

# Prospective randomized double-blind study of the wound-debriding effects of collagenase and fibrinolysin/deoxyribonuclease in pressure ulcers

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## Abstract

**Background:** proteolytic enzymes such as collagenase, fibrinolysin and deoxyribonuclease are used for debriding purulent or fibrinous pressure ulcers.

**Objective:** to test the hypothesis that collagenase debrides necrotic pressure ulcers more effectively than fibrinolysin/deoxyribonuclease.

**Methods:** we enrolled 135 elderly patients with pressure ulcers in a randomized, prospective double-blind trial. Patients were treated until complete wound debridement or for a maximum of 4 weeks with twice-daily applications of collagenase or fibrinolysin/deoxyribonuclease. The primary endpoint was percentage change in the yellow or black wound surface. Secondary endpoints were wound environment, margins, depth, pocketing, area and healing. Assessment was by two independent dermatologists who were unaware of the treatment administered and evaluated results from photographs taken at the beginning and end of treatment.

**Results:** on intention-to-treat analysis, collagenase gave slightly better results with regard to the primary endpoint in the 121 assessable patients, but this difference was not statistically significant ( $P=0.115$ ). Additional efficacy measures did not show any statistically significant difference between the groups.

**Conclusion:** there was no evidence of a difference between collagenase and fibrinolysin/deoxyribonuclease in the debridement of pressure ulcers.

**Keywords:** debridement, pressure ulcer, treatment

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## Introduction

Pressure ulcers in elderly patients are often covered with purulent detritus or a necrotic eschar. Wound debridement must be complete before healing can take place [1, 2]. It can be achieved by various techniques, including surgical, mechanical, autolytic and enzymatic [3].

Enzymatic debridement has the advantage of being quick and non-invasive [3]. Products available for enzymatic wound cleansing include collagenase, streptokinase, streptodornase, trypsin, fibrinolysin and deoxyribonuclease (DNAse) [4, 5].

Collagenase, obtained from *Clostridium histolyticum*, cleaves native and denatured collagen [4]. Numerous studies, including several double-blind trials, have demonstrated collagenase's wound-cleansing effects in

comparison with placebo [6–12]. Fibrinolysin, from bovine plasma, cleaves fibrin-containing blood clots, thereby promoting wound debridement by macrophages. DNAse, obtained from bovine pancreas, promotes wound cleansing by breaking down the deoxyribonucleoproteins and deoxyribonucleic acid present in the necrotic tissue of chronic wounds [4]. The outcomes of studies investigating the use of fibrinolysin/DNAse in chronic wounds differ [13–17]. It is unclear whether enzymatic products differ in their wound-debriding effects.

Here, we compare the wound debriding efficacy of collagenase 1.2 U/g ointment (Novuxol) and fibrinolysin/DNAse (Fibrolan) ointment administered for about 28 (26–30) consecutive days in patients with pressure ulcers.

**Materials and methods**

Between May 1993 and November 1995 we recruited patients with pressure ulcers in the pelvic region from 17 hospitals in Germany which provided acute care and rehabilitation services for elderly patients.

The study protocol received ethics committee approval. Patients or their caregivers gave written consent.

Patients with Seiler stage 2, 3 or 4 pressure ulcers with fibrinous and/or necrotic slough were eligible for inclusion. If several pressure ulcers were present, the worst ulcer was chosen as the reference ulcer by the treating physician and treated during the course of the study. All patients were over 54 years of age (range 55–94 years). Those with a history of alcohol or drug dependency, end-stage malignant disease, a history of hypersensitivity to collagenase or fibrinolysin/DNAse, planned co-medication with local antiseptics, antibiotics, occlusive wound dressings, hydrogels or hydrocolloids were excluded. Ulcers covered with black eschar only or whose localization did not permit parallel positioning of the reference scale (e.g. in the anal fissure) were also excluded. The ulcers had to be between 2 cm and 14.5 cm in diameter to allow photographic assessment.

