ORIGINAL ARTICLE

Protease-modulating polyacrylate-based hydrogel stimulates wound bed preparation in venous leg ulcers – a randomized controlled trial

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Abstract

Background Stringent control of proteolytic activity represents a major therapeutic approach for wound-bed preparation.

Objectives We tested whether a protease-modulating polyacrylate- (PA-) containing hydrogel resulted in a more efficient wound-bed preparation of venous leg ulcers when compared to an amorphous hydrogel without known protease-modulating properties.

Methods Patients were randomized to the polyacrylate-based hydrogel (n = 34) or to an amorphous hydrogel (n = 41). Wound beds were evaluated by three blinded experts using photographs taken on days 0, 7 and 14.

Results After 14 days of treatment there was an absolute decrease in fibrin and necrotic tissue of 37.6 ± 29.9 percentage points in the PA-based hydrogel group and by 16.8 ± 23.0 percentage points in the amorphous hydrogel group. The absolute increase in the proportion of ulcer area covered by granulation tissue was 36.0 ± 27.4 percentage points in the PA-based hydrogel group and 14.5 ± 22.0 percentage points in the control group. The differences between the groups were significant (decrease in fibrin and necrotic tissue P = 0.004 and increase in granulation tissue P = 0.0005, respectively).

Conclusion In particular, long-standing wounds profited from the treatment with the PA-based hydrogel. These data suggest that PA-based hydrogel dressings can stimulate normalization of the wound environment, particularly in hard-to-heal ulcers.

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Conflict of interests

S.A. Eming, J. Dissemond and G. Weyandt were consultants for the PAUL HARTMANN AG in the past. P. Humbert, B. Faivre, Y. Véran, C. Debure, F. Truchetet, P.-A. Bécherel, P. Plantin, J.-C. Kerihuel, D. Kaspar, H. Smola, and P. Zöllner: declare no conflict of interest.

Funding sources

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Introduction

Clinically and economically, venous leg ulcer treatment represents a major challenge to health care systems and significantly impairs the quality of life. Venous leg ulcer treatment focuses on the therapy of the underlying venous insufficiency, whereby compression therapy increased ulcer healing rates.[1] The reported healing of uncomplicated leg ulcers at 12 weeks ranges from 30% to over 75%.[2] More complex ulcers at risk of non-healing are characterized by long duration and a large wound surface area, accompanied by reduced patient mobility.[3] These hard-to-heal ulcers are typically covered by fibrin or necrotic tissue, and are complicated by concomitant infection, oedema and wound desiccation. To initiate healing, debridement is recommended to create a productive wound bed.

At the molecular level, uncontrolled protease activity was identified as a major underlying pathomechanism of non-healing wounds.[4–6] Indeed, wound fluids from chronic wounds contained considerably increased protease activities than those from acute wounds.[6] Therefore, targeting proteases represents a major therapeutic approach for wound bed preparation and the induction of productive granulation tissue formation.

We recently showed that certain polyacrylic acid (PA-) polymer types are potent matrix metalloproteinase (MMP) inhibitors.[7] Moreover, wound dressings containing these hydrated polymers removed large amounts of MMPs from the chronic wound environment.[7] These results complement various clinical results demonstrating excellent debriding activity and concomitant stimulation of granulation tissue formation by PA-containing wound dressings.[8,9] In this randomized controlled trial, we investigated whether wound bed preparation of venous leg ulcers using protease-inhibiting, PA-containing dressing is more efficient compared to an amorphous hydrogel lacking protease-inhibiting activity.

Material and methods

Study design

This was an open, prospective, randomized and two-arm parallel-group study with blinded outcome assessment conducted in 21 French hospitals, clinics and private practices with experience in the management of venous leg ulcers.

Patients

Main inclusion criteria:

- age >18 years
- performance of the Eastern Cooperative Oncology Group ECOG <2[10]
- leg ulcer minimum of 4 weeks
- Ankle-Brachial-Index ABI >0.8
- wound coverage > 70% fibrin and/or necrotic tissue
- wound size 8–100 cm^2
- concomitant compression therapy with stockings or bandages.

Main exclusion criteria:

- prior treatment with the study or the control dressing during the previous 4 weeks
- mechanical and enzymatic debridement as well as the use of gels < 2 weeks before inclusion
- surgical debridement <8 weeks before inclusion
- patient with severe concomitant disease
- blood haemoglobin ≤8 g/L
- serum albumin ≤25 g/L
- $HbA_{1c} \geq 8.5\%$.

Randomization

Randomization was performed on day 0. It was balanced by blocks of four patients and stratified per study centre. A randomization list using the PLAN procedure of the SAS® software (SAS Institute, Cary, NC, USA) was centrally generated, controlled and accessed through a secured website.

