Clin Exp Med
DOI 10.1007/s10238-011-0136-7

ORIGINAL ARTICLE

A new protocol for the treatment of the chronic venous ulcers of the lower limb

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Received: 11 January 2011 / Accepted: 16 April 2011

Abstract Venous leg ulcer is a pathological condition afflicting prevalently elderly patients, which has been found to have a major impact on individuals' health and social aspects of quality of life. Actually, the best practice treatment is recommended to include wound dressing and multilayer compression therapy. In this study, we have tested the effectiveness and safety of Vulnamin®, a novel dressing in the form of a metal cellulose gel containing the amino acids glycine, L-lysine, L-proline, L-leucine, and hyaluronic acid, and elastic compressive bandages in the treatment of chronic venous ulcers of the lower limbs. The study has been conducted in two groups of patients, one treated with Vulnamin® plus Ca-alginate (ulcer duration = 25.4 ± 6.2 weeks; mean baseline ulcer area = 13.9 ± 4.5 cm^2) and a control group treated with Ca-alginate alone (ulcer duration = 23.4 ± 4.2 weeks; mean baseline ulcer area = 15.1 ± 4.7 cm^2). Results have shown that after 70 days of treatment patients significantly ameliorate their pathological condition if they are treated with Vulnamin®, as compared with patients treated with Ca-alginate alone. In fact, at the end of the treatment, complete healing closure was 61% in the group treated with Vulnamin® and, respectively, 27% in the control group. Moreover, ulcer areas showed a significant reduction in patients treated with Vulnamin® (mean ulcer area = 3.04 ± 0.8 cm^2), as compared with controls (mean ulcer area = 10.96 ± 3.8 cm^2). Overall, the results of this study indicate that Vulnamin® together with elastocompression is safe and more effective than standard dressing together with elastocompression in inducing a faster healing in chronic venous ulcers of the lower limb.

Keywords Amino acids · Angiogenesis · Chronic venous ulcers · Wound healing

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Introduction

Chronic venous ulcers are the consequence of chronic venous hypertension, which is responsible for the disturbance of dermal microcirculation [1]. One of the major features in patients with chronic venous insufficiency is a leukocyte infiltration into the capillary and post-capillary venules [2]. The leg ulcer is a skin condition afflicting prevalently elderly patients, which has been found to have a major impact on individuals' health and social aspects of quality of life [3, 4].

Best practice treatment of venous leg ulcers is recommended to include wound dressing and multilayer compression therapy [5, 6]. The successful management of patients who have leg ulcers related to chronic venous disease requires optimal management of the wound bed, elimination of edema with compression, and correction of venous hypertension whenever possible.

A novel dressing (Vulnamin®) in form of a powder containing the amino acids glycine, L-lysine, L-proline, L-leucine, and hyaluronic acid, has been proposed for the management of chronic ulcers [7].

This report presents the results of a prospective randomized controlled study designed to test the effectiveness and safety of Vulnamin® and elastic compressive bandages

Published online: 11 May 2011

in the treatment of chronic venous ulcers of the lower limbs.

Materials and methods

The inclusion criteria have been the following: ankle brachial pressure index (ABI) ≥0.8; age 18–70 years; chronic venous ulcers of the lower limbs. The exclusion criteria have been the following: diabetes mellitus; treatment with systemic steroids, immunosuppressive or cytotoxic agents; history of bleeding disorders; delayed wound healing.

A total of 52 patients have been assessed, fulfilling the inclusion criteria. The patients were randomized into two different groups, using a computer-generated randomization list. Patients in group A (26, 14 men and 12 women, mean age 58.6) were treated with local application of Vulnamin® powder (in two capsules to avoid precipitation of the amino acids: the first one containing glycine 1.5 gr., lysine 0.15 gr., leucine 0.25 gr., and hyaluronic acid, and the second one containing proline 1.1 gr.) and Calcium-alginate (Ca-alginate), whereas patients in group B (26, 15 men and 11 women, mean age 57.9) were treated only with Ca-alginate. All patients received three-layer elastic compressive bandaging applied by medical professional, accordingly to the standard criteria for the treatment of the venous ulcers. The compressive bandage was removed at the beginning of each control visit, and a new one was applied at the end of the visit. The leg circumference was measured in cm at malleolus and calf.

