A Phase III Study to Evaluate the Safety and Efficacy of Recombinant Human Epidermal Growth Factor (REGEN-D™ 150) in Healing Dia

Authors

Issue: Volume 18 - Issue 7 - July 2006
ISSN:
Index: WOUNDS. 2006;18(7):186-196.

A skin ulcer occurs when an area of skin has broken down and the underlying tissue can be seen. Most skin ulcers occur on the lower legs or the feet. In a normal person, the skin ulcer heals quickly after the injury. However, in a person with diabetes, the healing process is impaired and takes more time even if the injury is minor.

About 1 in 6 people with diabetes develop a foot ulcer at some stage.[1,2] Such foot ulcers do not heal easily, are difficult to treat, and are more prone for serious infection. This calls for better treatment methods that address the causative problem instead of offering supportive therapy.

Over the last 10 to 15 years, a large number of trials have been performed to evaluate the safety and efficacy of growth factors in the healing of chronic wounds due to pressure (decubitus ulcers), diabetic neuropathy, and venous insufficiency.[3] The greatest potential for the use of growth factors in chronic wound care is that they can accelerate healing. Growth factors work by binding to specific cell surface receptors and can target cells in a number of recognized ways or modes. After binding to receptors, growth factors can have a profound influence on cell proliferation, chemotactic activity, and extracellular matrix synthesis.[4]

Epidermal growth factor (EGF) belongs to a family of growth factors that regulate cell proliferation, migration, and differentiation through binding to receptor kinases on target cells.[5] Epidermal growth factor has been shown to act as a mitogen and also a differentiation factor for many cell types.[5]

Mechanism of Action

Epidermal growth factor peptide induces cellular proliferation through the EGF receptor, which has a tyrosine kinase cytoplasmic domain, a single transmembrane domain, and an extracellular domain involved in EGF binding and receptor dimerization.

The proliferative effects of EGF are signaled through several pathways. Binding of EGF results in EGF receptor dimerization, autophosphorylation of the receptor, and tyrosine phosphorylation of other proteins.

Epidermal growth factor receptor activates MAP kinase pathway, ultimately causing phosphorylation of transcription factors, such as c-Fos, to create AP-1 and ELK-1 that contribute to proliferation.

Activation of STAT-1 and STAT-3 transcription factors by JAK kinases in response to EGF contributes to proliferative signaling. Phosphatidylinositol signaling and calcium release induced by EGF activate protein kinase C, another component of EGF signaling.

Recombinant human epidermal growth factor (rhEGF 150 mg/g; REGEN-D™ 150, Bharat Biotech

International Limited, Hyderabad, India) is a growth factor that mainly helps stimulate cell growth. The product safety study was established by conducting a complete preclinical toxicity study in rats and rabbits tested by acute, subacute, dermal, and ocular toxicity studies. The study inferred that rhEGF was safe in rats and rabbits (V.V., S.P., N.S., and G.S.R.M., unpublished data, 2002).
In the present trial, the efficacy of rhEGF 150 mg/g was evaluated in healing patients with diabetic foot ulcers.

Aim

The aim of the study was to evaluate the efficacy and safety of rhEGF gel applied topically in patients with Grade I or II (Wagner's classification) diabetic foot ulcers and to compare the time required for complete healing of the ulcer in the test group and control group.

Study Design

This phase III multicenter study was conducted at 3 centers under the guidance of the principal investigators. The Institutional Ethics Committees of each study center approved the clinical study protocols. The study was conducted in accordance with the provisions of the Declaration of Helsinki as amended in Hong Kong in 1989. Good clinical practice guidelines (ICH 1996) were adhered to during the entire course of the study.
Inclusion criteria. Patients had to be able to understand and sign the informed consent form. In the case of compromised mental capacity, a legal guardian approved and signed the consent form. Target ulcers were no less than 2 cm^2 and no more than 50 cm2 in area. Patients were expected to be available for the 15-week study period and had to be able to adhere to the treatment regimen. Healthy men or women between the ages of 18 and 65 years at the time of consent were included. Women had to be of non-child bearing potential (eg, surgically sterilized) or, if of child bearing potential, must have had a negative pregnancy test, must have used adequate contraceptive precautions (as confirmed by the investigator) 30 days prior to screening and baseline visit, and must have agreed to continue such precautions up to Week 15. Included patients had controlled diabetes mellitus (type 1 and 2) and foot ulcers. Ulcers that remained open without healing for more than 2–3 weeks (irrespective of the ambulatory treatment administered) were included. Patients had to have ankle brachial index (ABI) readings of ≥ 0.75.
Exclusion criteria. Patients with ≥ Grade III Wagner classification diabetic foot ulcers were excluded from the study. Those with life-threatening or serious cardiac failure (NYHA Grades III and IV), gastrointestinal, hepatic, renal, endocrine, hematological, or immunologic disorder were excluded. Additionally, patients were excluded from the study for any of the following factors: hypertension Grade III; known case of hypersensitivity to the incipient(s); uncontrolled diabetes mellitus (type 1 or 2), diabetic ketoacidosis or coma; pregnant women and nursing mothers; past history of/current acute or chronic autoimmune disease; chronic alcohol abuse (40 mL/day for at least 6 months); those who were receiving or had received within 1 month prior to the initial visit any treatment known to impair wound healing including but not limited to corticosteroids, immunosuppressive drugs, cytotoxic agents, radiation therapy, and chemotherapy; use of any marketed, investigational, or herbal medicine or non-registered drug for wounds or burns in the past 6 months; clinically relevant abnormal hematology or biochemistry values in the opinion of the investigator; any criteria that, in the opinion of

