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The Efficacy of a Polyhydrated Ionogen Impregnated Dressing in the Treatment of Recalcitrant Diabetic Foot Ulcers : a Multi-centre Pilot Study

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Key words. Diabetic foot ulcer ; polyhydrated ionogen ; matrix metallo proteinases (MMP) ; wound dressing.

Abstract. *Objective :* Assessing the efficacy of a polyhydrated ionogen impregnated dressing in the treatment of recalcitrant diabetic foot ulcers.
Summary Background Data : Diabetic Foot Ulcers (DFU) continue to present a formidable challenge in terms of morbidity and health care costs. Increasing evidence ascertains the important role of Matrix MetalloProteinases (MMPs) and their tissue inhibitors, TIMPs, in wound healing. Imbalance of MMPs in the DFU microenvironment has been associated with poor wound healing. Current research is directed towards therapeutic agents that could redress the imbalance of MMPs/TIMPs.
Poly Hydrated Ionogen (PHI) formulation is based on metallic ions and citric acid. PHI application aims to positively restore MMP ratios within chronic wounds. This initial multi-centre pilot study aimed to investigate the efficacy of the PHI formulation in achieving stable wound closure in recalcitrant DFUs.
Material and methods : Twenty patients with therapy resistant DFUs of at least 1 cm^2 and 3 months duration were treated with PHI formulation in an acetate carrier dressing. Wound debridement, digital imaging and wound perimeter tracing was performed weekly. Off-loading was performed by the use of appropriate shoe-wear (cut-out sandals) and crutches. Patient satisfaction was assessed with a questionnaire. A detailed evaluation sheet was kept for every patient and updated at each visit.
Results : Stable wound closure with high patient satisfaction was achieved in 16 (80%) DFUs. The mean time to full closure was 18 weeks. A stable wound epithelization was seen in all full closure patients up to latest follow-up of one year.
Conclusions : Encouraging results of this pilot study prompt us to further investigate the PHI efficacy in DFU treatment in a multi-centre, randomized controlled trial.

Introduction

Diabetic foot ulcers (DFU) continue to pose a major health burden in terms of costs and reduction of the quality of life of affected patients (1). A diabetic foot wound is the most common cause for hospitalization of patients with diabetes. Almost half (43%) of all diabetic patients have their first hospital admission or diagnosis of diabetes associated with either a foot ulcer or an infection. Up to 70% of all non-traumatic, major lower limb amputations are performed on diabetic patients amounting to approximately 82,000 limb amputations each year (2). The 5-year mortality after amputation in diabetes patients ranges from 39%-68% (3).

The expenses for patients and the health care system associated with diabetic foot ulcers are substantial and must not be underestimated (3). The direct medical cost for the treatment of a diabetic patient with a foot ulcer in a 2-year period has been estimated at approximately 28,000 USD (4). Amputation costs per patient annually range from 20,000-60,000 USD (5). These figures do not take into account the reduction in quality of life for these patients and the fact that DFU related morbidity poses significant social, personal and economic implications for each affected individual.

Successful treatment of diabetic ulcers continues to be a formidable challenge. These wounds are difficult to heal and closure is often unstable and difficult to maintain. Diabetic related wounds are highly susceptible to infection, which can spread quite rapidly, leading to major tissue destruction and subsequent amputation (6). The cornerstone of DFU treatment evolves around surgical debridement, adequate off-loading and moist wound healing (7).

A considerable amount of research is targeted towards strategies that aim to reduce ulcer formation and subsequent amputation and improve the quality of life of affected patients. Off-loading measures aimed at reducing the repetitive mechanical stress forces on the foot that have been recommended for DFU treatment include : bed rest, crutches, walking casts, various prefabricated walkers and braces, cut-out sandals and foam pads (8).

Topical application of wet-to-dry gauzes has traditionally been the standard of care of DFUs to which new treatment modalities are compared (7).

A plethora of new dressings are being marketed for the treatment of DFUs aiming to improve wound care and accelerate healing (9-10).

More recently, new treatment strategies brought about by tissue engineering and growth factor research have been evaluated for the more difficult to treat wounds. Biological materials such as Apligraf®, Dermagraft®, and Becaplermin have been evaluated within trials with varying degrees of success. None of these modalities has gained wide acceptance up to now (11-14).