At the beginning of treatment, the ulcer duration, the lesion area, and diameter overlapped between the groups and the differences in the mean values were not statistically significant ($P > 0.05$). The mean ulcer duration was 25.4 ± 6.2 weeks in the patients of the group A and, respectively, 23.4 ± 4.2 weeks in the patients of the group B; the mean baseline ulcer area was 13.9 ± 4.5 cm^2 in the patients of group A and, respectively, 15.1 ± 4.7 cm^2 in the patients of group B. Patients were followed-up for 70 days at regular intervals (days 10, 20, 30, 40, 50, 60, and 70); at each control visit, the wound area and its reduction were measured by means of digital camera Canon Digital Ixus 4000 (Canon Inc., Tokyo, Japan) and of computerized image software (Rhinoceros version 3.1, Robert Mc Nell & Associates, Seattle, USA).

All data were expressed as mean ± standard deviation (SD) and analyzed by Student's *t* test. To assess differences in both groups, a statistical analysis of variance and Wilk's lambda test were also performed (Starview, SAS Institute, Cary, IL). *P* values less than 0.05 were considered as significant.

The study protocol and informed consent of the patients were approved by the Ethics local Committee of the University of Bari Medical School.

Biopsy samples and histological analysis

There were no significant differences in terms of demographic and clinical features of patients in both groups.

After application of 2% xylocaine local anesthetic, 1 cm^2 biopsy samples were taken with a No. 15 blade scalpel from the edge of 10 ulcers at the beginning of the treatment and at the end of the treatment (5 for the group A and 5 for the group B). Bioptic specimens were fixed for 2 h in 2% paraformaldehyde and then processed with standard procedure for embedding in paraffin wax. Five-μm-thick sections were cut by microtome and stained with hematoxylin and eosin using a routine protocol.

Results

In Tables 2 and 3, the characteristics of the patients in the group treated with Vulnamin® and Ca-alginate (group A) and, respectively, of the patients in the control group treated with Ca-alginate alone (group B) in terms of ulcer areas expressed in cm^2 at the beginning and at the end of the treatment after 70 days are reported. At the end of the treatment, complete healing closure was 61% (16/26) in the group treated with Vulnamin® and, respectively, 27% (7/26) in the control group. Photographs of representative ulcers of group A and B are reported in Figs. 1 and 2.

Ulcer areas after 70 days of treatment showed a significant ($P < 0.05$) reduction in patients in group A (from 13.95 ± 4.5 cm^2 to 3.04 ± 0.8 cm^2), but not in patients of the group B (from 15.14 ± 4.7 cm^2 to 10.96 ± 3.8 cm^2) (Fig. 3). The differences in the mean values of the ulcer areas between group A and group B patients at baseline and after 10, 20, 30, 40, 50, 60, and 70 day of treatment have been also estimated (Fig. 4), and it has been demonstrated that Vulnamin® plus Ca-alginate treatment induced a more rapidly and progressive reduction in the ulcer areas as

Table 1 Demographic and clinical features of the patients of the group treated with Vulnamin® (Group A) and with Ca-alginate (Group B)

	Group A	Group B
Age	58.6	57.9
Gender (% female)	46.1	47.8
ABPI	1.2 ± 0.3	1.0 ± 0.2
Circumference at malleolus (cm)	24.5 ± 7.2	24.3 ± 7.2
Circumference at calf (cm)	27.4 ± 4.5	28.2 ± 5.3
DVT%	44. 4	40.2
ST%	23.5	21.2
Mean BMI	24 ± 3	25 ± 2

