

Patterned wound healing effect of topical recombinant human epidermal growth factor in trophic ulcers secondary to leprosy

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Abstract

The aim of this study was to evaluate the wound healing effect of topical recombinant human Epidermal Growth Factor (Rh EGF) in trophic ulcers in leprosy patients. 40 patients, 29 males and 11 females, were included in the study. Re-epithelialization started on the 7th day, most of it occurred from the 2nd week onwards. Symmetric concentric hyperkeratosis started from the periphery and migrated towards the centre. In 4th to 5th week, complete uniform healing was noted with shedding of the overlying hyperkeratotic plaque. There was faster wound healing with excessive uniform keratosis that eventually desquamated, Ulcers healed with minimal healthy scar formation (almost scar free closure) and subsequent longer ulcer free interval. Overall it can be concluded from this study that topical Rh EGF is effective in promoting faster and better wound repair.

Keywords: Wound healing, Epidermal growth factor, Trophic ulcer.

Introduction

Trophic ulcers secondary to Hansen's disease have been a challenge to treat. The healing process includes inflammatory responses, epidermal regeneration, connective tissue formation, wound shrinkage and remodeling. Different methods have been employed to reduce the duration of wound healing and produce a better scar; one being the topical application of EGF.¹

The objective of this study was to examine the effectiveness of Rh EGF in treating trophic ulcers in Hansen's disease, to record the sequential clinical pattern of wound healing and to analyze its efficiency in preventing their recurrence. This study has attempted to highlight the patterned healing effect of topical EGF resulting in scar free closure.

Materials and Methods

Type of Study: Prospective study- Interventional clinical trial without comparison group

The study was a prospective study conducted at the Department of Dermatology and Leprosy in a South Indian tertiary care hospital. 40 patients were included; inclusion criteria included Hansen's disease patients of all ages, both sexes, with culture negative (non-infected) trophic foot ulcers of Grades I & II (Wagner classification), with ankle brachial index (ABI) reading of ≥ 0.75 and willing for weekly follow up. Exclusion criteria included culture positive (infected) ulcers, deep complicated ulcers, trophic ulcers due to other diseases, ulcers \geq Grade III Wagner's classification and lepra reactions.

Forty patients with superficial non-infected ulcers of Grade I and II (Wagner's classification)² were selected for the study.

Measurement of Volume of ULCER: Measurement of ulcer was done by clock face method. Disposable scales

with centimeter markings were used. $VOLUME = length \times width \times 0.7854 \times depth$; Length: 12.00 to 6.00 with 12.00 towards the head; Width: 3.00 to 9.00 side to side and Depth: deepest point of wound bed to the level of normal skin surface at right angles to skin surface.

After obtaining informed written consent, photographs were taken and Rh EGF gel was applied topically and covered with sterile gauze. Applications were repeated on alternate days and closed with normal dressing. Post dressing advice included bed rest with restricted movement using microcellular rubber footwear. They were asked to come for weekly follow up. In the intervening period patients were given multivitamin supplements and if patients were on multidrug therapy asked to continue it.

Follow-up examination was done and serial photographs were taken every week until complete wound closure. Photographs were compared and ulcer volume reduction was assessed based on reduction rate at the end of 5th week. Epithelialization of wound, granulation of tissues, presence/ absence of exudates and volume of wound was documented at each visit. Complete healing was defined as full epithelialization of wound with absence of discharge. After complete wound healing patients were advised to avoid prolonged standing and use of appropriate off-loading MCR footwear while walking. Patients with ulcers over lateral malleoli were advised to avoid crossed leg sitting position.

Observations and Results

Study Population: This study included 40 Hansen patients with trophic ulcers of which 29 were male and 11 were female patients.

Table 1: Youngest age was 26 years and oldest was 60 years.

