

Epidermal Growth Factor in Healing Diabetic Foot Ulcers: From Gene Expression to Tissue Healing and Systemic Biomarker Circulation

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ABSTRACT

Lower-extremity diabetic ulcers are responsible for 80% of annual worldwide nontraumatic amputations. Epidermal growth factor (EGF) reduction is one of the molecular pillars of diabetic ulcer chronicity, thus EGF administration may be considered a type of replacement therapy. Topical EGF administration to improve and speed wound healing began in 1989 on burn patients as part of an acute-healing therapy. Further clinical studies based on topically administering EGF to different chronic wounds resulted in disappointing outcomes. An analysis of the literature on unsuccessful clinical trials identified a lack of knowledge concerning: (I) molecular and cellular foundations of wound chronicity and (II) the pharmacodynamic requisites governing EGF interaction with its receptor to promote cell response. Yet, EGF intra- and perilesional infiltration were shown to circumvent the pharmacodynamic limitations of topical application. Since the first studies, the following decades of basic and clinical research on EGF therapy for problem wounds have shed light on potential uses of growth factors in regenerative medicine. EGF's molecular

and biochemical effects at both local and systemic levels are diverse: (1) downregulation of genes encoding inflammation mediators and increased expression of genes involved in cell proliferation, angiogenesis and matrix secretion; (2) EGF intervention positively impacts both mesenchymal and epithelial cells, reducing inflammation and stimulating the recruitment of precursor circulating cells that promote the formation of new blood vessels; (3) at the subcellular level, upregulation of the EGF receptor with subsequent intracellular trafficking, including mitochondrial allocation along with restored morphology of multiple organelles; and (4) local EGF infiltration resulting in a systemic, organismal repercussion, thus contributing to attenuation of circulating inflammatory and catabolic reactants, restored reduction-oxidation balance, and decreased toxic glycation products and soluble apoptogenic effectors. It is likely that EGF treatment may rearrange critical epigenetic drivers of diabetic metabolic memory.

KEYWORDS Epidermal Growth Factor, diabetes, diabetes complications, wound healing, diabetic foot, amputation, ulcer, Cuba

INTRODUCTION

Diabetic foot ulcers (DFU) are one of the most feared complications of diabetes. It is a common cause of nontraumatic amputation, resulting in significant disability, morbidity and mortality.[1] An ulcer is the distal expression of an impaired healing process with a high rate of recurrence, so that patients who have temporarily achieved wound closure are considered to be remission rather than healed.[1]

The glycemic imbalance and other diabetes-related factors contribute to sculpt an epigenetic blueprint that results in a sort of “stagnant transcriptome”[2,3] in which precocious senescence, proliferative refractoriness, and apoptosis appear to be critical

drivers resulting in wound chronicity.[4] These biological deterrents have been related to a substantial reduction in availability and activity of several growth factors, as major players of internal and peripheral tissue repair.[5,6]

The diabetic wound microenvironment is hostile to the chemical integrity and bioavailability of local growth factors (GF) and ultimately, to their role in the healing process. Examples of these growth factors include EGF, Platelet-Derived Growth Factor (PDGF), Transforming Growth Factor beta-1 (TGF- β 1), and Insulin-Like Growth Factor I (IGF-1).[7–9] The expression and transduction signaling of EGF and PDGF receptors are also impaired within the diabetic environment.[9] Accordingly, as described for the molecular mechanisms operating in peripheral insulin resistance, it may be that diabetic wound cells exhibit reduced tyrosine kinase activity, accounting for loss of function of the growth factor receptor, which predisposes cells to proliferative arrest and senescence.[10]

In 1962, Stanley Cohen announced EGF isolation and purification from salivary glands. EGF was shown to induce precocious development and maturation of epidermal tissue and its appendages when injected into newborn mice. In other words, EGF induced maturational reprogramming of chronologically imprinted events. This is the most studied growth factor in wound healing, given its ability to promote epithelial and mesenchymal cell proliferation.[11] Yet, circulating EGF levels are reduced by