Other dressings are being impregnated with substances that are designed to modulate the wound microenvironment with a shift towards accelerated healing. The characterization of wound microenvironment and the processes that cause progression of acute wounds into chronic wounds have been the focus of considerable research (15-18). These studies suggest that high concentrations of proteases are induced by persistently high concentrations of pro-inflammatory cytokines in patients with chronic wounds (19-20). These proteases are instrumental in degradation of various growth factors, receptors and components of the extracellular matrix (ECM), which are essential for normal wound healing. Amongst these proteases are the matrix metalloproteinases (MMPs), a family of zinc-dependent endopeptidases capable of degradation of the entire ECM (21). The normal balance between MMPs and their tissue inhibitors (TIMPs) plays an important role in the well orchestrated events that lead to wound healing (22). Lobmann, Ambrosch, Schultz *et al.* studied the expression of MMPs and TIMPs in the wounds of diabetic and non-diabetic patients and concluded that the combination of increased MMPs and decreased TIMPs in chronic DFUs compared to healing wounds in normal patients suggests that the increased proteolytic activity contributes to the failure of healing in DFUs (23). These authors advocate treatment strategies aimed at reducing MMP concentration and increasing TIMP concentrations.

A formulation containing metal ions (polyhydrated ionogen, or PHI-5) and citric acid has been developed that is shown to reduce reactive oxygen species in cultures of polymorphonuclear leukocytes (24). The effect of PHI on gene expression, including synthesis of MMPs, was studied in cultures of dermal fibroblasts from normal and diabetic patients and showed that PHI decreased MMP-2 mRNA and increased TIMP mRNAs levels (25). Dermax® (Epimax® in U.S.A.) is an acetate carrier dressing impregnated with this PHI-5 formulation which is intended to promote wound healing in difficult to heal wounds such as DFUs by reduction of reactive oxygen species and by redressing the imbalance of the MMP/TIMP ratio in the wound microenvironment.

The aim of this phase I multi-centre pilot study was to initiate clinical investigations into the safety and efficacy of this PHI-5 impregnated dressing as preparation for larger randomized controlled trials.

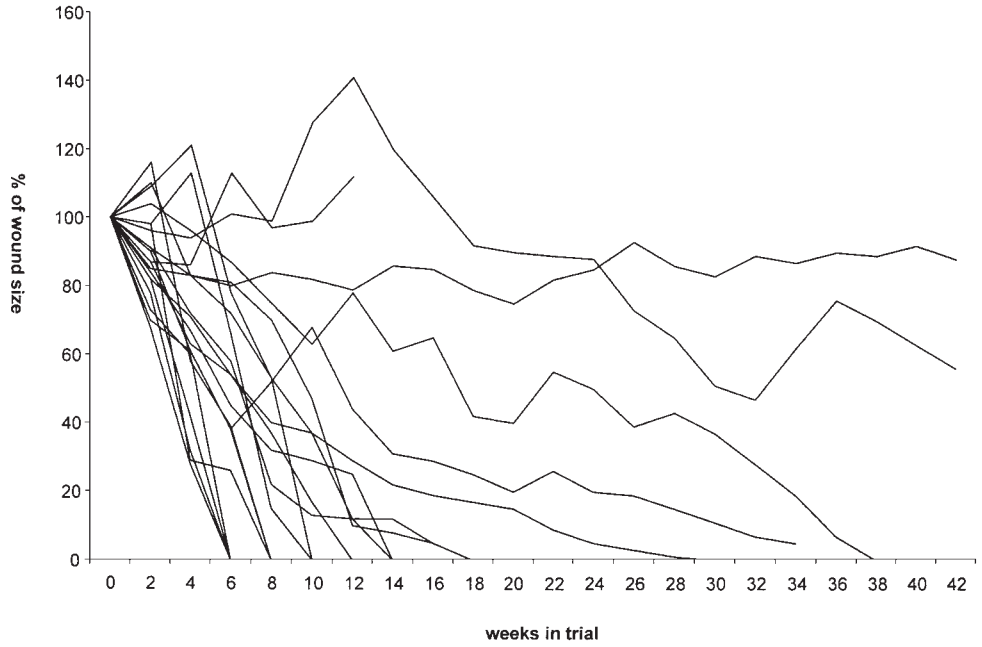
Material and Methods

Twenty patients with recalcitrant DFUs were recruited between January 2003 and January 2005. Research centres were plastic surgery departments in Ghent (Belgium), Verona (Italy), Rome (Italy) and East Grinstead (United Kingdom). All procedures, including obtaining informed consent, were conducted in accord with the ethical standards of the Committee on Human Experimentation of each centre.