Age group (in years)	20-30	31-40	41-50	51-60
Number of patients	3	12	18	7

Table 2: Spectrum of leprosy in study population

Leprosy spectrum	Borderline tuberculoid	Lepromatous leprosy	Borderline lepromatous	Pure neuritic
Number of patients	18	12	8	2

Table 3: ULCER site wise distribution in study population

Site of ulcer	Forefoot	Lateral aspect of sole	Heel	Mid-foot	Lateral malleolus	Medial aspect of dorsum of foot
Number of patients	21	5	4	2	6	2

Motor Deformity was present in 22 patients in our study

Table 4: Baseline ULCER volume included in the study

Ulcer volume	0 – 5 mm ³	5.1 – 10 mm ³	10.1 – 15 mm ³
Number of patients	5	30	5

Volume = Length x width x 0.7854 x depth

At the end of 4 weeks the percentage of wound healing based on volume reduction was graded as follows- Grade I (100%), Grade 2 (80 – 99%), Grade 3 (40 – 79%), Grade 4 (0 – 39%)

Table 5: Healing grade vs number of patients

Grades	Grade I (100%)	Grade 2 (80 – 99%)	Grade 3 (40 – 79%)	Grade 4 (0 – 39%)
Number of patients	25	13	2	0

Table 6: Number of weeks required for complete healing

Number of weeks taken for healing	1	2	3	4	5	6	7
Number of patients	0	2	3	14	12	7	2

H&E section in healed ulcer in our study showed well organized collagen with paucity of inflammatory cells in the dermis, comparable to the similar picture seen in the murine study. (r)

Human EGF treated collagen in our study- low power view (Fig 1)

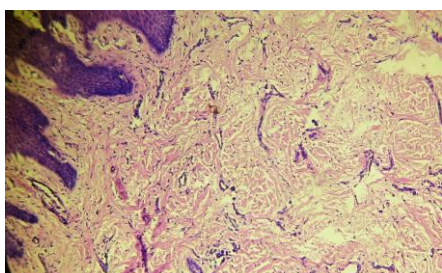


Fig. 1:

On average many patient showed the following pattern of healing like, at the end of first week, there was uniform healthy granulation tissue formation covering the floor of the ulcer with the formation of peripheral hyperkeratotic rim. (Fig. 2)



Fig. 2:

In second week, reduction in the depth of the ulcer was noted. (Fig 3)



Fig. 3:

At the end of third week keratotic tissue was layered down as concentric manner from periphery towards the centre. One ring within another resembling the step well with trough in the center. (Fig 4 & 5)



Fig. 4:



Fig. 5:

In fifth week hyperkeratotic layer separated (Fig. 6) and shed in toto leaving behind the healthy depigmented contracted scar. (Fig 6)



Fig. 6:

On further follow up size of the scar was smaller than the initial width of the ulcer and recurrence was almost not noticed

Discussion

Wound healing is complex, with three stages namely inflammation, proliferation and remodeling. Many growth factors, cytokines, proteases and adhesion molecules are involved. The efficacy of growth factors in enhancing wound healing has been demonstrated both in vivo and in vitro. Growth factors come under biological therapy which is constitutively present, usually released by few selected subset of cells and have a primarily trophic effect on cells.

Epidermal growth factor EGF is one of the key growth factors in wound healing, others being platelet-

derived, vascular endothelial, fibroblast, transforming, insulin-like, granulocyte, mast cell growth factors (PDGF, VEGF, FGF, TGF β , IGF, G-CSF, MCSF). The growth factors most studied are PGDF, EGF and FGF.^{4,5}

Physiological effects of EGF:⁶

1. Directs epithelialisation in an autocrine fashion evidenced by aged dermal fibroblast.
2. Stimulates fibroblast collagenase secretion – evidenced by increased EGF receptor expression.
3. Inhibits fetal wound contraction – evidenced by scar less repair seen in utero.
4. Accelerates fibronectin synthesis.
5. Increases myofibroblasts –wound contraction.

Rh EGF used since 1983 topically augments epithelialization of skin donor sites by stimulating epidermal and dermal repair.