Randomized patients received twice-daily treatment either with collagenase (1.2 U/g) or fibrinolysin/DNAse (1 U Loomis and 666 U Christensen/g). The ointments were applied by nurses in a 2 mm layer to the ulcer and covered with gauze. They were not irrigated between treatments. Treatment was continued until complete wound debridement or for a maximum period of 4 weeks. The physician determined the type of mattress and frequency of repositioning.

The treating physician took at least 12 photographs of the reference pressure ulcer under standard conditions at the beginning of the study and about every 4 days thereafter. The last photograph of the ulcer was taken within 2 days of the last application of study medication. Photographs were taken using a specific camera (Canon Eos 100 QD, Compact-Macro EF 50 mm lens, f/2.5) with a special flash (Canon Ringblitz Macro Ring Lite ML 3). Each physician was trained in the use of the camera. A scale displaying a range of colours was placed adjacent to the pressure sore to facilitate standardized evaluation of the lesions. An automatic distance meter ensured that the photographs were always taken from the same distance.

The primary efficacy objective was the change in the area of the necrotic layer from baseline to end of study (or premature withdrawal from study). The change of the necrotic wound area was clinically assessed by two independent dermatologists by means of 13×18 cm photographs of the wound and classified into five categories: marked increase by at least 100%, appreciable increase by at least 30%, no appreciable increase, appreciable reduction by at least 25%, and marked reduction by at least 50%. All percentage increases and

decreases were approximate. The size of the wound surface at the start of the study was taken as 100%. The dermatologists were blinded to the treatment used.

Additional efficacy criteria assessed subjectively by the two independent physicians were: environment of the wound, wound margins, wound depth, pocketing, area and wound healing. For the evaluation, the same photographs were used.

All patients underwent a physical examination at the start of the study. Concomitant medication, inter-current disease, Norton Scale, and other measures were recorded, as were details of their pressure ulcers (Tables 1 and 2). Assessment of laboratory indices was done routinely at the first and the final visit: haemoglobin, haematocrit, blood count (red blood cells, white blood cells, platelets), differential white blood

**Table 1.** Characteristics of patients and pressure ulcers

	Group	
	Collagenase (n=66)	Fibrinolysin/ DNAse (n=69)
Mean duration (and SD), months	1.3 (0.6)	1.4 (1.0)
No. (and %) of subjects		
Seiler decubitus stage <sup>a</sup>		
2	18 (27.3)	20 (29.0)
3	44 (66.7)	43 (62.3)
4	4 (6.1)	6 (8.7)
Surgical pretreatment		
Yes	24 (36.4)	23 (33.3)
No	41 (62.1)	46 (66.7)
No data available	1 (1.5)	0
Support		
Normal mattress	18 (27.3)	23 (33.3)
Extremely soft mattress	12 (18.2)	16 (23.2)
Other	36 (54.5)	30 (43.4)

<sup>a</sup>1, reversible erythema, epidermis and dermis intact; 2, skin damage, subcutaneous tissue intact; 3, damage to adipose tissue, muscles, ligaments and tendons; eschar in many cases; 4, as stage 3, but additional involvement of bone and periosteum, osteomyelitis.

**Table 2.** Patient characteristics

	Mean value (and SD), by treatment	
	Collagenase (n=66)	Fibrinolysin/DNAse (n=69)
Age, years	78.4 (8.9)	79.7 (8.1)
Weight, kg	62.1 (16.0)	60.5 (12.2)
Body mass index	22.2 (5.2)	22.1 (4.7)
Modified Norton scale score at baseline <sup>a</sup>	18.6 (4.5)	19.1 (4.7)

<sup>a</sup>Score of 1–4 points on ability to cooperate, condition of skin, concomitant diseases, physical condition, mental condition, activity, mobility and incontinence plus score of 1 or 2 points for index of age. (Best possible score 34 points; the worst possible 9 points.)

The 14 patients who were not eligible for inclusion in the assessment did not differ from the study patients.

cell count, prothrombin time and PTT, electrolytes, bilirubin, liver enzymes, fasting glucose, serum protein electrophoresis, urea, serum creatinine and erythrocyte sedimentation rate.

