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CLINICAL RESEARCH ARTICLE

Intralesional epidermal growth factor for diabetic foot wounds: the first cases in Turkey

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Background: Intralesional recombinant epidermal growth factor (EGF) was produced in the Centre for Genetic Engineering and Biotechnology (CIGB), Cuba, in 1988 and licensed in 2006. Because it may accelerate wound healing, it is a potential new treatment option in patients with a diabetic foot wound (whether infected or not) as an adjunct to standard treatment (i.e. debridement, antibiotics). We conducted the initial evaluation of EGF for diabetic foot wounds in Turkey.

Methods: We enrolled 17 patients who were hospitalized in various medical centers for a foot ulcer and/or infection and for whom below the knee amputation was suggested to all except one. All patients received 75 µg intralesional EGF three times per week on alternate days.

Results: The appearance of new granulation tissue on the wound site ($\geq$75%) was observed in 13 patients (76%), and complete wound closure was observed in 3 patients (18%), yielding a 'complete recovery' rate of 94%. The most common side effects were tremor ($n = 10$, 59%) and nausea ($n = 6$, 35%). In only one case,a serious side effect requiring cessation of EGF treatment was noted. That patient experienced severe hypotension at the 16th application session, and treatment was discontinued. At baseline, a total of 21 causative bacteria were isolated from 15 patients, whereascultures were sterile in two patients. The most frequently isolated species was *Pseudomonas aeruginosa*.

Conclusion: Thus, this preliminary study suggests that EGF seems to be a potential adjunctive treatment option in patients with limb-threatening diabetic foot wounds.

Keywords: *diabetic foot*; *intralesional epidermal growth factor*; *treatment*

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Among persons suffering from diabetes mellitus, the lifetime risk of developing a foot ulcer is estimated to be 15–25%, and it is believed that every 30 s a lower limb is lost somewhere in the world as a consequence of diabetes (1, 2). The high rates of diabetes in many parts of the world make foot ulcers a major and increasing public health problem. Foot ulcers cause substantial morbidity, impair quality of life, engender high treatment costs, and are the most important risk factor for lower extremity amputation (3). The mortality after 5 years in patients undergoing a lower limb amputation is 40% (4). Despite all standard therapies (debridement, antibiotics, wound care therapies, etc.), the rate of limb loss still remains high. Thus, new products are being investigated for the treatment of diabetic foot ulcers.

Epidermal growth factor (EGF) is a 53-aminoacid polypeptide isolated from adult mouse submaxillary glands that exerts potent mitogenic activity through binding to a specific cell membrane receptor (5, 6). Recombinant EGF was produced for the first time in Centre for Genetic Engineering and Biotechnology (CIGB), Cuba, in 1988. In 2006, it was licensed in Cuba as a new treatment option for patients with a diabetic foot wound, (whether infected or not) to accelerate wound healing, as

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an adjunct to standard treatment procedures. Intralesional EGF has been available as a medication in Turkey since 2012. We present our results on the first intralesional EGF applications performed in Turkey on patients with diabetic foot wounds.

Materials and methods

In total, four Turkish medical centers were included in this prospective preliminary study conducted from January 2012 to June 2013. Patients were screened for risk factors known to be associated with lower extremity complications (e.g. age, sex, duration of diabetes, previous hospitalization, previous amputation, previous foot infections, previous osteomyelitis, peripheral neuropathy, peripheral vascular disease, wound depth, and ulcer localizations). The data on enrolled subjects were recorded in patient follow-up forms. Foot pathology was assessed by a trained physician according to International Working Group on the Diabetic Foot criteria (PEDIS classification) (7). All patients had been hospitalized for various durations in one of several medical centers because of the same wound (whether infected or not). For all except one patient, below the knee (at any location below the knee joint) amputation had been recommended. On admission, specimens for culture were obtained following cleansing and the debridement of the wound by curettage, needle aspiration, or biopsy, depending on the wound depth.

All the patients received intralesional injections of 75 μg EGF three times per week on alternate days. The application site was cleansed by debridement of necrotic, infected soft-tissues, and infected bone tissues (only in patients with osteomyelitis) prior to administration of EGF by the physician. In all cases, intralesional EGF treatment was initiated following the infection control by surgical debridement and antibiotic therapy. The decision to start EGF administration was given without waiting for the termination of antibiotherapy. Both antibiotherapy and EGF administrations were continued during the treatment period. Patients were discharged from the hospital following clinical stability, and outpatient intralesional EGF administrations were performed. EGF vials were transferred to the medical centers in lyophilized form with cold chain procedures and stored at 4–8°C. EGF was dissolved with 5 ml of sterile water for injection. In each application, this volume was distributed throughout the lesion, in 0.5–1 ml injections, starting from the deeper zones. Previously described criteria were utilized for the evaluation of wound healing (8). These criteria measured efficacy by amount of the ulcer surface covered by granulation tissue: $\leq$25% (no response); 26–50% (minimal response); 51–75% (partial response); and $>$75% (complete response). The area of the granulation tissue was divided by the area of the whole wound to obtain a ratio. Adverse reactions were monitored daily during treatment. We did the measurements repeatedly until the formation of new granulation tissue. All the patients were followed up for various periods following the termination of EGF treatments.

