Intralesional administration of epidermal growth factor-based formulation (Heberprot-P) in chronic diabetic foot ulcer: treatment up to complete wound closure

José I Fernández-Montequín, Blas Y Betancourt, Gisselle Leyva-Gonzalez, Ernesto L Mola, Katia Galán-Naranjo, Mayte Ramírez-Navas, Sergio Bermúdez-Rojas, Felix Rosales, Elizeth García-Iglesias, Jorge Berlanga-Acosta, Ricardo Silva-Rodriguez, Marianela Garcia-Siverio, Luis H Martinez

Fernández-Montequín JI, Betancourt BY, Leyva-Gonzalez G, Mola EL, Galán-Naranjo K, Ramírez-Navas M, Bermúdez-Rojas S, Rosales F, García-Iglesias E, Berlanga-Acosta J, Silva-Rodriguez R, Garcia-Siverio M, Martinez LH. Intralesional administration of epidermal growth factor-based formulation (Heberprot-P) in chronic diabetic foot ulcer: treatment up to complete wound closure. Int Wound J 2009;6:67–72.

ABSTRACT

Previous studies have shown that an epidermal growth factor-based formulation (Heberprot-P) can enhance granulation of high-grade diabetic foot ulcers (DFU). The aim of this study was to explore the clinical effects of this administration up to complete wound closure. A pilot study in 20 diabetic patients with full-thickness lower extremity ulcers of more than 4 weeks of evolution was performed. Mean ulcer size was 16·3 $\pm$ 21·3 cm^2. Intralesional injections of 75 μg of Heberprot-P three times per week were given up to complete wound healing. Full granulation response was achieved in all 20 patients in 23·6 $\pm$ 3·8 days. Complete wound closure was obtained in 17 (85%) cases in 44·3 $\pm$ 8·9 days. Amputation was not necessary in any case and only one relapse was notified. The most frequent adverse events were tremors, chills, pain and ardour at site of administration and local infection. The therapeutic scheme of intralesional Heberprot-P administration up to complete closure can be safe and suitable to improve the therapeutic goal in terms of healing of chronic DFU.

Key words: Diabetic foot ulcers ● Epidermal growth factor ● Wound healing

Authors: JI Fernández-Montequín, National Institute for Angiology and Vascular Surgery, Havana, Cuba; BY Betancourt, Clinical Trials Division, Center for Biological Research, Havana, Cuba; G Leyva-Gonzalez, Health Lodging 'Manuel Fajardo'; EL Mola, Center for Genetic Engineering and Biotechnology, Havana, Cuba; K Galán-Naranjo, Health Lodging 'Manuel Fajardo'; M Ramírez-Navas, Health Lodging 'Manuel Fajardo'; S Bermúdez-Rojas, 'Hermanos Amejeiras' University Hospital, Havana, Cuba; F Rosales, Health Lodging 'Manuel Fajardo'; E García-Iglesias, Clinical Trials Division, Center for Biological Research, Havana, Cuba; J Berlanga-Acosta, Center for Genetic Engineering and Biotechnology, Havana, Cuba; R Silva-Rodriguez, Center for Genetic Engineering and Biotechnology, Havana, Cuba; M Garcia-Siverio, Center for Genetic Engineering and Biotechnology, Havana, Cuba; and LH Martinez, Center for Genetic Engineering and Biotechnology, Havana, Cuba
Address for correspondence: BY Betancourt, Clinical Trials Division, Center for Biological Research, PO Box 6332, Havana, Cuba
E-mail: blas.yamir@cigb.edu.cu

Key Points

- diabetic foot ulcers (DFU) are a significant health care problem affecting around 15% of the people with diabetes mellitus in their lifetime
- epidermal growth factor (EGF) plays an important role in the regulation of cell growth, proliferation and differentiation that can be useful to enhance wound healing
- however, the effect of topical EGF formulation can be abated, especially in high-grade wounds since an increased protease activity has been identified
- direct intralesional administration of an EGF-based formulation (Heberprot-P) can overcome this limitation, as has been reported in previous studies and recently confirmed in a randomised, double-blinded, placebo controlled phase III trial in DFU (data not yet published)
- this pilot study was performed to explore the safety profile and the clinical effects of Heberprot-P in chronic, non healing DFU up to complete wound closure

INTRODUCTION

Diabetic foot ulcers (DFU) are a significant health care problem affecting around 15% of the people with diabetes mellitus in their lifetime (1). The annual incidence of DFU is more than 2% in all diabetic patients (2) and largely rises when peripheral neuropathy is present (3). This condition is an important economic burden to medical care systems demanding 7–20% of the total expenditures on diabetes (4).

