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Matrix Protection Therapy in Diabetic Foot Ulcers: Pilot Study of CACIPLIQ20®

Abstract

We evaluated whether matrix protection therapy by CACIPLIQ20® promotes healing of chronic lower extremity wounds in diabetic patients. Ten diabetic patients with non-infected chronic skin wounds and with no evidence of healing were included. CACIPLIQ20® was applied topically twice a week for 5 minutes for up to 10 weeks. Wound surface area was measured at baseline then weekly during treatment. Wound closure, defined as complete reepithelialization, was the primary endpoint. Mean wound surface area decreased by 25% within the first week (p=0.021 vs. baseline) and by 47% after 4 weeks (p=0.001 vs. baseline). After 10 weeks, the wound was closed in 6 of the 10 patients and decreased over 80% in the other patients. Subsequently, the none healed patients returned to standard care. Six months later, complete wound healing was noted in one additional patient and no further change in the remaining 3 patients. Two patients were again treated with CACIPLIQ20® for one month: one healed, the other improved again by 50%. Nine months later, closed ulcers did not re-open. No evidence of intolerance to CALCIPLIQ20® was noted. Matrix protection therapy holds considerable promise for healing chronic refractory foot wounds in diabetic patients.

Keywords: CACIPLIQ20®, diabetic ulcer, matrix protection therapy, regeneration, wound healing.

Introduction

Diabetes-related lower-extremity amputations are largely preventable. Among them, 85% are preceded by a foot ulcer.[1] Overall, it has been estimated that 15% of diabetic patients develop a neuropathic ulcer in their lifetime and that 15% of neuropathic ulcers eventually lead to amputation. Among patients who undergo lower-extremity amputation, about half require an amputation on the other side within the next 3 years, and about half of these die within the next 5 years.[2]

The substantial morbidity and mortality associated with diabetic foot ulcers is of considerable concern, as many of these ulcers are refractory to even optimal use of the standard treatment strategy. In the Eurodiale prospective cohort study of 1088 diabetic patients with foot ulcers in 14 countries, 23% of the patients still had unhealed ulcers after 1 year.[3]

Research efforts focus on the development of treatments capable of promoting the healing of diabetes-related ulcers. Thus far, however, multiple techniques based on cells and growth factors produced controversial results.[4-8] In wounds exhibiting chronic inflammation, necrosis, and/or fibrosis, a high-level of enzyme activity causes nonspecific destruction of the multiple components involved in wound healing and tissue homeostasis. These include glycosaminoglycans (GAGs) and, more specifically, heparan sulfate (HS) proteoglycans. HS proteoglycans interact with major components of the extracellular matrix (ECM) such as fibronectin, collagen type IV, and laminin and with a multitude of polypeptides that are involved in wound healing and regulate the bioavailability and transduction pathways of HS-bound polypeptides released by the cells or ECM. These include inflammatory mediators, chemokines, angiogenic factors, morphogens, and growth-promoting factors responsible for keratinocyte proliferation and migration, as well as dermal substratum reformation.[9] These data suggest that introducing a glycanase-resistant biopolymer, engineered to mimic HS, into the ECM might improve tissue healing by halting the endless cycles of ECM destruction and reconstruction that characterize chronic wounds. These biopolymers, known as regenerating agents (RGTAs), protect ECM proteins from proteolysis and enhance their bioavailability. This effect would be expected to promote wound healing.[10-12] CACIPLIQ20® is a member of the RGTA family.

The objective of this pilot study was to determine the effect of topical CACIPLIQ20®treatment on healing of chronic foot ulcers in diabetic patients.

Methods

PATIENTS

This open-label observational study was conducted between July and December 2009. Patients potentially eligible for study inclusion were identified through a manual search of the patient files at the Endocrinology and Diabetology Department of the Farhat Hached University Hospital in Sousse, Tunisia. Inclusion criteria were as follows: controlled diabetes; single chronic lower-extremity ulcer; ulcer size no greater than 12 x 12 cm^2; negative cultures of specimens from the depths of the ulcer; and the absence of healing despite at least 4 weeks of standard care including dressing changes, local debridement, and relief of pressure. A history of surgical excision was not a selection criterion. Exclusion criteria were wound infection, age younger than 18 years, terminal illness, inability to attend our clinic twice a week, pregnancy or lactation, and allergy to heparin.

Our study protocol complied with the ethical guidelines of

the Declaration of Helsinki and was approved by our institution's human research review committee. All patients gave their written informed consent before study inclusion.

STUDY TREATMENT

Extensive debridement is a key step to have full effect of CACIPLIQ20® by giving access to the wound bed. Therefore, the wound was first debrided to remove necrotic tissue, exudates, bacterial and fibrin and then cleaned with saline. The HS mimetic used in this study was CACIPLIQ20® (Skin Regenerating Kit, OTR3, Paris France), a class 3 CE marked medical device, containg a sterile solution intended for topical application to wounds. A gauze pad impregnated with CACIPLIQ20® solution was applied to the wound for 5 minutes every 3-4 days either until complete healing or up to 10 weeks. After removal of the gauze, the wound was covered by a non-adherent dry secondary dressing.