Cooper et al⁷ showed that a number of growth factors were markedly reduced in wound fluid from chronic wounds compared with acute wounds.

Bennett and Shultz⁸ postulated increased destruction or inhibition of growth factors by elevated levels of pro-inflammatory cytokines and matrix metallo protein following repeated trauma and infection.

Local application of high concentration of growth factors in chronic wound is effective in promoting wound healing.⁹

Wounds treated with topical Rh EGF healed faster than control groups, width of the scar was smaller by 30% and the area was smaller by 20%. It was observed that the amount of collagen in the EGF treated group was larger. It was suggested that EGF reduced cutaneous scar by suppressing inflammatory reactions, decreasing expression of TGF- β and mediating the formation of mature collagen similar to normal skin.¹⁰

Epithelial portion of wound repair begins with cell mobilization and migration across the wound. Thereafter cellular numbers are augmented by mitosis and cellular proliferation, while cellular differentiation accounts for maturation into the normal epithelial appearance. The epithelial cells immediately adjacent to the wound initially undergo a mobilization process; they enlarge, flatten and detach from the neighbouring cells and basement membrane. An apparent loss of contact inhibition serves as stimulus for migration and flow of the flattened cells in a direction away from adjoining epithelial cells. As marginal cells begin their migration, cells immediately behind them also tend to flatten, break their cellular connection and drift along.^{11, 12} Epidermis thus flows across the gap of the wound, continues until the advancing cells meet cells coming from the opposite side of the wound whereupon motion stops abruptly – contact inhibition. As resurfacing of the wound proceeds, cells that have migrated start to divide and multiply. Increasing number of cells thicken the new epithelial layer.¹³ Rh EGF enhances all these processes.

Fig II: Wagner classification of diabetic foot ULCERS^(17,19)

Grade 0 skin intact but bony deformities produce a "foot at risk"

Grade 1: localized, superficial ulcer

Grade 2: deep ulcer to tendon, bone, ligament, or joint

Grade 3: deep abscess, osteomyelitis

Grade 4: gangrene of toes or forefoot

Grade 5: gangrene of entire foot

Dressing with topical Rh-EGF: Normal saline wash given, and sterile occlusive dressing done with topical Rh-EGF on alternate days.

Measurement of volume of ULCER⁽¹⁶⁾

1. Measurement of ulcer by clock face method.
2. Disposable scale with centimetre markings were used.
3. Length: 12.00 to 6.00 with 12.00 towards the head.
4. Width: 3.00 to 9.00 side to side
5. Depth : deepest point of wound bed to the level of normal skin surface at right angles to skin surface
Volume=length×width×0.7854×depth

Study in mice examined the role of epidermal growth factor (EGF) in the formation of cutaneous scars.²⁰²² Twenty -CrI: CD-1 (ICR) mice were used and 2 full-thickness skin wounds were made on the dorsum of each mouse. One of the wounds was treated with recombinant human EGF by local application and the other was treated with saline for control until complete healing was achieved. The EGF-treated group's wounds healed faster than the control group. The width of the scar was smaller by 30% and the area was smaller by 26% in the EGF-treated group. Inflammatory cell numbers were significantly lower in the EGF treated group. The expression of transforming growth factor (TGF)-b1 in the EGF treated group was increased. It was observed that the amount of collagen in the EGF - treated group was larger than the control group. In the EGF-treated group, the visible external scars were less noticeable than that in the control group. These results suggest that EGF can reduce cutaneous scars by suppressing inflammatory reactions, decreasing expression of TGF-b1, and mediating the formation of collagen.^{14,15}

Limitations of the Study:

1. No control group was used in the study
2. Inpatient admission was required for management and rest to the patient

Conclusion

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 micro environment, or that higher levels of the factors recruit other cytokines required to promote wound healing.

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.²³ Debridement enabled removal of necrotic tissue and allowed better surface contact with Rh EGF. Topical Rh EGF is a simple procedure - non invasive self applicable and cost effective.

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