Human Epidermal Growth Factor Enhances Healing of Diabetic Foot Ulcers

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OBJECTIVE — To study the healing effect of recombinant human epidermal growth factor (hEGF) on diabetic foot ulcers.

RESEARCH DESIGN AND METHODS — A total of 127 consecutive patients were screened and 61 diabetic subjects were recruited into this double-blind randomized controlled study. Predetermined criteria were used for diagnosis and classification of the diabetic wound. The patients were randomized into three groups. All patients attended our Diabetes Ambulatory Care Center every other week for joint consultation with the diabetologist and the podiatrist. Group 1 (control) was treated with Actovegin 5% cream (Actovegin), group 2 with Actovegin plus 0.02% (wt/wt) hEGF, and group 3 with Actovegin plus 0.04% (wt/wt) hEGF. The study end point was the complete closure of the wound. Failure to heal was arbitrarily defined as incomplete healing after 12 weeks.

RESULTS — Final data were obtained from 61 patients randomly assigned into three groups. The mean ages of the patients, wound sizes, wound duration, metabolic measurements, and comorbidities were comparable within groups, except that group 3 had more female patients. Mean follow-up for the patients was 24 weeks. Data were cutoff at 12 weeks, and results were analyzed by intention to treat. After 12 weeks, in group 1 (control) eight patients had complete healing, two patients underwent toe amputation, and nine had nonhealing ulcers. In group 2 (0.02% [wt/wt] hEGF) 12 patients experienced wound healing, 2 had toe amputations, and 7 had nonhealing ulcers. Some 20 of 21 patients in group 3 (0.04% [wt/wt] hEGF) showed complete wound healing. Healing rates were 42.10, 57.14, and 95% for the control, 0.02% (wt/wt) hEGF, and 0.04% (wt/wt) hEGF groups, respectively. Kaplan-Meier survival analysis suggested that application of cream with 0.04% (wt/wt) hEGF caused more ulcers to heal by 12 weeks and increased the rate of healing compared with the other treatments (log-rank test, \( P = 0.0003 \)).

CONCLUSIONS — Our data support the contention that application of hEGF-containing cream, in addition to good foot care from a multidisciplinary team, significantly enhances diabetic foot ulcer wound healing and reduces the healing time.

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Diabetic foot ulcer is a major complication of diabetes. People with diabetes show a 5- to 50-fold higher risk of nontraumatic amputation compared with individuals who do not suffer from diabetes (1). Between 1996 and 1998, diabetic patients accounted for 47% of all the lower-limb amputations performed in our hospital (M.W.T., K.M.L., G.K., unpublished data), comparable to published data (2). The major risk factor leading to diabetic foot ulcer is poor diabetes control, which results in neuropathic and vascular changes. In general, diabetic foot ulcers are difficult to heal and frequently lead to amputation if complicated by infection and gangrene. Several new treatment modalities, such as exposure to an oxygen chamber, the use of platelet-derived growth factor (PDGF), and the application of various local dressings, have produced various degrees of success. Currently, there are only a few interventions in which randomized control trials have documented efficacy. Among these is the use of PDGF, and the Food and Drug Administration has approved Becaplermin gel as an adjuvant therapy in diabetes foot ulcer management (3).