Initial evaluation of the patient included a full medical history and physical examination with special attention to the diabetic status of the patient including duration, type, management, diabetic control, activity level and ambulatory status. Patients were eligible who met the following inclusion criteria : 18 years or older with a diabetic foot ulcer of at least 3 months duration, Wagner grade 1 to 2 and at least 1 cm^2 (greatest length and width). Patients had an adequate circulation with an ankle /brachial index > 0.65. Doppler venous imaging of at least 6 months prior to referral was obtained to exclude thrombosis or deep venous insufficiency. Baseline blood tests including HBA1c, ureum and creatinine and liver function test were within normal ranges. HBA1c levels were evaluated at the initial visit at 6 weeks and at termination of the study to monitor glycemic control.

Exclusion criteria included carcinoma, vasculitis, known hypersensitivity to dressing components, radiation therapy, multiple ulcers on same foot, use of steroids, alcohol or drug abuse, non-compliance or inability to off-load or meet outpatient appointments on more than 2 subsequent weekly visits. Informed consent and local institutional board approval were obtained.

Weekly evaluations consisted of wound perimeter tracing, measurements and digital photography. Surgical debridement was carried out by the local coordinator once a week or more often if necessary and was



Graph 1
illustrates time to full closure of diabetic foot ulcers treated with Dermax®.

standardized at each centre. Dressing changes were standardized in the following manner : wounds were cleaned with isotonic saline and dressed with Dermax® as primary dressing and sterile gauze and bandage and tape as secondary dressing. The frequency of dressing changes depended on the level of exudate. At least one dressing change per 2 days was performed. Off-loading was performed by the use of appropriate shoe-wear (cut-out sandals) and crutches. At the final visit (week 14), a questionnaire was completed by the patient and the investigator, evaluating the satisfaction.

A detailed evaluation sheet was kept for every patient and updated at each visit. The following parameters were recorded : ulcer area, ulcer duration, ulcer grade, frequency of dressing changes, the presence of oedema, erythema, undermining, maceration, exudate, type of exudate, wound granulation and extent of granulation. Pain perception between and during dressing changes was scored on a 1-5 scale (with 1 being no pain and 5 being excruciating pain).

Results

Twenty patients with diabetic foot ulcers (12 men and 8 women) were treated with a mean age of 56 years (range 28 years to 78 years). Ulcers sizes ranged from 2,33 cm^2 to 19,8 cm^2 (mean 7,81 cm^2) with a mean duration of 5.25 months (range : 3 to 12 months). Haematological investigations were unremarkable and HBA1C stayed within acceptable ranges with a mean of 6,9% (range 6,3% to 7,7%).

Complete closure was defined as the completely epithelialized state at which the patient was able to take a shower. In 16 out of 20 patients (80%) complete wound closure was obtained with Dermax® treatment. The time required for complete healing ranged from 6 to 38 weeks, the mean time to full closure was 18 weeks (Graph 1). Follow-up examination for all full closure patients continued for at least 6 months. A stable wound epithelization was seen in all full closure patients up to latest follow-up of one year. Patients in which no wound closure was obtained showed the following characteristics : 2 patients developed purulent wound infection at the wound site causing further stagnation in wound healing process. Another patient underwent surgery and amputation of the forefoot after accidental severe burn wounds on the investigated foot. One patient showed no response to Dermax® application, arguably due to morbid obesity, fluctuating sugar levels and questionable compliance.

Overall patient satisfaction was very high and all the patients who had full wound closure would recommend the treatment to others. Patients and investigators were happy about the easy application of the product and the majority of patients experienced a reduction of pain during the period they used Dermax®. The mobility of all patients improved during the therapy which resulted in a higher quality of life for everyone (Fig. 1 & 2).

