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Research Article

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Recombinant Human Epidermal Growth Factor: Medical Experience of Use in Patients with Advanced Diabetic Foot Ulcers in Mexico

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Abstract

Background: Diabetic foot ulcer (DFU) is one of the most frequent severe complications of Diabetes mellitus, and timely diagnostic and intervention is a significant driver of reduction of major amputation rate, long-term prognosis, and patients' survival.

Methods: Implementation and deploy of advanced DFU treatment with intralesional infiltration of recombinant human epidermal growth factor (rhEGF) was performed in four hospitals of the Mexican Institute of Health and Social Insurance of State Workers (ISSSTE) from November 2018 to March 2019. Fifty-six in-hospital patients with neuropathic or ischemic ulcers, classified as grades 3 and 4, according to Wagner's scale, were included in a non-controlled pilot study. Demographic and clinical variables, such as total granulation, wound closure, healing time, and adverse reactions, were evaluated.

Results: Total granulation of DFU was obtained in 100.0% of patients. Wound closure was observed in 90.0% of patients, mostly in neuropathic DFUs, and average healing time was 6.4 weeks in these patients. Treatment with rhEGF was effective in both neuropathic and ischemic patients.

Conclusions: In this trial, percent of granulation, wound closure, and healing time were significantly high, regardless of wound location. Perilesional and intralesional injection of rhEGF is recommended in this report for treatment of advanced DFU, based on the efficacy and safety profile demonstrated. (Funded by Laboratorios Pisa S.A. de C.V.; gob.mx/cofepris number, 15CI09012060.)

Key words: *Diabetes mellitus; Diabetic foot ulcer; Wound, EGF; Healing.*

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Introduction

Diabetes mellitus (DM) remains as a major challenge for health systems, governments, and societies, [1,2] and probably the most concerning complication is the diabetic foot ulcer (DFU). [3,4] Timely diagnostic and intervention is a significant driver of reduction of major amputation rate, long-term prognosis, and survival of DFU patients. [5,6]

Prevalence of DFU in Mexico, and amputation rate were 9.1% of diabetics and 5.5% of DFU patients, respectively, in 2016. Both variables have kept a persistent growing trend from 2006, and attention to DFU accounts for 20.0% of total expense in DM. The cost of DFU treatment in Mexico has been reported in the [7] range between 2 806 and 5 361 USD/patient, depending on wound severity. [7,8]

Safety and efficacy of DFU treatment by intralesional and perilesional injection of recombinant human epidermal growth factor (rhEGF) have been demonstrated in clinical trials. [7-10] Post-marketing information from 2 702 patients confirmed results obtained in clinical trials. [7,8] This was taken into account to implement and deploy EGF treatment, and evaluating results in patients with DFU grades 3 and 4, according to Wagner's classification, [7] in four hospitals.

Methods

Trial Oversight

Four hospitals participated in a multicenter prospective implementation and deploy of the treatment. The trial was designed and overseen by a steering committee and was supported by Laboratorios Pisa S.A. de C.V., which had no influence on the design or conduct of the trial, and was not involved in data collection or analyses, in the writing of the manuscript or in the decision to submit it for publication. The trial protocol was approved by National Commission for Protection against Health Risks (COFEPRIS). The trial was performed in accordance the principles of the Declaration of Helsinki. [7] The authors assume responsibility for the accuracy and completeness of the data and analyses, as well as for the fidelity of the trial and this report to the protocol.

Patients

Fifty-six type 1 or 2 diabetes patients of both sex, ≥ 20 years old, Wagner's grade 3 or 4 DFU, wound size > 1 cm^2, more than 4 weeks of wound evolution, and signed informed consent to participate were eligible for enrollment (Tables 1 and 2). Patients who required revascularization, hemoglobin <100 g/l, uncompensated chronic diseases, diabetic coma or ketoacidosis and renal failure, malignancies, neurological diseases, immunosuppressor drugs or corticosteroid use, pregnancy, and nursing were excluded.

