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Original Research Article

Recombinant Human Epidermal Growth Factor in Healing of Pressure Ulcers

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ABSTRACT

Treating pressure ulcers is a frustrating challenge for any surgeon. We have studied the use of Recombinant Human Epidermal Growth Factor (rhEGF) for treating pressure ulcers that occur over bony points in 25 patients. This is prospective double blind randomized study with control group being placebo containing paraffin jelly. Ulcers are graded as per" Pressure Ulcer Grading System (according to the European Pressure Ulcer Advisory Panel Grading System" The period observed for healing of ulcer is 12 weeks. We have observed that there is no difference in the time for healing overall for all grades of ulcers in both the groups; bur there is significant percentage of complete healing of the ulcers in rhEGF group (p0.014). The literature also supports the use of rhEGF in chronic non healing ulcers with much more promising results. It is worth trying rhEGF in pressure ulcers and chronic non healing ulcers with aim to promote neovascularization which ultimately will help in healing of ulcers.

Key Words: Recombinant human epidermal growth factor, rhEGF, pressure ulcers, ulcer healing

INTRODUCTION

Pressure ulcers are a type of injury that breaks down the skin and underlying tissue when an area of skin is placed under constant pressure for certain period causing tissue ischemia, cessation of nutrition and oxygen supply to the tissues and eventually tissue necrosis. Constant pressure resulting in 'distortion or deformation damage' is probably the most accurate description of a pressure ulcer. Many factors contribute to the development of pressure ulcer but final pathway for ulcer formation is microcirculatory occlusion followed by tissue necrosis. Majority of people affected with pressure sores are those having health conditions (mental or physical) that encourage immobility, especially those who are confined to bed or chair for prolonged periods of time, or those with immune compromised states. Majority of the pressure ulcers frequently develop over a bony prominence. Majority of the cases reportedly are affected over the area where skin covers bones such as sacral, ischial and trochanteric pressure ulcers [1] and in the lower extremities they are seen in the malleolar, heel, patellar and pretibial locations - account for approximately 25% of all pressure sores. [2]

Grading of pressure ulcers:

Pressure Ulcer Grading System (according to the European Pressure Ulcer Advisory Panel Grading System. [3]

Grade 1 Non-blanching erythema of intact skin. Discoloration of the skin, warmth, oedema, induration or hardness may also be used as indicators, particularly on individuals with darker skin.

Grade 2 Partial thickness skin loss involving the epidermis, dermis or both. The ulcer is superficial and presents clinically as an abrasion or blister.
Grade 3 Full thickness skin loss involving damage to, or necrosis of subcutaneous tissue that may extend down to, but not through the underlying fascia.
Grade 4 Extensive destruction, tissue necrosis or damage to muscle, bone or supporting structures with or without full thickness skin loss. [3]

In 1962, Stanley Cohen purified a peptide from the saliva of mouse and showed it could disrupt the incisor eruption and eyelid opening in new born mice. This peptide was named as epidermal growth factor and members of this family have been identified in pathological and physiological context. Epidermal growth factor (EGF) binds to the cell receptor (EGFR) and creates biochemical modification (phosphorylation) of the receptor cytoplasmic tail. The phosphorylated receptor then activates the biochemical pathway within the cells by acting as a site for the enzymes that mediate signal transduction. Signals regulates process that effect cell division, differentiation and migration. In 1986 Stanley Cohn and Ritil Levi-Montalcini were awarded the Nobel Prize in Biochemistry. [4]

EGF is a single-chain polypeptide consisting of 53 amino acids with molecular weight of 6,200 daltons. The six cysteine residues in the sequence of EGF form three disulfide bonds, which are required for EGF to be biologically active.