Physiologically, wound healing can be divided into three stages: inflammation, proliferation, and remodeling (4). Wound repair is characterized by a series of complex cellular and molecular events. Numerous growth factors are involved in these processes and act by stimulating chemotaxis, cellular proliferation, extracellular matrix formation, and angiogenesis, with contraction and reestablishment of cellular integrity. Both in vivo and in vitro data have demonstrated the efficacy of growth factors in enhancing wound healing. The most studied growth factors are PDGF, fibroblast growth factor (FGF), transforming growth factor-β1, and epidermal growth factor (EGF) (5–7). Early experimental studies have shown the potential of EGF in promoting wound healing. EGF clearly stimulates epidermal repair in animal excisional and thermal injury models and may also stimulate dermal repair (8). Namney (9), using a pig partial thickness wound model, reported...
a dose-dependent increase in the thickness of granulation tissue and epithelialization with EGF. By means of Northern hybridization specific for the content of mRNA of type I and III procollagens, Laato et al. (10) confirmed that EGF is a potent dose-dependent mitogen for the granulation fibroblast. However, early clinical studies provided mixed data on the utility of exogenous growth factors in chronic wound healing. In a small study published in 1993, Lev-Ran and Hwang (11) reported an elevation of PDGF and EGF in the plasma of diabetic subjects compared with control subjects. However, Cooper et al. (12) showed that a number of growth factors were markedly reduced in wound fluid from chronic wounds compared with acute wounds. Bennett and Schultz (13) postulated increased destruction or inhibition of growth factors by elevated levels of proinflammatory cytokines and metalloproteinase following repeated trauma and infection. In the current study, we postulated there was a relative deficiency of growth factors in chronic wounds such as diabetic foot ulcers and aimed to determine whether local application of a high concentration of human EGF (hEGF) might be effective in promoting wound healing of diabetic foot ulcers.

**RESEARCH DESIGN AND METHODS** — A randomized double-blind controlled trial was used to study the efficacy of hEGF in promoting healing of diabetic foot ulcers. Between September 2000 and August 2002, 127 patients from our Diabetes Ambulatory Care center were screened. Predetermined criteria used for patient selection were 1) ulcer with grade I or II, as defined by the Wagner Classification (grade I, superficial ulcer; grade II, deep ulcer to tendon, capsule, or bone; grade III, deep ulcer with abscess, osteomyelitis, or joint sepsis; grade IV, localized gangrene of forefoot or heel; and grade V, gangrene of entire foot) (14), 2) ulcer located below the ankle, and 3) ulcer with adequate perfusion, as indicated by an ankle-brachial index (ABI) ≥0.7. Patients were excluded if they had very poor sugar control (HbA1c ≥12%) or had ulcers with severity equal to or greater than grade III. The patients were seen in the designated multidisciplinary diabetes foot clinic in our diabetes center. We accepted referral of new patients from the medical, orthopedic, and podiatrist clinics in our hospital and other hospitals in our region. Diabetes control was maximized by the diabetologist. Comprehensive foot assessment was carried out by the podistrist, including determination of ABI, vibration perception threshold, and 10-g monofilament and pin prick tests. Standard wound care consisted of debridement of necrotic tissue and reduction of callus. Wound parameters such as exudates, signs of infection, combined with granulation of tissues, and eschar were documented on each visit, and complete healing was defined as full epithelialization of the wound with absence of discharge. Wound swabs were taken if infection was suspected, and antibiotics were prescribed based on clinical judgment or on positive wound bacterial cultures.

After the initial consultation, patients were then evaluated 2 weeks later with a view to their inclusion in the trial. In the second consultation, we excluded patients whose ulcers healed >25% with conventional foot ulcer care. Informed consent was obtained, and randomization was performed by drawing envelopes. Patients were randomly assigned into three groups: group 1 (treated with Actovegin 5% cream only), Actovegin is a protein free calf blood extract manufactured by NYCOMED Austria [Linz Austria]. It is claimed to improve the utilization of oxygen and to promote the uptake of nutrient media into the cell. It is indicated for varicose ulcer and for any ulcers with a
smeary coat.) group 2 (treated with Actovegin plus 0.02% [wt/wt] hEGF), and group 3 (treated with Actovegin plus 0.04% [wt/wt] hEGF). The study protocol and consent form were approved by the ethics committee of the United Christian Hospital.

Throughout the study, ulcerated areas were overlaid with gridded paper for size reference in photography. The digital pictures were processed with Adobe Photoshop version 5.0, and the ulcerated areas were compared with reference areas. The sizes of the ulcerated areas were then calculated using Matrox Inspector version 2.1 (Matrox Electronic System) (Fig. 1).