IMPORTANCE

This article describes the molecular mechanisms of epidermal growth factor (EGF) pharmacological activity, and links gene response to organ system homeostasis. The depicted cascade of events may underlie the clinical efficacy of locally-infiltrated EGF in restoring the healing response of high-grade diabetic foot ulcers. To our knowledge, this is the first comprehensive description of how EGF reverts the chronic wound phenotype in a meaningful clinical scenario when properly delivered to responsive cells.

diabetes,[12] contributing to development of local and systemic complications.[13,14] Consequently, EGF and other deficient growth factors are exogenously administered as a replacement therapy in diabetes, as an attempt to restore physiological healing processes.[13,14]

Topical administration of recombinant human EGF dates back more than 30 years. Initially, it was thought to be an encouraging alternative to combat the torpid healing of problem wounds.[15] However, the history of GF pharmacology in wound healing suggests that EGF's clinical introduction was rather precocious, at a time when basic knowledge on the biology of chronic wounds remained elusive. Initial clinical trials proved disappointing, as topical EGF administration failed to enhance a healing response in chronic wounds,[16] even in acute, experimentally induced wounds in healthy volunteers.[17]

In line with the notion that EGF reverses the proliferative arrest that characterizes chronic wounds,[18,19] we introduced EGF administration through local infiltration to treat high grade DFU (for review see [18]). It was our hypothesis that intralesional infiltration could circumvent the limitations confronted during years of topical EGF administration.

The infiltration protocol calls for an EGF liquid formulation to be injected locally in the wound, at a depth of 6 mm to 10 mm, 3 times a week for 5 to 8 weeks, targeting the wound bottom and dermo-epidermal junction. The decision to use this delivery mode resulted from insights accumulated from animal models and *ex vivo* and *in vitro* experiments, further enriched by valuable conclusions obtained by others.[18,20–22] These studies were possible given the availability of high-purity recombinant human EGF manufactured at the Genetic Engineering and Biotechnology Center, Havana, Cuba.[23]

A nationwide clinical development program started in Cuba in 2001,[24] which ultimately included pharmacovigilance studies that confirmed the safety and efficacy of EGF delivery by intralesional infiltration. Almost 20 years of clinical practice have shown a 75% probability of complete granulation response, 61% of complete healing; 16% absolute and 71% relative reduction of amputation risk. Furthermore, recurrences were reported as an exceptional event upon a 12-month follow-up period.[25,26]

Despite years of international research, GF prescription for healing problem wounds remains controversial.[27] Although GF therapy is not yet included in International Working Group on the Diabetic Foot (IWGDF) recommendations, (www.iwgdfguidelines.org), EGF intralesional infiltration has nevertheless been internationally validated and recommended as adjuvant therapy for high-grade DFU, considering its benefits in resuming a normal healing process with reduction of amputation rates.[28–32]

This article summarizes the major molecular, cellular and biochemical findings supporting the clinical efficacy of EGF intralesional infiltration for DFU in the commercially available pharmaceutical formulation Heberprot-P. The drug is included in the Cuban national medication registry since June 2008 and has offered the only pharmacological alternative for the treatment of high-grade, complex diabetic ulcers.

