Intralesional Injections of Citoprot P® (Recombinant Human Epidermal Growth Factor) in Advanced Diabetic Foot Ulcers with Risk of Amputation

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Running title: Intralesional EGF administration in advanced diabetic foot ulcers.

Abstract

Aim. To investigate the efficacy and safety of recombinant human epidermal growth factor (rhEGF) in advanced diabetic foot ulcers (DFU). **Methods.** A double-blind trial was done to test two rhEGF dose levels in type 1 or 2 diabetes patients with Wagner's grade 3 or 4 ulcers. Subjects were randomised to receive 75 (group I) or 25 μg (group II) rhEGF through intralesional injections, 3 times per week for 5 to 8 weeks together with standardized good wound care. Endpoints were granulation tissue formation, complete healing and need of amputation. Safety was assessed by clinical adverse events (AEs) and laboratory evaluations. **Results:** Forty-one patients were included. After 5 to 8 weeks of treatment 83% of the patients in the higher dose group and 61% in group II achieved useful granulation tissue covering more than 98% of the wound area. At long-term assessment 13 (56.5%) patients healed in group I and 9 (50%) in group II. The mean time to complete healing in group I was 20.6 weeks (95% CI 17.0 – 24.2) and 19.5 weeks (16.3 – 22.7) in group II. After one year follow-up only one patient relapsed. Amputation was not necessary in 65% and 66.7% of groups I and II, respectively. The adverse events rates were similar. The most frequent were sepsis (33%), burning sensation (29%), and tremors, chills and local pain (25% each). **Conclusions:** rhEGF local injection enhances advanced diabetic foot ulcer healing and reduces the risk of major amputation. No dose-dependency was observed.

Keywords: epidermal growth factor, diabetic foot ulcers, wound healing.

List of abbreviations: ABI: ankle-brachial index; CI: confidence interval; DFU: diabetic foot ulcer; EGF: epidermal growth factor; HbA_1C: glycohaemoglobin; PDGF: platelet-

derived growth factor; QR: interquartile range; rhEGF: recombinant human epidermal growth factor; SD: standard deviation; $TcpO_2$: transcutaneous oxygen pressure

Introduction

The number of people with diabetes mellitus is expected to rise from 171 million in 2000 to 366 million in 2030 [1]. Major complications include foot ulcers, gangrene and amputation, which represent leading causes of hospitalization among those patients. Around 15% of people with diabetes mellitus develop a diabetic foot ulcer (DFU). This condition precedes 85% of major amputations in this population [2]. The annual incidence of DFU is more than 2% in all diabetic patients [3] and rises largely when peripheral neuropathy is present [4]. It is estimated that 7-20% of total expenditure on diabetes might be attributable to diabetes foot disease [5].

Metabolic control, wound care, debridement, pressure relief, dressings and antibiotics are among the basic interventions for DFU management. New therapies are emerging to promote wound healing and to reduce the incidence of lower-limb amputations, including recombinant human platelet-derived growth factor [6,7] and skin equivalents obtained by tissue engineering techniques [8,9]. However, these products have been only studied in small, neuropathic-origin wounds. At present, there is no specific treatment for advanced or ischemic DFU and amputation is the foreseeable outcome.

Epidermal Growth Factor (EGF) is a 53-aminoacid polypeptide, isolated by Stanley Cohen from adult mouse submaxillary glands [10] that exerts potent mitogenic activity through binding to a specific cell membrane, tyrosine kinase-type receptor on the target cells [11]. EGF topical application or subcutaneous injection produces skin keratinocytes and fibroblasts hyperplasia and hypertrophy, as well as corneous layer thickening [12,13]. Exogenous EGF can also play a significant stimulating role in peripheral nerve regeneration [14]. Some clinical trials have been conducted to evaluate

the efficacy and safety of topical application of EGF in different indications such as DFU [15,16], radiogenic ulcers [17], venous ulcers [18], burns [19,20], and graft donor sites [21].

The availability of the growth factors on the wound deeper layers is an important issue to obtain an adequate efficacy. This can be a limitation with topical formulations since active agent diffusion is affected by necrotic tissue, sepsis, inflammation, and by wound proteases [22]. Growth factor intralesional injection could take it to the desired region.

A preliminary clinical study, where rhEGF (25 μg thrice weekly for 5 weeks) was injected intralesionally in advanced DFU yielded encouraging positive results in terms of useful granulation tissue formation and major amputations prevention in more than 50% of the 29 patients treated [23]. The present study evaluated the efficacy and safety of this treatment to promote healing of advanced DFU and prevention of limb amputation at two dose levels in a randomised, double-blinded design.