The cream under study was applied locally and covered with sterile gauze. Patients were instructed to continue with the normal daily saline dressing, combined with local application of the cream, either in the government outpatient clinic or with the aid of the community nurse who provided home care for patients with ambulatory problems. The pharmacy department of our hospital prepared the creams, and both patients and physicians were blind to the hEGF concentrations.

**Wound measurement and validation**

A foot model was used to create 12 superficial wounds over the big toe, second toe, dorsum, sole, and heel regions to compare data from measurements by digital imaging and tracing. The Spearman range correlation coefficient was 0.858, comparable to that in an earlier study (15).

**Statistical analysis**

Analysis was based on intention to treat. A t test for continuous data and a Kruskal-Wallis test and χ² test for categorical data were used for comparison of baseline characteristics among the three groups. A Kaplan-Meier plot was used to examine the time to complete healing in each group. Data were censored at 12 weeks. A log-rank test was used to compare the time to complete healing among the different groups.

**RESULTS** — A total of 127 patients were screened between September 2000 and August 2002. Sixty-six patients were excluded for various reasons: 19 had ABI <0.7, 6 had high-grade foot ulcers, 9 refused to participate in the study, and 2 had ulcers above the malleoli. Some 30 patients were not included in the study because, under conventional treatment, their ulcers healed by >25% by the second visit. A flow chart of the experimental protocol is shown (Fig. 2).

![Figure 2—Diagram showing the flow of participants through the randomized control study.](image)
A total of 61 patients, 57 with type 2 and 4 with type 1 diabetes, were enrolled in the study and randomly assigned into the three groups defined above. The endpoint of treatment was defined as complete closure of the wound, whereas failure to heal was defined as incomplete closure after 12 weeks of treatment.

The baseline profile of the three groups was similar in regard to age, duration of diabetes, HbA1c, BMI, wound size, duration of ulcer, distribution of ulcer, vibration threshold, monofilament test results, and diabetic comorbidities, as shown in Table 1. The use of oral hypoglycemic agents and insulin was comparable in all groups. There was a difference in sex distribution ($P = 0.03$) between group 2 and group 3. However, there was no difference in median healing time between the two sexes.

Wound healing, by the Kaplan-Meier survival plot, is shown in Fig. 3. Data were analyzed at a 12-week cutoff. As indicated, 20 of 21 patients (95.3%) in the 0.04% (wt/wt) hEGF group achieved complete healing, which is a significantly higher value than those obtained for the 0.02% (wt/wt) hEGF and placebo groups in post hoc analysis. Patients in the 0.04% (wt/wt) hEGF group also healed more quickly than those in the other groups. The median time to complete healing in the 0.04% (wt/wt) hEGF group was 6 weeks (CI 4.22–7.78) ($P = 0.0003$) (Fig. 2). Healing rates were 42.10% and 57.14% for the control and the 0.02% (wt/wt) hEGF groups, respectively. There was no significant difference in healing time between the 0.02% (wt/wt) hEGF and control groups. As there was a sex difference between the 0.02 and 0.04% group, we therefore also analyzed the healing times of the two sexes. At 12 weeks, 21 female and 18 male patients had healed ulcers. The median healing times were 8 and 12 weeks, with a $P$ value of 0.599, for women and men, respectively. The mean follow-up time was 24 weeks. As depicted in Fig. 2, more ulcers healed with time: seven more patients from the placebo group and five more patients from the 0.02% (wt/wt) hEGF group had healed ulcers. The fact that most of the ulcers from the 0.04% (wt/wt) hEGF groups healed with a median healing time of 6 weeks supports the efficacy of 0.04% (wt/wt) hEGF in enhancing wound healing. As the extend of healing between the placebo and the 0.02% (wt/wt) hEGF group was of no statistical significance, despite a mean follow-up study of 24 weeks, there may exist a threshold for hEGF action. However, it must be pointed out that the original design was a randomized double-blind 12-week study, and after 12 weeks, the study was open and further conclusions therefore must be cautiously drawn.

