Effectiveness of topical application of amino acids to chronic wounds: a prospective observational study

• **Objective:** To evaluate whether the topical application of an amino acid dressing, Vulnamin, aids the management of chronic wounds.

• **Method:** A total of 160 patients with non-infected cutaneous chronic wounds were recruited. Before treatment, wound size was assessed using digital planimetry. Treatment lasted for a maximum of six weeks. Wound area measurements were repeated two and six weeks after starting treatment.

• **Results:** There was a significant reduction in the mean wound area after two weeks (7.4 ± 8.7cm^2) and six weeks (4.6 ± 6.3cm^2) of treatment, when compared with baseline (11.2 ± 12.1cm^2, $p<0.01$). At the final follow up, 23% of patients (n=36) healed and 34% (n=54) achieved a greater than 60% reduction in wound size. Seventy-six per cent (n=120) achieved positive outcomes (defined as a greater than 40% reduction in the ulcer size).

• **Conclusion:** Although further investigations on the potential effects of this product on chronic wound healing are required, these data suggest it may promote healing in venous, pressure and diabetic ulcers.

• **Declaration of interest:** This study was sponsored by Professional Dietetics, Milan (Italy).

amino acids; leg ulcers; pressure ulcers; neuropathic ulcers; wound bed preparation

The concepts of wound bed preparation and TIME have been developed to aid the assessment and management of chronic wounds.[1] However, in some cases wound healing is impaired by systemic conditions, such as malnutrition,[2-5] which therefore also need to be addressed.

Using this example, wound management would need to include an optimal supply of the nutrients that affect the wound healing process.[6-8] One such nutrient is protein, which plays a key role in inflammation, the immune response and the development of granulation tissue.[9,10]

The development of novel 'interactive' wound dressings, such as products based on collagen or hyaluronic acid scaffolds, led us to investigate the possibility of applying nutrients that aid healing, such as basic elements for protein synthesis, directly onto the wound bed, rather than through diet.

We were also inspired by evidence that a macromolecule such as albumin can be absorbed at tissue level.[11] It therefore seemed reasonable to speculate that small molecules, such as amino acids, can be absorbed easily into granulation tissue, which is rich in newly formed vessels.

Vulnamin (Professional Dietetics, Milan, Italy) is a medical preparation (regulation 93/42/CEE MDD) comprising a mixture of amino acids (glycine, L-lysine hydrochloride, L-Leucine, L-Proline) and hyaluronic acid. It is approved as an adjuvant wound treatment as it helps regenerate tissue and re-epithelialise cutaneous lesions.[12-15]

Studies on experimentally induced cutaneous wounds in aged and diabetic animals[13] demonstrated that topical Vulnamin stimulated fibroblast proliferation and the production of collagen fibres. Moreover, it can modulate the inflammatory phase by reducing inducible nitric oxide (i-NOS) expression, thereby decreasing nitric oxide (NO) levels, which prolong the inflammatory phase.[14] Preliminary patient data have suggested that Vulnamin may accelerate healing of chronic wounds.[15]

This prospective, observational study aimed to determine the effect of topical Vulnamin (the test product) on chronic wounds of varying aetiologies.

Materials and method

This non-randomised, multicentre, observational study was conducted between January 2007 and March 2008. Each of the 28 participating centres received a study protocol and dedicated data-collection forms, and were asked to recruit 3–10 patients.

Inclusion criteria were:

• The presence of a cutaneous chronic ulcer (of any aetiology) of over six weeks' duration.

• Wound bed preparation grade A or B (defined as mostly clean wounds — that is, more than 50% granulation tissue).[16]

Exclusion criteria were:

• Wound size greater than 15 x 15cm

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Table 1. Baseline patient demographic details

	Males	Females	
No. of patients (%)	66 (41.25)	94 (58.75)	
Age			
Mean age (years) ± SD (range)	72.9 ± 10.2 (37–91)	77.7 ± 9.6 (55–94)	p=0.0022
<65 years (n=20)	15.2%	10.6%	
66–74 years (n=48)	39.4%	23.4%	
>75 years (n=92)	45.5%	66.0%	p=0.0339