Basic treatment for good wound care in DFU includes strict metabolic control, debridement, offloading (i.e. relieving pressure from the wound area), dressings and antimicrobials. New therapies are emerging for low-grade, neuropathic ulcers such as recombinant human platelet-derived growth factor (5,6) and artificial skin substitutes (7,8). For high-grade ulcers, which are more likely to progress to amputation, advances in therapy have been scarce.

Epidermal growth factor (EGF) plays an important role in the regulation of cell growth, proliferation and differentiation that can be useful to enhance wound healing (9). Evidences of the beneficial effect of topical EGF application in low-grade, neuropathic ulcers have been shown in clinical trials (10–12). However, the effect of topical EGF formulation can be abated, especially in high-grade wounds since an increased protease activity has been identified (13–16). Direct intralesional administration of an EGF-based formulation (Heberprot-P®) can overcome this limitation, as has been reported in previous studies (17,18) and recently confirmed in a randomised, double-blinded, placebo-controlled phase III trial in DFU (data not yet published).

In these initial studies, however, Heberprot-P intralesional treatment was continued until a complete granulation response or up to a maximum of 8 weeks. Thus, the safety profile of this intervention modality under a more prolonged application schedule had not been characterised so far. Although with the 8 weeks scheme, complete wound healing and reduction in the amputation risk was attained, better results were expected if the treatment continues up to complete wound healing. This pilot study was performed to explore the safety profile and the clinical effects of Heberprot-P in chronic, non healing DFU up to complete wound closure.

MATERIALS AND METHODS

A pilot study was performed in 20 patients older than 18 years with diabetes mellitus and full-thickness lower extremity ulcer with more than 4 weeks of evolution. Informed consent to participate in the study was given by the patients. Exclusion criteria were foot ulcer area ≤ 1 cm^2, cardiopathy (recent acute myocardial infarction, unstable angina or uncontrolled heart failure), renal failure (serum creatinine >200 μmol/l and oligoanuria), malignancies, pregnancy and nursing. The exclusion criteria were evaluated during an initial period (2 weeks) when patients received only the standardised good wound care and no more than a 30% decrease in the ulcer size was required. Any sign or symptom of infection should be solved before inclusion as well. This study was approved by institutional review committee.

Patients were treated with intralesional injections of a lyophilised formulation of Heberprot-P containing 75 μg (one vial) of EGF, three times a week on alternate days up to complete wound healing. Recombinant, human EGF was obtained from a transformed *Saccharomyces cerevisiae* strain at the Center for Genetic Engineering and Biotechnology of Havana and contained a mixture of the EGF1-51 and EGF1-52 forms (19). The dose selection was based on a better risk-benefit balance observed with 75 μg in the accumulated clinical data with this product.

Heberprot-P was administered together with a standardised good wound care regimen. Ulcers were cleansed daily using saline or chlorhexidine in case of contamination or infection. Sharp debridement was indicated whenever necessary to remove necrotic tissue. Saline-moistened gauze dressing was used and the affected area was pressure off-loaded. Broad-spectrum antibiotics were used to treat infections, whereas metabolic control was managed with insulin alone or combined with oral hypoglycaemic drugs. Patients were initially hospitalised at 'Hermanos Amejeiras' Hospital up to favourable clinical response (granulation, wound area reduction, no infection or requirement for invasive procedure and adequate metabolic control). The follow up for treatment completion was carried out at the Health Lodging 'Manuel Fajardo', supervised by the same angiologist.

Table 1 Baseline characteristics of the patients

Characteristics	Results
Age (years)*	59·1 ± 7·4
Gender, *n* (%)	
Males	16 (80·0)
Females	4 (20·0)
Race	
White, *n* (%)	4 (20·0)
Non white, *n* (%)	16 (80·0)
Time with diabetes (years)*	14·2 ± 11·3
Ulcer duration (days)*	264 ± 294
Ulcer size (cm^2)*	16·3 ± 21·3
Wagner's classification, *n* (%)	
Grade 2	1 (5·0)
Grade 3	16 (80·0)
Grade 4	3 (15·0)
History of ulcer	13 (65·0)
History of amputation	10 (50·0)

*Values are expressed as mean ± SD.

