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# Efficacy of intralesional recombinant human epidermal growth factor in chronic diabetic foot ulcers

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**Abstract**

*Objective*: The aim of this study was to explore the clinical effects of intralesional administration of an epidermal growth factor (EGF) up to complete wound closure. *Methods*: Seventeen diabetic patients with full-thickness lower extremity ulcers of more than 4 weeks of evolution were enrolled in the study. Mean ulcer size was 15.5 +/− 7.5 $cm^2$. Intralesional injections of 75 μg of Heberprot-P three times per week for 5–8 weeks were given up to complete wound healing. *Results*: Full granulation response was achieved in all patients in 32.4 +/− 6.6 days. Complete wound closure was obtained in 16 (94.1%) cases in 53.1 +/− 4.7 days. The most frequent adverse events were burning sensation, tremors, chills and pain at the site of administration. After 1-year follow-up, only one patient relapsed. *Conclusions*: Intralesional EGF administration up to complete closure can be safe, effective and suitable to improve healing of chronic diabetic foot ulcer (DFU).

**Keywords**

Amputation and epidermal growth factor, diabetic foot ulcers, wound healing

**History**

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## Introduction

The prevalence of diabetes mellitus and its associated complications is growing at epidemic proportions worldwide. Diabetic foot is one of the common complications of diabetes affecting the quality of life of the patients. Around 20% of the total diabetic population has foot problems, while 5–10% have foot ulcers (Frykberg et al., 2006).

The treatment of diabetic foot ulcer (DFU) is complex. Even when properly managed, the wounds may not heal as expected; when they do heal, the closure is often temporary and difficult to maintain. Unfortunately, these foot ulcers do not heal easily, are difficult to treat and are more prone to secondary infections (Cruse & Foord, 1973). Neuropathy, high plantar pressure, poor glucose control and duration of diabetes contribute to the severity of foot ulceration (Boyko et al., 1999; Frykberg et al., 1998) and are the most common underlying cause of non-raumatic lower extremity amputations (Jeffcoate, 2005).

Novel therapeutic approaches such as topical use of honey, collagen, cryopreserved fibroblast implants or growth factors are being evaluated to treat diabetic foot ulcers. Human epidermal growth factor (hEGF) is one such factor, which plays a significant role in the regulation of cell growth, proliferation and differentiation. The most studied growth factors are PDGF, fibroblast growth factor (FGF), transforming growth factor-β1, and epidermal growth factor (EGF) (Buckley et al., 1987).

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Evidences of the beneficial effect of topical EGF application in low-grade, neuropathic ulcers have been shown in clinical trials (Hong et al., 2006; Tsang et al., 2003). However, the effect of topical EGF formulation can be abated, especially in high-grade wounds since an increased protease activity has been identified (Mast & Schultz, 1996; Medina et al., 2005). Direct intralesional administration of an EGF-based formulation (Heberprot-P) can overcome this limitation, as has been reported in previous studies (Berlanga et al., 2006; Fernández-Montequín et al., 2007).

In the current study, we postulated that there was a relative deficiency of growth factors in chronic wounds such as diabetic foot ulcers and aimed to determine whether local application of a high concentration of human EGF (hEGF) might be effective in promoting wound healing of diabetic foot ulcers.

## Materials and methods

This study was designed for the efficacy of hEGF in promoting healing of chronic DFUs. Between June 2013 and September 2014, 17 patients were screened. The wound area was determined by means of planimetry (greatest width × greatest length). Predetermined criteria used for patient selection were (1) ulcer with grade I or II, as defined by the Wagner Classification (grade I, superficial ulcer; grade II, deep ulcer to tendon, capsule or bone; grade III, deep ulcer with abscess, osteomyelitis or joint sepsis; grade IV, localized gangrene of forefoot or heel; and grade V; gangrene of entire foot) (Armstrong et al., 1998), (2) ulcer located below the ankle, and (3) ulcer with adequate perfusion, as indicated by an Ankle-brachial index (ABI) >0.7. Patients were excluded if they had very poor sugar control (HbA1c >12%) or had ulcers with severity equal to or greater than grade III. We accepted

referral of new patients from the medical, orthopedic and surgical clinics in our hospital and other hospitals in our region. Informed consent to participate in the study was given by the patients. Exclusion criteria were foot ulcer area $<1\,cm^2$, cardiopathy (recent acute myocardial infarction, unstable angina or uncontrolled heart failure), renal failure (serum creatinine >200 mmol/l and oligoanuria), malignancies and pregnancy.