Trials Procedures

Participants received rhEGF 75 µg intralesional and perilesional, three times per week on alternate days, during a maximum of eight weeks, and good wound care (GWC). Lyophilized rhEGF was dissolved with 5 ml of water for injection, and injected using a standard disposable syringe with a 27G x 0.5" insulin needle (5 – 10 injections of 0.5 – 1 ml), first into the dermo-epidermal junction at equidistant points all over the lesion contours and then deeply downward into the wound bottom in circles and centripetally to ensure a uniform distribution. Wounds were dressed with sterile gauze, and all patients were seen at follow-up visits three times per week until the end of the trial.

Outcomes

The primary outcome was wound closure. The secondary outcomes were per cent of granulation, time to appear granulation tissue, wound area, and healing time. Ankle-brachial index was measured at baseline, and end of treatment to evaluate vascular haemodynamic. Laboratory tests included blood cell counts, haemoglobin, haematocrit, globular sedimentation rate, glycohaemoglobin (HbA1C), creatinine, and aspartate aminotransferase. Blood glucose was measured for patients' metabolic control. Bacterial infection was monitored by wound cultures, before and during therapy.

Statistical Analysis

Continuous variables were expressed as mean ± standard deviation (SD) or median (25^{th} and 75^{th} percentiles). Categorical variables were given as absolute values and percentages. Absolute frequency, percent of granulation, and wound closure were estimated in categories: satisfactory and unsatisfactory. Normality (QQPlot), and fitness tests (Shapiro Wilk and Kolmogorov-Smirnov) were performed to verify if data were uncorrelated with one another, come from a normal distribution, and random component had fixed variation. A cross tabulation was performed including control variables and the response variable wound closure. Evaluation of safety was performed from frequency of patients with adverse events and frequency of adverse events. Two-sided P values of 0.05 or less were considered to indicate statistical significance. The Bayes Factor test was used for benefit-risk ratio analysis, considering wound closure as benefit criterion, and amputation and

interruption due to adverse events as risk criteria. [7,8] Data were double entered and validated on the free software OpenClínica [1] and imported to SPSS software version 15 (IBM SPSS Statistics, IBM, New York.) [8]

Results

Characteristics of the Patients

From November 2018 to February 2019, a total of 56 patients with advanced DFU were enrolled at four centers. Most patients suffered type 2 DM (94.6%), and received insulin treatment (51.8%), followed by Metformin (46.4%) and Glibenclamid (25.0%). Patients were predominantly males (66.0%), 61 years old on average, 33 (59.0%) with neuropathic, and 23 (41.0%) with ischemic DFU (Table 1). The average time with DM was 16 years. Ulcer size was in the range between 2 and 148 cm2, and most of them in fingers (Table 2). DFUs were classified as grade 3 (60.7%), and grade 4 (39.2%), according to Wagner's scale, and 60.7% of them located in the left foot.

Characteristic		Neuropathics	Ischemics	Total
Patients (%)		33 (59.0)	23 (41.0)	56 (100.0)
Gender (%)	males	22 (66.7)	15 (65.2)	37 (66.1)
	females	11 (33.3)	8 (34.8)	19 (33.9)
Age	mean ± SD	58 ± 13	66 ± 10	61 ± 12
	(minimum; maximum)	(28; 88)	(47; 82)	(28; 88)
Diabetes type (%)	1	1 (3.0)	2 (8.7)	3 (5.4)
	2	32 (97.0)	21 (91.3)	53 (94.6)
Diabetes evolution time (years)	media ± SD	15 ± 9	17 ± 11	16 ± 10
	(minimum; maximum)	(3; 45)	(3; 48)	(3; 48)
Glucose control treatment (%)	Insulin	19 (57.6)	10 (43.5)	29 (51.8)
	Metformin	15 (45.4)	11 (47.8)	26 (46.4)
	Glibenclamid	8 (24.2)	6 (26.1)	14 (25.0)

Table 1: *Demographic and baseline characteristics of patients. SD: standard deviation.*

Follow-Up and Outcomes

Follow-up of data for all outcomes were available through March 2019. Complete treatment compliance was reported in 52 (93.0%) patients. Interruption was reported in one patient due to renal failure, other two had extensive lesions, and amputation was necessary in three patients. Complete granulation response was achieved in all patients, including abandoners, at a mean time of 26.7 days. Wound closure was obtained in 50 patients (89.2%). Mean time to complete closure was in the range between 6.4 and 7.0 weeks (Table 3). Wound closure was observed in 18 out of 23 ischemic patients (78.0%).

