

Skin ulcer healing enhanced by brain-derived endothelial cell growth factors

Tang-De Zhang
Xian-Wen Qiu
Hong-Bing He

Abstract

Endothelial cell growth factors (ECGFs) are potent mitogens of endothelial cells and fibroblasts which are important cells in the granulation tissue of wounds. The effects of bovine brain-derived ECGFs on skin ulcer healing were investigated in 14 patients with 42 skin ulcers using a self-control design. The skin ulcers were varicose, decubitus and vasculitis ulcers.

The mean time to reach a 50 per cent healing was significantly shorter with ECGFs than with the control. The healing rate was approximately 1cm² per week in ECGFs treated ulcers. The skin ulcers treated with ECGFs also demonstrated a fast growth of granulation tissue. At the time of reaching 100 per cent healing, vasculitis ulcers had taken longer than non-vasculitis ulcers (varicose ulcer and decubitus ulcer). These results suggest that ECGFs can accelerate the healing of skin ulcers. It also suggests that the degree of vessel injury in skin ulcers has an impact on the effect of ECGFs on healing.

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Introduction

Skin ulcer healing is a complicated process involving multiple factors. In clinical settings, it is often seen that slow growth of granulation tissue postpones skin ulcer healing. It is known that endothelial cell growth factors (ECGFs) can accelerate the proliferation of endothelial cells and fibroblasts *in vitro* and are reported to have an angiogenic effect on irradiated wounds¹. Bovine brain-derived ECGFs have been purified²⁻⁴. We investigated the application effect of ECGFs on various kinds of skin ulcers in patients.

Tang-De Zhang

Xian-Wen Qiu

Hong-Bing He

Department of Dermatology/Surgical Laboratory
Zhujiang Hospital, First Military Medical
University
Guangzhou 510282 P.R.China

Tel: (86) 20 8514 3761/3555

Fax: (86) 20 8514 2983

E-mail: zhtangde@public.guangzhou.gd.cn1

Method

In this study, self-controlled skin ulcers served as the control (self-control design), so the observed objects were selected from the patients with two or more skin ulcers. The ulcers due to special infection or malignant tumours were not included. Fourteen patients with three kinds of skin ulcers, varicose, decubitus and vasculitis, were observed. There were six males and eight females with an age range of 18-71 and a mean age of 44.8. The total number of skin ulcers was 42. The time taken for the ulcers to heal was carefully monitored and their area and depth precisely measured. The mean healing time, area and depth of ulcers between the ECGF group and the control group did not differ significantly (Table 1).

Ethics

All patients signed an informed consent document and were treated under a research protocol approved by the Human Studies Ethics Committees of the First Military Medical University.

Extraction and purification of ECGFs

Rough ECGFs were extracted from fresh hypothalamus and pituitary of bovine. Purified ECGFs were obtained using

Table 1. Clinical data for three kinds of skin ulcers.

	Varicose (7 cases)		Decubitus (4 cases)		Vasculitis (5 cases)	
	ECGFs	Control	ECGFs	Control	ECGFs	Control
	X±SD	X±SD	X±SD	X±SD	X±SD	X±SD
Number of ulcers	7	6	6	6	9	8
Area (cm ²)	4.0±3.8	3.9±3.0	5.5±3.7	4.8±2.3	6.5±7.9	6.2±3.2
Depth (mm)	3.1±0.4	3.2±0.4	4.5±1.0	4.3±1.5	3.9±1.4	4.1±1.0
Deviation (weeks)	15.9±6.7	17.5±6.2	3.8±2.3	3.1±1.9	30.3±30.2	30.6±31.5

CM-cellulose C50, heparin-agarose 6B and high-pressure liquid chromatography (HPLC) column. The brain-derived ECGFs had a mitogenic effect on endothelial cells and fibroblasts. An anti-ECGF antibody inhibited the mitogenic response.

All skin ulcers were debrided until there was mild blood exudation on the surface of ulcer and treated with a furacin hydrophatic compress for 15 minutes. The skin ulcers were covered compactly by a monolayer of ulcer shaped gauze with solution of brain-derived ECGFs (2µ/ml) and then covered by petrolatum gauze. Finally, the skin ulcers were bandaged with antiseptic gauze. Self-control ulcers were treated with 0.9 per cent NaCl without ECGFs. All skin ulcers, with ECGFs and 0.9 per cent NaCl, had a dressing change every day until each skin ulcer was completely healed. Ulcers were evaluated and ulcer area was measured every week.

To compare healing time of the three kinds of skin ulcers, we used the one-tail student t-test.

Results

The ulcers with the treatment of ECGFs had growth of fresh granulation tissue within a shorter time. The ulcer surface bled easily yet mildly when touched. The area of ulcer contracted gradually and the epidermis migrated concentrically from the rim of the ulcer until it covered the ulcer completely.