ABPI ankle brachial systolic ratio, *BMI* body mass index, *DVT* deep venous thrombosis, *ST* superficial thrombosis

Table 2 Ulcer areas at the beginning and at the end of treatment of the patients treated with Vulnamin® (Group A)

Patient number	Ulcer area at day 1 (cm^2)	Ulcer area at day 70 (cm^2)
1	10.15 ± 2.22	2.93 ± 0.22
3	3.88 ± 0.52	0.68 ± 0.12
4	4.11 ± 1.45	–
5	36.89 ± 7.48	–
6	9.34 ± 2.32	6.67 ± 0.23
9	4.36 ± 1.23	–
11	2.51 ± 0.15	0.23 ± 0.11
12	7.81 ± 2.45	–
15	22.25 ± 7.35	–
16	3.73 ± 0.78	–
18	6.11 ± 2.39	–
22	17.97 ± 5.56	–
25	8.80 ± 2.35	–
26	3.31 ± 1.22	–
27	7.34 ± 3.68	–
28	28.89 ± 6.78	4.81 ± 0.23
29	39.41 ± 8.12	11.36 ± 2.23
30	7.57 ± 5.23	0.89 ± 0.11
32	16.01 ± 3.43	–
35	3.17 ± 0.35	–
36	32.56 ± 9.15	1.46 ± 0.10
37	24.64 ± 5.23	–
38	15.39 ± 2.34	–
39	8.78 ± 3.23	1.01 ± 0.23
40	28.69 ± 7.11	–
52	9.23 ± 4.22	0.37 ± 0.10

Table 3 Ulcer areas at the beginning and at the end of treatment of the patients treated with Ca-alginate (Group B)

Patient number	Ulcer area at day 1 (cm^2)	Ulcer area at day 70 (cm^2)
2	5.61 ± 1.21	7.44 ± 1.22
7	37.43 ± 6.23	10.81 ± 2.22
8	11.33 ± 3.49	11.27 ± 4.25
10	52.94 ± 10.56	30.75 ± 7.44
13	7.38 ± 2.41	10.24 ± 2.39
14	7.61 ± 1.89	–
17	16.76 ± 4.55	–
19	23.43 ± 8.10	22.44 ± 5.78
20	25.16 ± 8.54	–
21	19.14 ± 7.13	–
23	4.52 ± 1.10	1.83 ± 0.11
24	2.05 ± 1.23	–
31	10.56 ± 5.44	6.12 ± 2.10
32	20.05 ± 8.34	5.94 ± 1.72
33	6.00 ± 1.21	–
41	36.21 ± 8.87	28.69 ± 8.93
42	12.21 ± 3.45	7.52 ± 2.28
43	7.25 ± 1.23	4.98 ± 0.98
44	12.30 ± 5.56	8.69 ± 2.54
45	21.23 ± 7.85	19.00 ± 5.45
46	13.40 ± 4.34	–
47	8.60 ± 2.39	7.00 ± 1.73
48	11.60 ± 4.34	8.00 ± 1.15
49	7.60 ± 2.10	6.60 ± 2.31
50	4.90 ± 0.87	4.20 ± 0.43
51	8.45 ± 2.34	6.80 ± 2.30

compared with Ca-alginate alone. These data have been confirmed by a statistical analysis of variance ($P < 0.05$) and by Wilk's lambda test ($P = 0.0001$ as referred to the time not related to the modality of treatment, and $p = 0.0386$, as referred to the time related to the modality of treatment).

Microscopic analysis showed that in the patients of group A after 70 days of treatment, a re-epithelization of the lesions associated with a marked reduction in the inflammatory infiltrate and a colonization of the lesions by newly formed vessels (Fig. 5c, d), as compared with the same lesions at the beginning of treatment (Fig. 5a, b).