Patients and Methods

Trial design and patients

A randomised, double-blinded trial was done at 5 centres. Diabetic patients (type 1 or 2), both genders, older than 18 years were included if they had a grade 3 or 4 foot ulcer according to Wagner's classification [2] and gave their written, informed consent to participate. Exclusion criteria were foot ulcer area ≤1 cm^2, haemoglobin <100 g/l, uncontrolled chronic diseases (coronary or heart disease, diabetic coma or ketoacidosis, renal failure defined as a serum creatinine >200 μmol/l and oligoanuria), malignancies, psychiatric or neurological diseases that could impair proper reasoning

for consent, pregnancy and nursing. The trial protocol was approved by the Ethics Committee at each investigation site and by the Cuban Regulatory Authority.

Study medication and interventions

Recombinant, human EGF (rhEGF) was obtained from a transformed *Saccharomyces cerevisiae* strain at the Centre for Genetic Engineering and Biotechnology, Havana [24]. For the trial it was produced as lyophilized powder containing 75 or 25 μg per vial (Citoprot-P®, Heber Biotec, Havana). Both vials were indistinguishable in order to guarantee blindness during preparation and manipulation.

Patients were randomised to receive intralesional injections of rhEGF at 75 μg (group I) or 25 μg (group II), 3 times per week on alternate days. The product was dissolved in 5 ml of physiological saline. This volume was distributed throughout the lesion at each administration. The treatment lasted until complete response or 5 weeks. If a partial response (see below for response definitions) was observed at this point, treatment continued for 3 additional weeks. Patients were hospitalised during the treatment.

The study medication was administered together with a standardised good wound care regimen. Ulcers were sharply debrided, gangrenous and necrotic tissue removed whenever necessary, saline-moistened gauze dressing was used, and the affected area was pressure off-loaded. Broad-spectrum antibiotics were used to manage infections and metabolic control was strictly followed.

Evaluation and follow-up

Evaluation consisted in baseline and weekly wound clinical examination. Ankle/brachial index, digital pletismography, and transcutaneous oxygen pressure ($TcpO_2$) were

measured at baseline, end of treatment, and 12 months follow-up to evaluate vascular haemodynamics. Ulcers were classified regarding their etiopathogeny and in grades according to Wagner. Laboratory tests (at baseline, 3 weeks, end of treatment, and 3, 6 and 12 months after the end of treatment) included blood cell counts, haemoglobin, haematocrit, globular sedimentation rate, glycohaemoglobin (HbA_1C), creatinine, and aspartate aminotransferase, which were done by usual clinical laboratory methods. Blood glucose was measured more frequently for the patients' metabolic control. Wound cultures were done before and during therapy to monitor bacterial infections.

The main efficacy endpoint was the percent of the ulcer surface covered by granulation tissue. The protocol previewed to classify it as covering ≤ 25% (no response), 26-50% (minimal response), 51-75% (partial response) or >75% (complete response) of the ulcer surface. However, since all the complete responses obtained consisted in more than 98% of the wound area covered by granulation tissue, this was the actual variable evaluated. Secondary efficacy endpoints were time to obtain complete response, complete healing (no exudates or need of dressing), time to get it, and need for amputation. Recurrence up to one year follow-up was also assessed.

Biopsy samples were collected before, after one week, three weeks and at the end of treatment, preferably from a wound zone macroscopically suggesting granulation tissue. Samples were fixed in 10% buffered formalin, paraffin embedded and stained with Haematoxylin / Eosin and Masson's trichrome staining for collagen.

Safety was monitored by daily adverse events evaluation during treatment. The severity of the adverse events was classified as (1) mild, if no therapy was necessary; (2) moderate, if specific treatment was needed, and (3) severe, when hospitalization or its

prolongation was required, if the event was life-threatening or contributed to patient's death.

Statistics and data management

Sample size was estimated for each group considering that the treatment would represent a clinically significant benefit if it provides a 30% advantage in granulation tissue formation rate. Under this hypothesis 20 individuals per group was estimated that could provide this result with a 0.05 alpha error and a 0.2 confidence interval precision.

Data were double-entered and validated on Microsoft Visual FoxPro version 5.0 and then imported to SPSS version 11.5 for further analysis. Quantitative variables were expressed as mean ± standard deviation (SD) or median ± interquartile range (QR) and minimum and maximum values. Qualitative variables were given as absolute values and percentages. The probabilities of response were estimated using a Bayesian logistic model for fixed effects in WinBUGS14 package. The influence of baseline and demographic variables on response was tested using univariate analyses by the χ^2 or Fisher's exact tests as well as the odds ratio between the conditions compared and their 95% confidence interval. Then the variables more likely to be significant were tested in a multivariate analysis with a logistic regression model.