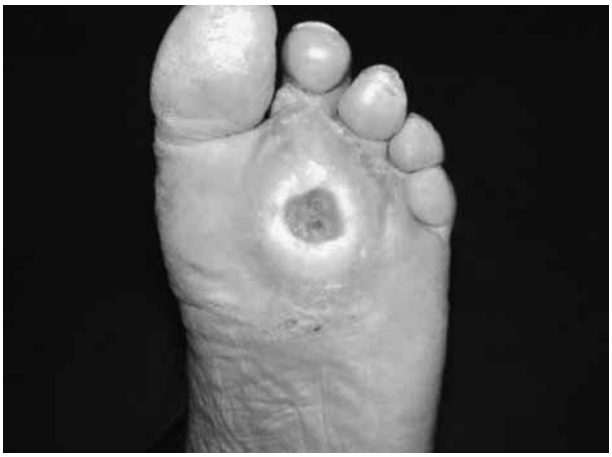


Fig. 1
A 33-year old woman with a plantar diabetic foot ulcer, existing already for 12 months, not responding to classic wound dressings and off-loading.

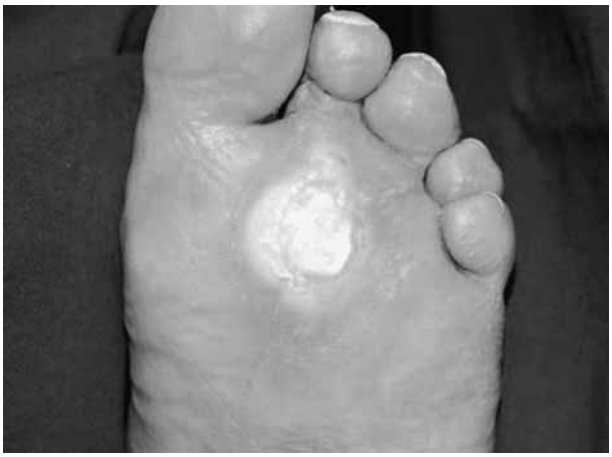


Fig. 2
Full closure after 18 weeks with daily application of a PHI-5 formulation dressing.

Discussion

Numerous factors contribute to impaired wound healing in diabetic patients. On a vascular level, there is decreased blood flow and oxygen levels due to atherosclerosis impeding wound healing by stagnation of collagen formation and oxidative bactericidal mechanisms (26).

Diabetic patients with sensory neuropathy have reduced nerve fibers. Their chronic wounds are not only associated with repetitive trauma but also by a diminished trophic effect caused by neuropeptides, which are essential for normal skin homeostasis (27-28).

Metabolic disruption of wound healing due to sustained hyperglycaemia is associated with alteration of cellular $Na^+/K+$ activity and increase in Protein kinase C activity which induces diabetic vascular complications (29-31). Moreover hyperglycaemia is associated with endothelial cell and extracellular matrix dysfunction. Hyperglycaemia also induces granulocyte dysfunction, particularly in the inflammatory phase with impairment of neutrophil chemotaxis and cytokine release (32). Delayed phagocytosis of micro-organisms causes accumulation of debris within the extracellular tissue and thus prevents formation of granulation tissue essential for healing (33). Fibroblast dysfunction causes impaired collagen synthesis and reduced tissue strength that is prone to brittle, unstable scar formation (34).

Many studies have shown that impaired wound healing in diabetic patients is related to dysfunctional wound cells and by imbalances in key proteases, cytokines and growth factors. The protracted inflammatory reaction in these patients generates a correspondently high protease response, especially of matrix metalloproteinases (35-36). Bacterial contamination and repetitive trauma sustain this inflammatory reaction which is shown by high levels of neutrophil granulocytes. Granulocytes produce pro-inflammatory cytokines such as tumour necrosis factor alpha (TNF-α) and interleukin 1 (IL-1), both capable of stimulation of MMP synthesis (37-38). Thus, wound healing requires a well orchestrated balance of growth factors, cytokines, proteases and extracellular matrix. High levels of proteases, such as MMPs in chronic wounds may cause excessive degradation of matrix proteins and growth factors that are essential for wound healing (39-40).