### Table 1—Baseline characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>Group 1: control</th>
<th>Group 2: 0.02% (wt/wt) hEGF</th>
<th>Group 3: 0.04% (wt/wt) hEGF</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>19</td>
<td>21</td>
<td>21</td>
<td>NS</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>10/9</td>
<td>13/8</td>
<td>6/15</td>
<td>0.03*</td>
</tr>
<tr>
<td>Age (years)</td>
<td>64.37 ± 11.67</td>
<td>68.76 ± 10.45</td>
<td>62.24 ± 13.68</td>
<td>NS</td>
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<tr>
<td>ABI</td>
<td>0.99 ± 0.16</td>
<td>1.03 ± 0.22</td>
<td>1.05 ± 0.19</td>
<td>NS</td>
</tr>
<tr>
<td>Vibration threshold</td>
<td>&lt;25</td>
<td>6</td>
<td>8</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>&gt;25</td>
<td>13</td>
<td>13</td>
<td>NS</td>
</tr>
<tr>
<td>10-g monofilament test</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>13</td>
<td>11</td>
<td>11</td>
<td>NS</td>
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<tr>
<td>Abnormal</td>
<td>6</td>
<td>10</td>
<td>10</td>
<td>NS</td>
</tr>
<tr>
<td>Ulcer area (cm$^2$)</td>
<td>3.48 ± 0.82</td>
<td>2.78 ± 0.82</td>
<td>3.40 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>Site (distribution of ulcer)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Sole</td>
<td>2</td>
<td></td>
<td></td>
<td>NS</td>
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<td>Toe</td>
<td>11</td>
<td>11</td>
<td>10</td>
<td>NS</td>
</tr>
<tr>
<td>Ankle</td>
<td>2</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Others</td>
<td>4</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Duration of ulcer (weeks)</td>
<td>12.00 ± 15.47</td>
<td>8.24 ± 5.55</td>
<td>11.48 ± 14.68</td>
<td>NS</td>
</tr>
<tr>
<td>History of diabetes (years)</td>
<td>10.11 ± 8.29</td>
<td>9.85 ± 7.79</td>
<td>9.05 ± 6.19</td>
<td>NS</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.97 ± 1.81</td>
<td>8.69 ± 1.99</td>
<td>8.5 ± 1.34</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>25.69 ± 5.21</td>
<td>23.33 ± 3.11</td>
<td>23.83 ± 3.17</td>
<td>NS</td>
</tr>
<tr>
<td>Use of insulin</td>
<td>9</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Use of oral OHA</td>
<td>9</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Serum creatinine &gt;2 mg</td>
<td>3</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
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<td></td>
<td>NS</td>
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<tr>
<td>(proteinuria &gt;300 mg/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Diabetic retinopathy</td>
<td>9</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Comorbidities†</td>
<td>17</td>
<td></td>
<td></td>
<td>NS</td>
</tr>
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</table>

Data are means ± SE. *Sex distribution between groups 2 and 3; †including hypertension, coronary heart disease, and hyperlipidemia. OHA, oral hypoglycemic agent; NS, not significant.
**CONCLUSIONS** — The main conclusion of this study is that 20 of 21 diabetic foot ulcers healed with daily application of 0.04% (wt/wt) hEGF for 12 weeks under the management of a multidisciplinary team. The difference in healing rate compared with that seen without the hEGF cream was statistically significant, and the curve from control diverged at the beginning of the fourth week. The healing with 0.04% (wt/wt) hEGF was accompanied by a significant reduction in median healing time.