Discussion

Vulnamin® is a new dressing containing four amino acids (glycine, leucine, proline, lysine) and sodium hyaluronate, used to accelerate the wound healing process of venous ulcers of the lower limb. In this study, we have demonstrated for the first time that patient suffering from chronic venous ulcers of the lower legs significantly ameliorate their pathological condition if they are treated with Vulnamin®, as compared with patients treated with Ca-alginate alone. In fact, at the end of the treatment, complete healing closure was 61% in the group treated with Vulnamin® and, respectively, 27% in the control group. Moreover, ulcer areas showed a significant reduction in patients treated with Vulnamin®, as compared with controls. Taking into account the characteristics of managed ulcers (their size, duration, etc.), the choice of Ca-alginate as the control dressing seems appropriate.

Wound healing is characterized by the formation of a granulation tissue consisting of inflammatory cells, newly formed blood vessels, and fibroblasts embedded in a loose collageneous extracellular matrix. A variety of growth factors has been reported to participate in the process of wound healing, including epidermal growth factor (EGF), platelet-derived growth factor (PDGF), transforming growth factor β (TGF-β), insulin-like growth factor (IGF), and granulocyte–macrophage colony-stimulating factor

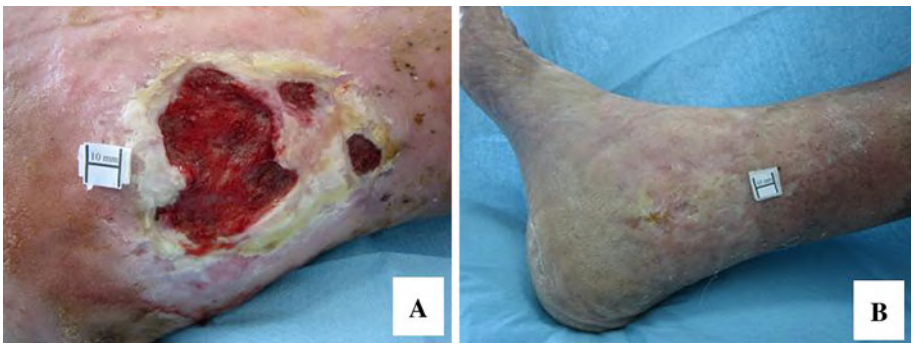

Fig. 1 Photographs of representative ulcer of a group A patient treated with Vulnamin® plus Ca-alginate. **a** shows the patient's ulcer at baseline, while **b** shows the same ulcer at the end of the treatment after 70 days, when completely healing closure has occurred

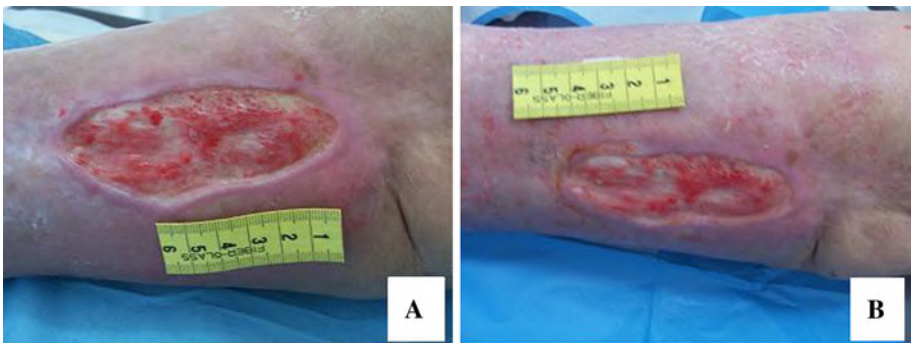

Fig. 2 Photographs of representative ulcer of a group B patient treated with Ca-alginate. **a** shows the patient's ulcer at baseline, while **b** shows the same ulcer at the end of the treatment after 70 days, when healing closure has not yet occurred