MMPs play an important role in wound debridement, angiogenesis, epithelialization and final scar remodelling. The MMP family comprises of 20 enzymes that are grouped in subclasses (collagenases, gelatinases, stromelysins and membrane type MMPs). MMPs are produced by inflammatory cells, fibroblasts, endothelial cells and keratinocytes in different phases of wound healing. The controlled, specific release of each MMP is critical for successful wound healing. MMP-1 is associated with keratinocyte migration (22, 41-42), MMP-3 plays a pivotal role in formation of a basement membrane whereas MMP-2 and MMP-9 are essential for degradation of denatured collagen and granulation tissue formation (43-44). TIMPs bind covalently to MMPs and inhibit their (excessive) activity. Synthesis, activation and inhibition of MMPs is regulated on the following levels : cytokines such as EGF, PDGF, IL-1 and TNF-α regulate transcription and MMP production (45-48). TGF-β can inhibit transcription and MMP production (49-51). MMPs are produced as inactive pro-

enzymes that must be activated by proteases including kalikrein, plasmin, elastase (52). MMP activity is also regulated and controlled by their tissue inhibitors TIMPs (45).

Several studies have shown imbalance of MMP/TIMP ratios in chronic wounds. Treatment modalities that are aimed at redressing this imbalance are evolving and being evaluated in clinical practice (20, 23, 53-54).

One approach towards reduction of excessive protease activity is the application of a dressing with high concentration of gelatine which is a substrate for MMPs (10). Cullen, Smith, McCulloh *et al.* reported reduced elastase and plasmin activity by local application of a dressing containing collagen to oxidized collagen ratio of approximately 55 :45 called Promogran®. In a large randomized study on DFUs however no significant benefit was seen with Promogran® therapy as compared to standard moist gauze application.

In our study, we set out to initiate clinical investigations into the efficacy of a formulation synthesized from an initital botanical source, the red oak bark tree. This formulation contains the metal ions zinc, potassium, calcium, rubidium and citric acid (PHI-5). It has been shown to reduce reactive oxygen species in cultures of polymorphonuclear leukocytes (24). The effect of PHI on gene expression, including synthesis of MMPs, was studied in cultures of dermal fibroblasts from normal and diabetic patients and showed that PHI decreased MMP-2 mRNA and increased TIMP mRNAs levels (25).

Dermax® (Epimax in U.S.A.) is an acetate carrier dressing impregnated with this PHI-5 formulation which is intended to promote wound healing in difficult to heal wounds such as DFUs by reduction of reactive oxygen species, control of complement activation and preservation of nitric oxide metabolism and by redressing the imbalance of the MMP/TIMP ratio in the wound microenvironment.

In our study, stable full closure was obtained in 16 out of 20 patients with difficult to treat DFUs that were referred to our department by endocrinologists. These patients had ulcers with a mean duration of 5.25 months that was refractory to standard DFU treatment : 80% of these ulcers healed within 18 weeks therapy with Dermax®.

Wound dressings should ensure moist wound healing and protect the wound against adverse reactions such as infection, maceration and allergic reaction. No adverse reactions were found concerning safety of Dermax® treatment and the results of our evaluation questionnaire showed that at least 80% of patients and care workers were satisfied with this treatment as far as comfort, ease of application, exudate control and pain control was concerned.

A myriad of treatment methods are under clinical investigation for the management of DFUs These include growth factors, synthetic and biological skin equivalents and cultured autogenic cells. None of these modalities can replace the role of dressings. Wound bed preparation has become an integral part of the battle against difficult to heal wounds and interactive dressings can increase the efficacy of above mentioned treatment modalities.

Effective approach to DFU treatment should include : radical surgical debridement, lower limb revascularization when necessary, effective off-loading regimen, bacterial control and judicious use of dressings that promote wound healing. Critical attention to all these principles was given to patients enrolled in this study which may also have contributed to the high full closure rate. Off-loading and dressing change intervals were not standardized but left to the discretion of the investigators reflecting the current practice. These modalities should be standardized in future trials by use of Camwalkers® and daily dressing changes. Cost and quality of life issues need to be clarified in further trials with appropriate cost utility analysis.

Proof of efficacy of the PHI-5 has been shown in vitro and on a genomic level. Clinical evidence can be satisfactorily substantiated when MMP tissue quantification with Elisa techniques show a significant difference in MMP/TIMP ratio with Dermax® treatment compared to controls.

The promising results of this initial pilot study with Dermax® strongly encourage us to advocate further randomized controlled trials, including MMP quantification on a tissue level to further substantiate the efficacy of this formulation.

Aknowledgements

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