EGF triggers cell proliferation via signal transduction pathways involving EGF-R, adapter proteins (Grb2, 5hc and 50S), Ras, Rafl and MAP (Erk1/Erk2) kinases, which, after translocation to nuclei, activate transcription factors and cell proliferation. Cell migration requires cytoskeletal rearrangements and is controlled by growth factors via Rho/Rac and signaling pathways involving PLC-g, PI-3 K and phosphorylation of focal adhesion proteins. Granulation tissue develops at the ulcer base. It consists of connective tissue cells: fibroblasts, macrophages and proliferating endothelial cells forming micro vessels under the control of angiogenic growth factors: FGF, VEGF and angio proteins, which all promote angiogenesis capillary vessel formation, essential for the restoration of microvascular network.

A number of in-vivo models have extensively documented that EGF is involved in embryonic, fetal and neonatal development, differentiation and maturation.

In lieu of the various roles of EGF in cell biology and wound healing, recombinant human epidermal growth factor (rhEGF) has become one of the most attractive growth factor as a therapeutic modality for chronic wound healing.

In this double blind randomized study we studied the efficacy of rhEGF in pressure ulcers comparing with placebo

MATERIALS AND METHODS

Study design: This is a prospective, randomized (1:1), double blind, comparative (Group I: rhEGF and Group II: Placebo) study to evaluate the safety and efficacy of recombinant Human Epidermal Growth Factor (rhEGF) in patients aged between 18 years and 75 years with Bed Sores (Pressure ulcers).

Study duration: The maximum and expected duration of study of treatment and follow ups will be up to 12 weeks in pressure ulcers/ bed sores.

Number of patients – 25

The ulcers are categorized into two types based on the ulcer area

Category -1: $<10cm^2$

Category - 2:$>=10cm^2$ but $< 200\ cm^2$

Patients with above ulcer sizes were randomized to the test and control groups. Treatments and the observations were recorded.

For the purpose of this study the ulcers were classified by grade as follows:

Grade 1-Non-balanceable erythema of intact skin. Discoloration, warmth,

induration, or hardness of skin may also be used as indicators, particularly in people with darker skin

Grade 2-Partial-thickness skin loss, involving epidermis, dermis, or both. The ulcer is superficial and presents clinically as an abrasion or blister

Grade 3-Full-thickness skin loss involving damage to or necrosis of subcutaneous tissue that may extend down to, but not through, underlying fascia

Grade 4-Extensive destruction, tissue necrosis or damage to muscle,bone, or supporting structures, with or without full-thickness skin loss

Method of blinding

The study drug- rh EGF and placebo are packed in similar tubes and are similar in color, smell, taste, consistency and appearance. All supplies are pre labeled with subject numbers. After selecting a patient he is assigned a subject code and given the corresponding tubes for the period of the study at regular intervals. Neither the patient nor the investigator knows whether the subject code is that of the drug or placebo. Decoding was done after the completion of the entire study.

Criteria for patient selection

Inclusion criteria

- Patient is able to understand and has signed the informed consent form. In case ot compromised mental capacity, approval and signature of a legal guardian is required.
- A diagnosed case of pressure ulcer based on clinical evaluation.
- The target ulcer is no less than 2 cm^2 and n6 more than 50 cm^2
- Patients are expected to be available for the 12 week study period and are able to adhere to the treatment regimen.
- Patients (male or female) aged between 18 and 75 years at the time of consent.
- If the patient is female:

She must be of non-children potential (e.g. surgically sterilized) or if of child bearing potential, she must have used adequate contraceptive precautions (as confirmed by the investigator) 30 days prior to screening & baseline visit or must be negative on pregnancy test and must agree to continue such precautions till the end of study. The pregnancy test will be done regularly on these patients if these are outdoor (ambulatory) patients.