Despite that the objective of the trial was not to compare the efficacy between dose levels, it was done to look if there was any evidence of dose dependence effect. Normality (Shapiro-Wilks test) and homogeneity of variances (Levene's test) assumptions were tested and depending on whether verified or not, differences between groups were assessed by the Student's t or Mann-Whitney's U test, respectively. Qualitative variables comparison between groups was assessed by the χ^2

or Fisher's exact tests. The level of significance chosen was $p < 0.05$. All analyses were done on intention-to-treat basis.

Results

Course of the trial

From September 2003 to December 2004 forty-one patients with diabetic foot ulcer were recruited (23 in the high dose group I and 18 in group II). A flow chart of the trial course and the reasons for treatment discontinuation are shown in Figure 1. Of the 41 patients included, 13 (46%) did not complete the treatment schedule: 5 (22%) in group I and 8 (44%) in group II. The interruptions in group I were due to adverse events (sepsis) in 3 (13.0%), and wound progression in 2 (8.7%). In group II there was wound progression in 4 patients (22%), voluntary abandon in 2 (11%), and sepsis in 2 (11%). Four patients (2 in each group) prolonged the treatment because of partial response at week 5. One from each group withdrew before week 8 due to adverse reactions (sepsis in the group I case and exclusion criteria in the group II subject). However, these four patients achieved total response at the end. The 30 patients that achieved total response were followed during at least one additional year.

Demographic and baseline characteristics of the patients

Table 1 shows the baseline characteristics of the treatment groups. Patients in both groups were predominantly males and white. They suffered diabetes, mostly type 2, for a long time. Wound size medians were 22.5 and 25.0 cm^2 in groups I and II, respectively; three patients had >100 cm^2 ulcers. Lesions were located on all foot regions, more frequently on the toes. Ulcer etiopathogeny was predominantly ischemic.

Although all Wagner's grade 4 cases were in group I this difference did not reach statistical significance (p=0.056). There were no significant differences between groups in any of the other baseline characteristics.

Efficacy

More than 30% granulation tissue covered wound area was obtained since the first week of treatment in most patients of both groups. The granulation response rate is shown in Table 2. By the 5th week, complete response was achieved in 73.9% of group I patients and 50.0% in group II. The four partial responders who were evaluated at 8 weeks attained complete response, so the final complete response rates were 83% in group I and 61% in group II. The probability to attain 50% of the wound area covered by granulation tissue (total and partial response) was higher for group I and complete response was attained one week earlier. None of these variables were statistically different between both groups.

The univariate analysis of the relationship of different baseline variables with complete response rate at 5 weeks showed significantly higher probability of complete response (odds ratio; 95% confidence interval) for age ≤ 65 (10.9; 2.3 – 50.0), history of cardiopathy (5.1; 1.1 – 25.0), neuro-infectious ulcer (14.1; 1.6 – 125.0), and having received ≥ 15 rhEGF doses (5.2; 1.3 – 21.1). However, using multivariate logistic regression analysis, the only variables that had or were close to a significant effect on complete response at 5 weeks were male gender (9.5; 0.9 – 100), age ≤ 65 years (34.1; 2.8 – 409), and ≥ 15 rhEGF doses (15.3; 1.4 – 165).

The final outcome is shown in Table 3. Complete wound healing was obtained in 13 (56.5%) and 9 (50.0%) patients in groups I and II, respectively after approximately 20 weeks since the onset of treatment. Skin graft was used in one case after the complete granulation response. Ulcer relapse occurred in one of the subjects in group I. Besides, one group II patient did not reach complete healing although the lesion had improved considerably. Some examples of healed patients are shown in Fig. 2. Granulation response, wound contraction and complete epithelization can be observed.

Amputation was necessary in 8 (34.8%) and 6 (33.3%) patients in groups I and II, respectively taking into account the whole treatment and follow-up period. During the treatment period amputations occurred in 3 and 5 patients in groups I and II respectively. However, 5 and 1 patients were further amputated, despite having had complete granulation response. Mean time to amputation in group I was 15.6 months (CI 11.9 – 19.3) and 13.9 months (CI 9.3 – 18.5) in group II. No statistically dependence was found between the amputation and the patients' demographic characteristics.

Histological studies confirmed that useful granulation tissue was obtained since the first week of treatment, with more than 25% of the images with neoformation vessels and a significant increase of collagen fibres and fibroblast proliferation (Fig. 3).

Safety

Adverse events are listed in Table 4. The most frequent were sepsis, burning sensation, and local pain. Most of the adverse events were classified as mild or moderate. Only two (4.9%) patients had severe events. One anaemia and chest pain that required blood transfusions and medication to resolve it. The other, a woman with history of cardiopathy, had interrupted the treatment due to local sepsis and one week after

developed an acute abdomen and a fatal arrhythmia during emergency surgery. There were no alterations of the clinical laboratory measurements done.