EGF INFILTRATIVE INTERVENTION: IMPACT ON GENE EXPRESSION, TISSUE REPAIR AND CIRCULATING BIOMARKERS

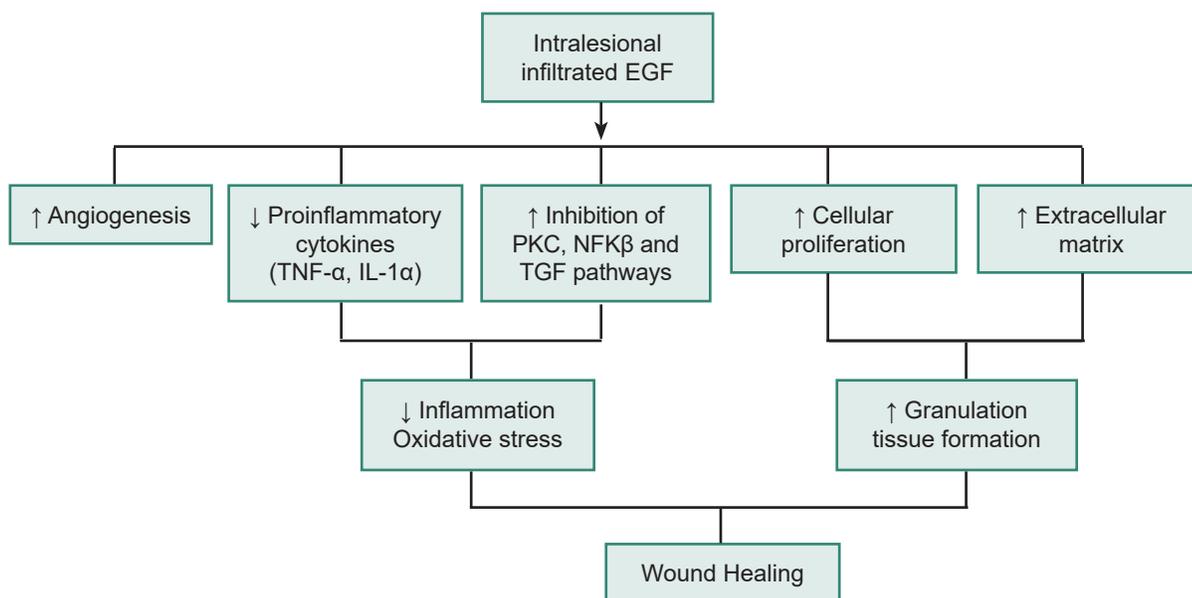
Gene transcriptional response in granulation cells Although not found on hematopoietic cells, the EGF receptor is widely expressed in mammals and has been implicated in the expression of a myriad of genes during various stages of embryonic development of both epithelial and mesenchymal tissues.[33–37] Accordingly, EGF administration modifies the course of the cutaneous healing process by promoting migration and proliferation of both epithelial and mesenchymal skin cells where its receptor expression is enhanced.[15,38]

Camacho and colleagues[39] described changes in the expression of several genes encoding proteins involved in wound healing. The investigation was part of a clinical trial (IG/FCEI/PD/0911 in the Cuban Public Registry of Clinical Trials, <http://registroclinico.sld.cu/en/trials/RPCEC00000117-En>) and included paired granulation tissue biopsies from 29 patients meeting the following criteria: Wagner grade 3–4 lesions, clinical responders with complete re-epithelialization at the end of treatment, and high-quality RNA samples for differential expression studies. Of the 29 patients, 10 were randomly chosen as the minimum sample size able to detect a 1.5-fold RNA expression difference relative to the basal constitutive value (paired control) just before treatment (biopsy identified as T0). EGF (75 µg dose) was infiltrated intralesionally 3 times/week. A second biopsy (T1) was collected at the end of treatment week 2. Paired comparisons between T1 and T0 biopsies revealed a significant increase in cell proliferation modulators Cyclin-Dependent Kinase 4 (CDK4), P21 and TP53, in collagen synthesis and Extracellular Matrix remodeling gene products (Collagen type I, alpha 1 chain, Matrix Metalloproteinase 2 and TIMP2), and a concomitant reduction of some inflammation markers, including NFκB, Tumor-Necrosis Factor-alpha (TNF-α) and interleukin 1 alpha (IL-1α). Local cell proliferation, synthesis and secretion of wound matrix proteins, and downregulation of inflammation mediators such as TNF-α, are critical events for physiological healing.[10]

The authors concluded that the observed increase in P21 and TP53 is a cellular feedback mechanism limiting the intensity and duration of the EGF-induced proliferative signal. A molecular action mechanism was postulated from these findings (Figure 1).[39] Irrespective of the differences between samples collected from diabetic ulcers and neonatal keratinocytes cultured from healthy donors, the data from Blumenberg[40] on EGF effects on transcriptomes validate the induction of keratinocyte proliferation and motility associated with feedback mechanisms controlling EGF effects. In concurrence with Blumenberg's study, our data indicate that EGF effects are modular and multifaceted rather than all-or-nothing events. This is the first clinical study addressing the transcriptomic effect of EGF in a model of human diabetic ischemic ulcers.