Statistical analysis

Normally distributed parameters were given as mean and standard deviation, whereas others were given as median and 25th–75th percentile.

Results

In total, 17 patients were enrolled in the study, all of whom had type 2 diabetes mellitus and were receiving insulin therapy. Most patients were late middle-aged elderly men who had their foot infection for about 3 months. The demographic characteristics of the patients are shown in Table 1.

All of the patients underwent surgical soft-tissue debridement or minor amputations (transmetatarsal or toe amputation). Microbiological assessment was performed in all patients. At baseline, cultures were sterile in two patients, whereas a total of 21 causative bacteria were isolated from the other 15 patients. The most frequently isolated species was *P. aeruginosa* ($n=7$, 33%) (Table 2).

In total, 306 intralesional EGF administrations were performed in 17 patients by the end of the study. Granulation tissue on the wound site ($\geq$75%) was observed in 13 (76%) patients. Among this group, an autologous skin graft was performed in two cases. Complete wound closure was observed in one of these patients (Fig. 1), whereas graft failure was experienced in the other patient, who had undergone three dialysis sessions each week. EGF applications were performed on alternate days except dialysis days. In three (18%) patients, complete wound closure was observed by the end of the treatment. The outcomes in 16 patients (94%) were defined as complete recovery.

Major lower extremity amputation was not required in any case. Secondary bacterial infection did not develop in any case during the EGF applications. One patient withdrew before the ninth application session because of the need for urgent coronary angiography, but the wound had already developed granulation tissue ($>$75%) at application session 9. Serious side effects requiring cessation of treatment were not observed during EGF applications except in one patient (Table 3), who experienced severe hypotension at the 16th application session, and the treatment was discontinued. However, formation of granulation tissue was observed at 50% of the wound site (partial response) in this patient. On the other hand, premedication with paracetamol and antiemetic drugs was administrated in patients who experienced tremor and nausea/vomiting at the first application. The most common side effects were tremor ($n=10$, 59%) and nausea ($n=6$, 35%); both began soon after the application and continued for a period of 10–15 min and 20–30 min, respectively.

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Table 1. Demographical and clinical characteristics and outcome of the patients

Characteristics	
Age mean (±SD) (years)	62.24±11.14
Gender (male)	9 (53)
Duration of diabetes (year) median (25th–75th percentile)	15 (12–19)
Duration of diabetic foot infection (months) median (25th–75th percentile)	3 (3–6)
Previous minor amputation	13 (76)
Renal failure	1 (6)
Peripheral vascular disease	
Grade 1	7 (40)
Grade 2	7 (40)
Grade 3	3 (20)
Neuropathy	17 (100)
Infection	
Grade 1	0 (0)
Grade 2	1 (6)
Grade 3	13 (76)
Grade 4	3 (18)
Wound size (cm^2) median (25th–75th percentile)	20 (13–30)
Wound localizations	
Hallux	1 (6)
Other toes	2 (12)
Metatarsal	8 (46)
Plantar foot	3 (18)
Heel	2 (12)
Two or more regions	1 (6)
Osteomyelitis	13 (76)
Number of intralesional EGF injections median (25th–75th percentile)	18 (14–21)
Follow-up period (months) median (25th–75th percentile)	4 (1–8)
Outcome	
Partial response (granulation tissue 51–75%)	1 (6)
Complete response (granulation tissue >75%)	13 (76)
Wound closure	3 (18)

During 4 (range: 1–14) month follow-up, none of the patients whose ulcers healed had a recurrent ulcer or need for major lower extremity amputation following the termination of the treatment.

Discussion

Patients included in this report are the patients who underwent the first intralesional EGF in Turkey. EGF has a mitogenic and motogenic role and cytoprotective actions in wound healing. It stimulates 1) the migration of productive cells to the ulcer area, 2) formation of granulation tissue including extracellular matrix accumulation, maturation, and *de novo* angiogenesis, 3) wound contraction by myofibroblast activation and proliferation, and 4) resurfacing of damaged area by epithelial cells migration and proliferation (9). EGF plays a dominant early role in wound healing by stimulating keratinocyte proliferation and migration (10).

Table 2. Microorganisms isolated from foot infections

Causative bacteria	*N* (%)
Gram-positive aerobic cocci	11 (52)
Staphylococcus aureus	5
Methicillin sensitive	2
Methicillin resistant	3
Coagulase-negative staphylococcus	4
Methicillin resistant	3
Methicillin sensitive	1
Enterococcus spp.	1
Group D *streptococcus*	1
Gram-negative *aerobic bacilli*	10 (48)
Pseudomonas aeruginosa	7
Serratia marcescens	2
Acinetobacter spp.	1
Total	21 (100)