- Ulcers which remained open without healing for more than 2-3 weeks (irrespective of the ambulatory treatment administered).
- Exclusion criteria :
  - Life threatening or serious cardiac disease (NYHA grades lIl-IV), gastro-intestinal, hepatic, renal, endocrine, hematological or immunologic disorder.
  - Malignant ulcers.
  - Uncontrolled Hypertension (Grade III).
  - Known case of hypersensitivity to the incipient(s).
  - Uncontrolled diabetes mellitus (type [or II), diabetic ketoacidosis
  - Pregnant woman and nursing mothers.
  - History of acute or chronic auto-immune disease.
  - Chronic alcohol abuse (40ml/day for at least 6 months).
  - Patient receiving or has received within one month prior to visit. Any treatment known to impair wound healing including but not limited to: corticosteroids, immunosuppressive drugs, cytotoxic agenls, radiation therapy & chemotherapy.
  - Use of any marketed or investigational or herbal medicine or nonregistered drug for wounds 30 days prior to the screening.
  - Any criteria which in the opinion of the investigator, suggests that the patient would not be complaint with the study.
  - Presence of a systemic or deep local infection such as: purulent drainage, osteomyelitis or as non-viable 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.
- 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 should exclude the patient from the study.

- Patient requests to be discontinued from the study.
- The patient requires any treatment/therapy that would compromise
- the evaluation of the test product
- The patient missed two consecutive weekly clinic visits.
- There is a lack of adherence to the study protocol
- When an adverse event occurs, whether or not treatment related, this precludes continued treatment
- Any female patient who becomes pregnant during course of investigation.
- If the patient's compliance is found to significantly outside this range at two consecutive visits, such subjects will be excluded from efficacy analysis because of non-compliance to treatment.

IDENTITY OF TEST DRUG AND PLACEBO

Study Drug Generic Name: rhEGF; gel base; Strength: 60 pg/gm

Manufacturer: Bharat Biotech International Ltd. Ingredients:

- Active: rhEGF
- Inactive: Propyl Paraben, Methyl Paraben, Carbopol ultrez, Glycerol, Mannitol, Lysine HCL,

Placebo: Gel base

Manufacturer: Bharat Biotech International Ltd. Ingredients:

- Active: None
- Inactive: Propyl Paraben, Methyl Parabcn, Carbopol ultrez, Glycerol, Mannitol, Lysine HCL

Procedure

A detailed history and physical examination was carried out on each patient. After evaluation of inclusion and exclusion criteria, the patients were enrolled and a subject and a code was allotted. The wound was debrided and once the wound was free of necrotic tissue and slough, study medication was applied. In most of the cases, the drug was applied 3times daily, in small and superficial ulcers, once daily. After application of the medication the wound was dressed with saline soaked gauze. Photographs were taken once every week with specially designed square type plastic scale. Routine blood investigations (complete blood picture), liver function tests, urine examination, blood sugar, urea, Serum creatinine were done at the beginning and at the end of the study.

RESULTS

Twenty one patients with 25 pressure sores were selected for the study and randomly assigned to receive either the study drug or placebo.

Two patients dropped out of the study after one week and one patient died due to his underlying disease (head injury). The remaining 18 patients (had 21 pressure ulcers) completed the study.

For all the grades put together, the rate of complete healing in the rhEGF group was 91% compared to 55% in the placebo group. Out of the eight ulcers in the placebo group, five healed completely, three showed a decrease in size and one ulcer increased in size. In comparison, 11 out of 12 ulcers healed completely in the rhEGF group and 1 showed increase in size

Table-1: the comparison between placebo and rhEGF groups for the parameter Healing time (in days) for all grades

Groups	*N*	*Range*	*Mean*	*SD*	*P-value*
Placebo	8	20 to 80	50.75	21.80	0.871
rhEGF	11	27 to 96	52.36	20.53	

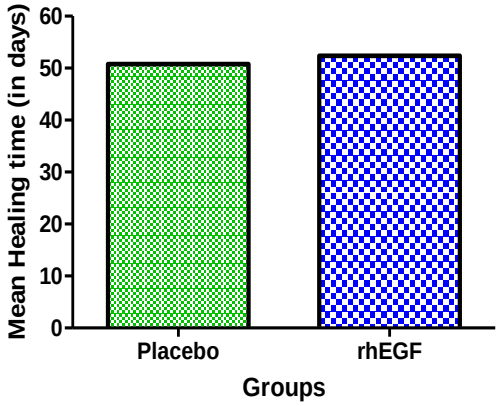

Figure-1: the simple mean bar diagram for the comparison between placebo and rhEGF groups for the parameter Healing time (in days) for all grades

Statistical test: "Unpaired t-test".
Conclusion: there is no significant difference between placebo and rhEGF groups for the parameter Healing time (in days).