Study treatments

HydroClean® (alternative trade name for the same proprietary product: TenderWet®; Paul Hartmann AG, Heidenheim, Germany) is a PA-based hydrogel available in different sizes. Depending on wound size, the following dressings were used: 5.5 cm diameter, 7.5 × 7.5 cm^2 and 10 × 10 cm^2.

IntraSite® (Smith & Nephew, Hull, UK) is an amorphous hydrogel available in applicator packs of 15 or 25 g. For this study, 15-g packs were used.

These dressings were applied according to manufacturers' recommendations. The PA-based hydrogel was changed every 24 h, and the amorphous hydrogel every 24–72 h according to wound status. Between visits, the dressings were changed following the product instructions.

Study protocol

Patients were screened for up to 3 days to confirm selection criteria (Fig. 1). If all inclusion criteria were met, patients were randomized (day 0) for treatment with the PA-based hydrogel or the amorphous hydrogel for 2 weeks, including a detailed wound evaluation on days 7 and 14.

Both dressings were used in direct contact with the wound. Secondary dressings, such as gauzes, were used with the amorphous hydrogel. The investigators were free to use other topical treatments at dressing change, including sodium chloride solution for wound rinsing, and compresses and tapes for fixation. Throughout the 2-week study duration, no debridement procedure was performed except autolysis. All patients received compression therapy using bandages or stockings. Outpatients documented all dressing changes and related procedures at

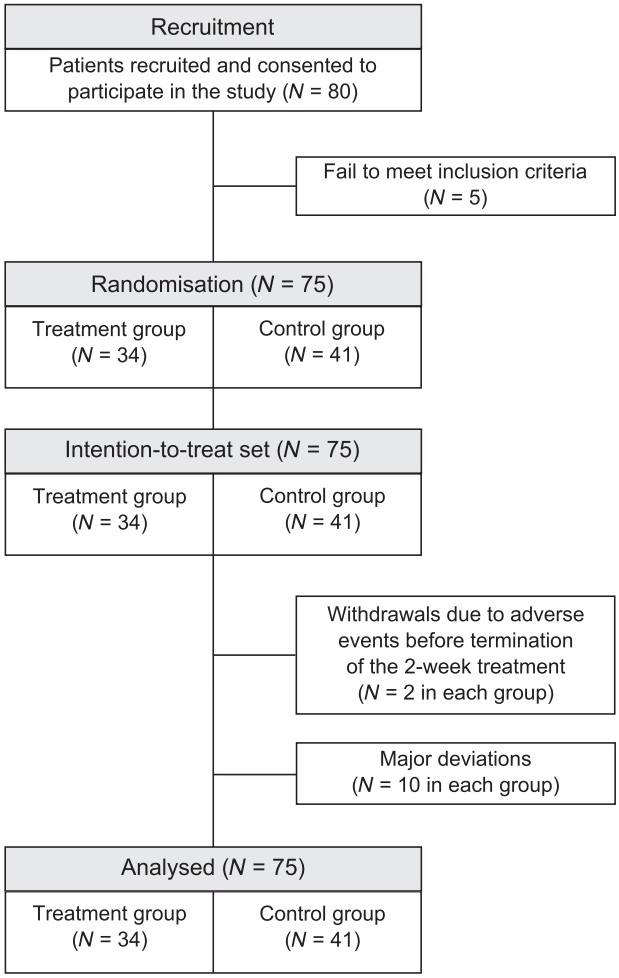

Figure 1 Flow diagram of the patients involved in the study. The results shown in the present report were based on the ITT-population.

home. Concomitant drug treatment and adverse events (AE) were recorded.

Outcomes

- Absolute reduction in the proportion of fibrin and necroses covering the wound area on day 14 (or earlier for early withdrawal) compared to the baseline (for definition of 'absolute reduction' see Fig. 2). For further analysis, wounds were grouped into those with <50% surface coverage with slough and those with ≥50% slough and necroses.
- Absolute increase in the proportion of red granulation tissue covering the wound area on day 14 compared to the baseline. For further analysis, the wounds were grouped into those with >50% surface coverage with granulation tissue and those with ≤50% granulation tissue.
- AE occurrences were assessed at each visit.

Outcome measures

Wound bed assessment was performed by three blinded, independent experts who assessed photographs of all wounds from all 21 sites by recording the proportions of wound surface covered by fibrin and necrotic tissue, and granulation tissue.