the investigator, suggest that the patient would not be compliant with the study; evidence of systemic or local infection, such as purulent drainage, osteomyelitis, or nonviable tissue that cannot be removed by debridement; treatment with a dressing containing any other growth factors or other biological dressings within 30 days prior to the screening visit; or participation in another clinical study within 30 days prior to the screening visit or during the study.

Discontinuation criteria. During the study period, the following events excluded the patient from the study: the patient requested to be discontinued from the study; the investigator decided that it was not in the best interest of the patient to continue the study; the patient required any treatment/therapy that would compromise the evaluation of the test product; the patient missed 2 consecutive weekly clinic visits; lack of study protocol adherence; or an adverse event, whether or not treatment related, occurred and precluded continued treatment. A final evaluation was carried out within 7 days of all premature discontinuation from the study.

Study Samples

The study samples of rhEGF 150 µg/g were obtained from 3 batches—REN04, REN05, and REN06. The quality control department tested the batches, and a batch analysis certificate was issued to the clinical and regulatory affairs department for the conduct of the phase III study.

Study Medication

The active drug was rhEGF; the placebo was water based and did not include the active ingredient. The medication was supplied in 30-g tubes to the study centers. Each tube was affixed with a randomization number.

Study Protocol

The study was a multicenter, double-blind, randomized (1:1), parallel phase III trial. Subjects were screened after the investigator discussed with the subject the nature of the study, its requirements, and restrictions, and written informed consent was obtained. Prior to study allocation, each subject was assigned a unique screening number that served to identify laboratory specimens and all documentation. At enrollment, subjects who complied with all inclusion/exclusion criteria were randomized and enrolled for the study.

The subjects enrolled were allowed to take their normal dose of insulin prescribed to them during the trial and were also given oral and intravenous antibiotics for prevention of infection. The antibiotics used were regular antibiotics prescribed for patients with diabetes and foot ulcers.

Randomization Procedure

Both the patient and the investigator were blinded to the treatment arm. To ensure this, the tubes containing either rhEGF or placebo were similar. Each tube was identified with a randomization number that had a unique code containing information regarding the tube contents. This code was sealed and placed in the investigator site/master file at the site and only was to be broken in emergencies. The allocation ratio was 1:1 (rhEGF:placebo).

Wound Measurements

Wound healing typically has been expressed as a change in area over time. However, these methods are inaccurate when applied to wounds of varying size and shape. A relatively small amount of healing in a large wound will produce a greater change in area than in a smaller wound.

Accurate measurement of the physical size of wounds was vital for assessment of the healing process. As 3-dimensional objects, wounds are difficult to measure. Wound measurements were divided into 3 major groups: ruler-based assessment schemes, transparency tracings, and optical methods.

Transparency tracings. The practice of tracing the outline of a wound through a transparency is the most popular and practical method for area measurements. A sterilized transparent sheet was placed on the wound and its perimeter was traced using a permanent marker pen. The tracing was made on the upper sheet. The lower sheet, which was in contact with the wounds, was disposed after use. The transparency with the tracing was then placed on a piece of metric graph paper and the area was measured by counting the number of 1 mm^2 squares.

Photography. Measuring wound area using tracings of photographs has the advantage of avoiding direct contact with the wound. The photographs were arranged sequentially according to patient visits, and healing progress was monitored throughout the study process.

Skin biopsy. Skin biopsy is one of the most important diagnostic tests for skin disorders. Punch biopsy is considered the primary technique for obtaining diagnostic full-thickness skin specimens. Skin biopsy was done at baseline and after treatment to evaluate the degree of healing.

Drug Administration

The study drugs were administered by topical application as per the procedure set forth in the study protocol. The visit at Day 0 constituted the study medication administration day. The test group received rhEGF and the control group received placebo. The study drug was provided in a gel base to allow for even application (topically) on the ulcer using a sterile cotton swab. This was done twice daily until the wound healed or until the end of Week 15, whichever was earlier.

Safety Reporting

All observed or volunteered adverse events regardless of the treatment group or suspected causal relationship to study drug were recorded on the adverse event page(s) of the case report form. Events involving adverse reactions, illness with onset during the study, or exacerbations of pre-existing illnesses were recorded. The recorded adverse events were 1 case of rash, 3 cases of pain, and 2 cases of skin irritation.

Results

The study included 60 cases at 3 centers. There were 3 dropouts: patient 29 (placebo) dropped out in the third week; patient 30 (placebo) dropped out in fifth week; and patient 60 (rhEGF) discontinued in the fourth week (Table 1).

Statistical analysis. A parametric distribution analysis and a nonparametric analysis were conducted to determine the differences between the groups.

