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Heberprot-P: A Novel Product for Treating Advanced Diabetic Foot Ulcer

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ABSTRACT

Diabetic foot ulcer is a principal diabetic complication. It has been shown that diabetic patients have decreased growth factor concentrations in their tissues, particularly epidermal growth factor. Growth factor shortage impairs wound healing, which leads to chronic nonhealing wounds and sometimes eventual amputation. Ischemic diabetic foot ulcer is the most difficult to treat and confers the highest amputation risk.

Injecting epidermal growth factor deep into the wound bottom and contours encourages a more effective pharmacodynamic response in terms of granulation tissue growth and wound closure. Epidermal growth factor injected into the ulcer matrix may also result in association with extracellular matrix proteins, thus enhancing cell proliferation and migration.

Heberprot-P is an innovative Cuban product containing recombinant human epidermal growth factor for peri- and intra-lesional infiltration; evidence reveals it accelerates healing of deep and complex ulcers, both ischemic and neuropathic, and reduces diabetes-related amputations.

Clinical trials of Heberprot-P in patients with diabetic foot ulcers have shown that repeated local infiltration of this product can enhance healing of chronic wounds safely and efficaciously. As a result, Heberprot-P was registered in Cuba in 2006, and in 2007 was included in the National Basic Medications List and approved for marketing. It has been registered in 15 other countries, enabling treatment of more than 100,000 patients.

Heberprot-P is a unique therapy for the most complicated and recalcitrant chronic wounds usually associated with high amputation risk. Local injection in complex diabetic wounds has demonstrated a favorable risk–benefit ratio by speeding healing, reducing recurrences and attenuating amputation risk. Further testing and deployment worldwide of Heberprot-P would provide an opportunity to assess the product's potential to address an important unmet medical need.

KEYWORDS Diabetic foot ulcer, Heberprot-P, amputation, healing, unmet medical need, rhEGF, Cuba

DIABETIC FOOT ULCER: A SERIOUS COMPLICATION

Diabetes mellitus (DM) is a noncommunicable endocrine disease increasing in global incidence. Lower extremity ulceration is a main complication and often leads to amputation.[1]

In DM, failure in the repair process of distal peripheral soft tissues leads to the characteristic appearance of chronic wounds. These exhibit protracted cellular and noncellular inflammatory reactions that hinder transition to the granulation phase, inhibiting edge contraction and slowing re-epithelialization. Hyperglycemia is the proximal trigger of numerous processes that lead to a pro-inflamed, pro-oxidant and pro-degradative phenotype in such diabetic wounds.[2,3]

Evidence shows that diabetic patients have decreased concentrations of growth factors in their tissues, notably epidermal growth factor (EGF). This shortage impairs natural wound healing and leads to chronic nonhealing wounds, diabetic foot ulcers (DFU), which in later stages can require limb amputation. More than half of DFU patients also have peripheral vascular disease, characterized by impaired lower limb blood circulation that leads to lack of oxygenation in the foot (known as ischemic foot). Ischemic DFUs are the most difficult to treat and at highest risk of amputation.

DM is the leading cause of nontraumatic amputation in the US,[4] resulting in more than 70,000 amputations in 2008.[5] In Brazil, DM is now thought to affect more than 7% of the adult population, and many of these patients find it difficult to maintain good glyce-mic control.[6] The estimated diabetic population in Cuba is about 450,000; and there are 15,000 new cases of DFU every year. Between 3000 and 5000 of these patients are at risk of amputation.[7]

In 2007, treatment of DM and its complications in the USA generated some \$116 billion in direct and \$58 billion in indirect costs.[8] At least one third of direct costs were linked to DFU treatment.[9] There, estimated two-year followup costs for a DFU amputee range from \$80,000 to \$110,000.[10]

Antimicrobial agents, surgical techniques and a broad variety of therapeutic approaches based on drugs and devices have been applied to DFUs.[11–14] These interventions have shown limited clinical success, even when included in a comprehensive wound care program,[15] and there is no evidence of impact on amputation rates. Short-term recurrences remain a problem hampering clinical effectiveness of some contemporary therapies.[16]