Patients (%)		Neuropathics	Ischemics	Total
		33 (59.0)	23 (41.0)	56 (100.0)
Wagner's classification grade (%)	3	31 (93.9)	3 (13.0)	34 (60.7)
	4	2 (6.1)	20 (87.0)	22 (39.2)
Affected lower limb (%)	left	21 (63.6)	13 (56.5)	34 (60.7)
	right	12 (36.4)	10 (43.5)	22 (39.3)
Wound location (%)	finger	17 (51.5)	11 (47.8)	28 (50.0)
	sole	14 (42.4)	3 (13.0)	17 (30.3)
	dorsum	7 (21.2)	5 (21.7)	12 (21.4)
	internal edge	2 (6.1)	4 (17.4)	6 (10.7)
	calcaneus	1 (3.0)	4 (17.4)	5 (8.9)
	extreme edge	3 (9.1)	1 (4.3)	4 (7.1)
	transmetatarsal	0 (0.0)	3 (13.0)	3 (5.3)
	stump	0 (0.0)	1 (4.3)	1 (1.8)
Wound evolution time (days)	median ± QR	97 ± 169	120 ± 168	110 ± 152
	(minimum; maximum)	(3; 730)	(28; 395)	(3; 730)
Wound area (cm^2)	median ± QR	5.0 ± 15.0	17.0 ± 31.0	6.5 ± 22.5
	(minimum; maximum)	(2.0; 105.0)	(3.0; 65.0)	(2.0; 105.0)
Indication of minor surgical procedures (%)	yes	15 (45.4)	8 (34.8)	23 (41.1)
	toilette	12 (80.0)	7 (87.5)	19 (82.6)
	disarticulation	4 (26.7)	2 (25.0)	6 (26.1)

Table 2: *Wound classification and data. QR: quartile range.*

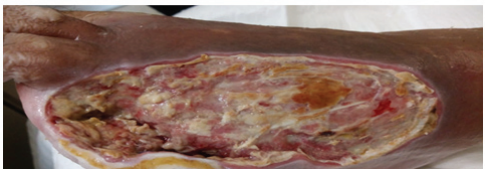
Patients (%)		Neuropathics	Ischemics	Total
		33 (59.0)	23 (41.0)	56 (100.0)
rhEGF applications	Mean ± QR (minimum; maximum)	10 ± 5 (4; 24)	14 ± 13 (3; 24)	12 ± 8 (3; 24)
Complete granulation (%)	yes	33 (100.0)	23 (100.0)	56 (100.0)
Wound closure (%)	yes	32 (97.0)	18 (78.2)	50 (89.2)
	no	1 (3.0)	5 (21.8)	6 (10.8)
Major amputation (%)	yes	1 (3.0)	2 (8.6)	3 (5.3)
	no	32 (97.0)	21 (91.4)	53 (94.7)
Wound closure time, mean ± SE (weeks)	6.4 ± 0.6		7.0 ± 1.0	6.6 ± 0.5
CI (95%)	(5.3; 7.5)		(5.1; 8.9)	(5.6; 7.6)
Log Rank (χ^2 = 1.025; p = 0.311)				

Table 3: *Granulation response to treatment with intralesional rhEGF, and final outcomes. QR: quartile range, and SE: standard error.*

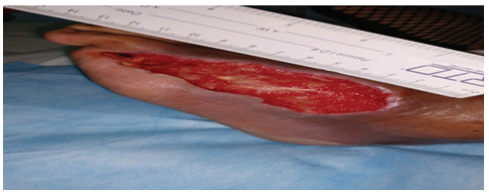
Figure 1 shows two examples of wounds' clinical aspects. Photo A of the case 1 shows the ulcer of a 54 year old male with diabetes for 4 years and an extensive ischemic wound (area 57 cm^2) on the dorsum. Wound evolution was 60 days, and after revascularization, amputation had been indicated. After 2 weeks of rhEGF treatment with 6 interventions, complete granulation was observed. Twenty interventions with rhEGF were necessary to obtain wound closure after 7 weeks. Photo A of the case 2 shows the most complicated ulcer treated with rhEGF. This patient was a 50 year old male with diabetes for 14 years and an extensive ischemic wound (area 148 cm2) on the dorsum. Infection and osteomyelitis were also present and amputation had been previously indicated as the only alternative. After soft tissue debridement, bone resection within the necrotic area and broad-spectrum antibiotics, rhEGF intervention was thereafter performed. Complete granulation response was achieved in 3 weeks, after seven rhEGF interventions. He was re-evaluated thereafter and complete wound closure was confirmed at week 8, and 21 interventions with rhEGF.

