease activity from a wound fluid swab sample taken from wounds.

We used a standardized specimen collection protocol (Serena's technique) to ensure an optimal collection of the wound fluid.²⁸ The test took ~15 min to provide results.

Negative pressure wound therapy

Owing to the hospital availability, KCI V.A.C. Ultra™ Negative Pressure Wound Therapy System

Figure 1. Schematic diagram of the study design.

(Kinetic Concepts Incorporated, San Antonio, TX) was used during patient admission. During KCI V.A.C. Ultra application, the supplied black sponge was used and sealed at 125 mmHg of continuous pressure. All NPT systems were applied according to the KCI guidelines and changed two times per week. All NPT systems were ongoing for 2 weeks.

RESULTS

A total of four patients were included. This case series included three men and one woman (mean age 67.25 years).

All patients had type 2 diabetes with a disease duration of ≈ 20 years. A1c was 79.5 ± 15.3 mmol/mol. Ulcer area was $>5 \text{ cm}^2$ in all cases. TcPO₂ values were 42.7 ± 11.7 mmHg. Percutaneous angioplasty had been previously performed in all patients. The patients' characteristics are listed in Table 1.

All wounds showed EPA at the baseline. After 2 weeks of NPT, all patients with EPA showed normalization of MMPs activity.

DISCUSSION

Over the past years, several researches have been focused on the usefulness of NPT in the treatment of chronic wounds, including DFUs.^{29–34}

Nevertheless only few articles described the NPT influence on MMPs activity.^{22–25}

Greene *et al.* described the reduction in MMP-9/neutrophil gelatinase-associated lipocalin, MMP-9, latent, and active MMP-2 activity by 15% to 76% determined by zymography in three patients (1 DFU) after 2 weeks of NPT.²² Moues *et al.* showed significantly lower levels of pro-MMP-9 and higher

ratio of MMP-9/TIMP-1 in 15 NPT-treated wounds than in 18 conventional treated wounds. Wound fluid samples were collected daily for up to 10 days using a sterile porous filter, and quantitative measurements of pro-MMP-9 and TIMP-1 levels were analyzed using enzyme-linked immunosorbent assay (ELISA).²³

Stechmiller *et al.* reported significant decreased tumor necrosis factor- α levels after 7 days of NPT in six patients with pressure ulcers. Instead, they did not find decreased levels of interleukin-1 β (IL-1 β), MMP-2, MMP-3, or TIMP-1 from the baseline. The wound fluid was collected directly from the drainage tube and quantitative measurements were performed by ELISA.²⁴

Otherwise, Shi *et al.* reported a reduction in MMP-2 levels in chronic wounds treated with NPT for 7 days.²⁵

In our knowledge, studies regarding the role of NPT on MMPs modulation in DFUs are not available.

In our small pilot study, we evaluated MMPs activity in chronic DFUs detected by a noninvasive diagnostic test.

In this study, we applied NPT in patients with EPA. After 2 weeks of treatment, we repeated the protease status diagnostic test that showed, in all cases, normal MMPs activity.

Our data seems to confirm the role of NPT in the modulation of MMPs activity in patients affected by DFUs.

According to a recent study, EPA is associated with 90% probability of nonhealing without an appropriate intervention.³⁵

This finding is in line with our previous study, in which we found a strict relationship between MMPs activity and the dermal graft integration.³⁶

The evaluation and modulation of MMPs activity are a key point in the concept of “wound bed preparation.”

In addition to NPT, several researches are focused on dressings able to modulate MMPs activity. Oxidized regenerated cellulose/collagen matrix (ORC/collagen matrix) has been reported to improve the wound microenvironment by adsorbing wound exudate and retaining proteases within the dressing structure.^{7–13} A randomized controlled trial demonstrated the superiority on modulating proteases of a wound dressing based on the Lipido-Colloid Technology, impregnated with a Nano-Oligo Saccharide Factor compared with an ORC/collagen matrix.¹⁴

A systematic review of the clinical evidence on protease-modulating dressing and researches that compare these devices would be useful in providing

Table 1. Demographics and baseline wound characteristics

Sex (% males)	75
Age (years)	67.25 $\pm$ 5.9
Type 2 diabetes (%)	100
Duration of diabetes (years)	23.5 $\pm$ 3.1
A1c (mmol/mol)	79.5 $\pm$ 15.3
Ulcer size $>5 \text{ cm}^2$ (%)	100
TcPO ₂ (mmHg)	42.7 $\pm$ 11.7
VPT	26 $\pm$ 19.8
Previous PTA (%)	100
Serum creatinine (mg/dL)	4.4 $\pm$ 3.0
Dialysis patients (%)	50
Serum albumin (g/dL)	2.4 $\pm$ 0.2
Transferrin (mg/dL)	124.5 $\pm$ 29.2
Prealbumin (mg/dL)	11.8 $\pm$ 3.1
Retinol binding protein (mg/dL)	4.7 $\pm$ 1.3
Triglycerides (mg/dL)	164.2 $\pm$ 38.3

PTA, percutaneous transluminal angioplasty; VPT, vibration perception threshold.

an overview of the appropriate treatment to achieve optimal healing for chronic wounds.

INNOVATION

DFUs are among the most expensive wounds to treat. The analysis of wound bed environment, particularly MMPs activity, needs to be considered before to define the therapeutic strategy. In case of EPA wounds, protease-modulating therapy needs to be applied. NPT is a valid option to normalize MMPs activity to make a suitable environment to expensive dressing such as dermal graft. This study confirms the usefulness of NPT to modulate MMPs activity in chronic DFUs. Further studies with larger samples are needed to support this evidence.

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AUTHOR DISCLOSURE AND GHOSTWRITING

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KEY FINDINGS

- EPA has been correlated with delayed wound healing in chronic wounds of various etiologies.
- The analysis of wound bed environment, particularly MMPs activity, needs to be considered before planning the therapeutic strategy.
- Several works have described the usefulness of NPT in the treatment of DFUs.
- Our work shows the skill of NPT to reduce EPA also in chronic DFUs.

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Abbreviations and Acronyms

DFUs = diabetic foot ulcers
 ECM = extracellular matrix
 ELISA = enzyme-linked immunosorbent assay
 EPA = elevated protease activity
 HbA1c = glycated hemoglobin
 IL-1 β = interleukin-1 β
 MMPs = matrix metalloproteinases
 NPT = negative pressure therapy
 ORC = oxidized regenerated cellulose
 TcPO₂ = transcutaneous oximetry
 TIMPs = tissue inhibitors of metalloproteinases