EGF intervention to ameliorate the histological aspect of neuropathic and ischemic lesions Ischemic diabetic lesions are characterized by a hyaline aspect matrix and paucity of functional neovessels, as well as angiogenesis defects (Figure 2A). In sharp contrast, neuropathic lesions appear to granulate earlier, exhibiting a poor collagen matrix deposition, an image similar to a spider web of thin collagen fibers, which react weakly to Mallory

Figure 1: Changes in gene expression upon EGF infiltrations



Patients who responded to EGF intralesional infiltrations exhibited gene expression changes that assisted in resuming and sustaining wound healing, and a reversion of the chronic phenotype. The clinical response was mediated by an elevation of angiogenesis, cell proliferation, and matrix fibrogenic ingredients coding genes and a reduction in inflammation related genes, stimulating the tissue repair process. (Reproduced from [39] under CC4 license).

staining. Additionally, reduced density of extracellular matrix-producing cells has also been noted (Figure 2B). As opposed to ischemic ulcers, small capillaries are observed, often surrounded by peripheral fibrin cuffs, suggesting hyperpermeability.[41] Following 9 to 12 EGF infiltration sessions (third/fourth week of treatment), granulation tissue of both ischemic and neuropathic origins exhibited substantial clinical amendment, with a consistent increase of functional small-caliber vessels across the ischemic tissue (Figure 2C). The granulation tissue matrix of neuropathic lesions becomes densely indurated by thicker and compact collagen bundles accompanied by increased productive cellularity (Figure 2D). In both scenarios, the inflammatory infiltrate appears substantially reduced.[42] Therefore, EGF positively impacts the local microenvironment of both pathogenic classifications of DFU.

It is of relevant therapeutic significance that EGF infiltration changes the biology of ischemic ulcers. Given its angiogenic effect, in addition to creating *de novo* vessels,[43] EGF acts as a cytoprotective agent, enhancing cell and tissue survival in otherwise lethal episodes like ischemia/reperfusion and hypoxia. [21,44–47] This drives a hypothesis that agonistic stimulation of EGF receptor (EGFR) triggers survival signals that may depend on translational modifications, with tyrosine phosphorylation being the most common.[48,49]

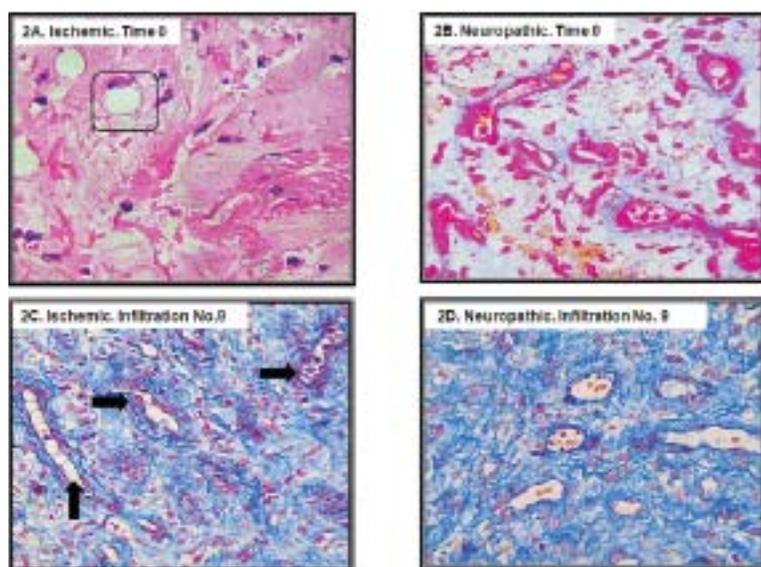
Zhang and colleagues recently conducted a thorough characterization of molecular mechanisms underlying EGF's effect on diabetic wounds.[50] The authors implemented a full-thickness wound model in type-2 diabetic rabbits. The EGF-induced effect after one month of daily dermal delivery is reminiscent of the microscopic outcomes identified in patient biopsies: (1) increased granulation tissue with elevation of clustered fibroblasts, (2) abundant extracellular matrix, indurated by dense and ordered collagen bundles, (3) increased active vessels and (4) attenuation

