Placental angiogenic and growth factors in the treatment of chronic varicose ulcers: preliminary communication

H Burgos MPH PhD **A Herd** MB BS **J P Bennett** FRCS
Blond McIndoe Centre for Medical Research, Queen Victoria Hospital, East Grinstead, Sussex RH19 3DZ

Keywords: skin ulcers; growth substances; angiogenic factors; granulation tissue; wound healing

Summary
A short-term clinical study on the effect of purified angiogenic and growth factors from human term placenta in the treatment of chronic varicose ulcers was carried out in 18 patients. Patients were randomly allocated to receive a maximum of two dressings containing or not containing these factors. The amount of granulation and epithelial tissue was clinically estimated 48 hours after each application. Patients treated with placental angiogenic and growth factors showed increased granulation and epithelial tissue. These results indicate that placental factors may be used for acceleration of wound healing.

Introduction
Previous studies have demonstrated that application of human amnion membranes for the treatment of chronic venous ulcers caused the formation of a profusely vascular granulation tissue[1,2]. The use of amnion membranes, however, demands certain laboratory procedures which fall outside routine activities of a hospital. Incorporation of placenta angiogenic and growth factors (PGFs) in a hydrocolloid membrane carrier, that replaces the amnion membrane itself, affords a convenient way for local application of these factors[3]. A short-term, double-blind initial clinical trial of PGFs incorporated in Geliperm was designed to study the effect of these factors on the granulation of chronic varicose ulcers.

Patients and methods
Approval for this trial was obtained from the Hospital Ethical Committee. Patients, admitted via outpatient referrals, agreed to inclusion in a controlled study, and understood they might not be treated with PGFs. All patients, however, would receive adequate treatment, including surgery and skin grafts, following these studies. Patients with evidence of arteriopathy or aetiology other than venous origin, and patients less than 40 years old and more than 85 years old were excluded from the trial.

Preparation of dressings
PGFs from human term placenta were purified by chromatography methods[4,5], and dressings prepared as previously described[3]. Briefly, pieces of Geliperm-dry, a gel dressing made of 1% agar and 3.5% crosslinked polyacrylamide (Geistlich, Wolhunsen, Switzerland), were cut to appropriate size and placed in a sterile dish. Solution of PGFs in buffered saline was evenly dispensed over the gel at a ratio 26 μg protein/cm^2 gel, and maintained at room temperature (22°C) for 2 hours to allow PGF incorporation. Control dressings were prepared in a similar way with buffered saline devoid of PGFs.

Protocol
Patients were randomly allocated to receive Geliperm dressings with or without PGFs. Details of the randomization were not disclosed to the clinical team. Initial ulcer cleansing was carried out with half-strength Eusol followed by Ringer's solution wash and daily dressing with Jelonet, Aserbine or a combination of the two until clean and free of pathogens. Geliperm dressing (with or without PGFs) was then applied and left for 48 hours. Application was repeated if no change had occurred in the ulcer bed. After two applications any ulcer that had still not formed sufficient granulation tissue for skin graft was treated with Jelonet-based dressings until ready for surgery. Procedures following Geliperm dressings were out of the scope of the present studies. Amount of granulation was estimated by visual observation of the ulcer and 35 mm colour photographs taken under standard conditions, and graded as follows: 1, amount of granulation insufficient to allow skin graft; 2, amount of granulation adequate for skin graft; and 3, overgranulation. Initial epithelialization was also recorded. Clinical evaluation was carried out by the same member of the clinical team throughout the duration of the studies.

Results
Table 1 summarizes the main clinical details and results obtained. Most patients had a history of deep venous thrombosis and treatment by vein injection and sclerotherapy or ligation and stripping followed by skin grafts. More females than males entered the trial (ratio 2.6 : 1). Randomization procedures allocated 11 patients to receive PGF-Geliperm dressings (experimental group) and 7 patients to receive saline-Geliperm dressings (control group). Mean age (±standard deviation) was 63.73±13.78 years in the experimental group, and 71.57±8.28 years in the control group. Mean ulcer duration was 11.64±7.35 years in the experimental group, and 24.57±15.34 years in the control group. All patients but one (patient number 3) in the experimental group showed increased granulation and initial epithelialization after one PGF-Geliperm dressing, except patient number 11 who needed two applications. None of the patients in the control group showed any change in granulation or epithelialization even after two saline-Geliperm dressings, except patient number 12 presenting a marginal increase in granulation without initial epithelialization.