We decided on a central assessment of wound photos by blinded experts to counter the potential risk of bias. Three experts with long-standing experience in wound management were selected for reliable wound assessment and because reliability is increased when more than one observer rated the wound.[11] Because of the multicentric nature of the study, an assessment by three observers was only feasible by central wound photo evaluation. We preferred a visual segmentation of wound tissues by estimating the proportion of the different tissue types within the wound vs. computer-based tissue image analysis, because computer-based systems require image acquisition skills in the patient room where lighting may not be appropriately con-

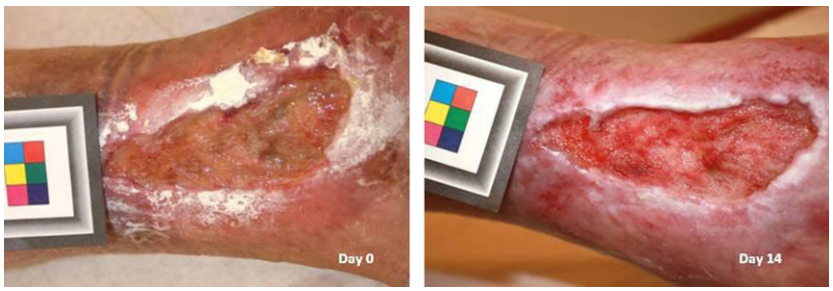

Figure 2 At day 0 the wound was 90% covered with fibrin and necrotic tissue related to the total wound area (= 100%). At day 14, the proportion of fibrin and necrotic tissue was reduced to 8% of the total wound area. This decrease (90%–8%) corresponds to a difference of 82 percentage points (synonymic with the term 'absolute reduction' used in the present report). This difference is equivalent to a percent reduction in fibrin and necrotic tissue of 91.1% related to the proportion of 90% fibrin and necrotic tissue on day 0.

trolled.[12] In addition, different structures within the wound may have similar colour spectra and potentially be misinterpreted by computer-based systems.[13] Furthermore, visual quantification of tissue segments using computer-based systems can result in high interrater variability when quantifying granulation tissue and slough.[14] Wound rating evaluation was also performed on-site, by local investigators.

Wound photos

All study sites were equipped with high quality cameras (Canon EOS 40D; Macro-objective: Canon EF 2,5/50 mm; Macro-flash: Canon MT-24 EX; Tripod: Stativ Bilora Pro 27; Memory card: 1GB CF; Canon, Krefeld, Germany). At each wound evaluation, six photos were taken, which were recorded by fixing the distance and the angle between the camera and the patient to control for optical deformations. Wound scale and colour were adjusted using a colour checker. To avoid colour artefacts, a neutral tissue (avoid white) should cover the background of the image as well as the patient's clothes. If necessary, wounds were dry-patted to reduce reflections. Photographs of each individual patient were taken by the same person throughout the study.

An independent study coordinator selected the best photograph from the six taken at each visit, for analysis by the experts. Bloemen *et al.* (2011) reported that one photograph was sufficient for reliable ratings when performed by experienced observers.[11] Photos were identified using a unique code number and were mixed randomly. A CDROM was generated containing all the photographs for evaluation. Each reviewer independently determined the colorimetric aspect of each wound.

Outcome assessment

Experts (photo assessment) as well as investigators (on-site-inspection) estimated the proportion of tissue type on days 0 and 14. The absolute change in a specific tissue was the difference between the proportion on days 0 and 14, expressed in percentage points (see Fig. 2 for clarification).

Adverse events (AE)

AE and patient complaints about discomfort were recorded at each visit.

Statistical analysis

All analyses were conducted using an independent data management centre (International Clinical Trials Association ICTA, Fontaine les Dijon, France).

Sample size calculation and interim analysis A sample size of 86 patients allowed a statistical power of ≥80% for the detection of a clinically meaningful difference in the mean absolute change in fibrin and necrotic tissue between Hydroclean® and Intrasite® Gel of at least 12% on day 14, considering a standard deviation of 25% and using a two-tailed test on the $\alpha = 2.5\%$-significance level, allowing an interim analysis after about half of the patients. To allow for a withdrawal rate of approximately 15%, 102 patients were required for randomization into this trial (51 per group). Once 52 patients were enrolled, an interim analysis was performed as planned before while enrolment continued. Due to the interim results, the study was stopped and the final analysis was made using 75 patients.

Analyses The analyses were performed on the ITT-population, defined as all randomized patients with at least one data point of the wound colorimetric aspect. To adjust for baseline data, a covariance model (ANCOVA; SAS® procedure: PROC GLM, Cary, NC, USA) was used that included the following factors:

- change in the proportion of the particular tissue on day 14 respectively (dependent variable)
- treatment group (PA-based hydrogel vs. amorphous hydrogel) (explicative variable)
- baseline value: percentage of proportion of particular tissue on day 0 (covariate).

When data distribution was non-Gaussian, a transformation was performed to obtain normalized data or a non-parametric test was used. The analyses were performed based on the median of the blinded expert assessments. In addition, a sensitivity analysis was performed using the mean values of the evaluation by both the experts and the investigators.

The proportion of wounds with <50% of slough or >50% granulation tissue at the final evaluation was calculated using the χ^2-test. Analyses of tolerability and safety were descriptive.

Ethics

This study complied with the European and ISO guidelines as well as current French regulations. The study protocol was approved by the Ethics Committee of the Besançon Teaching Hospital, France (CPP Est II) and all required documents were authorized by the responsible French authority (AFSSAPS, now ANSM). Informed consent was obtained from all the patients.