of the inflammatory infiltrate. Interestingly, and aside from the histological findings, EGF treatment induced the transcription of its own gene with an increased EGF-mRNA accumulation.[50]

The EGF-induced modifications in problem wounds with different pathogenic ingredients suggest that locally-infiltrated EGF stimulates both mesenchymal and ectodermal cell responses, expressed by proliferation, migration, secretion, angiogenesis and survival. Accordingly, EGF infiltration is a DFU-specific therapy that may synchronize local cellular behaviors, thus reversing the chronicity phenotype.[51]

EGFR intracellular trafficking: EGF induces its own receptor expression in granulation tissue fibroblasts By means of immunoelectron microscopy of ulcer fibroblasts, Falcón-Cama[52] characterized EGFR time-point kinetic intracellular trafficking. EGF locally infiltrated into Wagner's 3 and 4 neuropathic ulcers translated into:

- (a) Significant increase of EGFR membrane expression 15 minutes after EGF infiltration as compared to T0;
- (b) Immediate EGFR endocytosis;
- (c) Translocation and biodistribution to different cytoplasmic organelles from 15 minutes to 24 hours after infiltration;
- (d) Nuclear translocation of EGFR and its binding to DNA, which appeared to last from minute 45 to 24 hours after treatment;
- (e) Concomitant activation of proliferating cell nuclear antigen (PCNA) gene transcription which appeared to last for about 24 hours after treatment;
- (f) Substantial EGFR accumulation in mitochondria, which peaked between hours 6 and 24 after infiltration; and
- (g) EGFR accumulation bound to extracellular matrix-secreted collagen fibers, along with abundant appearance of exosomal extracellular vesicles.

Figure 2: Wound matrix transformation by locally infiltrated EGF

Source: Jorge Berlanga-Acosta. Archives of the laboratory of experimental pathology. Center for Genetic Engineering and Biotechnology. Havana. Cuba.

Histological images of granulation tissue biopsies collected prior to the initial EGF infiltrative intervention and after the 9th intervention. Images are representative of the two major etiopathogenic forms of diabetic lower extremity disease: ischemic and neuropathic. 2A: Representative of a clean, ischemic diabetic granulation tissue bed before the first local EGF infiltration. Granulation tissue exhibits a “hardened” hyaline matrix with a general scarceness of functional neovessels. Nonfunctional capillaries are seen since early stages (enclosed). 2B: Representative of an early granulation tissue matrix, collected from a neuropathic lesion exhibiting poor extracellular matrix accumulation, scarce collagen deposition and a limited productive cellularity before EGF treatment. These are all histological hallmarks of protracted, poor healing of neuropathic wounds. 2C: Image showing the transformation of the wound matrix composition, with substantial angiogenic response induced by the local EGF infiltration with patent large vessels (arrows) across the microscopic field of an ischemic lesion. 2D: Accumulation and organization of a substantial amount of new extracellular matrix material is conspicuous. There are functional vessels across the wound area after EGF infiltration. Biopsies from 2C and 2D were collected upon the 9th EGF infiltration session. Figure 2 conclusively denotes that EGF infiltration may positively impact on the healing biology of both ischemic and neuropathic wounds. All samples are 5 μ m sections and Mallory stained X 40. Original unpublished images.