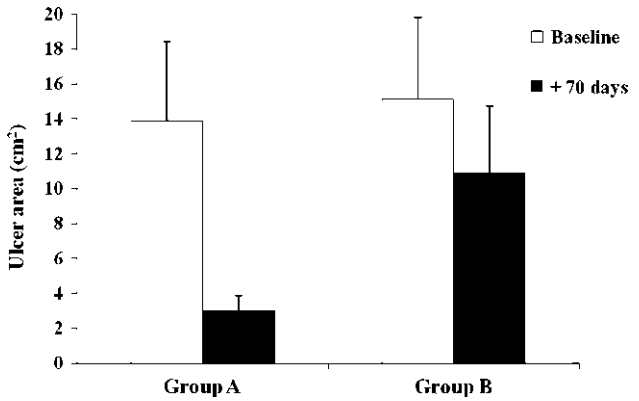

Fig. 3 Mean values of the ulcer areas at baseline and after 70 days of treatment in the patients of the group A, treated with Vulnamin® plus Ca-alginate, and in the patients of the group B, treated with Ca-alginate alone

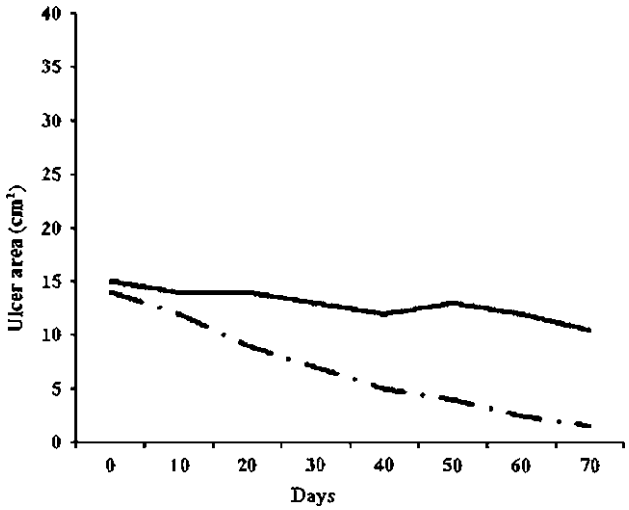

Fig. 4 Time course of the mean values of the ulcer areas at baseline and after 10, 20, 30, 40, 50, 60, and 70 days of treatment in the patients of the group A, treated with Vulnamin® plus Ca-alginate (*dotted line*), and in the patients of the group B, treated with Ca-alginate alone (*continuous line*)

(GM-CSF) [8]. Another group of active compounds important to wound healing process are vitamins and mineral supplements including vitamin A, C, E, as well zinc and copper [8].

Re-epithelization, angiogenesis, and matrix deposition are critical events controlling this process [8]. Angiogenesis is confined to the wound site and plays a pivotal role for successful wound healing [9]. Indeed, re-vascularization is required to furnish the new tissue and to dispose the waste products of metabolism. Wound angiogenesis is believed to be initiated by the early release of preformed growth factors, such as fibroblast growth factor-2 (FGF-2) and vascular endothelial growth factor (VEGF) [10, 11]. We have previously demonstrated that another dressing, identical in its composition to Vulnamin®, exterted a strong angiogenic activity in vivo in the chick embryo chorioallantoic membrane assay and induced the expression of VEGF in human fibroblasts in vitro [12].