Evaluation consisted of baseline and weekly clinical examinations. Data on demography, personal pathological history, type and duration of diabetes and its current treatment, peripheral neuropathy, peripheral vascular disease and wound examination were documented. Wound area was determined by planimetry using a wound measurement system (Visitrak™; Smith & Nephew, Mull, UK). Ankle/brachial index was taken at baseline. Ulcers were classified in grades according to Wagner (1). Laboratory tests were performed at baseline and thereafter whenever required, including blood cell count, haemoglobin, haematocrit, globular sedimentation rate, creatinine and aspartate aminotransferase, which were performed by routine clinical laboratory methods. Blood glucose was measured more frequently for the patients' metabolic control. Wound cultures were performed before and during therapy if necessary to monitor infections. Foot infection was defined clinically based on the presence of purulent secretions or at least two signs or symptoms of inflammation (20).

The primary efficacy endpoint was complete wound closure defined as skin reepithelialisation without drainage or dressing requirements. Other variables recorded were complete granulation response, time to complete closure, time to complete granulation response and indication of amputation. Safety was monitored by daily adverse events evaluation during treatment.

Data were double entered and validated on Microsoft Visual FoxPro version 5.0 and then imported to SPSS version 13.0 for further analysis. Continuous variables were expressed as mean ± SD. Categorical variables were given as absolute values and percentages. The confidence intervals (CI) for the probabilities of complete granulation and complete wound closure were estimated using a Bayesian logistic model for fixed effects in WinBUGS14 package. Kaplan–Meier curves for time to complete granulation and complete wound closure were also calculated.

RESULTS

Patients demographic and baseline characteristics are shown in Table 1. They all suffered type 2 diabetes mellitus and five (25%) patients received insulin. Mean ulcer size was 16·3 ± 21·3 cm^2. In nine (45%) patients, wounds were localised on the sole, two of them embracing calcaneous. Other localisations were toes in eight (40·0%), foot external edge in two (10·0%) and internal edge in one (5·0%) patients. The principal risk factors were previous history of ulcer in 13 (65·0%) patients, history of amputation in 10 (50·0%) and foot deformity in 10 (50·0%) patients.

Complete treatment compliance was reported in 17 (85%) patients. Voluntary interruption was reported in three (15%) cases. Complete granulation response was achieved in all patients, including the three abandoners, at a mean time of 23·6 ± 3·8 days (95% CI: 15·6–31·5). Complete wound closure was obtained in 17 (85%) patients (95% CI: 0·64–0·95). The mean time to complete closure was 44·3 ± 8·9 days (95% CI: 26·9–61·8). Amputation was not necessary in any case and relapse was reported in one patient after 6 months of complete closure.

Figure 1 shows examples of wounds' clinical aspects. Photos D, E and F show the ulcer of the most complicated patient. This was a 63-year-old male with diabetes for 30 years and an extensive wound (area 52·4 cm^2) on the calcaneous. Infection, ischaemia and osteomyelitis were also present and amputation had been previously indicated by other specialists as the only alternative. After soft tissue debridement, bone resection within the necrotic area and

Key Points

- the primary efficacy endpoint was complete wound closure defined as skin epithelialisation without drainage or dressing requirement

Key Points

- this study shows that continuity of the treatment with intralesional Heberprot-P up to complete wound closure is feasible and safe to promote healing of chronic DFU
- treatment was well tolerated, adverse events were easily manageable and no significant safety concern was reported