Results

Study participants From March 2008 to June 2010, 80 patients were selected and 75 were randomized for treatment using the PA-based hydrogel (34 patients) or the amorphous hydrogel (41 patients) (Fig. 1). Two patients in each group discontinued the study before day 14 because of an AE. Ten patients had a major deviation from the protocol, with two patients in each group failing to perform adequate compression therapy.

Most patients were women (70.7%) and the majority were followed as outpatients (61.3%) (Table 1). The mean patient age was 74.4 ± 10.5 years. Dressings were changed on average every 1.1 days (min 0.9 days, max 2.0 days) in the PA-based hydrogel group and every 1.4 days (min 0.8 days, max 2.3 days) in the amorphous gel group.

Table 1 Patients' characteristics

	PA-based hydrogel *n* = 34	Amorphous hydrogel *n* = 41	*P*-value
Outpatients	19 (55.9%)	27 (65.9%)	0.377**
Sex (F/M)	21(62%)/13 (38%)	32 (78%)/9 (22%)	0.123**
Age (years) ± SD	74.8 ± 11.7	73.7 ± 9.6	0.427*
Age >80 years	13 (38.2%)	9 (22.0%)	0.123**
BMI (kg/m2) ± SD	29.6 ± 7.70†	30.6 ± 7.12	0.585*
BMI >30 kg/m2	14 (42.4%)	22 (53.7%)	0.337**
Diabetes	4 (11.7%)	6 (14.6%)	0.716**
HbA_{1c} (%) ± SD	5.95 ± 0.50	5.96 ± 0.43†††	0.967***
ABI ± SD	1.08 ± 0.18††	1.07 ± 0.18	0.755*
Compression			0.901**
Bandages	29 (90%)††	35 (90%)††††	
Stockings	3 (9%)††	4(10%)††††	

†*n* = 33; ††*n* = 32; †††*n* = 40; ††††*n* = 39.
*Wilcoxon-Mann–Whitney; **χ2-test; ***Student's *t*-test.
SD, standard deviation.

Table 2 Baseline characteristics of leg ulcers

	PA-based hydrogel *n* = 34	Amorphous hydrogel *n* = 41	*P*-value
Ulcer duration in years ± SD***			
Mean	2.32 ± 3.22	3.32 ± 4.37	0.557*
Median	1.7	1.5	
Range	(0.1–16.5)	(0.1–22.6)	
Ulcer > 6 months ± SD***	22 (64.7%)	30 (73.2%)	0.429**
Wound area in cm^2 ± SD***			
Mean	31.0 ± 28.9	26.1 ± 20.1	0.563*
Median	21.0	18.0	
Area >10 cm2	30 (88.2%)	34 (80.5%)	0.518**
Wound surface covered with			
Fibrin and necrotic tissue in% ± SD			
Mean of medians of three blinded experts	83.3 ± 15.4	74.2 ± 19.5	0.036*
Mean of means of three blinded experts	81.9 ± 14.3	73.3 ± 18.0	0.030*
Investigators' assessment	83.5 ± 9.5	83.7 ± 9.7	0.951*
Granulation tissue in% ± SD			
Mean of medians of three blinded experts	14.3 ± 12.0	22.8 ± 18.9	0.057*
Mean of means of three blinded experts	16.0 ± 12.1	24.4 ± 17.8	0.040*
Investigators' assessment	15.3 ± 9.3	13.9 ± 8.9	0.613*

*Wilcoxon-Mann–Whitney test (two-sided), *t*-approximation, because non-Gaussian variable; **χ2-test; ***Investigators' assessment.
SD, standard deviation.

Baseline characteristics of wounds The median duration of ulcer treatment was 1.7 and 1.5 years in the PA-based hydrogel and amorphous hydrogel groups respectively (Table 2). More than 80% of the ulcers had a surface >10 cm^2. In 69 patients (92.0%), the wound had received a dressing prior to inclusion. The most frequently used dressings were contact layers (52%), hydrofibre/alginate dressings (42.7%), silver-releasing dressings (22.7%) and foam dressings (20.0%).

Thirty-five patients (46.7%) received a concomitant medication at baseline. Analgesics were the most frequently prescribed drugs (19 patients, 25.3%). Systemic antibiotics were used in 8.3% of patients.