Table-2: the comparison between placebo and rhEGF groups for the completely healed ulcers for all grades

Groups	*Completely Healed Ulcers*	*P-value*
Placebo	5 (62.5%)	
rhEGF	10 (90.9%)	0.014
Total	***15***	

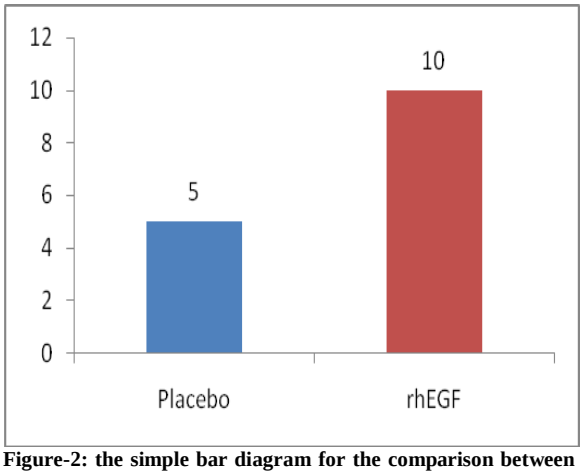

Figure-2: the simple bar diagram for the comparison between placebo and rhEGF groups for the completely healed ulcers for all grades

Statistical test: "Proportion test".
Conclusion: there is significant difference between placebo and rhEGF groups for the parameter completely healed ulcers.

Table-3: the comparison between placebo and rhEGF groups for the parameter Healing time (in days) for grade 1

Groups	*N*	*Range*	*Mean*	*SD*	*P-value*
Placebo	3	20 to 37	26.33	9.29	***0.034***
rhEGF	2	51 to 54	52.50	2.12	

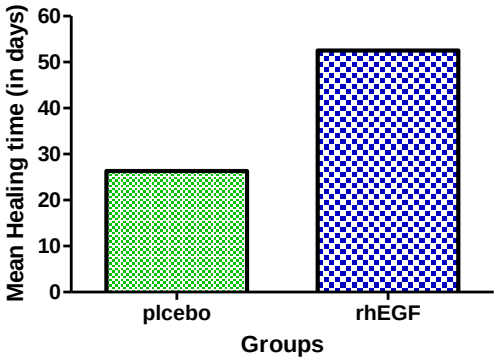

Figure-3: the simple mean bar diagram for the comparison between placebo and rhEGF groups for the parameter Healing time (in days) for grade 1

Statistical test: "Unpaired t-test".
Conclusion: there is significant difference between placebo and rhEGF groups for the parameter Healing time (in days) in grade 1.

Table-4: the comparison between placebo and rhEGF groups for the completely healed ulcers for grade 1

Groups	*Completely Healed Ulcers*	*P-value*
Placebo	2 (66.7%)	
rhEGF	2(100.0%)	0.041
Total	***4***	

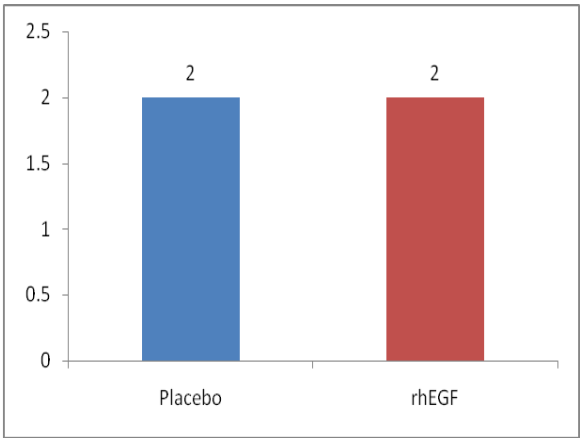

Figure-4: the simple bar diagram for the comparison between placebo and rhEGF groups for the completely healed ulcers for grade 1

Statistical test: "Proportion test".
Conclusion: there is significant difference between placebo and rhEGF groups for the parameter completely healed ulcers in grade 1.