Informed consent for participation in the study was obtained according to the guidelines of our institutional review board and the local ethics committee, which approved the study.

Patients were treated with intralesional injections of a lyophilized formulation of Heberprot-P containing 75 μg (one vial) of EGF, three times a week on alternate days up to complete wound healing. Recombinant, human EGF was obtained from a transformed *Saccharomyces cerevisiae* strain at the Center for Genetic Engineering and Biotechnology of Havana and contained a mixture of the EGF1-51 and EGF1-52 forms (Cinza et al., 1991).The dose selection was based on a better risk-benefit balance observed with 75 mg in the accumulated clinical data with this product.

Heberprot-P was administered together with a standardized good wound care regimen. Ulcers were cleansed daily using saline or chlorhexidine in case of contamination or infection. Sharp debridement was indicated whenever necessary to remove necrotic tissue. Saline-moistened gauze dressing was used and the affected area was pressure off-loaded. Broad-spectrum antibiotics were used to treat infections, whereas metabolic control was managed with insulin alone or combined with oral hypoglycemic drugs.

The observations of the treatment were recorded after every 2 weeks. At each inspection, the following points were recorded:

- Type of wound discharge, i.e. purulent/seropurulent
- Amount of wound discharge, whether increasing or decreasing
- Granulation tissue formation as percentage of the total surface area
- Changes in the size of wound as the largest transverse diameter and largest vertical diameter
- Presence of slough, if any – as a percentage of the total surface area
- Any cellulitis in the surrounding area

Data on demography, personal pathological history, type and duration of diabetes and its current treatment, peripheral neuropathy, peripheral vascular disease and wound examination were documented. Ankle/brachial index was taken at baseline. Ulcers were classified in grades according to Wagner (Armstrong et al., 1998).

Laboratory tests were performed at baseline and thereafter whenever required, including blood cell count, hemoglobin, hematocrit, globular sedimentation rate, creatinine and aspartate aminotransferase, which were performed by routine clinical laboratory methods. Blood glucose was measured more frequently for the patients' metabolic control. Wound cultures were performed before and during therapy if necessary to monitor infections. Foot infection was defined clinically based on the presence of purulent secretions or at least two signs or symptoms of inflammation.

The primary efficacy endpoint was complete wound closure defined as skin re-epithelialization without drainage or dressing requirements. Other variables recorded were complete granulation response, time to complete closure, time to complete granulation response and indication of amputation. Safety was monitored by the evaluation of daily adverse events during the treatment.

## Results

Patients' demographic and baseline characteristics are shown in Table 1. They all suffered type 2 diabetes mellitus and nine (52.9%) patients received insulin. Mean ulcer size was $15.5 \pm 7.5\,cm^2$. In seven (41%) patients, wounds were localized on the sole, two of them embracing calcaneous. Other localizations were toes in 5 (29%), foot external edge in two (11%) and internal edge in one (5%) patients. The principal risk factors were previous history of ulcer in 10 (58%) patients, history of amputation in 16 (35%) and foot deformity in 8 (44%) patients.