Most importantly, ultrastructural characterization of the fibroblast-like cells 24 hours after EGF exposure revealed significant changes, suggesting organelle repair as compared to T0.[52] Figures 3A and 3B reflect how EGF resulted in effective treatment for control of the rough endoplasmic reticulum (RER) dilation. At 24 hours after EGF intervention, RER tubules and cisternae appeared far less dilated as compared to T0. Similarly, mitochondria were also a target of EGF effect (Figures 3C and 3D). The latter show a far less dilated organelle in which matrix cristae are observed. The presence of two adjacent organelles may suggest an active process of mitochondrial fission.

Although prior evidence had indicated that EGF can induce the expression of its own receptor,[53] current research provides the first evidence concerning EGFR transcriptional induction, internalization and intracellular trafficking kinetics in response to a therapeutic intervention with an EGFR ligand in a clinical setting.[52] This intense EGF-induced cellular response is

consistent with its broad biological activity. *In vitro* models have documented that EGFR activation upon EGF binding induces the phosphorylation of 2244 proteins at 6600 catalytic sites,[54] the expression of 3172 genes and 596 proteins which are significantly altered in epithelial cells.[55]

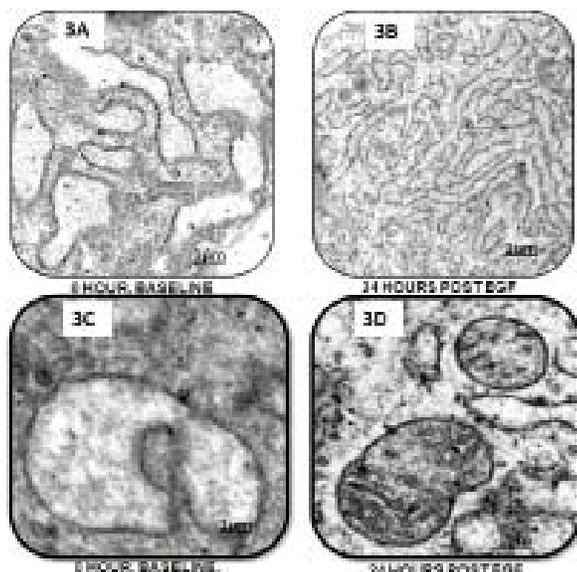
In vitro evidence shows that full length EGFR translocates to the cell nucleus after ligand binding,[29,56] where several functions are performed.[57] First, EGFR operates as a co-transcription factor regulating the expression of cyclin D1, a proximal driver of cell proliferation.[58,59] EGFR interacts with DNA-dependent protein kinase, leading to the repair of DNA double-strand breaks.[60] Furthermore, nuclear EGFR phosphorylates chromatin-bound PCNA, thus increasing its stability and eventually enhancing cell proliferation.[61] Intracellular PCNA is related to anti-apoptotic activity, which may act as one of the multiple mechanisms mediating EGF pro-survival effects in a variety of cell populations.[62] Supporting this notion is the identification of the mitochondrion as another EGFR translocation compartment. Mitochondria are the hub of cellular metabolism, survival and death; they modulate not only apoptosis, but also autophagy. EGFR translocates to mitochondria where it phosphorylates cytochrome c oxidase subunit II, resulting in decreased cyclooxygenase activity, thus eventually preventing apoptosis.[63] EGF is also involved in mitochondrial fission,[64] fusion[65] and ultimately, in control of cellular response to stress, where it plays a pro-survival role.[37]

EGF infiltration sequentially activates EGFR in dormant ulcers, fibroblasts, and in its intracellular trafficking, promotes fibroblast proliferation, migration and survival.[59,66,67] The fact that EGF may reduce RER dilation, ameliorate mitochondrial damages, and stimulate proliferation of fibroblasts in DFU drives speculation that EGFR stimulation may mitigate senescence-related traits. Although this hypothesis has yet to be experimentally verified, evidence from our group and others support this possibility.[68,69]