Table 3 Sensitivity analysis of the changes of the proportion of fibrin and necrotic tissue from day 0 to day 14

	PA-based hydrogel *n* = 34	Amorphous gel *n* = 40	*P*-value
Mean of means of three blinded experts			
Absolute change in pp* ± SD	39.0 ± 27.3	17.9 ± 20.4	0.002*
Relative change in% ± SD	46.7 ± 32.3	24.8 ± 32.6	0.011*
Relative change in% [CI]	46.3 [34.9; 57.7]	25.1 [14.6; 35.6]	0.009**
Mean of medians of three blinded experts			
Absolute change in pp* ± SD	37.6 ± 29.9	16.8 ± 23.0	0.004*
Relative change in% ± SD	43.4 ± 36,7	21.9 ± 39.4	0.018*
Relative change in% [CI]	41.9 [28.6; 55.1]	23.2 [11.0; 35.4]	0.046**
Mean of investigators			
Absolute change in pp ± SD	45.0 ± 28.5	28.5 ± 25.16	0.016*
Relative change in% ± SD	53.1 ± 32.5	34.2 ± 29.6	0.015*

*Wilcoxon-Mann–Whitney test (two-sided), *t*-approximation, because non-Gaussian variable; **Covariate is baseline% of fibrin and necrotic tissue covering ulcer area.
CI, confidence interval; pp, percentage points; SD, standard deviation.

Decrease in the proportion of fibrin and necrotic tissue at day 14 Both the PA-based and amorphous hydrogel dressings reduced the proportion of slough and necrotic tissue within 14 days. The proportion of ulcer area covered by slough and necroses decreased by 37.6 ± 29.9 percentage points in the PA-based hydrogel group and by 16.8 ± 23.0 percentage points in the amorphous hydrogel group compared to the baseline, (P = 0.004) (Fig. 3a). These changes corresponded to a relative decrease of 43.4 ± 36.7% in the PA-based hydrogel group and of 21.9 ± 39.4 percentage points in the amorphous hydrogel group (P = 0.018) (Table 3). According to the cumulative distribution, PA-based hydrogel-treated wounds displayed a larger reduction in fibrin and necrotic tissue compared to wounds treated with the amorphous hydrogel at day 14 (Fig. 3b). On day 14, 17 of 34 (50.0%) ulcers treated with PA-based hydrogel had < 50% of their surface covered by slough compared to 11 of

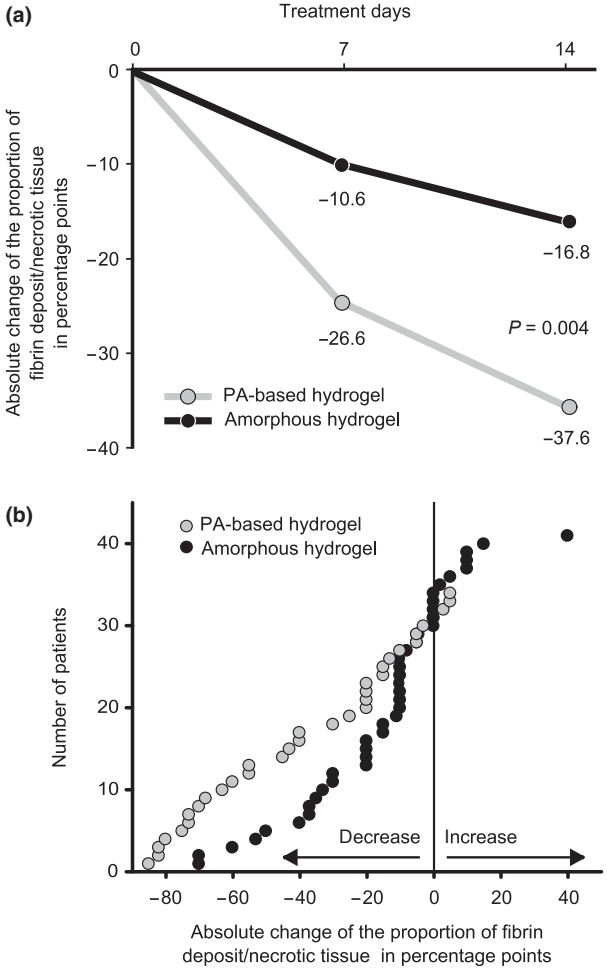

Figure 3 Absolute decrease in fibrin and necrotic tissue (a). Cumulative distribution of the absolute change in the amount of fibrin and necrotic tissue at day 14 after treatment with either the PA-based hydrogel or the amorphous hydrogel (b). The graph representing the wounds treated with PA-based hydrogel is clearly shifted to the left compared to the graph representing wounds treated with the amorphous hydrogel. Each point represents one wound.