Topical application of human growth factor dates back almost 30 years, when it sparked hopes of a 'magic bullet' for tissue healing. Two main factors quenched that initial excitement: the almost simultaneous finding from basic science that growth factors were involved in malignant growth[17] and disappointing results from a rigorous clinical trial in which EGF was topically administered to acute, experimentally-induced, controlled wounds in healthy volunteers.[18] The need to precondition the chronic wound bed and to ensure local growth factor bioavailability for subsequent receptor stimulation and downstream signaling activation emerged as paradigmatic concepts.[19,20]

RATIONALE FOR GROWTH FACTOR WOUND INFILTRATION

In Cuba, epithelial response to daily topical administration of three different EGF concentrations formulated in a semisolid cream was examined; results suggested a possible reduction of EGF bioavailability by proteases derived from noninfected,

acute, controlled wounds.[21] This was somewhat surprising as other studies had already established proteolysis affecting growth factors and their receptors in chronic circumstances.[22,23] It is worth noting that previous studies had also documented the need for prolonged interaction between EGF and its receptor to achieve a significant granulation tissue response in controlled wounds in mice.[24] Our initial research indicated ^{125}I -EGF was rapidly cleared from the application site, probably by protease-driven cleavage and receptor-mediated endocytosis. Mean residence time values suggested that over 60% of the amount administered could have disappeared as early as two hours after administration.[25] The message of these studies was that even acute, clean and controlled wounds may not represent a hospitable substrate for growth factor physical and chemical integrity. Previous disappointing clinical results may have been due to local bioavailability limitations.[26,27]

Such knowledge prompted the hypothesis that injecting EGF deep into the wound base and walls would allow for greater pharmacodynamic response in terms of granulation tissue growth and wound closure. In further studies, single or repeated EGF systemic or local injections produced clear-cut cytoprotective and proliferative responses, suggesting an intrinsic ability of EGF at supraphysiological concentrations to trigger biological events necessary for tissue repair.[28–30]

Injecting EGF into the tissue, down and inside the base and walls (including the dermo-epidermal junction), possibly also reduces its degradation following topical application and contact with wound exudate. These experiments identified three layers of cellular response potential along the longitudinal axis of granulation tissue. Fibroblasts populating the more superficial stratum expressed far more prohibitin and far less EGF receptor. Advanced glycosylated endproducts and elastase also appeared overexpressed next to the wound surface than in deeper cells strata. It is likely that topographic positioning along the wound bed axis dictates fibroblasts' intrinsic ability to respond to a mitogenic signal. Notably, prohibitin is a renowned inhibitor of cell cycle progression.[31] Contemporary evidence supports that EGF injected into the ulcer matrix may result in an association complex with extracellular matrix proteins, thus enhancing cell proliferation and migration.[32]

Classic studies have shown that growth factor effectively counteracts senescence of chronic ulcer-derived fibroblasts—including diabetic ulcer fibroblasts—and stimulates proliferation.[33,34] Appropriate wound bed preparation through sharp debridement and infection elimination is required prior to infiltration.

HEBERPROT-P IMPROVES HEALING AND REDUCES AMPUTATIONS IN PATIENTS WITH SEVERE DFU

Following earlier research, scientists at the Center for Genetic Engineering and Biotechnology (CIGB, the Spanish acronym) in Havana developed Heberprot-P, a patented pharmaceutical composition whose parenteral formulation is based on rhEGF. The product is administered in DFU patients by intralesional infiltration to accelerate healing of deep and complex ulcers, either neuropathic or ischemic.[35]

Based on the rationale that rhEGF can enhance healing of chronic wounds following repeated local infiltrations,[36] various clinical trials using Heberprot-P in DFU patients have been conducted, demonstrating safety and efficacy.[37–43] Infiltration with rhEGF 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 in 2001–2002, the first clinical evidence using EGF infiltration for diabetic foot ulcers and amputation residual bases emerged.[37] All lesions were chronic, complex and recalcitrant, Wagner scale stages 3 and 4.[36] 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.[39]