SD = standard deviation

Table 2. Wound characteristics at baseline

	Gender		Age		
	Males (n=66)	Females (n=94)	≤65 years (n=20)	66–74 years (n=48)	≥75 years (n=92)
Wound location	**(p=0.3335)**		**(p=0.0159)**		
Sacrum (n=18)	9.1%	12.8%	0%	16.7%	11.3%
Trocanter (n=5)	6.1%	1.1%	10%	4.2%	3.1%
Ischium (n=3)	3%	1.1%	10%	2.1%	1.9%
Leg (n=82)	47%	54.3%	40%	47.9%	51.3%
Malleolus (n=16)	13.6%	7.4%	10%	12.5%	10%
Heel (n=11)	4.5%	8.5%	5%	2.1%	6.9%
Foot (plantar) (n=9)	7.6%	4.3%	15%	8.3%	5.6%
Other (n=16)	9.1%	10.6%	10%	6.3%	10%
Wound aetiology	**(p=0.4773)**		**(p=0.0558)**		
Venous (n=31)	22.7%	17%	20%	18.8%	19.6%
Arterial (n=31)	16.7%	21.3%	5%	18.8%	22.8%
Mixed (n=16)	12.1%	8.5%	0%	8.3%	13%
Lymphatic (n=1)	0%	1.1%	0%	2.1%	0%
Pressure (n=43)	22.7%	29.8%	25%	29.2%	26.1%
Neuropathic (n=9)	9.1.%	3.2%	15%	10.4%	1.1%
Other (n=29)	16.7%	19.1%	35%	12.5%	17.4%
Wound depth	**(p=0.0808)**		**(p=0.2407)**		
Superficial (n=64)	48.5%	34%	45%	47.9%	34.8%
Medium (n=87)	43.9%	61.7%	55%	50%	56.5%
Deep (n=9)	7.6%	4.3%	0%	2.1%	8.7%

- Wounds presenting with eschar and/or infected tissue
- Traumatic wounds and wounds whose aetiology could not be determined
- Patients aged under 18 years
- Terminally ill patients
- Pregnant or breastfeeding patients
- Patients who were allergic to any of the dressing components.

Patients with sloughy wounds were not excluded due to the potential debriding abilities of the test product.

The study was approved by all of the participating hospitals' institutional review boards and performed in accordance with the ethical principles stated in the Declaration of Helsinki.

All eligible patients were fully informed of the study aims, that no physical, social or legal risks were involved and that participation was voluntary. Written consent was obtained.

Treatment protocol

The wounds were treated with the test product for a maximum of six weeks. The product is available as either a sterile powder or cream; wounds could be treated with either version, depending on the investigator's preference, although the powder is recommended for highly exuding wounds (but can also be used on other exudate levels). The composition of the powder and cream is the same, with both having the same carrying medium of hyaluronic acid, which is believed to have no effect on healing. (Most patients received both powder and cream, so it was not possible to give separate results for the two formulations.)

Dressing change frequency was determined by the condition of the wound, based on the practitioner's clinical judgment.

The management regimen comprised:

- Cleansing with saline
- Drying with sterile gauze

- Application of the test product to the wound bed followed by a secondary wound dressing, selected on the basis of the wound aetiology and the centre's protocol.

Before enrolment, patients had previously received various advanced dressings/technologies, to which they had not responded; some were new referrals and had not received prior treatment.

Assessment

At the study start, each wound was assessed to determine the wound aetiology (with a view to ensuring appropriate management) and given a wound bed preparation score.[16]

Any reduction in wound area was measured objectively using digital planimetry (Visitrak) two and six weeks after starting treatment. Adverse events were also recorded.

All data collected by each participating centre during the study period were sent to the study research office for chart review and data extraction.

Based on the extent of reduction in wound area at the end of the treatment period, wound outcomes were classified as:

- Considerable improvement, defined as a reduction in wound size of over 60%
- Moderate improvement, defined as a reduction in wound size of 40–60%
- No improvement, defined as a reduction in wound size of less than 40%
- Worsening, defined as an increase in wound size.

The primary outcome measure was the reduction in wound area. We also aimed to assess any correlation between the test product and patient demographic and wound-associated factors on healing outcomes.

Statistical analysis

Continuous variables are reported as mean values ± standard deviations. Statistical analysis of independent variables was performed using the Student's t-test and variance analysis and, in case of multiple comparisons, using the Bonferroni correction for the critical value of statistical significance.

Discrete variables were analysed using non-parametric methods such as the chi-square test, with the Yates correction where appropriate.[17-19]

Results

In all, 160 patients were evaluated. Baseline patient demographic details are summarised in Table 1. The difference in the mean age of men and women (72.9 versus 77.7 years), although moderate, was statistically significant, with a significantly higher percentage of females (66% versus 45.5% males) in the subgroup of patients aged over 75 years.