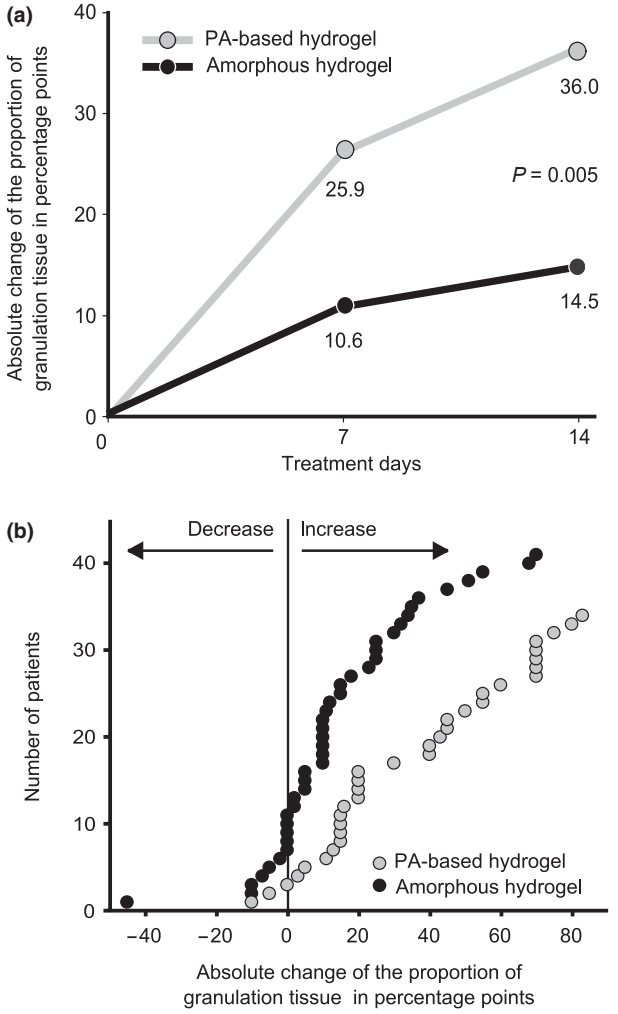

Figure 4 Absolute increase in granulation tissue (a). Cumulative distribution of the absolute change in the amount of granulation tissue at day14 after treatment with either the PA-based hydrogel or the amorphous hydrogel (b). The graph representing the wounds treated with PA-based hydrogel is clearly shifted to the right. Each point represents one wound.

41 (26.8%) in the amorphous hydrogel group ($P = 0.0389$; χ^2- test). In the two treatment groups, the probability of reducing the amount of fibrin and necrotic tissue to < 50% of the total wound area was 2.7 times higher for wounds treated with the PA-based hydrogel (OR = 2.72; 95% CI = 1.31–9.66) (Fig. 5).

Increase in the proportion of granulation tissue at day 14 Both the PA-based and amorphous hydrogel dressings increased the proportion of granulation tissue within 14 days. The proportion of ulcer area covered by granulation tissue increased by 36.0 ± 27.4 percentage points in the PA-based hydrogel group and by 14.5 ± 22.0 percentage points in the amorphous hydrogel group compared to the baseline ($P = 0.005$) (Fig. 4a). The cumulative distribution indicates a preferential induction of granulation tissue formation (Fig. 4b).

On day 14, 16 of 34 (47.1%) ulcers treated with PA-based hydrogel had >50% surface coverage with granulation tissue compared to 9 of 41 (23.1%) in the amorphous hydrogel group ($P = 0.0217$; χ^2-test) (Fig. 4b). In the two treatment groups, the probability of having >50% covered with granulation tissue on day 14 was 3; 16 times higher for wounds treated with the PA-based hydrogel (OR = 3.16; 95% CI = 1.16–8.59) (Fig. 5).

Response rates of hard-to-heal ulcers of more than 6 months On day 0, no hard-to-heal ulcer to be treated with

PA-based hydrogel had <50% surface coverage by fibrin and necrotic tissue compared to 2 of 30 ulcers (6.67%) in the amorphous hydrogel group (Fig. 6a). After 14 days of treatment, 12 of 22 (54.5%) hard-to-heal ulcers treated with PA-based hydrogel had <50% surface coverage by fibrin and necrotic tissue compared to 7 of 30 ulcers (23.3%) in the amorphous hydrogel group ($P = 0.0209$; χ^2-test) (Fig. 6a).

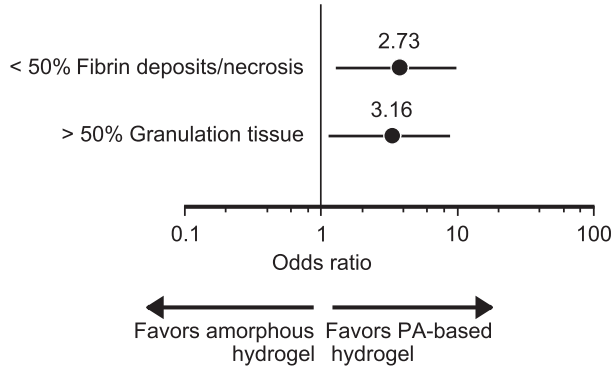

Figure 5 Odds ratio for the possibility of having <50% fibrin and necrotic tissue or having >50% granulation tissue after 14 days of treatment with either the PA-based hydrogel or the amorphous hydrogel compared to each other.