Table-5: the comparison between placebo and rhEGF groups for the parameter Healing time (in days) for grade 2

Groups	*N*	*Range*	*Mean*	*SD*	*P-value*
Placebo	2	60 to 66	63.00	4.24	***0.048***
rhEGF	7	27 to 60	42.14	11.63	

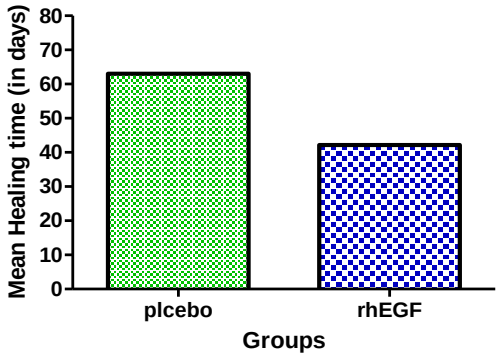

Figure-5: the simple mean bar diagram for the comparison between placebo and rhEGF groups for the parameter Healing time (in days) for grade 2

Statistical test: "Unpaired t-test".

Conclusion: there is significant difference between placebo and rhEGF groups for the parameter Healing time (in days) in grade 2.

Table-6: the comparison between placebo and rhEGF groups for the completely healed ulcers for grade 2

Groups	*Completely Healed Ulcers*	*P-value*
Placebo	1 (50.0%)	
rhEGF	7 (100.0%)	0.061
Total	***8***	

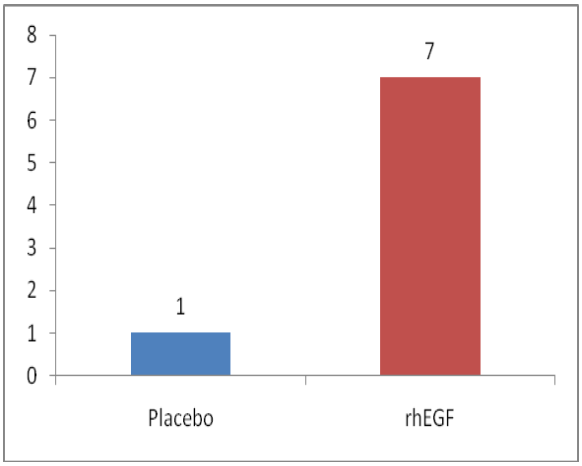

Figure-6: the simple bar diagram for the comparison between placebo and rhEGF groups for the completely healed ulcers for grade 2

Statistical test: "Proportion test".

Conclusion: there is significant difference between placebo and rhEGF groups for the parameter completely healed ulcers.

For Grade 3 ulcers,1 ulcer out of 3(33%) healed completely in the placebogroup with a healing time of 60 days.1 ulcer showed 78% decrease in size and 1 ulcer increased in size. In the rhEGF group the complete healing rate was 88% (7 out of 8ulcers) with a mean healing time of 42 days.