Complete treatment compliance was reported in 16 (94.1%) patients. Voluntary interruption was reported in one case. Complete granulation response was achieved in all patients, including the one abandoner, at a mean time of $32.4 \pm 6.6$ days. The mean time to complete closure was $53.1 \pm 4.7$ days. In the second week of the treatment, 14 patients had an increase in granulation tissue but two patients were resistant to treatment. Fifteen patients continued to show increasing trend in the fourth week. Moreover, six patients had an increase in the surface area covered with granulation tissue in the eighth week of the treatment (Table 2). The time taken for patients to reach end point is shown in Figure 1. Amputation was not necessary in any case and relapse was reported in one patient after 6 months of complete closure.

Figures 2 and 3 show examples of wounds' clinical aspects. Figure 2 was of a 65-year-old female with diabetes

Table 1. Baseline characteristics of the study population.

| Variables | Results |
|---|---|
| Age | $63.2 \pm 9.4$ |
| Sex (Male/Female) | 10/6 |
| Ankle brachial index | $0.88 \pm 0.17$ |
| Ulcer area ($cm^2$) | $15.5 \pm 7.5$ |
| Site (Distribution of ulcer) | |
| Sole | 7 (41.1%) |
| Toe | 5 (29.4%) |
| Calcaneus | 2 (11.7%) |
| Foot | 3 (17.6%) |
| Duration of ulcer (weeks) | $13 \pm 4.6$ |
| History of diabetes (years) | $11.5 \pm 2.8$ |
| HbA1c (%) | $7.91 \pm 1.12$ |
| BMI ($kg/m^2$) | $26.2 \pm 2.25$ |
| Wagner's classification, *n* (%) | |
| Grade 2 | 2 (11.7%) |
| Grade 3 | 12 (70.5%) |
| Grade 4 | 3 (17.6) |
| History of ulcer | 7 (41%) |
| History of amputation | 6 (35.2%) |
| Use of insulin | 9 (52.9%) |
| Serum creatinine >2 mg | 2 (11.7%) |
| Comorbidities* | 15 (88.2%) |

Values are expressed as mean ±SD. *including hypertension, coronary heart disease and hyperlipidemia.

for 25 years and an extensive wound on the foot. Infection, ischemia and osteomyelitis were also present and amputation had been previously indicated by other specialists as the only alternative. After soft tissue debridement, bone resection within the necrotic area and broad-spectrum antibiotics, Heberprot-P intervention was thereafter instituted. Complete granulation response was achieved in 41 days when the patient withdrew from treatment. He was re-evaluated thereafter and complete wound closure was confirmed at day 155.

In our patient group, two patients underwent grafting, 15 had completely healed ulcers, two patients had their wounds sutured, nine patients required debridement, incomplete healing occurred in one patient and one patient was excluded from the study because of cardiovascular complication in the form of heart failure.

The rate of adverse events is shown in Table 3. The most frequent were tremors, chills, pain and burning at the site of administration, and local infection. Most of the adverse events were classified as mild or moderate. The treatment was not interrupted because of adverse events.

Table 2. The trends in granulation tissue formation.

| Weeks | 2 | 4 | 6 | 8 |
|---|---|---|---|---|
| Granulation | (%) | (%) | (%) | (%) |
| Resistant | 2 (11.7) | 1 (5.8) | 2 (11.7) | 0 (0) |
| Increasing | 14 (82.3) | 14 (82.3) | 8 (52.9) | 6 (35.2) |
| Decreasing | 0 (0) | 1 (5.8) | 0 (0) | 0 (0) |