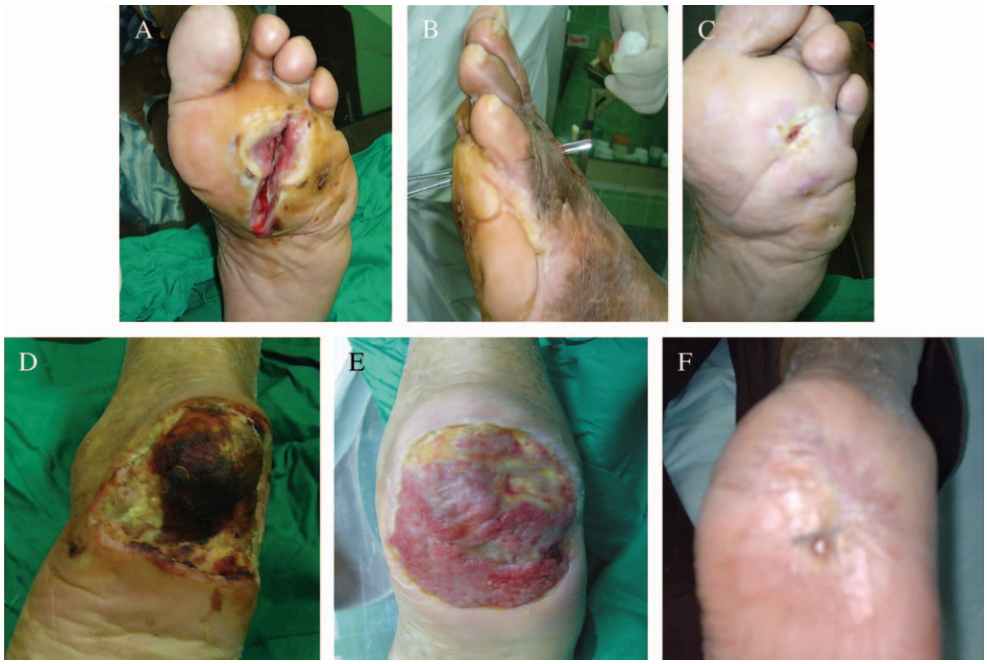

Figure 1. Comparative photos of some patients. (A, B, C) show an extensive neuropathic ulcer with abscess. (A) Before treatment, (B) evidence of dorsum-plantar communication and (C) after treatment. (D, E, F) show an extensive ischaemic ulcer with osteomyelitis and infection. (D) Before treatment, (E) response at the end of treatment (F) after follow up (for more details see the text).

broad-spectrum antibiotics, Heberprot-P intervention was thereafter instituted. Complete granulation response was achieved in 63 days when the patient withdrew from treatment. He was re-evaluated thereafter and complete wound closure was confirmed at day 143.

The rate of adverse events is shown in Table 2. The most frequent were tremors, chills, pain and burning at site of administration, and local infection. Most of the adverse events were classified as mild or moderate. The treatment was not interrupted because of adverse events.

Table 2 Adverse events frequency

Events	*n* (%)
Tremors	11 (55·0)
Chills	8 (40·0)
Pain at site of administration	5 (25·0)
Burning at site of administration	5 (25·0)
Local infection	4 (20·0)
Weakness	1 (5·0)
Fever	1 (5·0)
Headache	1 (5·0)
Loss of consciousness	1 (5·0)
Hypotension	1 (5·0)
Sweat	1 (5·0)

DISCUSSION

The primary objective of treatment for DFU is to obtain complete wound closure as expeditiously as possible. Therapy with a growth factor should be maintained up to achieve this goal. In this sense, this study shows that the continuity of the treatment with intralesional Heberprot-P up to complete wound closure is feasible and safe to promote healing of chronic DFU. Treatment was well tolerated, adverse events were easily manageable and no significant safety concern was reported.

These results are better than those reported in previous trials (17,18). After Heberprot-P was administered in high-grade DFU in an up to 8-week treatment schedule, a complete granulation response appeared in 73% of the patients. Complete wound healing was reached in 54% of the patients after 20 weeks since the beginning of the treatment (18). In contrast, the analysis of present study showed that the continuity of treatment was associated to improvement in the rate of both granulation response and complete wound closure. Generally, when complete

granulation occurs following administration of the formulation, a partial epithelisation is also present that continue until complete closure, although treatment had ceased. It seem that the stimulation of granulation response by Heberprot-P treatment is an important step to enhance healing, but while the ulcer does not reach complete closure the risks for infection and amputation cannot be neglected.

Recent clinical trials with an EGF-based topical formulation support the efficacy of this growth factor for enhancing DFU healing (10–12). All these studies were conducted in low-grade, neuropathic DFU. Similarly, another growth factor, such as recombinant human platelet-derived growth factor-BB (becaplermin) has been topically used in neuropathic and small-sized lesions (5,6). There is no evidence, however, of beneficial effect of these growth factors in high grade and ischaemic ulcers.

Moreover, the treatment with growth factors in DFU may be of limited success because they are susceptible to degradation by proteases that have been recognised as a significant impediment for wound healing (13–16). This may explain the beneficial effect of the strategy based on protease modulating combined with autologous growth factors to enhance the efficacy in DFU (21). The still active factor may be unavailable for biologic activity because of trapping or binding to molecules such as fibrinogen, macroglobulin or albumin (22,23). Another strategy to overcome the effect of the mentioned limitation is the intralesional injection of the growth factor to the desired region, which is the base of the rationality of this intervention.