On day 0, no hard-to-heal ulcer of the PA-based hydrogel group had >50% surface coverage by granulation tissue compared to 2 of 30 ulcers (6.67%) in the amorphous hydrogel group (Fig. 6b). On day 14, 11 of 22 (50.0%) hard-to-heal ulcers treated with PA-based hydrogel had >50% surface coverage by granulation tissue compared to 6 of 30 ulcers (20.0%) in the amorphous hydrogel group ($P = 0.0227$; χ^2-test) (Fig. 6b).

Tolerability of dressings The investigators reported 58 AEs in 33 of 75 (44.0%) patients during this study, with the same frequency in each treatment group. A total of 18 (24.0%) AEs were possibly related to the treatment (Table 4). This relatively high number of AEs may be attributed to the age of the patient population and the duration of the wounds giving rise to a high susceptibility to skin irritations and infections as well as pain

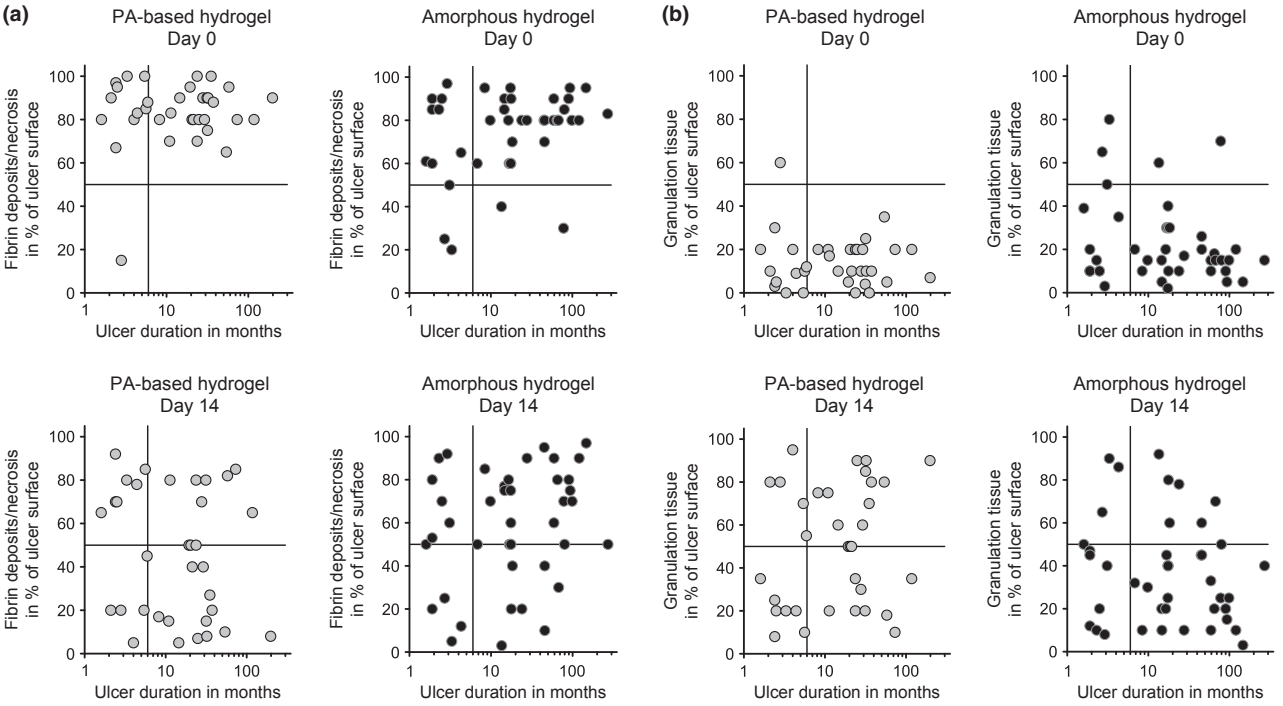

Figure 6 Distribution of ulcers related to the coverage with fibrin and necrotic tissue and to ulcer duration (a). In the PA-based hydrogel group, none of the hard-to-heal ulcers (>6 months duration) had <50% surface coverage with fibrin and necrotic tissue at day 0. After 14 days, the proportion of fibrin and necrotic tissue had decreased to <50% of the wound surface in 12 of 22 wounds. In the amorphous hydrogel group, 2 of 30 wounds had <50% surface coverage with fibrin and necrotic tissue at day 0. After 14 days, 7 of 30 wounds were covered with <50% fibrin and necrotic tissue. Distribution of ulcers related to the coverage with granulation tissue and to the duration (b). In the PA-based hydrogel group, none of the hard-to-heal ulcers (>6 months duration) had >50% surface coverage with granulation tissue at day 0. After 14 days, in 11 of 22 wounds the proportion of granulation tissue had increased to >50%. In the amorphous hydrogel group, 2 of 30 wounds had >50% surface coverage with granulation tissue at day 0. After 14 days, the number increased to 6 of 30 wounds.