Ulcer aetiology comprised:

- Venous (19.4%)

Fig 1. Overall outcomes according to the percentage reduction in wound size

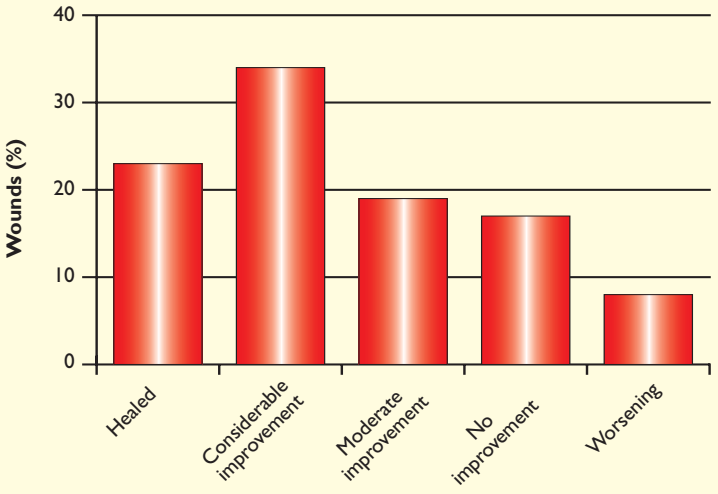

Table 3. Relationship between change in wound area (cm²) and patient demographic data and wound characteristics

	Mean size at baseline	Mean size on day 14	Mean size on day 42
All patients	11.2 ± 12.1	7.4 ± 8.7*	4.6 ± 6.3*†
Sex (p=0.769 between subgroups)			
Men (n=66)	11.2 ± 11.3	7.6 ± 11.3*	5.2 ± 9.1*†
Women (n=94)	11.1 ± 12.6	7.2 ± 8.5*	4.3 ± 4.9*†
Age (p=0.673 between subgroups)			
<65 years (n=20)	10.0 ± 10.4	5.7 ± 5.4*	4.2 ± 5.0*
66–74 years (n=48)	9.8 ± 7.4	6.7 ± 6.0*	4.2 ± 4.2*
>75 years (n=92)	12.1 ± 8.1	8.1 ± 10.3*	5.0 ± 7.5*†
Wound aetiology (p=0.775 between subgroups)			
Venous (n=31)	10.4 ± 8.7	6.3 ± 6.1*	3.9 ± 5.3*
Arterial (n=31)	10.7 ± 9.7	6.3 ± 5.1*	3.6 ± 3.8*
Mixed (n=16)	18.9 ± 25.5	15.3 ± 18.7*	9.5 ± 13.0*
Pressure (n=43)	11.6 ± 10.1	8.2 ± 7.6*	5.5 ± 5.3*
Neuropathic (n=9)	8.7 ± 7.0	5.2 ± 6.5*	3.3 ± 5.5*
Other (n=30)	8.4 ± 9.0	4.8 ± 5.2*	3.1 ± 4.5*

Results are given as mean ± SD;
* p<0.01 versus baseline;
† p<0.01 versus day 14

Table 4. Relationship between percentage reduction in wound size and patient demographic and wound characteristics

	Wound healed	>60% area reduction	>40% area reduction
Sex	**p=0.526**	**p=0.275**	**p=0.266**
Men (n=66)	25.8%	62.2%	69.7%
Women (n=94)	20.2%	52.1%	78.7%
Age	**p=0.537**	**p=0.992**	**p=0.713**
≤65 years (n=20)	15%	55%	75%
66–74 years (n=48)	25%	56.3%	70.8%
≥75 years (n=92)	22.8%	56.6%	77.2%
Wound aetiology	**p=0.337**	**p=0.649**	**p=0.544**
Venous (n=31)	25.8%	61.3%	74.2%
Arterial (n=31)	19.4%	64.6%	87.1%
Mixed (n=16)	12.5%	43.8%	62.5%
Pressure (n=43)	16.3%	48.9%	72.1%
Neuropathic (n=9)	44.4%	55.5%	77.8%
Other (n=30)	30%	60%	73.3%
Wound depth	**p=0.044**	**p=0.002**	**p=0.052**
Superficial (n=64)	31.3%	71.9%	81.3%
Medium (n=87)	18.4%	48.3%	73.6%
Deep (n=9)	0%	22.2%	44.4%
Baseline area	**p=0.000**	**p=0.002**	**p=0.140**
<5cm² (n=54)	42.6%	75.9%	85.2%
5–9cm² (n=41)	12.2%	36.6%	65.9%
10–19cm² (n=40)	15%	52.5%	75%
≥20cm² (n=25)	8%	52%	68%

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- Arterial (19.4%)
- Mixed venous/arterial (10%)
- Lymphatic (0.6%)
- Pressure (26.9%)
- Neuropathic (5.6%)
- Other (18.1%)

All patients with venous leg ulcers received compression both before and during the trial.