Planning of the study was based on an estimated probability of 0.69 that collagenase reduces the necrotic wound surface to a greater extent than fibrinolysin/DNase. A sample size of 50 patients per treatment arm was calculated in order to identify the supposed difference between the products with 90% probability ( $\beta=0.1$ ) at a specified error probability of 5% ( $\alpha=0.05$ ) using Wilcoxon's test. Taking an assumed dropout rate of about 30% into account, the required sample size was set at 130 patients.

Efficacy in terms of the primary efficacy criterion was assessed for two populations:

1. Intention-to-treat analysis—including all patients who have received study medication and for whom data concerning the primary efficacy criterion have been generated after starting treatment with the study medication. This population was evaluated by end-point analysis—i.e. the principle of 'last observation carried forward' was applied.
2. Per-protocol analysis—including only patients who met all the criteria for inclusion and none of those for exclusion, and who completed the study without major protocol violations; especially, patients who were treated with the study medication for 26–30 days, and for whom the photographic documentation covered this period. Patients who discontinued the trial prematurely and whose withdrawal was related to therapy were included in the analysis. SAS software (version 6.11) was used for statistical evaluation.

## Results

All 135 enrolled patients received the study medication at least once and were consequently considered for safety analysis (safety population). Fifty-one percent of subjects in the collagenase group and 47% of those in the fibrinolysin/DNase group were women. Details of all the patients and their pressure ulcers are given in Tables 1 and 2.

In 14 patients, no data on the primary efficacy criterion were available, because the respective pictures of the wounds were not assessable. Therefore, these patients were excluded from the intention-to-treat population. Of the 121 patients in the intention-to-treat population, 16 from the collagenase group and 27 from the fibrinolysin/DNase group were excluded from the per-protocol population because of protocol violations. Therefore, the per-protocol population consisted of 78 patients. The disposition of study patients is displayed in Figure 1.

The statistical evaluation of the change of necrotic wound area in the intent-to-treat population showed

slightly better results for the collagenase group compared to the fibrinolysin/DNase group, however this difference was not statistically significant ( $P=0.115$ , Cochrane Mantel–Haenzel test; see Table 3). Analysis of the per-protocol population also revealed no statistically significant difference between the treatment arms ( $P=0.164$ ). Nor was there any statistically significant difference ( $P>0.1$ ) between the treatment groups in terms of the secondary endpoints, i.e. wound environment, wound margins, wound depth, pocketing, area and slough, and wound healing.

A total of 118 adverse events was reported in 45 patients (68.2%) of the collagenase group and 103 adverse events in 34 patients (49.3%) of the fibrinolysin/DNase group (Table 4). Seventy-eight

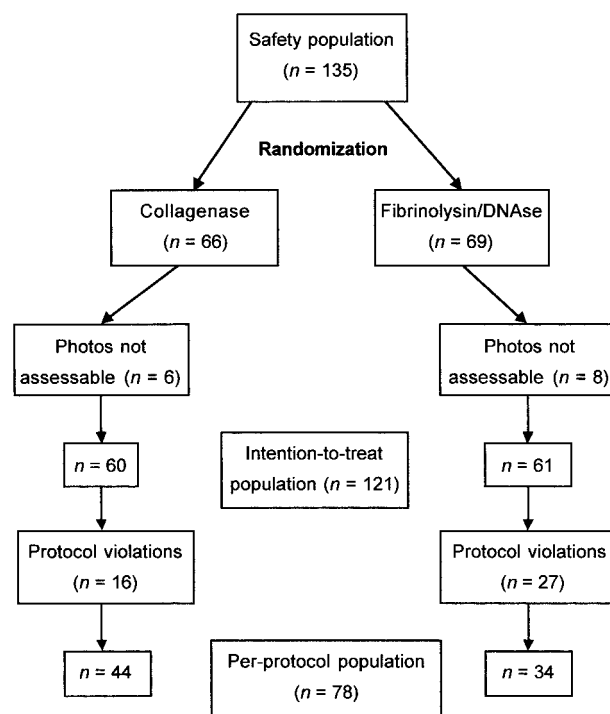


Figure 1. Study population.