The mean time taken for 50 per cent of the ulcers with ECGFs to heal was significantly shorter than with 0.9 per cent NaCl (control) (Table 2). In the former, the time taken to achieve 50 per cent healing was accelerated by 3.4 weeks.

There was a time dependent relation between the area change of the ulcer and the application time of ECGFs. Ulcer areas showed gradient diminution as the treatment time of ECGFs

extended. The healing of the ulcer area reached 1cm² per week. The speed of ulcer healing was rapid and obvious 1-2 weeks before complete healing (Figure 1).

We also observed the time taken to heal 50, 80 and 100 per cent of the area of the ulcer (Table 3). Comparing the time taken to heal 100 per cent of the three kinds of skin ulcers, healing time of vasculitis ulcer was longest (7.7 weeks), varicose ulcer was second (5.3 weeks) and decubitus ulcer was the shortest (4 weeks). There was a significant difference in time of 100 per cent healing between vasculitic ulcers and non-vasculitic ulcers (varicose and decubitus) ($t=2.467$, $p<0.05$).

Discussion

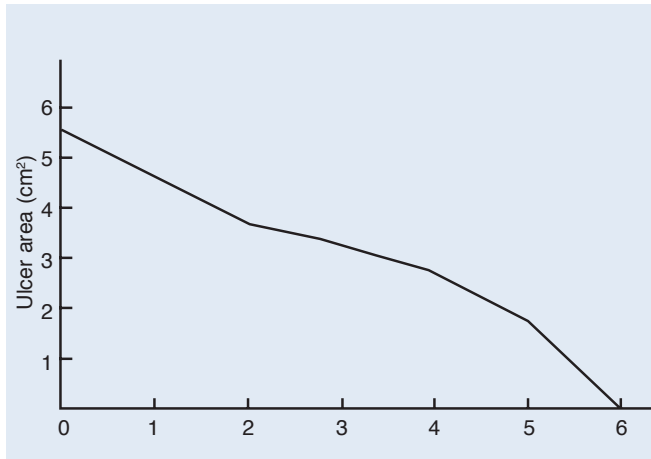
Skin ulcer healing is a complex biologic process including four phases; inflammation, proliferation-migration, deposition and maturation. At the proliferation-migration phase, the quantity of endothelial cells and fibroblasts increases and these cells began to migrate towards the centre of the ulcer. This phase is very important to skin ulcer healing.

It was known that some growth factors, such as basic fibroblast growth factor (BFGF), transforming growth factor (TGF) and platelet derived growth factor (PDGF) improved cutaneous wound healing⁵. The ECGFs we used were extracted from bovine brain and purified using HPLC

Table 2. Time to heal 50 per cent of skin ulcers.

	Number of ulcers	50% healing (weeks)
ECGFs	22	3.1±1.3*
Control	20	6.5±2.7
* $t=4.635$, $p<0.001$		

Figure 1. Ulcer healing over the course of treatment.



column. Brain-derived ECGFs can elevate mitosis capacity of endothelial cells and fibroblasts; a key mechanism in ulcer healing.

We observed the effect of ECGF treatment on skin ulcers. The time taken for 50 per cent of the area of ulcers with ECGFs to heal was reduced by half in comparison to the control. It was therefore indicated that ECGF was a potent biological factor improving skin ulcer healing. Topical application of ECGFs resulted in appearance of fresh granulation with mild bleeding. It was a morphologic manifestation that ECGFs stimulated fibroblast division and angiogenesis⁶.

We also found that the epidermis migrated towards the centre from the ulcer margin which implied that ECGFs might induce migration or proliferation of keratinocytes. Both ECGF and FGF- α were reported to have similar source, molecular weight, isoelectric point, even immunologic and receptor properties^{7, 8}. FGF- α is a potent mitogen for endothelial cells, dermal fibroblasts and keratinocytes⁹.

The clinical presentation of cutaneous vasculitis may be purpura, papules, necrosis and ulceration. In vasculitis ulcers, the blood vessel is injured and the endothelial cell is exhausted or destroyed. We found that healing time of vasculitic ulcers was significantly longer than non-vasculitic ulcer, suggesting a degree of vessel injury has a direct correlation with the effect of ECGF on ulcer healing. In

Table 3. Time to heal 50, 80 and 100 per cent of ulcer area in three kinds of skin ulcers (weeks: $\bar{X} \pm SD$).

	50%	80%	100%
Vasculitis	4.1 \pm 1.1	6.1 \pm 1.6	7.7 \pm 2.2
Varicose	2.9 \pm 1.1	4.1 \pm 1.5	5.3 \pm 1.7
Decubitus	1.8 \pm 0.8	3.0 \pm 0.9	4.0 \pm 0.9

addition, insufficient levels of ECGF receptors in ulcer tissue may contribute to the molecular mechanism of chronic wound formation.

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Editors' note

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Please contact Gary Davidson on (08) 9382 3911 or email gary@cambridgemediacom.au