Case 1

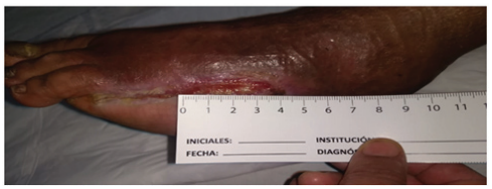
A)



B)

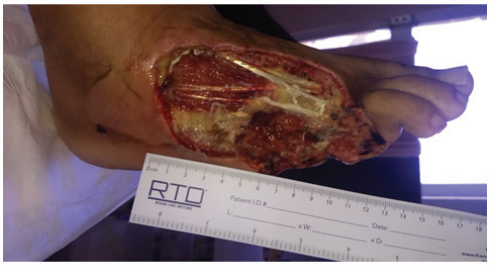


C)

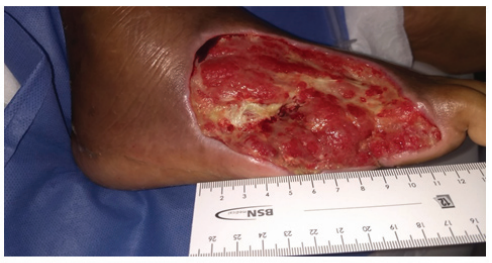


Case 2

A)



B)



C)

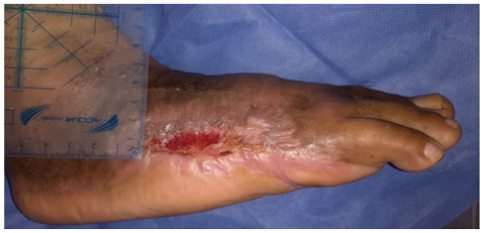


Figure 1: *Cases of wounds' clinical evolution during treatment with rhEGF.*

Safety

Adverse events (164) are listed in Table 4. The most frequent adverse events were local pain, chills, tremors, and nauseas. More than 90.0% of adverse events were classified as mild, and 3.2% as severe. Adverse events in neuropathic patients account for 58.5% of the total, 41.5% in ischemic patients, and none of them were attributable to rhEGF treatment. The benefit/risk ratio is presented in Figure 2. In both neuropathic and ischemic patients, absence of interceptions between probability distribution functions for benefit (wound closure) and risk (amputation and interruption) were evident. Bayes Factor, representing ratio of the likelihood of benefit (red) to the likelihood of risk (blue), was 12.9 for neuropathic and 6.3 for ischemic patients.