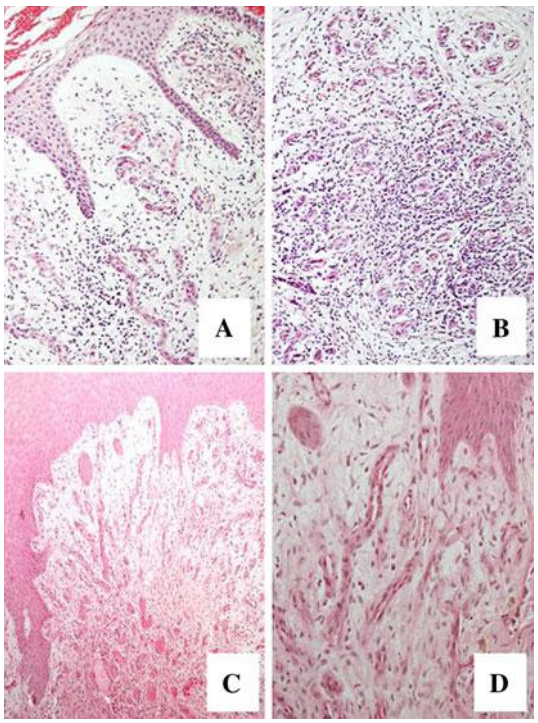

Fig. 5 Histological aspects of the same lesion in a patient of the group A at the beginning **a**, **b** and the end of the treatment with Vulnamin® plus Ca-alginate **c**, **d**. Note in **c** and **d** a re-epithelization of the lesions associated with a marked reduction in the inflammatory infiltrate and a colonization of the lesions by newly formed vessels as compared with the same lesions at the beginning of treatment **a**, **b**. Original magnification: **a**, **c** ×160; **b**, **d** ×200

Wound management implies to obtain the healing in the shortest time, with minimal pain, discomfort, and scarring for the patient, leading to the wound closure with a flexible and fine scar with high tensile strength. Several factors could impede tissue repair and re-generation such as hypoxia, infection, tumors, metabolic disorders such as diabetes mellitus, the presence of debris and necrotic tissue, whereas certain types of medications and a diet deficient in protein, vitamins, or minerals could delay this process.

It is generally accepted that a high availability of amino acids is necessary in the wound repair due to an increased metabolic activity. Hyaluronic acid is also involved in wound healing. It is a glycosaminoglycan composed of repeating disaccharide units on D-glucoronate and N-acethylglucosamine and is one of the most abundant constituents of the extracellular matrix. Hyaluronic acid is involved in a number of developmental processes, has been shown to promote cell proliferation, differentiation, and motility, is naturally biocompatible, biodegradable and lack immunogenicity [13]. Hyaluronic acid-modified liposomes as bioadhesive carriers for delivering growth factors to wound sites have been studied and reported [14], and hyaluronic acid demonstrated effectiveness for managing acute wounds particularly in terms of its safety and efficacy [15].

Deposition of newly synthesized collagen necessary for wound closure may be enhanced by availability of amino acids. For example, in wound fluid, proline concentration is 50% higher than in plasma [16]. In the extracellular matrix, proline is provided by prolidase, a cytosolic enzyme that splits imido peptides with C-terminal proline [17] and that may be a rate-limiting factor in collagen production [18]. Providing the diet with additional proline to enhance its bioavailability for collagen biosynthesis does not result in increased collagen accumulation, while arginine and ornitine supplementation seems to be effective in collagen deposition [16].

We have recently studied the effects on human fibroblasts in vitro of a gel composed by the same amino acids of Vulnamin® [19], and we have demonstrated that fibroblasts increased their proliferative activity, collagen I and III, fibronectin synthesis and the expression of TGF-β, connective tissue growth factor, interleukin-6 and -8, assayed by real time–polymerase chain reaction (RT–PCR) [19].

Finally, it might be hypothesized that the aminoacids present in the Vulnamin® formulation modulate the secretion of pro-inflammatory cytokines by leukocytes and their mechanism of action in healing of the chronic venous ulcers might involve an antiinflammatory activity associated with a pro-angiogenic stimulus, both favoring a more rapid closure of the wound. This is confirmed by the histological analysis, which has demonstrated in bioptic samples obtained by patients treated with Vulnamin® as compared with those treated with Ca-alginate alone a significantly reduction in inflammatory infiltrates at the margin of the lesions associated with an increase in microvascular density.

Overall, the results of this study indicate that Vulnamin® together with elastocompression is safe and more effective than standard dressing together with elastocompression in inducing a faster healing in chronic venous ulcers of the lower limb.

Acknowledgments This study was supported by Errekappa Euroterapici, Milan, Italy.

Conflict of interest None.

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