Since then, EGF local injection has been used for complex diabetic wounds in various Cuban clinical trials, demonstrating a favorable risk–benefit balance by speeding healing, reducing recurrences and attenuating amputation risk.[43] 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 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 μg and 75 μg) and in the pooled analysis; intensity was mild to moderate and symptom appearance was not associated with treatment suspension.[39]

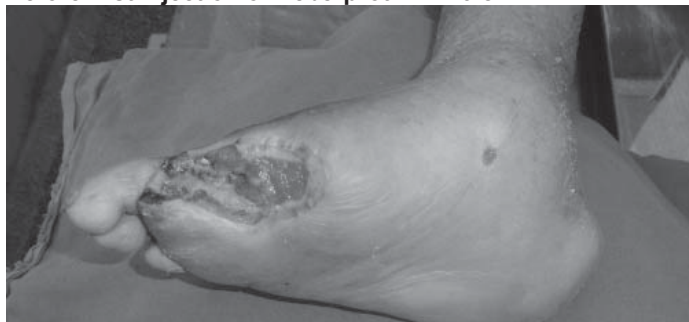
EGF infiltration increased and accelerated healing in poor-prognosis wounds toward a rapid and sustained response (Figure 1). More than 80% granulation was obtained globally with Heberprot-P, in comparison with less than 60% with standard care alone. Of patients treated with Heberprot-P at 75 μg , three 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.[40] Seminal clinical trials are summarized in Table 1.

As a result, Heberprot-P was registered in Cuba in 2006, and in 2007 was included in the National Basic Medications List and approved for marketing. Heberprot-P has also been registered in 15 other countries (Table 2) enabling treatment of over 100,000 patients. Registration and market approval submissions are in process in countries such as Brazil, Russia, China, South Africa, and the Arab states of the Persian Gulf. A Spanish phase II clinical trial for the DFU indication, approved by the Spanish Drug Agency under European Good Clinical Practices, concluded recently (publication pending), with the aim of moving to a pivotal phase III clinical trial in Europe.

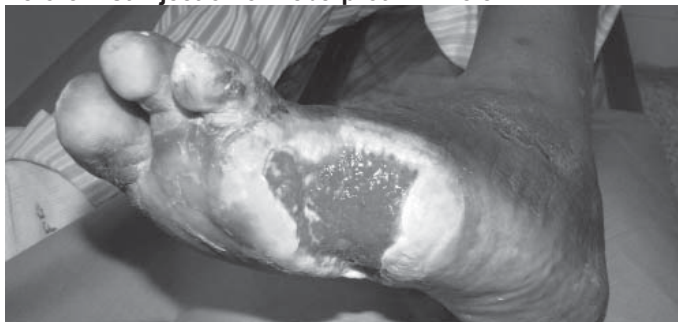
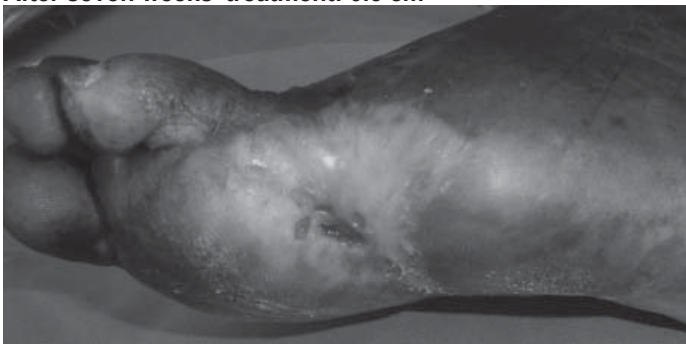
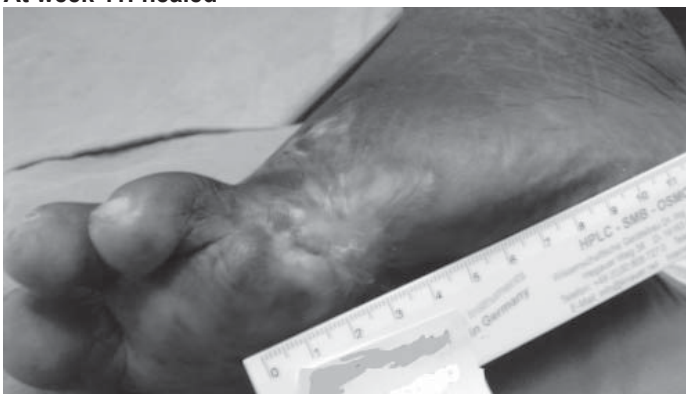
EFFECTIVE COMPREHENSIVE DFU TREATMENT: AN UNMET MEDICAL NEED