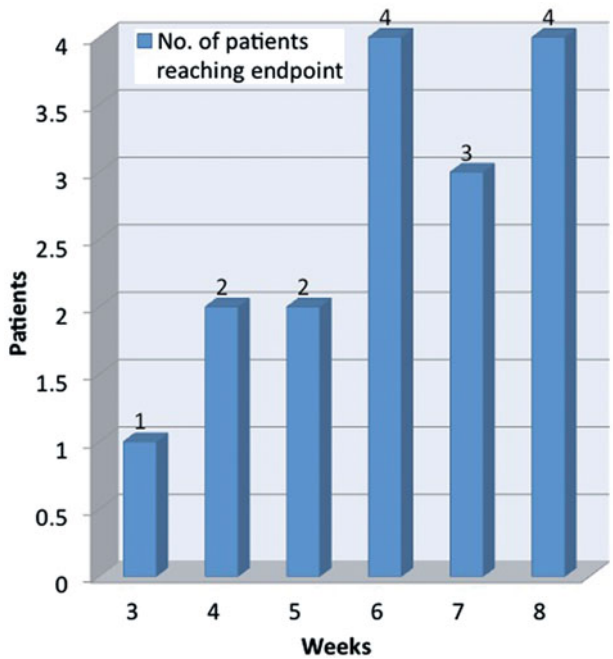


Figure 1. The time taken by patients to reach end point.

## Discussion

Prevention and treatment of diabetic foot ulcers is extremely complicated. Many factors are important for the development of diabetic foot ulcers and all of them have to be considered. The current available evidence is not satisfying for clinicians who are forced to make a choice. Even in recommendations for standard wound care there are variations. The existing evidence concerning alternative therapies is still weaker. Hence, further studies about the treatment of diabetic foot ulcers with growth factors alone or in combination with other technologies of high methodological quality with adequate sample sizes are necessary, especially regarding the demographic change and the growing prevalence of diabetes mellitus leading to higher prevalence rates of diabetic foot ulcers.

Antimicrobial agents, surgical techniques and a broad variety of therapeutic approaches based on drugs and devices have been applied to DFUs (Armstrong & Lipsky, 2004; Dalla & Faglia, 2006). These interventions have shown limited clinical success, even when included in a comprehensive wound care program and there is no evidence of impact on amputation rates. Short-term recurrences remain a problem hampering clinical effectiveness of some contemporary therapies (Gregor et al., 2008).

The primary objective of treatment for DFU is to obtain complete wound closure as expeditiously as possible. Therapy with a growth factor should be maintained until this goal achieved. In this sense, this study shows that the continuity of the treatment with intralesional Heberprot-P up to complete wound closure is feasible and safe to promote healing of chronic DFU. Treatment was well tolerated, adverse events were easily manageable and no significant safety concern was reported. These results are better than those reported in

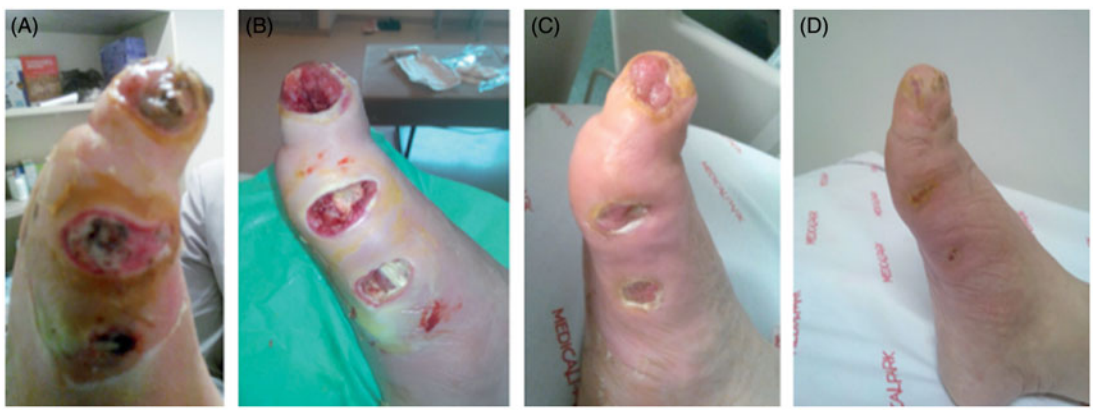


Figure 2. Extensive ischemic ulcer with osteomyelitis and infection. (A) Before treatment; (B) ulcer Immediately after debridement; (C) complete granulation at the end of the treatment; (D) complete re-epithelization after the follow-up.