Table 3. Primary end-point: change of the necrotic wound area (intent-to-treat population,  $n=121$ )

	Score	Treatment	
		Collagenase ( $n=60$ )	Fibrinolysin/ DNase ( $n=61$ )
No. (and %) of subjects showing change in necrotic wound area			
Clear increase ( $\geq 100\%$ )	1	3 (5.0)	7 (11.5)
Increase ( $\geq 30\%$ )	2	1 (1.7)	4 (6.6)
No change	3	19 (31.7)	15 (24.6)
Decrease ( $\geq 25\%$ )	4	9 (15.0)	13 (21.3)
Clear decrease ( $\geq 50\%$ )	5	28 (46.7)	22 (36.1)
Mean score of change (and SD)	–	4.0 (SD 1.2)	3.6 (SD 1.3)

## Wound-debriding effects of collagenase in pressure ulcers

**Table 4.** Adverse events classified by body system (safety population,  $n=135$ )

	No. of adverse events and patients			
	Collagenase ( $n=66$ )		Fibrinolysin/DNAse ( $n=69$ )	
	Events	Patients	Events	Patients
Whole body	57	30	45	24
Cardiovascular system	13	11	11	6
Digestive system	10	8	10	7
Nervous system	5	4	1	1
Haematological and lymphatic system	3	2	8	6
Metabolic and nutritional disorders	5	4	2	2
Musculo-skeletal system	1	1	0	
Respiratory system	12	8	11	7
Skin	6	5	5	5
Urogenital system	6	6	10	8
Total	118	45	103	34

adverse events were assessed by the investigator as serious: 54 in 16 patients in the collagenase group and 24 in 11 patients in the fibrinolysin/DNAse group. Twelve (18.2%) of the patients of the collagenase group and 11 (15.9%) of those in the fibrinolysin/DNAse group died. No serious adverse event was evaluated as probably or possibly related to study medication.

### Discussion

From the 121 patients (intention-to-treat population) the decrease of the necrotic wound area was slightly more pronounced in the collagenase group. A decrease in necrotic wound area was reported for 37 (61.7%) of the patients in this group, compared with 35 (57.4%) of those in the fibrinolysin/DNAse group.

For 35 patients in the collagenase group and 40 patients in the fibrinolysin/DNAse group the difference between the first picture and the last picture available for evaluation purposes was less than the required 26–30 days.

The patients in this study constituted a typical older patient population with multiple pathologies. This explains the large number of adverse events and the high mortality during the study. Both drugs were very well tolerated. No severe side effects attributable to either drug were observed.

The patients in the two treatment groups did not differ significantly as regards age, intercurrent diseases, concomitant therapy, modified Norton Scale or type of mattress.

The choice of the primary efficacy objective is of great importance in studies investigating the treatment of chronic wounds. Total wound closure is the only endpoint currently accepted by the US Food and Drug Administration [18]. This criterion is frequently impracticable, for many reasons. One is the long duration until total wound closure—as long as 6 months in stage 3 and 4 pressure ulcers [19]. Wound debridement was the selected endpoint in this study, as this criterion provides

a useful reflection of the effects of the drugs studied. Total wound healing is affected not just by wound debridement, however, but is also dependent on other factors such as nutritional status. Therefore, wound debridement is of limited value as a basis for assessing wound healing in general.

An earlier multicentre trial involving 258 patients also failed to show a significant difference in the clinical efficacy of three topical enzymatic products (fibrinolysin/DNAse, clostridiopeptidase with chloramphenicol and trypsin with famycetin) [20]. In spite of the difficulties involved in pressure ulcer therapy studies in elderly patients, further studies need to be carried out to provide an evidence base for treating pressure ulcers.

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