Details on the baseline wound characteristics are given in Table 2. Distribution of wound types and grade did not vary significantly with sex or age, although location appeared to vary significantly with age. However, due to the small size of the subgroups, such a result should only be interpreted in a descriptive manner.

The product was well tolerated and no adverse events were experienced.

Wound area reduction

Fig 1 outlines the reduction in wound area. Statistical analysis revealed a significant reduction in the mean wound area after two (7.4 ± 8.7cm²) and six weeks (4.6 ± 6.3cm²) compared with baseline (11.2 ± 12.1cm², p<0.01) (Table 3).

Interestingly, the mean wound area had also significantly reduced after six weeks when compared with after two weeks of treatment (p<0.01). No statistically significant difference in the trend between aetiologies was observed (p=0.775, Anova test with Bonferroni correction).

No significant relationship was observed between the reduction in wound area and gender (p=0.769, Anova test with Bonferroni correction) or age (p=0.673, Anova test with Bonferroni correction) (Table 3).

At the final follow-up:

- 36 patients (23%) had healed
- 54 (34%) had improved considerably
- 30 (19%) had moderately improved
- 27 (17%) were stagnating
- 13 (8%) were worsening.

Just over three-quarters of the patients (n=120, 76%) had positive outcomes (>40% reduction in ulcer size).

Fig 2 shows examples of wounds before treatment and at the final follow-up.

More men (25.8%) than women (20.2%) healed completely (Table 4). Although healing outcomes varied with wound aetiology, no statistically significant differences were observed; none of the deep wounds healed, with only 44.4% achieving a >40% reduction. Conversely, 31.3% of the superficial and 18.4% of the medium-sized wounds healed, while 81.3% of the superficial and 73.6% of the medium-sized wounds achieved a positive outcome.

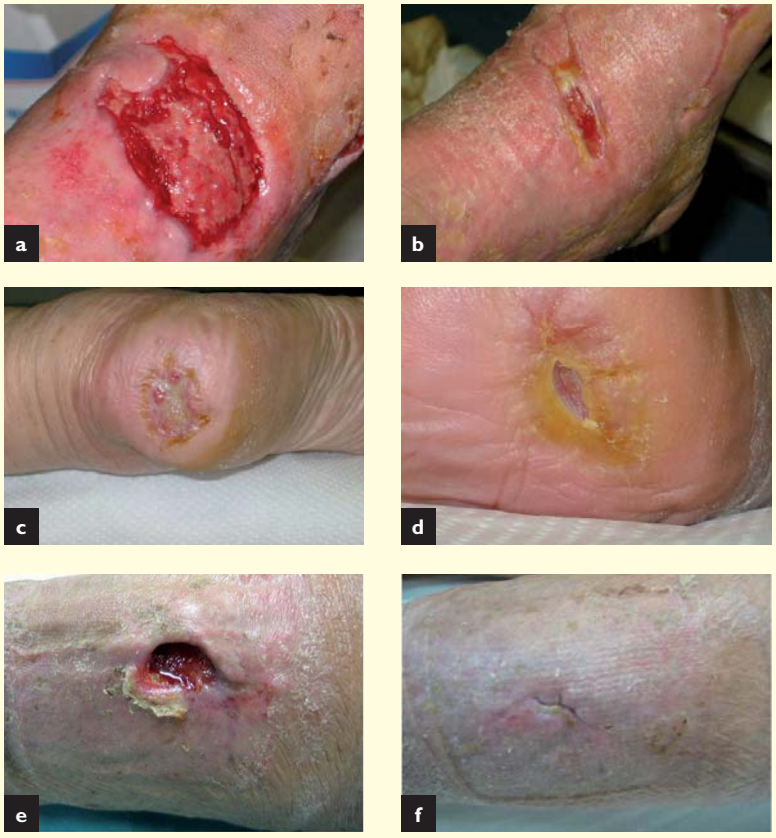
Cost-analysis

From a sample of 97 patients, we collected data on the number and quantity of dressings used for a preliminary pharmaco-economic evaluation (Table 5). The sample comprised the first 100 patients enrolled into the study minus three drop-outs.