The present study is limited by the small number of patients and by the absence of a concurrent group for a proper comparison. The selected patients enter in a initial enrolment period when they received only the standardised wound care and did not had more than a 30% decrease in the ulcer. This approach has been proposed to minimise the variability because of the improvement in chronic ulcer healing by standard treatment (24). However, it is difficult to quantify the exact effect because of the study treatment from those caused by standard therapy. Anyhow, this result offers a proof of concept that intralesional Heberprot-P administration up to complete closure can be safe and suitable to improve healing of chronic DFU and also provide the basis for further clinical trials design.

In summary, intralesional administration of Heberprot-P up to complete wound closure in DFU, in association to good wound care measures, accelerates wound healing without any evidence of safety limitations. A large controlled study is needed to confirm these results.

ACKNOWLEDGMENTS

BYB and EG-I are employees of the Center for Biological Research, which is part of the Center for Genetic Engineering and Biotechnology, Havana's network, where Heberprot-P is produced and the new formulation was developed. JB-A, ELM, RS-R, MG-S and LHM are employees of the Center for Genetic Engineering and Biotechnology. The rest of the authors have no conflict of interests. The study was financed by Heber Biotec, Havana, Cuba (product) and the Ministry of Public Health of Cuba (hospital facilities and general medical care of the hospitalised patients). The authors are grateful to Dr Pedro Lopez-Saura for his revision of the manuscript as well as to the personnel involved in the patients' care.

REFERENCES

1 Frykberg RG, Zgonis T, Armstrong DG, Driver VR, Giurini JM, Kravitz SR, Landsman AS, Lavery LA, Moore JC, Schuberth JM, Wukich DK, Andersen C, Vanore JV. American College of Foot and Ankle Surgeons. Diabetic foot disorders. A clinical practice guideline (2006 revision). J Foot Ankle Surg 2006;45(5 Suppl):S1–S66.

2 Abbott CA, Carrington AL, Ashe H, Bath S, Every LC, Griffiths J, Hann AW, Hussein A, Jackson N, Johnson KE, Ryder CH, Torkington R, Van Ross ERE, Whalley AM, Widdows P, Williamson S, Boulton AJM. The North-West Diabetes Foot Care Study: incidence of, and risk factors for, new diabetic foot ulceration in a community-based patient cohort. Diabet Med 2002;19:377–84.

3 Abbott CA, Vileikyte L, Williamson S, Carrington AL, Boulton AJM. Multicenter study of the incidence of and predictive risk factors for diabetic neuropathic foot ulceration. Diabetes Care 1998;21:1071–5.

4 Boulton AJ, Vileikyte L, Ragnarson-Tennvall G, Apelqvist J. The global burden of diabetic foot disease. Lancet 2005;366:1719–24.

5 Wieman TJ, Smiell JM, Su Y. Efficacy and safety of a topical gel formulation of recombinant human platelet-derived growth factor-BB (becaplermin) in patients with chronic neuropathic diabetic ulcers. A phase III randomized placebo-controlled double-blind study. Diabetes Care 1998;21:822–7.

6 Margolis DJ, Bartus C, Hoffstad O, Malay S, Berlin JA. Effectiveness of recombinant human platelet-derived growth factor for the treatment of diabetic

Key Points

- the present study is limited by the small number of patients and by the absence of a concurrent group for a proper comparison
- this result offers a proof of concept that intralesional Heberprot-P administration up to complete closure can be safe and suitable to improve healing of chronic DFU and also provide the basis for further clinical trials design
- in summary, intralesional administration of Heberprot-P up to complete wound closure in DFU, in association to good wound care measures, accelerates wound healing without any evidence of safety limitations
- a large controlled study is needed to confirm these results

neuropathic foot ulcers. Wound Repair Regen 2005;13:531–6.

7 Veves A, Falanga V, Armstrong DG, Sabolinski ML; Apligraf Diabetic Foot Ulcer Study. Graftskin, a human skin equivalent, is effective in the management of noninfected neuropathic diabetic foot ulcers: a prospective randomized multicenter clinical trial. Diabetes Care 2001;24:290–5.