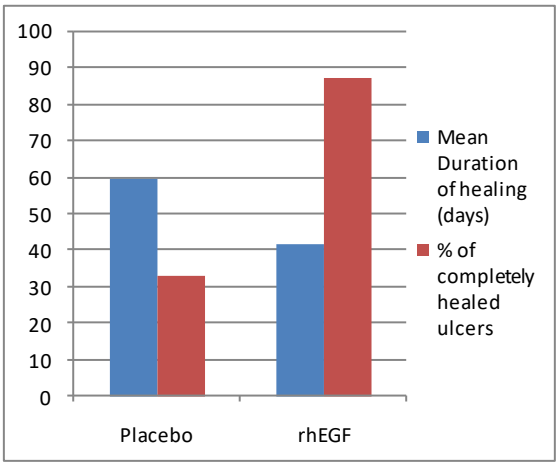

Fig7--the simple bar diagram for the comparison between placebo and rhEGF groups for the completely healed ulcers for grade 3

For Grade 4 ulcers, the complete healing rate in placebo group was 66.7% (2 out of 3 ulcers) with a mean healing time of 70 days. In rhEGF group both the ulcers (2 out of 2) healed completely with a mean healing time of 88days.

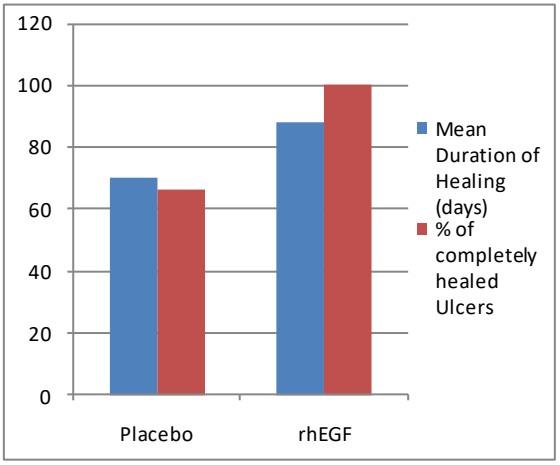

Fig8--the simple bar diagram for the comparison between placebo and rhEGF groups for the completely healed ulcers for grade 4

The mean healing time mainly depended upon the grade of the ulcer. The mean healing time was 40.5 days for grade II ulcers, 44.5 days for grade III ulcers, and 79 days for grade IV ulcers.

There were no adverse side effects either local or systemic in any of the patients in this study.

From these results it is evident that there was no significant difference in the rate of healing between the drug and placebo

groups, but in the drug group there was higher percentage of complete healing of ulcers in all the ulcer grades.

DISCUSSION

Brown et al studied nine patients (including four with diabetes) suffering from longstanding refractory lower extremity ulceration and found that eight patients managed to heal after topical treatment with EGF. [5] There was no control group in this study. More recently, Tsang and colleagues randomized 61 diabetic patients with foot ulcer of moderate severity (Wagner score 1 or 2) and adequate blood flow to placebo, topical application of 0.02% EGF, and topical application of 0.04% EGF. The authors found that treatment with 0.04% EGF accomplished healing in 20 out of 21 patients with a significant reduction in median healing time. [6]

In another study, on diabetic foot, Larijani et al. found that after four weeks of treatment ,mean closure was significantly higher in the EGF group compared with placebo (71.2% vs 48.9%,p<0.03). [7] rhEGF was also found to be beneficial in the healing of deep partial thickness burns wounds(p<0.05), (Liu.x et al,2005). [8]

Recombinant human platelet-derived growth factor–BB has been examined in a 16-week clinical trial. Twenty-three percent of the group treated with 100 mi.g of recombinant human platelet-derived growth factor. [9] –BB, 19% of the group treated with 300mi.g, 3% of the group treated with 100mi.g twice daily, and 0% of the group treated with vehicle control healed. The negative dose–response effect, the fact that the rate of healing was considerably lower than reported rates seen with other standard treatments, and the finding that no ulcer healed in the vehicle control group make interpretation of this study problematic.