Patients (%)			Neuropathics	Ischemics	Total
			96 (58.5)	68 (41.5)	164 (100.0)
Events					
Pain at site of administration (%)	Frequency (%)		38 (39.6)	56 (82.4)	94 (57.3)
	Intensity	mild	37 (97.4)	54 (96.4)	91 (96.8)
		severe	1 (2.6)	2 (3.6)	3 (3.2)
	Severity	non severe	38 (100.0)	56 (100.0)	94 (100.0)
Shivering (%)	Frequency		25 (26.0)	2 (2.9)	27 (16.5)
	Intensity	mild	23 (92.0)	2 (100.0)	25 (92.6)
		moderate	2 (8.0)	0 (0.0)	2 (7.4)
	Severity	non severe	24 (96.0)	2 (100.0)	26 (96.3)
		hospitalization or extend hospitalization	1 (4.0)	0 (0.0)	1 (3.7)
Tremor (%)	Frequency (%)		18 (18.8)	5 (7.4)	23 (14.0)
	Intensity	mild	17 (94.4)	5 (100.0)	22 (95.7)
		severe	1 (5.6)	0 (0.0)	1 (4.3)
	Severity	non severe	18 (100.0)	5 (100.0)	23 (100.0)
Nausea (%)	Frequency (%)		3 (3.1)	2 (2.9)	5 (3.0)
	Intensity	mild	2 (66.7)	2 (100.0)	4 (80.0)
		severe	1 (33.3)	0 (0.0)	1 (20.0)
	Severity	non severe	3 (100.0)	2 (100.0)	5 (100.0)
Burning at site of administration (%)	Frequency (%)		4 (4.2)	0 (0.0)	4 (2.4)
	Intensity	mild	3 (75.0)	0 (0.0)	3 (75.0)
		severe	1 (25.0)	0 (0.0)	1 (25.0)
	Severity	non severe	4 (100.0)	0 (0.0)	4 (100.0)
Local infection (%)	Frequency (%)		3 (3.1)	1 (2.9)	4 (3.0)
	Intensity	mild	2 (66.7)	0 (0.0)	2 (50.0)
		moderate	1 (33.3)	0 (0.0)	1 (25.0)
		severe	0 (0.0)	1 (100.0)	1 (25.0)
	Severity	non severe	3 (100.0)	0 (0.0)	3 (75.0)
		Hospitalización o la prolonga	0 (0.0)	1 (100.0)	1 (25.0)

Headache (%)	Frequency (%)	2 (2.1)	1 (1.5)	3 (1.8)	
	Intensity	mild	1 (50.0)	1 (100.0)	2 (66.7)
		severe	1 (50.0)	0 (0.0)	1 (33.3)
	Severity	non severe	2 (100.0)	1 (100.0)	3 (100.0)
Diarrhoea (%)	Frequency (%)		2 (2.1)	1 (1.5)	3 (1.8)
	Intensity	mild	1 (50.0)	1 (100.0)	2 (66.7)
		moderate	1 (50.0)	0 (0.0)	1 (33.3)
	Severity	non severe	2 (100.0)	1 (100.0)	3 (100.0)
Pneumonia (%)	Frequency (%)		1 (1.0)	0 (0.0)	1 (0.6)
	Intensity	severe	1 (100.0)	0 (0.0)	1 (100.0)
	Severity	threat to life	1 (100.0)	0 (0.0)	1 (100.0)

Table 4: *Adverse events reported in DFU patients treated with intralesional rhEGF.*