The average number of test-product applications changes per week varied from 2.2 (for superficial wounds) to 2.9 (deep wounds), with no statistically significant difference regarding depth.

The average cost per product (excluding nursing costs) was €3.58 for superficial wounds, €3.53 and €4.40 for medium and deep wounds, respectively; the average cost for each test product was €3.63.

Fig 2. Healing of chronic wounds during treatment with the test product



Vascular ulcer at the dorsum of the foot before treatment (a) and at final follow-up (b);

heel pressure ulcer at the initial visit (c) and at final follow-up (d);

leg ulcer after surgical removal of nodular neoformation before (e) and after six weeks of treatment (f)

Discussion

Preclinical evidence of the test product as a modulator of the inflammatory phase and preliminary results, presented at a meeting, from a small number of patients provided the rationale for this observational study. The primary outcome was the change in ulcer area during the six-week treatment period. A significant mean reduction was reported after only two weeks.

The efficacy of early healing as a prognostic indicator is not unanimously supported,[20,21] although some believe that it is a strong predictor of the healing trajectory,[21-26] and a percentage reduction after two weeks of treatment has been suggested as a reliable prognostic index for the healing of deep venous leg ulcers.[27,28]

Unfortunately, there were too few (11/160) deep ulcers in out study for the latter point to be relevant here. Nevertheless, we believe that the significant reductions in ulcer area achieved after two weeks of treatment in wounds that had been 'stuck' for six months — a duration that is associated with poor healing[29] — might indicate a 'reawakening' of the healing mechanisms.

In our experience, controlling persistent inflammation in chronic open wounds may restart the healing process, an observation that is strengthened by our results. Indeed, ulcers that achieved good results within 15 days had the best outcomes. This suggests that, as well as providing nutrients topically to the wound, the product may have helped to modulate inflammation.

In our study, 23% of the wound healed completely within six weeks and 53% reduced by over 40%, resulting in 76% having positive outcomes. While we believe these results are encouraging, it is difficult to compare them with most other clinical studies as they assessed healing over a longer period (12–24 weeks).

Our data are consistent with those of Jones et al.,[30] who found that neither sex, age nor wound aetiology were associated with healing.

In our study, smaller wounds achieved the highest rates of healing, whereas deeper wounds achieved the poorest outcomes, which is consistent with other studies.[30-34]

Study limitations include the lack of a control group. However, literature data, along with our clin-

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Table 5. Cost-analysis data

	Superficial wounds	Medium wounds	Deep wounds	Total
No. of ulcers	47	44	6	97
Treatment duration (total, days)	1597	1580	252	3429
Mean duration of treatment (days)	33.9 ± 10.6	35.9 ± 10.5	42.0 ± 0*	
No. of medications (total)	517	577	105	1199
Mean no. of medications	11.0 ± 5.2	13.1 ± 5.4	17.5 ± 5.6†	
No. of dressing changes per week	2.2 ± 1.0	2.7 ± 1.3	2.9 ± 0.9	
Total cream used (g)	1120	607	80	1807
Total amount of powder used (no. of blisters)	213	309	77	599
Cost of cream (€)	716.80	388.48	51.20	1156.48
Cost of powder (€)	1136.00	1648.00	410.67	3194.67
Total cost of medication (€)	1852.80	2036.48	461.87	4351.15
Average cost/medication (€)	3.58	3.53	4.40	3.63

Results reported as mean ± SD (standard deviation)
*p<0.003 versus superficial and medium wounds (Student's t-test)
†p<0.01 versus superficial wounds (Student's t-test)

ical experience, indicated that the test product may promote wound healing. In addition, it is difficult to establish the causality of some of the significant associations as subdividing the subgroups — for example, comparing different sized wounds for different age groups — resulted in small sample sizes that lacked analytical power.

Finally, we only used the reduction in wound size to monitor the progression towards healing, although it should be noted that this was objectively assessed. Other outcome measures, such as clinical judgment[35] and wound bed preparation score,[16] are also of value in this regard. However, we are in the process of evaluating such parameters: our goal is to provide a more comprehensive overview of the potential benefits of the topical use of this product.

Conclusion

The data reported in this study indicate that the topical application of a product that applies amino acids directly to the wound bed might help to promote healing of chronic wound such as venous, pressure and diabetic ulcers. The treatment was shown to be well tolerated, and is similarly priced to other advanced wound therapies.

Further evaluations need to be conducted using other outcome measures, plus a control group, to provide a more comprehensive overview of its benefits for clinical practice. This is essential to provide a research-based rationale to support its use in the clinical setting. ■

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