Discussion

This study shows that treatment with intralesional rhEGF, associated to good wound care measures, can benefit patients with advanced DFU for which otherwise there is no available specific therapy. A complete granulation response appeared after 5 weeks of treatment in more than 60% of the patients. Complete wound healing was reached in more than 50% of the patients after 20 weeks since the onset of an up-to-8-weeks treatment schedule. Interestingly, this result was obtained despite that the ulcers were Wagner's grade 3 or 4, mostly larger than 20 cm^2, predominantly ischemic, and had a high risk of amputation. Only one recurrence was observed during a one-year follow-up after treatment, which supports the durability of the effect.

The study, although randomised and double blind, was not placebo-controlled, which limits the external validity of the results. A placebo group was not accepted by the Ethics Committee considering the high risk of amputation in these patients and the possibility to avoid it given the previous results obtained with this procedure [23]. The study was not powered to determine differences between the two dose levels studied, but to compare each of them with a 30% response that was considered to be clinically significant. However some dose effect was suggested by the tendency to an earlier response in the higher dose group. More studies are required to further elucidate this aspect.

Other growth factors such as recombinant human platelet-derived growth factor-BB (becaplermin) have been used topically but in neuropathic and smaller lesions [6,7]. In a meta-analysis of those studies Smiell et al. conclude that within the setting of a comprehensive wound management program, treatment with becaplermin gel at a dose of 100 μg/g once daily increases the incidence of complete healing [25]. However, 95% of the patients included in those trials had ulcers ≤ 10 cm^2 (median 1.4 – 3.5 cm^2) and an adequate blood supply (defined as $TcpO_2$>30 mm Hg) was a requisite for inclusion. On the contrary, this study treated more advanced, larger (median > 20 cm^2) and both neuropathic and ischemic wounds.

Previous use of EGF topically, on DFU has been reported. A randomised, double blind, placebo-controlled trial evaluated two doses of a rhEGF-containing cream in patients with diabetic foot ulcers compared to a protein-free calf blood derivative cream used as control. Healing rate was significantly enhanced by rhEGF 0.04% but not by the lower dose (0.02%). This trial also included much less severe ulcers given by Wagner's grades 1 and 2, ≤ 4 cm^2 size and only neuropathic [15]. Another non-controlled trial treated patients with grade 2-3, resistant to advanced dressing alone, neuropathic ulcers, mean size 4.8 cm^2 with a topical formulation containing 0.005% rhEGF added to the dressing. In the treated patients, complete healing was noted in 76% (52/68) of patients within an average of 46 days (range from 2 to 14 weeks) [16].

A limitation to the efficacy of topical formulations can be that the growth factor cannot steadily reach the deeper layers of the wound. Diffusion of the active agent is affected by necrotic tissue, sepsis, inflammation, and by the action of wound proteases [22]. It has been demonstrated that chronic wounds have elevated proinflammatory cytokines,

high protease activity, decreased levels of natural metalloproteinase inhibitors, and diminished growth factor activity [26-28]. The still active factor may be unavailable for biologic activity because of trapping or binding to molecules such as fibrinogen, macroglobulin, or albumin [29,30]. Additionally, the ever-present tissue level of bacteria in chronic wounds produce higher levels of proteases and other metalloproteinases that further degrade the growth factors and their receptors [31]. These facts can contribute to explain the lack of efficacy of topical EGF and PDGF at lower doses.

Intralesional injection of the growth factor could bring the active agent into the desired region and avoid the inactivating agents. The results shown here confirm the previous proof-of-concept study where 86% of 29 treated patients exhibited a productive granulation with substantial wound matrix transformation, granulation tissue cell population and angiogenesis enhancement. Seventeen of the patients (58.6%) were rescued from amputation [23]. Interestingly, more severe wounds were treated in both trials with the intralesional injection procedure, with an essentially satisfactory outcome.

Treatment was well tolerated. Besides local symptoms, most of the adverse events were mild and easily manageable. The two severe adverse events, including the patient who deceased, do not seem to be related to the EGF treatment. One of the major concerns of the use of exogenous EGF at concentrations much higher than physiological is that it could promote the development of malignant neoplasia. An accurate assessment of this event was included in the long term follow-up in this study. It was not observed in any of the patients. Nevertheless additional studies should be done with a larger number of patients as long as the use of this product is extended.