8 Marston WA, Hanft J, Norwood P, Pollak R; Dermagraft Diabetic Foot Ulcer Study Group. The efficacy and safety of Dermagraft in improving the healing of chronic diabetic foot ulcers: results of a prospective randomized trial. Diabetes Care 2003;26:1701–5.

9 Boonstra J, Rijken P, Humbel B, Cremers F, Verkleij A, van Bergen en Henegouwen P. The epidermal growth factor. Cell Biol Int 1995;19:413–30.

10 Tsang MW, Wong WKR, Hung CS, Lai KM, Tang W, Cheung EYN, Kam G, Leung L, Chan CW, Chu CM, Lam EKH. Human epidermal growth factor enhances healing of Diabetic foot ulcers. Diabetes Care 2003;26:1856–61.

11 Hong JP, Jung HD, Kim YW. Recombinant human epidermal growth factor (EGF) to enhance healing for diabetic foot ulcers. Ann Plast Surg 2006;56:394–8.

12 Viswanathan V, Pendsey S, Sekar N, Murthy GSR. A phase III study to evaluate the safety and efficacy of recombinant human epidermal growth factor (REGEN-D™ 150) in healing diabetic foot ulcers. Wounds 2006;18:186–96.

13 Berlanga J, Lodos J, Reyes O, Infante JF, Caballero E, López-Saura P. Epidermal growth factor stimulated re-epithelialization in pigs. The possible role of acute-wound proteases. Biotecnología Aplicada 1998;15:83–7.

14 Trengove NJ, Stacey MC, MacAuley S, Bennett N, Gibson J, Burslem F, Murphy G, Schultz G. Analysis of the acute and chronic wound environments: the role of proteases and their inhibitors. Wound Repair Regen 1999;7:442–52.

15 Medina A, Scott PG, Ghahary A, Tredget EE. Pathophysiology of chronic nonhealing wounds. J Burn Care Rehabil 2005;26:306–19.

16 Mast BA, Schultz GS. Interactions of cytokines, growth factors, and proteases in acute and chronic wounds. Wound Repair Regen 1996;4:411–20.

17 Berlanga J, Savigne W, Valdez C, Franco N, Alba JS, Del Rio A, López-Saura P, Guillén G, Lopez E, Herrera L, Férnandez-Montequín J. Epidermal growth factor intra-lesional infiltrations can prevent amputation in diabetic patients with advanced foot ulcers. Int Wound J 2006;3:232–9.

18 Fernández-Montequín JI, Infante-Cristiá E, Valenzuela-Silva C, Franco-Pérez N, Savigne-Gutiérrez W, Artaza-Sanz H, Morejón-Vega L, González-Benavides C, Eliseo-Musenden O, García-Iglesias E, Berlanga-Acosta J, Silva-Rodríguez R, Betancourt BY, López-Saura PA, Cuban Citoprot-P Study Group. Intra-lesional injections of Citoprot-P® (recombinant human epidermal growth factor) in advanced diabetic foot ulcers with risk of amputation. Int Wound J 2007;4:333–43.

19 Cinza AM, Quintana M, Lombardero J, Poutou R, Pérez E, Pérez LC, Castellanos L, Estrada R, Morales J. A batch process for production of human epidermal growth factor in yeast. Product characterization. Biotecnología Aplicada 1991;8:166–74.

20 Lipsky BA, Berendt AR, Deery HG, Embil JM, Joseph WS, Karchmer AW; Infectious Diseases Society of America. Diagnosis and treatment of diabetic foot infections. Clin Infect 2004;39:885–910.

21 Kakagia DD, Kazakos KJ, Xarchas KC, Karanikas M, Georgiadis GS, Tripsiannis G, Manolas C. Synergistic action of protease-modulating matrix and autologous growth factors in healing of diabetic foot ulcers. A prospective randomized trial. J Diabetes Complications 2007;21:387–91.

22 Falanga V, Eaglstein WH. The trap hypothesis of venous ulceration. Lancet 1993;341:1006–8.

23 Robson MC, Smith PD. Topical use of growth factors to enhance healing. In: Falanga V, editor. Cutaneous wound healing. London: Martin Dunitz Limited, 2001:379–98.

24 Guidance for industry: chronic cutaneous ulcer and burn wounds—developing products for treatment. US Department of Health and Human Services, Food and Drug Administration Web site [WWW document]. URL www.fda.gov/cber/gdlns/ulcburn.pdf [accessed on 7 April 2008].