Locally infiltrated EGF reduces diabetic dyshomeostasis

Oxidative stress not only promotes the onset of diabetes but also exacerbates the disease and its complications. Brownlee[70] proposed oxidative stress as a major operator in the pathophysiology of diabetes and its complications.[71] Hyperglycemia has been invoked to promote oxidative stress through free radical generation and ensuing deterioration of antioxidant defense systems.[71] Chronic wounds are considered a pro-oxidative organ superimposed upon a preexisting dysmetabolic host (the diabetic patient).[18,72] In a small cohort of diabetic neuropathic ulcers, García-Ojalvo and colleagues addressed whether an improved systemic reduction-oxidation (redox) balance is associated with healing response in patients infiltrated with EGF.[72] The rationale for the above study was supported by previous experiments demonstrating that EGF reduced levels of oxidative stress biomarkers, ultimately attenuating cytotoxic damage.[73–76] After 3 to 4 weeks of EGF treatment (9 to 12 infiltration sessions), 4 circulating biomarkers (erythrocyte sedimentation rate, IL-6, soluble FAS and pentosidine) were significantly reduced, while antioxidant parameters increased.

Figure 3: Impact of EGF treatment on ulcer-fibroblasts organelles repair



3A. Electron microscopy image of the rough endoplasmic reticulum (RER) at time 0. Sample collected before EGF infiltration. The dilation and distortion of the RER cisternae is evident.

3B. Effect of EGF intervention after reverting cisternae dilation and ameliorating the preexisting distortion after 24 hours of treatment.

3C. Electron microscopy image of a ballooned mitochondria showing its notable dilation and disappearance of internal cristae before the first EGF intervention. A fine granulation is dispersed within the mitochondrial matrix.

3D. Twenty four hours after the EGF treatment, it is noticeable the substantial transformation of the mitochondria. Mitochondria are far less ballooned with matrix content and exhibiting some cristae.

Source: Falcón-Cama V, et al.[52]

Notably, at least 50% of patients showed a favorable response for each evaluated marker. EGF’s molecular effect was simultaneously associated with a positive clinical response in terms of granulation, contraction and re-epithelialization. This was the first clinical validation of *in vitro* and animal data indicating that EGF’s cytoprotective effect is at least partially mediated by correcting the redox balance.[18,73,76–78]

A more recent study by García-Ojalvo and colleagues[79] confirmed previous observations concerning the systemic impact of locally-infiltrated EGF on reestablishment of a physiological redox balance. Moreover, the new data indicates that EGF’s effect extends to reduction of diabetic endovascular pro-inflammatory markers. Within three weeks of treatment, patients showed significant reduction of: erythrocyte sedimentation rate, IL-6 circulating levels, soluble FAS and the glycoxidation product pentosidine, as well as a significant reduction of oxidative and nitrosilative stress markers (Table 1).

The fact that EGF infiltration reduced circulating levels of IL-6 is highly significant in diabetes. IL-6 is perhaps the best-reputed bona fide cytokine, pathogenically involved in the primary event of insulin resistance, in the morbidity caused by multiorgan complications, and in the onset of a poor healing

Table 1. Systemic effects of locally infiltrated EGF in patients with diabetic foot ulcers

System	Systemic effect
Redox balance	↓ Total oxidative capacity ↓ MDA ↓ AOPP ↓ Total organoperoxides ↓ Nitrite/Nitrate ratio ↑ Total antioxidant capacity ↑ SH groups
Anti-inflammatory mechanism	↓ Erythro sedimentation ↓ C Reactive Protein ↓ IL-6 ↓ MIP1-α ↓ sFAS
AGE pathway	↓ Pentosidine ↑ sRAGE
Extracellular matrix	↓ MMP-9 ↓ TIMP-1