Mustoe and colleagues studied patients with stage 3 or stage 4 pressure ulcers who were randomly assigned to varying doses of recombinant human platelet-derived growth factor–BB. In these patients, the time to 50% healing and ulcer volume at the end of treatment (adjusted for initial volume) did not differ among groups. Recombinant human platelet-derived growth factor–BB failed to improve the rate of complete healing of pressure ulcers in another trial, although a 15% decrease in percentage of ulcer volume was observed in the treatment group. [10]

Robson and associates studied topical recombinant human interleukin-1 in 26 patients with pressure ulcers who were treated using 1 of 3 different doses or placebo. No difference in healing was observed among the 4 groups. [11]

In another study, Robson and associates used a recombinant basic fibroblast growth factor to treat 50 patients with stage 3 or stage 4 pressure ulcers in 8 dosing regimens, and a trend toward faster healing was observed in 6 of the 8 groups. A reduction in volume of 70% was almost statistically significant ($P < 0.05$). [1]

Sequential use of growth factors has also been attempted in treating pressure ulcers. Robson and associates studied 61 patients randomly assigned to receive granulocyte- macrophage colony-stimulating factor for 35 days, basic fibroblast growth factor for 35 days, granulocyte-macrophage colony-stimulating factor for 10 days followed by basic fibroblast growth factor for 25 days, or placebo. The mean change in volume of the pressure ulcer at 35 days did not differ among groups. [2]

Landi and colleagues reported on topical treatment of pressure ulcers with nerve growth factor. Topical nerve growth factor has been extensively studied in vitro, in animal models, and in human corneal and vasculitic ulcers. In Landi and colleagues' trial, 18 patients with pressure ulcers of the foot were randomly assigned to receive topical nerve growth factor daily for 6 weeks and were compared with 18 persons who received a balanced salt solution (vehicle control) without nerve growth factor. The results seem impressive. The pressure ulcers healed completely in eight

patients in the active treatment group but in only one patient in the vehicle control group. Improvement was greater (based on wound size) in the active treatment group than in the vehicle control group. The data are somewhat limited by study design. First, only pressure ulcers of the foot (mostly heel ulcers) were studied. In the vehicle control group, only one foot ulcer healed and none improved by three or more stages during the six weeks of treatment. Thus, a great deal of the difference between the two study groups can be accounted for by failure of treatment in the vehicle control group. The six week healing rate was 44% in the topical nerve growth factor group. The healing rate of pressure ulcers of the heel is not well described in the literature. However, in an observational study that included all body sites, 40% of pressure ulcers healed completely during a six week period. Thus, the rate of healing in the nerve growth factor–treated pressure ulcers was approximately the same as previously reported healing rates. While topical nerve growth factor was clearly superior to vehicle control in Landi and colleagues' study, whether it is superior to other conventional therapy remains to be demonstrated. [12]

In our study, we found that rhEGF improved the percentage of complete ulcer healing although the mean healing time did not improve when compared to placebo.

CONCLUSION

Pressure ulcers are difficult to heal. The main challenge is to relieve excess, prolonged pressure on bony prominences by frequent postural changes. This may not be possible always and therefore there is no 100% effective method in preventing pressure ulcers. Pressure sores are inevitable. Therefore strategies involved in treating pressure sores are equally important.

Patient factors are the most important determinants of the rate of healing of pressure ulcers, the most important being the ability of the patient to turn in the bed and his underlying condition. Old age, diabetes, peripheral vascular disease, smoking, excessive alcohol intake, malnutrition, malignancy, all negatively influences pressure ulcer healing.

The presence of these confounding variables makes interpretation of any therapeutic trial on pressure sores difficult, especially if the sample size is small wherein matching between study and control subjects is difficult to achieve.

Any novel methods to treat pressure ulcers are worth trying, while at the same time improving on the existing strategies. The overall approach to the patient should be holistic taking into consideration the limitations of the patient and the health care system.

The emergence of growth factors has given new hope. Initial trials have been promising and growth factors are likely to become effective tools in the surgeon's armamentarium in dealing with pressure ulcers. However they are at best only adjuncts to the basic well established treatment guidelines like postural changes, early wound debridement, control of infections and management of incontinence and cannot replace them. rhEGF improved the percentage of complete ulcer healing although the mean healing time did not improve when compared to placebo.

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