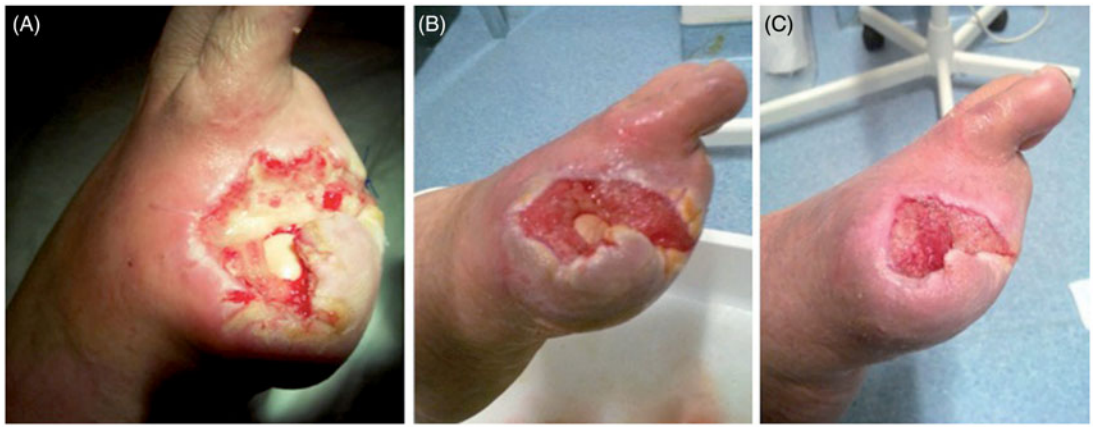


Figure 3. Extensive ulcer with abscess. (A) Before treatment; (B) 5 weeks after the treatment; (C) complete granulation at the end of treatment; (D) ulcer healed completely after 7 weeks of application of Heberprot.

Table 3. Adverse events.

| Events | $N = 17$ (%) |
|---|---|
| Tremors | 2 (11.7%) |
| Chills | 1 (5.8%) |
| Pain at the site of administration | 2 (11.7%) |
| Burning at the site of administration | 2 (11.7%) |
| Local infection | 0 (0%) |
| Weakness | 1 (5.8%) |
| Fever | 0 (0%) |
| Headache | 2 (11.7%) |
| Hypotension | 0 (0%) |
| Sweat | 1 (5.8%) |

previous trials (Berlanga et al., 2006; Fernández-Montequín et al., 2007). In Fernández-Montequín et al.' study, after Heberprot-P was administered in high-grade DFU for a 8-week treatment schedule, a complete granulation response was appeared in 73% of the patients. Complete wound healing was reached in 54% of the patients after 20 weeks since the beginning of the treatment (Fernández-Montequín et al., 2007). In contrast, the analysis of present study showed that the continuity of treatment was associated to improvement in the rate of both granulation response and complete wound closure. Generally, when complete granulation occurs following administration of the formulation, a partial epithelization is also present that continues until complete closure, although treatment had ceased. It seems that the stimulation of granulation response by Heberprot-P treatment is an important step to enhance healing, but while the ulcer does not reach complete closure the risks for infection and amputation cannot be neglected.

Based on the rationale that hEGF can enhance healing of chronic wounds following repeated local infiltrations (Berlanga-Acosta, 2011), various clinical trials using Heberprot-P in DFU patients have been conducted, demonstrating safety and efficacy (Acosta et al., 2006; Berlanga et al., 2006). Infiltration with hEGF for diabetic wound healing does not replace standard procedures but should be incorporated into comprehensive wound care along with medical interventions to correct patients' glycemia and creatinine. In a compassionate study with terminal ulcer patients during 2001–2002, the first clinical evidence using EGF infiltration for diabetic foot ulcers and amputation residual bases emerged (Acosta et al., 2006). All lesions were chronic, complex and recalcitrant, Wagner scale stages 3 and 4. Efficacy demonstrated in these types of wounds paved the way for solid clinical development, which culminated in a nationwide, double-blind, placebo-controlled phase III clinical trial, duly registered with the appropriate Cuban regulatory agency (Fernández-Montequín et al., 2009).