The parametric analysis showed that it took 13 weeks for ulcers to heal in the control group versus 9 weeks in the test group. In the test group, 90% of ulcers healed in 15 weeks compared with 22 weeks

in the control group (Figures 1, 2, and 3; Table 2). The nonparametric analysis showed that the chances of nonhealing within 15 weeks were 14% in the test group and 50% in the control (Tables 3, 4, and 5; Figure 4).

The healing parameters were compared between the 2 treatment groups by classifying them into 2 groups: patients with ulcer area £ 6 cm^2 and those with > 6 cm^2. In patients with ulcer area £ 6 cm^2, the difference in healing was equal. This difference can be attributed to the small number of patients in the tested population. However, in those with an ulcer area > 6 cm^2, patients in the test group exhibited better healing ($P < 0.002$) when compared with the control group. In the control group, 2 of 16 patients with ulcer size > 6 cm^2 healed, whereas 11 of 15 patients in the test group healed. Hence, a statistically significant difference in the wound healing pattern was noted between the 2 groups in patients with an ulcer size > 6 cm^2 (Figure 5). Photographic representation of healing time in several selected cases is illustrated in Figures 6–9.

Discussion

The effectiveness of rhEGF 150 µg/g in healing diabetic foot ulcers was determined in this trial. Subjects enrolled from 3 different centers were randomly allocated to placebo and treatment with rhEGF. The results were subjected to survival data analysis using both parametric and nonparametric methods.

The results of the analyses of these methods were in close agreement with each other. The gel was found to be effective in healing foot ulcers by taking less than half the time taken for healing in the placebo group.

Healing occurred in about 13 weeks for placebo-treated ulcers and 9 weeks for the rhEGF gel-treated ulcers. The percent of completely healed ulcers in the gel-treated population in Week 5, Week 10, and Week 15 was roughly 18%, 66%, and 84%, respectively. Studies on EGF in diabetic foot ulcers have documented similar results.[6] A study in 30 patients with diabetes from Tehran[6] showed that after 4 weeks of study treatment, mean closure was significantly higher in the EGF group compared with placebo (71.2% versus 48.9%, $P < 0.03$). In this study, 100% closure was observed in 7 patients treated with EGF and in 1 patient treated with placebo. This study showed that EGF was significantly superior to placebo in ulcer healing.[6]

A study by Tsang et al[7] showed that the 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 used in the study (log-rank test, $P = 0.0003$).

Laato[8] confirmed that EGF is a potent dose-dependent mitogen for the granulation fibroblast. Thus, EGF was found to be a practical treatment solution for diabetic foot ulcers. Treatment with EGF is not cumbersome and does not involve complicated dressing procedures.

Summary of Phase IV, Post-marketing Surveillance Study of rhEGF

In addition to the phase III study, a phase IV, post-marketing surveillance study of rhEGF was undertaken to study the efficacy of rhEGF in diabetic foot ulcers. Parameters evaluated included ulcer outcome; percentage of healing; duration of healing; and quality of healing and epithelization. The study and its results will be discussed briefly.

All patients enrolled in the phase IV, post-marketing surveillance study were given the study

medication. These patients were evaluated on a weekly basis for the said parameters.

The patients enrolled were in the age of 35–70 years with type I or type II diabetes mellitus. These patients presented with chronic nonhealing ulcers of different sizes at various locations. The population for the study geographically covered the entire country and the middle to lower economic strata.

On local examination of an ulcer, the following characteristics were noted:

• Shaggy and dirty ulcer floor

• Abundant seropurulent and foul smelling discharge

• Presence of slough or unhealthy granulation tissue

• Edematous and painful surrounding tissue.

During the study, both topical and parenteral antibiotics were used. Some of the parenteral antibiotics were third generation cephalosporins, amoxicillin and clavulanate, linezolid. Topical ointments, such as muprocin and betadine, were also used.

All patients (n = 54) who were enrolled for the study resulted in good clinical outcome, ie, ulcers had significantly improved in terms of both percentage of wound closure and quality of healing (Table 6). The average time required for the healing of an ulcer in this cohort was around 5.5 weeks. An average of 83% wound closure in the cohort was documented. Moreover, the quality and epithelization was excellent.

The phase IV, post-marketing surveillance study supports that rhEGF enhances healing of chronic diabetic foot ulcers by significantly reducing the duration of healing in addition to providing excellent quality of healing and reepithelization, resulting in faster wound closure.

Conclusion

This phase III multicenter study established the safety and efficacy of rhEGF formulated gel and found the gel healed diabetic foot ulcers faster than treatment with placebo. This study advocates the use of growth factor therapy in chronic wounds, such as diabetic foot ulcers, where healing of the ulcer is a major hindrance. Growth factor therapy would lead to prevention of leg amputations and would serve as a major treatment therapy to facilitate faster healing of chronic wounds and, thereby, wound closure. A clinical trial comparing rhEGF with other commercialized growth factors would be helpful in further assessment of its efficacy.

Acknowledgment

The authors thank Bharat Biotech International Limited for the scientific input, study supplies, and financial support for the conduct of the phase III clinical study.