Table 4 Adverse events (AE)

	PA–based hydrogel *n* = 34	Amorphous hydrogel *n* = 40
Number of patients with one or more AEs (%)	15 (44.1%)	18 (45.0%)
Number of patients with one or more AEs unrelated to the treatment (%)	4 (11.8%)	11 (27.5%)
Number of patients with one or more AEs possibly related to the treatment (%):	11 (32.3%)	7 (17.5%)
Skin maceration	1 (2.9%)	3 (7.3%)
Eczema	1 (2.9%)	2 (4.9%)
Dermatitis contact	1 (2.9%)	–
Pruritis	1 (2.9)	–
Skin burning sensation	1 (2.9)	–
Pain	7 (20.6%)	–
Malaise	–	1 (2.4%)
Wound infection	–	2 (4.9%)
Erysipelas	–	1 (2.4%)
Septic shock	–	1 (2.4%)
Device intolerance	1 (2.9%)	–

which might be caused by the wound itself rather than by the dressing.

Discussion

Targeting proteases is a potentially important therapeutic approach to treating chronic wounds. We demonstrated that wound bed preparation with the protease-modulating PA-based hydrogel induced more effective granulation formation compared to an amorphous hydrogel without known protease-modulating properties in venous leg ulcer treatment.

The study had limitations: study blinding was impossible because of the different aspect of the two dressing types. Taking into account the practice requirements for outcome studies in wound management, which postulate the highest level of blinding[15] we chose an observer-blinded design to counter the potential risk of bias (See Material & Methods for discussion.). The outcome measure of assessment of wound photographs by visual segmentation of tissue types may be considered subjective but was the most reliable method at the time of the study performance (See Material & Methods for discussion.)

The study was stopped early after an interim analysis that showed positive results and may thus bear the risk of overestimation of the benefit. To adjust for this bias we considered the following parameters (Bassler, 2008 #6836)[16]: The interim analysis was planned at the beginning of the study based on a sample size calculation allowing a statistical power of 80% for the detection of a statistically and clinical relevant difference of at least 12% between groups regarding the primary endpoint. The significance level was fixed at 2.5% and thus inflated to a global risk equal to 5% for the final analysis (Bonferroni correction for multiplicity). The interim analysis was performed while enrolment continued. In particular, wounds defined as hard-to-heal ulcers profited from treatment with the PA-based hydrogel. Risk factors associated with non-healing venous leg ulcers are an ulcer size >10 cm^2, a duration of >6 months and the presence of fibrin on >50% of the wound surface.[17] Therefore, most of the wounds treated in this study can be characterized as hard-to-heal ulcers because of a duration of >6 months (69%), an ulcer surface of >10 cm^2 (85%) and a wound bed that was covered by >70% with slough and necrotic tissue (73%). We demonstrated that the wounds with >6-month duration benefited most from PA-based hydrogel treatment compared to the amorphous hydrogel when considering fibrin and necrotic tissue reduction to <50% or increased granulation formation to >50%. Therefore, we suggest that the PA-based hydrogel is primarily suited for the autolytic debridement phase in hard-to-heal ulcers, in particular, with regard to the high prevalence of leg ulcers in community settings, where structural, financial and educational constraints frequently limit adequate treatment options[18] such as surgical debridement.

Different dressings have been developed and clinically applied to reduce protease activity in chronic wound fluids of different etiologies, in particular, dressings composed of collagen and oxidized regenerated cellulose or containing a nano-oligosaccharide factor.[19–23] Both dressings reduced venous leg ulcer wound size compared to non-protease modulating dressings within 12 or 8 weeks respectively.[19,23] At baseline, the treated wounds had a considerably smaller ulcer surface area, a shorter ulcer duration and mean granulation tissue coverage was >70% of the wound surface compared to the wounds in this study. It is fair to assume that once granulation tissue forms, the balance between inflammation and granulation tissue formation has shifted towards the latter, and that matrix deposition becomes possible. These wounds were thus in a more advanced healing phase, and by definition were not at risk of failure.

It was of particular interest to analyse the clinical impact of PA-based hydrogels at a stage when proteases can be assumed to be unbalanced and granulation tissue had not formed. In these wounds, a chronic type of inflammation is prevalent, which is perpetuated by a number of different triggers released from damaged or necrotic tissue, slough with or without bacterial contamination and inflammatory cells culminating in uncontrolled protease levels in the wound area.[4,24–29] The elimination of factors which trigger high protease activity levels and the reduction in protease activity itself are fundamental targets in treating non-healing venous ulcers. In the light of a small patient population and an early study end the PA-based hydrogel, which possesses protease-inhibiting properties, effectively eliminated slough and necrotic tissue, and induced granulation tissue for-

mation, particularly, in hard-to-heal venous leg ulcers that had largely remained in a chronic non-healing state.

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