Another concern with the intralesional route of administration could be the risk of inoculating or spreading bacterial infection. This was minimized by the concomitant good wound care practices, broad-spectrum antibiotic coverage, and adequate aseptic injection procedures. Around 20% of the patients developed sepsis, which accounted for most of the therapeutic failures. Infections control remains a critical problem in such advanced DFU.

In summary, intralesional administration of rhEGF improved granulation tissue formation in both neuropathic and ischemic advanced DFU. Wound healing was thus stimulated. Future controlled studies are needed to further assess the possible impact of this promising intervention, since this condition is still an unsolved medical problem and an important economic burden to medical care systems.

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Annex

Cuban Citoprot-P Study Group: (* Steering Committee)

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Table 1. Baseline characteristics of the study population

Characteristic		Group I (N=23)	Group II (N=18)
Age in years: median ± QR (min; max)		63.0 ± 12.0 (21; 76)	67.5 ± 19.5 (46; 82)
Gender	Males	12 (52.2%)	10 (55.6%)
	Females	11 (47.8%)	8 (44.4%)
Race	White	15 (65.2%)	13 (72.2%)
	non-white	8 (34.8%)	5 (27.8%)
History of heart disease		6 (26.1%)	3 (16.7%)
Diabetes Mellitus	Type 1	2 (8.7%)	2 (11.1%)
	Type 2	21 (91.3%)	16 (88.9%)
Time with diabetes in years		20.1 ± 8.5	17.5 ± 10.1
Ulcer duration in months: median ± QR (min; max)		1.0 ± 1.5 (0.3; 120)	1.0 ± 1.5 (0.4; 12)
Ankle-brachial index (ABI) > 0.8		7 (30.4%)	4 (22.2%)
ABI < 0.8	Femoral-popliteal	7 (30.4%)	1 (5.6%)
	Distal	9 (39.1%)	13 (72.2%)
Ulcer size (cm^2): median ± QR (min; max)		22.5 ± 35.0 (6; 300)	25.0 ± 10.9 (10; 110.5)
Predominant Etiopathogenic feature	Neuropathic	6 (26.1%)	8 (44.4%)
	Ischemic	17 (74.9%)	10 (55.6%)
Wagner's classification	Grade 3	18 (78.3%)	18 (100.0%)
	Grade 4	5 (21.7%)	0 (0%)
Ulcer location	Toes	15 (65.2%)	12 (66.7%)
	Internal edge	1 (4.3%)	--
	External edge	3 (13.0%)	4 (22.2%)
	Dorsum	4 (17.4%)	2 (11.1%)
	Sole	5 (21.7%)	4 (22.2%)
	Transmetatarsal	3 (13.0%)	2 (11.1%)
	Ankle	3 (13.0%)	2 (11.1%)

Table 2. Granulation response to treatment with intralesional rhEGF

		Group I (N=23)	Group II (N=18)
Response at 5 weeks (Percentage of ulcer area covered by granulation tissue)	Complete (75-100%)	17 (73.9%)	9 (50.0%)
	Partial (50-75%)	2 (8.7%)	2 (11.1%)
	Minimal (25-50%)	0 (0%)	2 (11.1%)
	No response (< 25%)	4 (17.4%)	5 (27.8%)
Response at 8 weeks	Complete (75-100%)	19 (82.6%)	11 (61.1%)
	Minimal (25-50%)	0	2 (11.1%)
	No response (< 25%)	4 (17.4%)	5 (27.8%))
Probability to obtain > 50% of ulcer area covered by granulation tissue (95% CI)		0.62 – 0.93	0.38 – 0.79
Time to obtain complete response (weeks) mean ± SD (95% CI)		3.8 ± 2.2 (2.8 – 4.8)	4.9 ± 2.2 (3.6 – 6.1)

Table 3. Outcome of the patients (including treatment and follow-up periods)

Endpoint			**Group I (N=23)**	**Group II (N=18)**
Complete healing			13 (56.5%)*	9 (50.0%)
Lesion persisted at the end of follow-up			0	1(5.1%)
Amputations			8 (34.8%)	6 (33.3%)
Withdrawals			2 (deceased)	2 (voluntary abandoners)
Probability of complete healing (95% CI)			(0.362 – 0.738)	(0.289 – 0.709)
Type of amputation	Above-knee		3	1
	Below-knee		2	4
	Transmetatarsian		2	1
	Toes		1	0
Time to healing (weeks)		Mean ± SD	20.6 ± 1.8	19.5 ± 1.6
		95% CI	(17.0 – 24.2)	(16.3 – 22.7)
Time to amputation (months)		Mean ± SD	15.6 ± 1.9	13.9 ± 2.4
		95% CI	(11.9 – 19.3)	(9.3 – 18.5)

* In one case healing was reached after skin graft;

Table 4. Adverse events frequency.