Since then, EGF local injection has been used for complex diabetic wounds in various clinical trials, demonstrating a favorable risk–benefit balance by speeding healing, reducing recurrences and attenuating amputation risk (López-Saura et al., 2011). Adverse effects were preponderantly mild to moderate (65.6% mild, 28.6% moderate and only 3.7% severe), with pain and burning sensation at administration site being the most frequent. Pain reported was mild to moderate in intensity and was not associated with treatment suspension. A dose-effect relation associated with appearance of shivering and chills was consistently obtained in all trials at both doses used (25 and 75 μg) and in the pooled analysis; intensity was mild to moderate and symptom appearance was not associated with treatment suspension (Fernández-Montequín et al., 2009). In our study, the most frequent adverse events were tremors, chills, pain and burning at the site of administration. The treatment was not interrupted because of adverse events.

The EGF infiltration increased and accelerated healing in poor-prognosis wounds toward a rapid and sustained response. More than 80% granulation was obtained globally with Heberprot-P, in comparison with less than 60% with standard care alone. Of patients treated with Heberprot-P at 75 μg, three times per week until complete granulation (or during 8 weeks) in association with standard care, 77% healed; while only 56% healed with placebo injections and standard care (Fernández-Montequín et al., 2007, 2009; López-Saura et al., 2011).

Adjuvant therapies and advanced technologies can be used in addition to standard care as a second line of treatment when appropriate. These include some topical drugs but are mostly medical devices: living skin equivalents, specialized dressings, hyperbaric oxygen therapy and negative pressure devices. These interventions provide moderate improvement over standard treatments, generally only 15 to 20% healing in less than 20 weeks, and may be expensive and time

consuming (Blume et al., 2011). In ischemic patients, surgical revascularization is not suitable for all cases and some authors consider its effectiveness to be overestimated in any case (Taylor et al., 2011). The regulatory process for surgical procedures does not require the same degree of clinical detail as demanded for biological products. Their remains a clear need for adequate comprehensive therapy to improve healing in severe wounds, for which Heberprot-P has demonstrated clear beneficial potential.

In these initial studies, however, Heberprot-P intralesional treatment was continued until a complete granulation response or up to a maximum of 8 weeks. Thus, the safety profile of this intervention modality under a more prolonged application schedule had not been characterized so far. Although with the 8 weeks scheme, complete wound healing and reduction in the amputation risk was attained, better results were expected if the treatment continues up to complete wound healing.

The present study is limited by the small number of patients and by the absence of a concurrent group for a proper comparison. The selected patients were entered with an initial enrolment period when they received only the standardized wound care and did not had more than a 30% decrease in the ulcer. This approach has been proposed to minimize the variability because of the improvement in chronic ulcer healing by the standard treatment. However, it is difficult to quantify the exact effect because of the study treatment from those caused by the standard therapy. Anyhow, this result offers a proof of concept that intralesional Heberprot-P administration up to complete closure can be safe and suitable to improve healing of chronic DFU and also provide the basis for further clinical trials design.



## Conclusions

Despite many major advances in health care delivery to patients with diabetes, foot problems continue to extract a heavy toll on the quality of life of diabetic patients. The high morbidity and mortality, loss of working hours, and expenditure associated with diabetic foot problems necessitate the need for a prompt and proper approach to foot ulcer management. Our data support the contention that, Intralesional administration of Heberprot-P up to complete wound closure in DFU, in association with good wound care measures, accelerates wound healing without any evidence of safety limitations and reduced hospital stay, in addition to a good foot care with a multidisciplinary team approach, enhances diabetic ulcer wound healing and significantly reduces the healing time.

## Declaraton of interest

All authors declare that there is no conflict of interest to be disclosed, including specific financial interests and relationships and affiliations relevant to the subject of their manuscript.

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