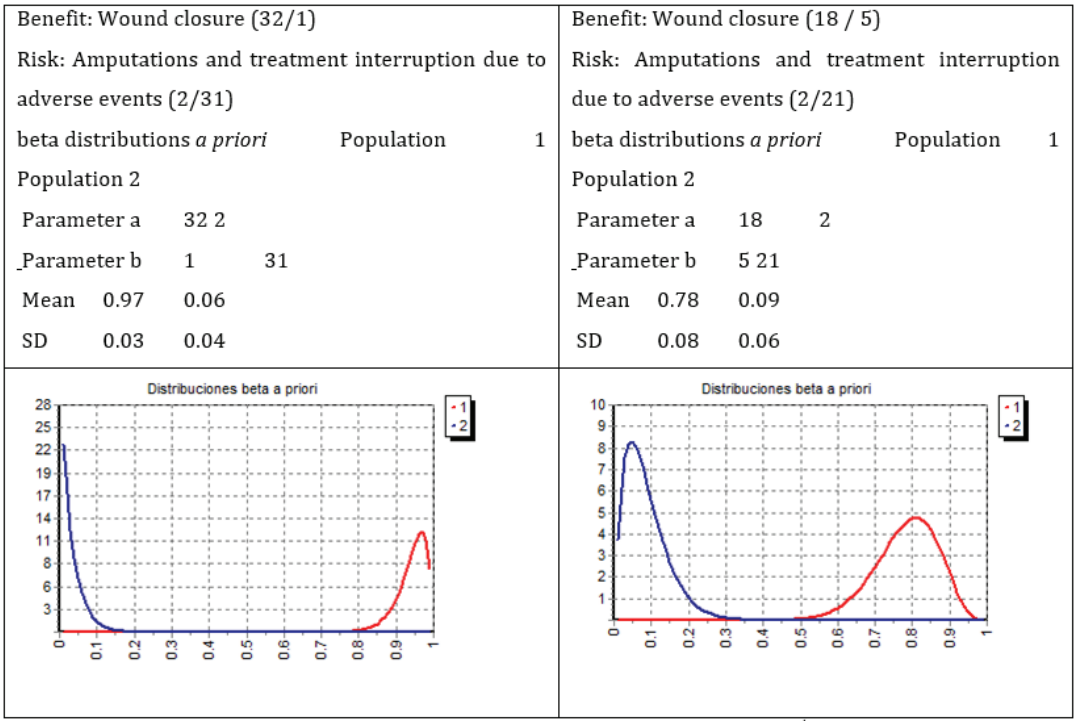


Figure 2: *Risk-benefit analysis of neuropathic and ischemic patients. Benefit (red; wound closure) and risk (blue; amputations and treatment interruption due to adverse events) probability distributions of the outcome of DFU patients treated with intralesional rhEGF. Left graph: neuropathic patients, Bayes Factor = 12.9. Right graph: Ischemic patients, Bayes Factor = 6.3.*

Discussion

We found that the most affected anatomic region was finger. Average area of these wounds was 20 cm^2, which is a condition with low probability to develop granulation tissue in a time as short as two weeks. The amount of toilettes (19) and disarticulations (6) indicated before rhEGF treatment is evidence of the high complexity of the treated DFUs (Table 2). Data obtained from post-marketing pharmacosurveillance suggested favorable results of healing time (14.0 weeks), compare to GWC alone (21.4 weeks). [17,18]

The high percentage of patients with effective granulation response observed in this study (100.0%) could be explained on the basis of the contribution of rhEGF to the healing process, since this protein is locally reduced in the surface of chronic wounds. [8] rhEGF injections support its ability to activate tissue repair, [9,10] avoiding degradation action of proteases against growth factors and their cellular receptors, [9,10] reducing diffusion barriers, [9] reaching deeper strata of fibroblasts where EGF receptors are more abundant than at wound surface cell layer, [9] and increasing EGF interaction time with cell receptors. [11-14]

Amputation rate was low (5.3%), which shows that was possible avoiding amputation. Ischemia in the affected limb represents a significant hindrance for DFU healing, but the beneficial impact of rhEGF treatment in ischemic patients was significant (closure time = 7.0 weeks). The median healing time of DFU in ischemic patients had previously been reported in the range between 5 and 16 months, irrespective of surgical interventions. [8]

Results of statistical analysis were strong evidence in favor of the benefit of the rhEGF treatment in neuropathic patients (Bayes Factor = 12.9), and moderate evidence in ischemic patients (Bayes Factor = 6.3). The strength of the statistical hypothesis in favor of the benefit of rhEGF treatment was higher than previously reported in clinical trials (Bayes Factor=3.2) and post-marketing pharmacosurveillance (Bayes Factor=5.4). Cases shown in Figure 1 confirmed the finding previously reported on granulation tissue development after 2 weeks as useful predictor of wound healing. [8] In addition, safety and efficacy of this treatment in patients with large ischemic wounds for which endovascular and surgical revascularization did not reduce major amputation rate at middle and long-term was confirmed. [8,9]

The International Working Group of the Diabetic Foot (IWGDF) evaluated results of the clinical trial with rhEGF as promising. [8] This method was compared with the state-of-the-art in a systematic review, [8] and a random effects meta-analysis stratified by the types of administration route has supported the use of rhEGF in the treatment of DFU. [8] In conclusion, in our trial, efficacy and safety of the treatment of advanced DFU with perilesional and intralesional injection of EGF were equally demonstrated in neuropathic and ischemic patients.

Acknowledgments

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Disclosure of Conflict of Interests

Julio E. Baldomero-Hernández, MD, Marel A. Valdés, MS., and Ángela D. Tuero-Iglesias, MS are employees of the Center for Genetic Engineering and Biotechnology, Havana, owner of the product's patent and sanitary registrations, where product Heberprot-P® is produced. The rest of the authors have not conflict of interest to declare.

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