Events	Group I (N=23)	Group II (N=18)	Total
Sepsis	5 (21.7%)	3 (16.7%)	8 (19.6%)
Burning sensation	5 (21.7%)	2 (11.1%)	7 (17.1%)
Local pain	4 (17.4%)	3 (16.7%)	7 (17.1%)
Tremors	5 (21.7%)	1 (5.6%)	6 (14.6%)
Chills	4 (17.4%)	1 (5.6%)	5 (12.2%)
Fever	3 (13.0%)	1 (5.6%)	4 (9.8%)
Anaemia	1 (4.3%)	1 (5.6%)	2 (4.9%)
Enterocolitis	2 (8.6%)	-	2 (4.9%)
Chest pain	1 (4.3%)	1 (5.6%)	2 (4.9%)
Facial paralysis	1 (4.3%)	-	1 (2.4%)

Figure legends

Figure 1. Study flowchart.

Figure 2. Comparative photos of some patients: A, B, and C: patient IA-01; D, E, and F: patient IA-11. A and D: before treatment, B and E: at the end of treatment; C and F: after one year follow-up.

Figure 3. Representative histological image of neuropathic (A, B) and ischemic (C, D) ulcers. Samples were collected just before the first Citoprot-P infiltration (A, C) and after the 5th week of treatment. (B, D). Haematoxilin/eosin staining, magnification X10. **A:** Neuropathic lesion before treatment. At this stage an acute inflammatory infiltration is observed as the only ingredient of the granulation process. Inflammation is diffuse and not polarized. No evidences of matrix production or neovessels are detected. **B:** At the 5^{th} week the matrix appeares dense, consolidated and indurated by compact collagen material. The presence of fibroblastoid cells is largely increased. **C:** Ischemic lesion before treatment. Wound matrix appears fibrinoid, non-organized and rich in cell and tissue debris. Granulation and inflammatory polarization processes have not yet set forth. **D:** At the 5^{th} week of treatment the wound matrix looks consolidated, organized and with well-shaped vessels showing luminal blood (indicated by arrows).