A Medline search using the key phrases “epidermal growth factor” and “clinical study” did not yield a placebo control study with EGF for the management of diabetic foot ulcers. To our knowledge, this is the first study to demonstrate a significant and positive effect of hEGF in the healing of diabetic foot ulcers in a randomized double-blind controlled trial. However, our data suggest that hEGF at 0.02% (wt/wt) in cream does not offer significant benefits over conventional foot care practice (Fig. 2). The dose sensitivity observed in our study is in accordance with a requirement for the sustained presence of EGF in the promotion of wound repair and reports of a dose-dependent effect of EGF on the formation of granulation tissue (7,16). Also, in a recent study on the effects of PDGF on neuropathic diabetic foot ulcers, a statistically significant improvement in ulcer healing was shown with a cream with 100 µg/g in PDGF but not when the cream contained 30 µg/g of the peptide (17). We suggest that the threshold effect for growth factor action seen in these studies may reflect the presence of growth factor inhibitors, or proteases, in the wound microenvironment, or that higher levels of the factors recruit other cytokines required to promote wound healing.

The reported diabetic ulcer healing rates ranged from 33 to 57.5% at a cutoff time of 12–20 weeks, when the growth factors PDGF or FGF were used in controlled trials (17,18). In a small pilot randomized double-blind study reported by Richard et al. (18), nine chronic neuropathic plantar foot ulcers (Wegner grade I–III) were treated with topical FGF for a total of 18 weeks. The healing rates for the treated and placebo group were 33 and 63%, respectively, with no statistically significant difference. In a large multicenter study, PDGF, at a concentration of 100 µg/g in cream, gave a better healing rate (50%) than the placebo group (35%) in a 20-week study (P = 0.007) (17). In an open-label study, Embil et al. (19) reported a complete healing rate of 57.5%, with a daily local application of PDGF cream, in chronic diabetic foot ulcer patients. The wide healing ranges shown above probably reflect differences in study design, duration of study, and patient and ulcer characteristics. It is difficult to compare these studies with our work. Our study included all patients with ulcers from the malleoli to toe apices. All patients in our study had an ABI >0.7, and the vibration threshold varied between 11 and 55 V. We excluded those with wound healing of >25% during the run-in period and thus deliberately selected relatively difficult cases. On the whole, the pretrial durations of ulcers in our study (<20 weeks), were shorter than those in the reported cases.

We suggest that a possible explanation for the successful use of hEGF in our study is due to the fact that we used a hEGF concentration higher than that used in earlier studies. In general, the dose of growth factors employed topically in previous studies was 1–100 µg/g (20). Another explanation may lie in differences in the sources and hence the potencies of the hEGF samples. The average time required for healing of ulcers in our work is shorter than that seen in other reported studies (17,20,21). It should be noted, however, that the average BMI of our patients was lower than those patients in other studies, and thus the average pressure on the ulcers was less. Most of our patients were ambulatory. No off-loading or total contact casting was prescribed in this study, but bespoke insoles were prescribed when appropriate.

It must be stressed that meticulous wound care such as debridement, callus reduction, and control of infection also played a vital role in promoting wound healing. Debridement enabled removal of necrotic tissue, maintained drainage, and allowed better surface contact with hEGF. Callus reduction also helped to reduce excessive pressure on the wound. Antibiotics were prescribed upon clinical suspicion of infection or positive culture results. However, even with all these measures, two patients from each of the placebo and the 0.02% EGF group had either toe or ray amputation as a result of chronic osteomyelitis or gangrene, which may partly reflect the limitation of the Wagner classification of foot ulcer.

There are several limitations to the present study. First, the small sample size limits significance. Second, the diabetic ulcers were heterogeneous in nature. Although ABI in our patients is >0.7, due to
the high prevalence of medial calcinosis in diabetic vessel, this value may not be reliable in ruling out cases of peripheral vascular disease. Third, some 50% of our patients had toe ulcers. It is generally appreciated that ulcers in different regions may have different etiologies or aggravating factors, making comparison between studies difficult. Lastly, this is a single center study and needs validation with a multicenter study.

In conclusion, our data support the contention that hEGF application, in addition to good foot care with a multidisciplinary team approach, enhances diabetic ulcer wound healing and significantly reduces the healing time. Further diabetic ulcer wound healing and significant implications in the context of wound healing and amputation prevention in the future.

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References