The efficacy and safety of EGF have been tested in three exploratory and one confirmatory randomized, double-blind, placebo-controlled clinical trial (8, 10, 11). Berlanga et al. administered 25 µg EGF to 29 patients and obtained some granulation response in 25 cases (86%) and complete response and wound closure in 17 cases (59%, one of them through skin graft). Amputation was prevented in all cases (10). Another study by Fernandez-Montequin et al. compared EGF doses of 75 µg ($N=23$) and 25 µg ($N=18$) and found a higher and faster granulation response with the higher dose (83 vs. 61%) (11). In another double-blind randomized and multicenter study, Fernandez-Montequin et al. compared two different doses of EGF (75 and 25 µg) with a placebo (8). The rate of complete granulation following 8 weeks treatment was 87% (75 µg EGF), 73% (25 µg EGF), and 58% (placebo). The difference between intralesional EGF administrations and placebo was statistically significant in favor of EGF. In another study consisting of 20 patients, complete granulation was observed in all cases and complete wound closure was observed in 17 patients (85%) by

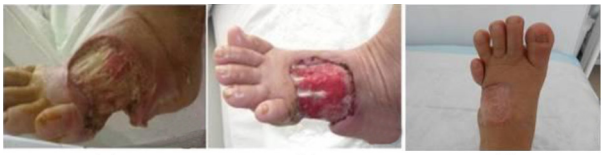

Fig. 1. The first case in Turkey. Comparative photos of patient: (a) before treatment, (b) after 18th intralesional EGF, and (c) complete wound closure following autologous skin graft.

Table 3. Side effects and premedication

Side effect	*N* (%)
Tremor	10 (59)
Nausea	6 (35)
Pain on the application site	5 (29)
Vomiting	3 (18)
Hypotension	3 (18)
Weakness	2 (12)
Chest pain	2 (12)
Requirement of premedication[a]	13 (76)

[a]Paracetamol and anti-emetic drugs.

the end of the treatment (8). In all these studies, patients were over 60 years old, ulcers area exceeded 20 cm^2, and they were grade 3 or 4 according to the Wagner classification (chronic, deep, large sized, infected, and necrotic ulcers); yet, a high rate of successful results (80%) was obtained with 75 μg EGF.

In our study, all of the patients had been hospitalized for various times and periods because of their diabetic foot ulcer with or without infection, and in all but one, a below the knee amputation was recommended. Among our patients, 13 (76%) had grade 3 and 3 (18%) had grade 4 foot infections, according to the PEDIS Classification. Our study group was similar with the studies described above. As we obtained wound healing in 16 of 17 patients (treatment was discontinued in one patient due to serious hypotension), our results are similar to those previously published.

Cultures of specimens obtained by debridement of infected bone or soft-tissue demonstrated that the distribution of causative bacteria was in concordance with the previous studies performed in our country, with a high prevalence of antibiotic resistant bacteria and *P. aeruginosa* (Table 2) (12–14). This was an expected result as these patients had longstanding and advanced grade diabetic foot infections. We could not make a comparison with the microbiological data of the previous studies because of lack of information on this issue in these other studies. The wounds were sharply debrided in order to remove callus, fibrin, and necrotic material and washed with saline solution prior to the treatment. All our patients received appropriate systemic antibiotics according to the susceptibility tests and standard wound care procedures, as well as any diabetes-associated medical treatment.

In most of our patients, we noted granulation tissue formation was achieved by continuous treatment with EGF. During the initial administrations, hyperemia and local heat increased around the application site, but this stopped following the fourth to sixth sessions. Although the amount of bleeding was slight during the initial sessions, it increased later, particularly after the sixth and following sessions. This is probably because of the effect of EGF on angiogenesis, although this is not proved by histopathology.

Angiogenesis at the wound site has a positive effect on wound healing, but it also increases the amount of the drug in the systemic circulation. That is why the most common two side effects, tremor and nausea, worsened toward the end of the treatment. In these cases, premedication with paracetamol and antiemetic drugs were initiated. The severity of side effects decreased, but did not disappear completely, following the premedication. Except for one case, all of the patients experienced various side effects, the most common being tremor (59%) and nausea (35%). Treatment was discontinued in two cases, one for severe hypotension and the other because of urgent need of coronary angiography (unrelated to EGF administration). The rate of side effects in our study was higher than those reported in the literature. Tremor (20–55%) was the most common side effect in the previous studies and chills (17–40%) the second most common. On the other hand, nausea was not a common side effect (8, 11). Previous studies, as well as ours, included limited number of patients. There is lack of information on the mechanisms of general side effects of EGF therapy including dizziness, nausea, and vomiting, and we could not make any comments on this issue. A large series of patients is needed to observe the adverse reactions.

Conclusion

Despite the limitations of the data, the most important outcome of our study is the prevention of major amputation in all cases. Advanced and longstanding foot wounds were probably the main reason that other clinicians had suggested major amputations for most of our patients. Intralesional EGF administration was initiated as soon as the infection was under control. EGF is a potential adjunctive treatment option for diabetic foot ulcer. It may be an alternative medical therapy to amputation, and the results are promising. During the follow-up period as outpatients, no patient had an ulcer recurrence or needed major lower extremity amputation. The small number of the cases and the lack of control group are the limitations to generalize the outcomes of our study. It is a new agent in clinical use and needs more clinical trials to get detailed information.

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