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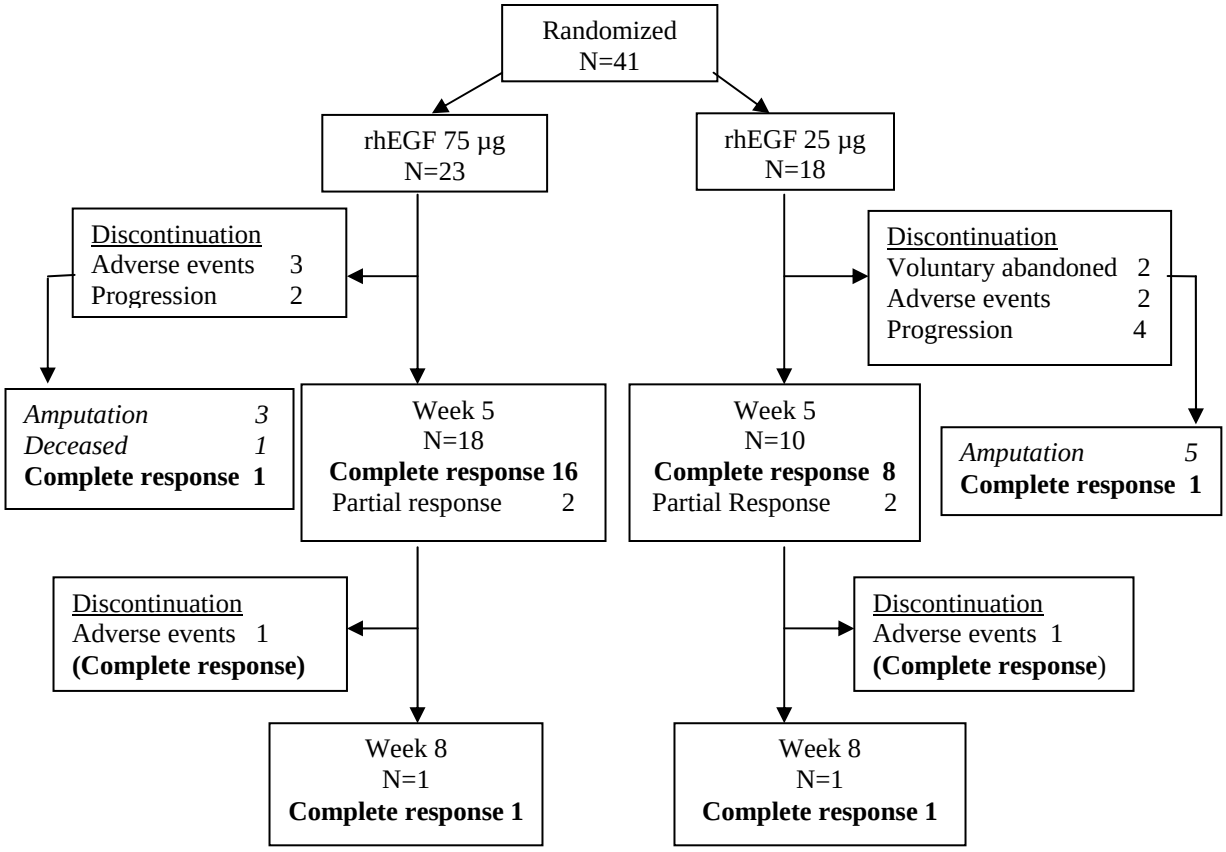
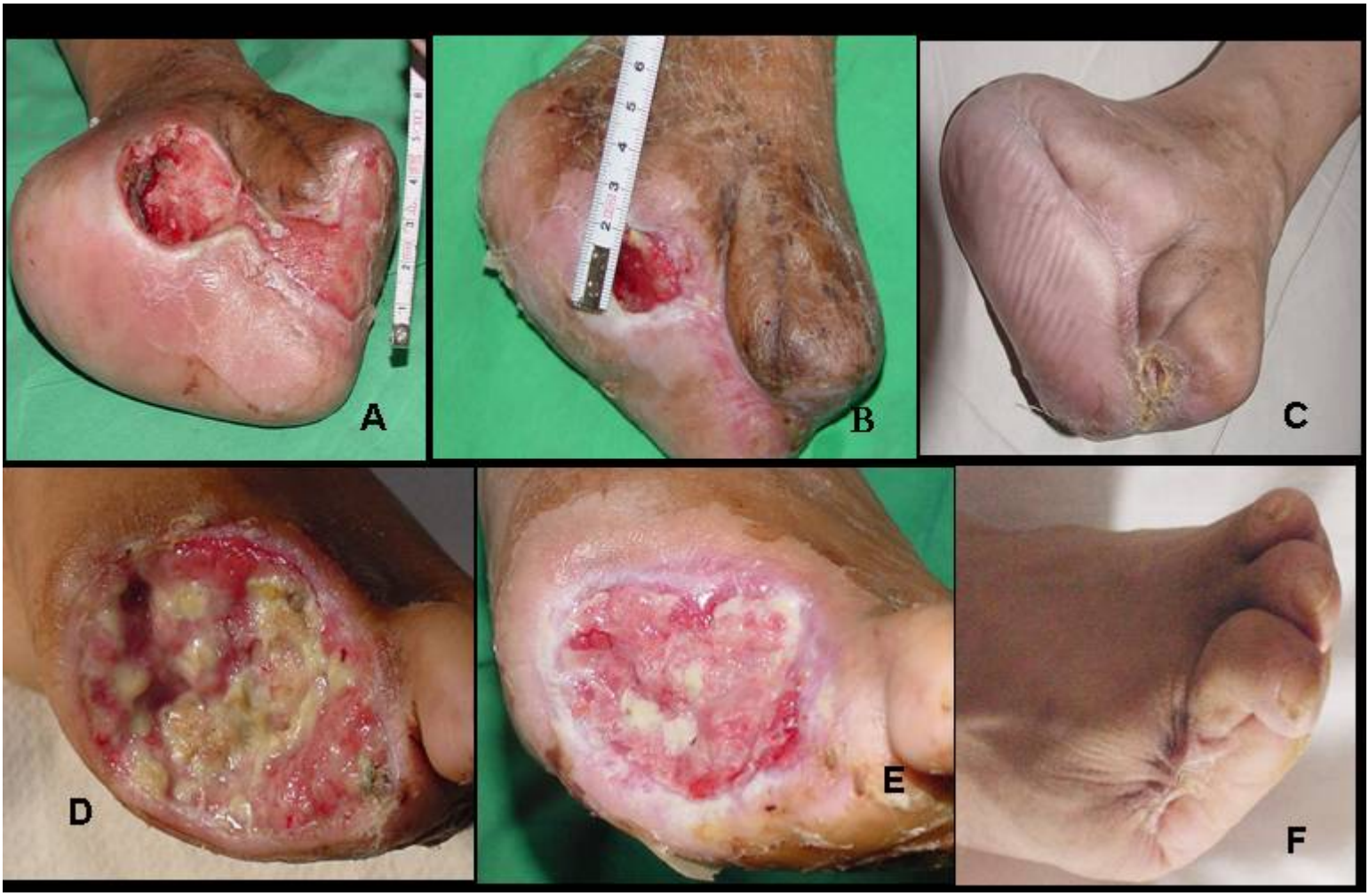
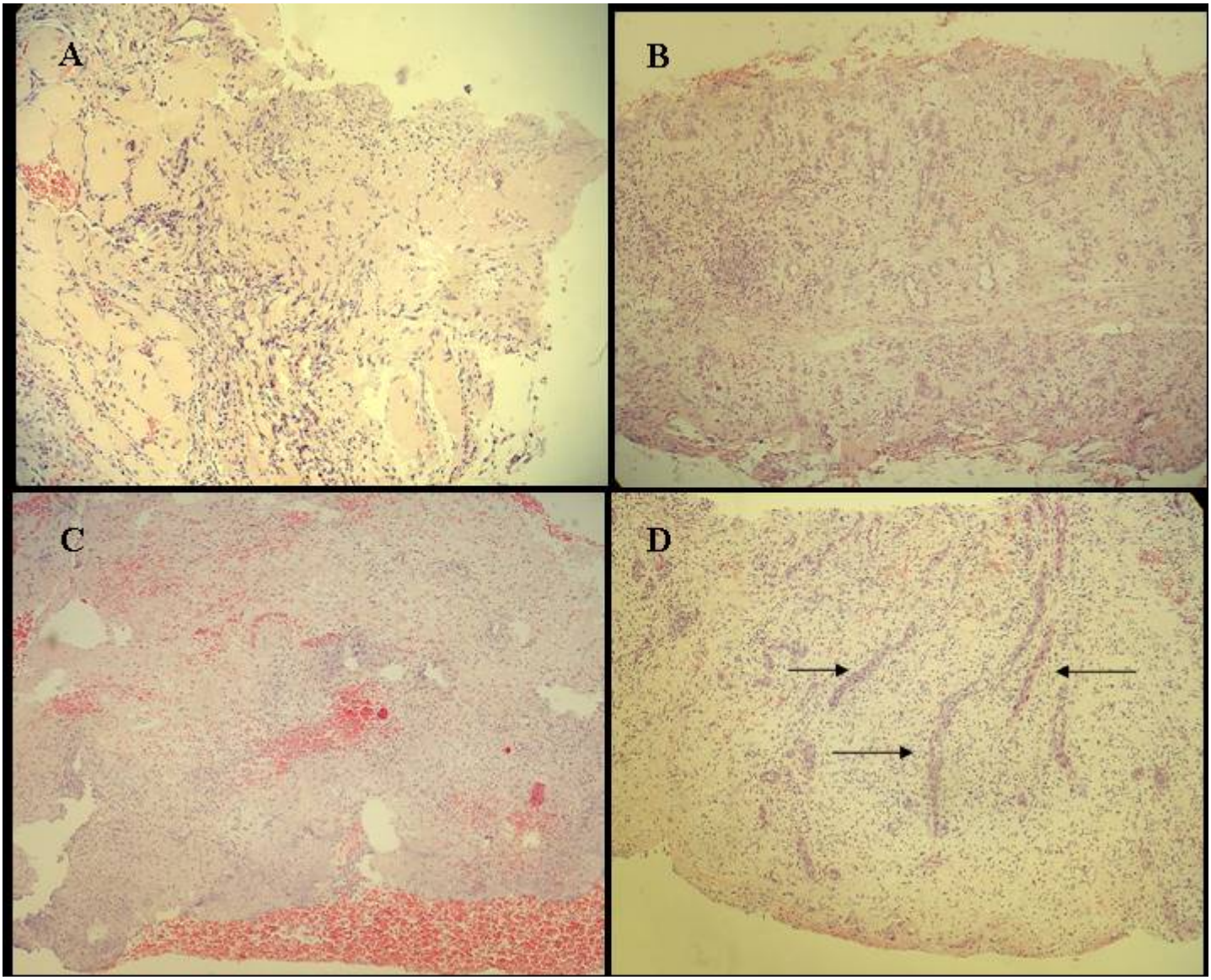

Randomized
N=41
rhEGF 75 µg
N=23
rhEGF 25 µg
N=18
Discontinuation
Adverse events 3
Progression 2
Discontinuation
Voluntary abandoned 2
Adverse events 2
Progression 4
Amputation *3*
Deceased *1*
Complete response 1
Week 5
N=18
Complete response 16
Partial response 2
Week 5
N=10
Complete response 8
Partial Response 2
Amputation *5*
Complete response 1
Discontinuation
Adverse events 1
(Complete response)
Discontinuation
Adverse events 1
(Complete response)
Week 8
N=1
Complete response 1
Week 8
N=1
Complete response 1


A
B
C
D
E
F


A
B
C
D