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 treat-

Figure 1: Severe diabetic foot ulcers treated with Heberprot-P

Ischemic Patient A**Before first injection of Heberprot-P: 11.9 cm²****After seven weeks' treatment: 1.1 cm²****At week 11: healed**

Photos: Dr A del Río Martín

Ischemic Patient B**Before first injection of Heberprot-P: 21.8 cm²****After seven weeks' treatment: 0.5 cm²****At week 11: healed**(available in color online at www.medicc.org/mediccreview/berlanga.html)**Table 1: Seminal clinical trials with Heberprot-P**

Trial	Details	Results	Ref.
Phase I: Exploratory	Patients with poor prognosis, 25 µg three times a week, until granulation or 8 weeks	Good safety pattern Promising results in granulation, healing and amputation avoidance	37
Phase II: Treatment dose determination	25 µg or 75 µg three times a week, until granulation or 8 weeks	Good safety pattern in both doses Trend to greater efficacy at 75 µg	38
Phase II: Treatment dose determination	25 µg or 75 µg three times a week, until healing or 8 weeks	Trend to greater efficacy when treatment is prolonged until healing	39
Phase III: Confirmatory trial	Double-blind placebo-controlled multicenter study, 25 µg versus 75 µg versus placebo (all 3 times a week)	Confirm efficacy (granulation and healing) and safety in patients with Wagner 3–4 DFUs	40
Phase IV: Pharmacovigilance	Pharmacovigilance study in 1835 patients	Confirmation of safety profile	Unpublished

DFU: diabetic foot ulcer

ments, generally only 15% to 20% healing in less than 20 weeks, and may be expensive and time consuming.[44] In ischemic patients, surgical revascularization is not suitable for all cases and

some authors consider its effectiveness to be overestimated:[45] in any case, the regulatory process for surgical procedures does not require the same degree of clinical detail as demanded for biological products. There remains a clear need for adequate comprehensive therapy to improve healing in severe wounds, for which Heberprot-P has demonstrated clear beneficial potential.

In the USA, 8.3% of the population—25.8 million people—have DM,[46] and therefore an estimated 25% lifetime risk of developing a DFU.[47] The International Diabetes Federation predicts that the number of people with DM in the USA will be 36 million in 2030, 12% population prevalence.[48]

The estimated number of US DFU patients in 2010 was between 3.9 and 4.6 million. Among these, 2.5 million patients had concomitant ischemia and hence were at greater risk of complications.[49] Heberprot-P would address the therapeutic needs of this population, as well as those at risk globally, especially patients with ischemic wounds that are the most difficult to heal.

Further testing of Heberprot-P—a unique and first-in-class therapy to treat the most complicated and recalcitrant chronic wounds with a high risk of amputation—would provide an opportunity to assess the product's potential to address this vast unmet medical need in different populations and settings worldwide.



Table 2: Heberprot-P registration year by country

Country	Registration Year
Cuba	2006
Algeria	2008
Argentina	2009
Uruguay	2009
Dominican Republic	2009
Venezuela	2010
Ecuador	2010
Mexico	2010
Paraguay	2010
Libya	2010
Colombia	2011
Guatemala	2011
Georgia	2011
Ukraine	2011
Vietnam	2012
Philippines	2012

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