Diabetic patients with lower limb wounds were treated with intralesional infiltrations of EGF (75 µg), three times per week during 3-4 weeks [72, 79]. *Systemic antioxidant effects:* decrease of circulating levels of total oxidative capacity, malondialdehyde (MDA), advanced oxidation protein products (AOPP), total organoperoxides, and nitrite/nitrate ratio; increase of total antioxidant capacity and sulfhydryl (SH) groups. *Anti-inflammatory effects:* reduction in erythro sedimentation, C reactive protein, interleukin-6 (IL-6), macrophage inflammatory protein 1-alpha (MIP1-a), and soluble FAS (sFAS). *Systemic attenuation of the advanced glycation end (AGE) pathway:* decrease in pentosidine, increase in the soluble receptor for AGE (RAGE) circulating levels. *Extracellular matrix elements modulated at the systemic level:* reduction of matrix metalloprotease 9 (MMP-9) and tissue inhibitor of MMP 1 (TIMP-1).

response. *In vitro* studies by our group reproducibly show that DFU-derived fibroblasts exposed to lipopolysaccharides exhibit a highly significant increase of IL-6, which returned to basal levels, similar to those of untreated cells, after adding EGF (Yssel Mendoza-Marí, manuscript in preparation, April 2020). Simply said, dampening IL-6 circulating levels could contribute to restoration of metabolic homeostasis in diabetic patients.[80–82] Aside from IL-6, EGF intervention also reduced serum levels of soluble FAS and the chemokine Macrophage Inflammatory Protein (MIP1-α). Although further studies are clearly needed, collectively this evidence suggests that EGF may have assisted in reduction of insulin resistance, attenuation of endovascular inflammation and reduction of apoptotic rates; thus attenuating premature diabetic organ senescence.[83] Conclusively, EGF treatment exhibits broad systemic pharmacodynamics that go beyond the reestablishment of redox balance.

CONCLUSIONS

The discovery of growth factors initiated a new era in wound healing biology and held out hope for recalcitrant wound treatment. EGF, the prototypic and founding member of the EGFR ligand family, led to use of topical administration of growth factors for wound healing. Evidence suggests its role in tissue repair was already apparent in the early 1960s in Stanley Cohen’s work subjecting rabbits to corneal burns followed by treatment with homemade natural EGF eye drops.[11] Despite the initial promise and years of research, growth factors have not garnered a definitive acceptance in the

clinical toolbox for wound management. Lessons learned over the past decades reinforce the importance of growth factor stability, which allows for sufficient residence time within the wound matrix to achieve the expected pharmacodynamic response. Cleverly engineered formulations are emerging that may yet vindicate growth factors' intrinsic biological potential. The intralesional infiltrative procedure, despite its simplicity, safeguards EGF bioactivity for prolonged periods, thus emphasizing the concept that spatio-temporal control of EGF availability is fundamental for clinical success.

This pioneer growth factor has proved to modify gene and protein expression, phosphorylate catalytic sites, modulate organelle homeostasis and, at an organismal level, reverse changes in inflammatory markers involved in progression of diabetic complications. The latter may represent the systemic effects of EGF, accompanied by amelioration of the wound chronicity

phenotype. Again, wound-host bidirectional communication is underscored.

A research challenge is elucidation of the molecular foundations that may explain the unusual EGF trait of helping to prevent ulcer recurrence over the long term.[25,26] We hypothesize that infiltrated EGF exerts a local 'rejuvenating' effect by replacing senescent cells or by dismantling or reversing the fibroblasts' epigenetic senescence program. Thus, EGF may potentially act as a senolytic agent for diabetic wounds, promoting neodermal resilience and tolerance to physical and mechanical stress.

In conclusion, two decades of clinical and basic research on EGF therapy for problem wounds have shed light on the utility of growth factors with broad pharmacological potential in regenerative medicine; the time has come to focus on how, when and where to deliver their messages to their targets. 

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Submitted: February 17, 2020

Approved for publication: June 3, 2020

Disclosures: The authors are employed by CIGB, Havana, Cuba, which owns the patent for the use of EGF local infiltration to reduce the risk of lower-limb amputation in diabetic populations. Jorge Berlanga-Acosta is the lead author on the patent, also authored by Gerardo Guillén-Nieto and Luis Herrera-Martínez.