Discussion
Higher incidence of ulcers in females has been found in the past[1], and this was not considered unusual. Age was not significantly different between experimental and control groups. Randomization

0141-0768/89/100598-02/$02.00/0

Table 1. Granulation and epithelial tissue in chronic ulcers treated with placental angiogenic and growth factors

Patient number	Sex	Age (years)	Before dressing G●	Before dressing E■	After 1st dressing G	After 1st dressing E	After 2nd dressing G	After 2nd dressing E	Ulcer duration (years)	Cause or complication and previous treatment
Experimental Group										
1	F	42	1	−	3	+			14	Deep venous thrombosis, pernicious anaemia, split skin graft×5
2	F	62	1	−	2	+			25	Vein injection, veins stripped
3	M	53	1	−	1	−	1	−	18	Deep venous thrombosis, oedema, eczema
6	M	75	1	−	2	+			3	Chronic icthyopia, cellulitis
9	F	74	1-2	−	3	+			17	Rheumatoid arthritis, split skin graft
10	M	54	1	−	2	+			5	Deep venous thrombosis, eczema, vein injection
11	F	44	1	−	1	−	2	+	14	Deep venous thrombosis, split skin graft×2
13	F	84	1	−	3	+			2	Deep venous thrombosis, phlebitis, vein injection
14	F	66	1	−	2	+			15	Rheumatoid arthritis, chemical sympathectomy, split skin graft
17	F		1	−	1-2	+			11	Veins stripped, pinch graft×2, split skin graft
18	F	76	1-2	−	2	+			4	Deep venous thrombosis, pinch graft×3, split skin graft
Control group										
15	M	64	1	−	1	−	1	−	12	Deep venous thrombosis, pinch graft×3
4	F	67	1-2	−	1-2	−	1-2	−	11	Diabetes, split skin graft
5	F	64	1	±	1	±	1	±	37	Phlebitis, split skin graft×2
7	F	68	1	±	1	±	1	±	15	Deep venous thrombosis, pinch graft
8	F	84	1	−	1	−	1	−	53	Veins stripped, split skin graft
12	M	82	1	−	1	−	1-2	−	21	Deep venous thrombosis, veins stripped, split skin graft
16	F	72	1-2	−	1-2	−	1-2	−	23	Deep venous thrombosis, split skin graft×2

●Granulation: 1, insufficient to allow skin graft; 2, adequate for skin graft; 3, overgranulation
■ Presence of initial epithelialization. +present; −absent; ±marginal

procedures resulted in the control group having twice the average ulcer duration than the experimental group. This probably had little bearing upon the results of the study but may be a cause for criticism. Continued recruitment of patients into a full-scale clinical trial will clarify this issue. Local application of purified PGFs produced a remarkably accelerated formation of granulation tissue. One application of PGFs was, in most cases, enough to produce adequate amounts of granulation for skin graft. Initiation of epithelialization was an additional effect found in the experimental group. Eventually all patients, both experimental and control, received split skin grafts.

The present studies were designed to find trends on the effect of PGFs in the treatment of chronic ulceration. Results obtained indicate that purified PGFs accelerate the formation of granulation and epithelialization in chronic venous ulcers. Further studies, however, will be needed to find out optimum dose and frequency of application. The search for different combinations of growth factors that would speed wound healing have, so far, been confined to the most well known and characterized growth factors, epidermal growth factor, fibroblast growth factor, platelet derived growth factor, transforming growth factors[6]. Results of the present studies suggest that PGFs, a family of angiogenic peptides with different molecular characteristics than those proteins[5], may offer a unique combination of growth factors for acceleration and modulation of wound healing.

Acknowledgments: This work was supported by grants from the Blond McIndoe Medical Research Trust. Geliperm was kindly supplied by Geistlich-Chester.

References

1 Bennett JP, Matthews RN, Faulk WP. The treatment of chronic ulceration of the legs with human amnion. *Lancet* 1980;i:1153-6
2 Faulk WP, Matthews RN, Stevens PJ, Bennett JP, Burgos H, Hsi BL. Human amnion as an adjunct in wound healing. *Lancet* 1980;i:1156-8
3 Burgos H. Incorporation and release of placental growth factors in synthetic medical dressings. *Clin Mater* 1987;2:133-9
4 Burgos H. Angiogenic and growth promoting factors in human amnio-chorion and placenta. *Eur J Clin Invest* 1983;13:289-96
5 Burgos H. Angiogenic factor from human term placenta. Purification and partial characterization. *Eur J Clin Invest* 1986;16:486-93
6 Brunt JV, Klausner A. Growth factors speed wound healing. *Biotechnology* 1988;6:25-30

(Accepted 16 February 1989)