CACIPLIQ20® treatment contraindicates the combined use of products containing polycationic salts (e.g., povidone iodine, silver, gold, and copper zinc) or topical aminoglycosides (e.g., neomycin and gentamicin). None of these products were used during the study.

Patients received no specific advice about their diet or glucose control. They were instructed to continue following the recommendations made by their dietician and endocrinologist and to protect the affected foot from weight bearing.

The treatment was planned for ten weeks. However a second course of 4 weeks of treatment was proposed to the patients that remained non-completely healed after 6 months.

BASELINE ASSESSMENT

At baseline, we collected the following data: age, sex, glucose control, co-morbidities, wound characteristics, and wound duration. Ankle-brachial systolic pressure index (ABPI) was assessed in seven patients using a Doppler ultrasound blood flow detector and a sphygmomanometer. The wound was measured on the two perpendicular axes, of which one was the largest axe of the wound; the product of these two values was the wound surface area (WSA). The depth of the wound was also measured by introducing a sterile cotton ear stick in the deepest part of the wound.

Finally, descriptions were written of the following wound characteristics: amount of exudates, appearance, odor and presence of necrotic tissue, fibrin, budding tissue and/or re-epithelialization.

ASSESSMENTS DURING THE STUDY TREATMENT

WSA was measured once a week throughout the treatment period. Exudates and odor were recorded and scored. Pain was recorded using a Visual Analog Scale (VAS). Photographs were taken directly above the wound at each weekly assessment.

Primary endpoint was the rate of complete wound healing and defined as the reepithelialization of the entire wound surface area. It was verified 2 weeks later that the closure was effective and no reopening had occurred. Any adverse events, such as infection or hypersensitivity to heparin, were also recorded. Patients with unhealed wounds at the study completion were reevaluated 6 and 9 months later and after the second course of 4 weeks of treatment.

STATISTICAL ANALYSIS

Data were analyzed using SPSS 17.0 for Windows (SPSS, Chicago, IL). Continuous variables were described as mean ± SD. To compare variables at different time points, we used Student's t-test for continuous variables and the chi-square test for categorical variables. p values ≤0.05 were considered significant. →

Patient #	Sex	Age (years)	Type of diabetes (1 or 2)	Diabetes duration (years)	HbA1C (%)	Diabetic complications/ Co-morbidities	PN	ABPI
1	M	66	2	15	9.5	Retinopathy, hypertension, stroke	5	2.00
2	M	20	1	15	8.0	Retinopathy	5	–
3	F	57	2	7	10.0	Hypertension	6	1.30
4	M	50	2	9	9.2	–	2	1.19
5	M	51	2	15	8.5	Retinopathy, renal failure, hypertension, smoking	7	–
6	M	79	2	20	7.2	Myocardial infarction	4	–
7	M	56	2	17	7.0	-	1	0.70
8	M	24	1	2	9.0	Obesity, smoking	2	1.30
9	M	48	2	6	8.5	Obesity, smoking, alcohol abuse	2	0.70
10	M	57	2	12	8.0	Retinopathy, smoking	3	1.25

M, male; F, female; HbA1c, glycated hemoglobin; PN, painful peripheral neuropathy using the DN4 score [13]; ABPI, ankle-brachial systolic pressure index [14]

Table 1. Baseline characteristics of the 10 patients with diabetes-related ulcers.

Patient #	Location	Duration (weeks)	Surface area (cm^2)	Depth (cm)	Volume (cm^3)	Necrosis (%)	Fibrin (%)	Granulation tissue (%)
1	Lateral aspect of the right great toe	10	1.56	0.30	0.46	5	60	35
2	Anterior aspect of the leg	4	7.20	0.20	1.44	20	40	40
3	Sole (under second metatarsal head)	12	1.20	0.25	0.30	0	30	70
4	Sole (under second metatarsal head)	77	3.75	0.40	1.50	0	10	90
5	Right great toe amputation stump	12	5.25	0.70	3.67	0	50	50
6	Plantar aspect of the right second toe	77	2.00	0.20	0.40	0	10	90
7	Sole (under second and third metatarsal heads)	308	13.20	0.25	3.30	0	10	90
8	Sole	51	33.60	0.40	13.44	0	20	80
9	Right foot amputation stump	77	4.90	0.25	1.22	0	10	90
10	Left leg amputation stump	25	2.00	0.10	0.20	0	10	90
Mean ± SD		65.3 ± 90.4	7.46 ± 9.86	0.30 ± 0.16	2.59 ± 4.00			

Table 2. Baseline characteristics of the diabetic patient's wounds

Results

We included 10 patients with diabetes-related neuropathic ulcers, including 3 with ulcers on amputation stumps. Baseline data are given in Tables 1 and 2. One patient had a wound duration of 308 weeks; in the other 9 patients, mean wound duration was 38 weeks (ranging from 4 to 77 weeks).

CHANGES IN WOUND SURFACE AREA DURING THE STUDY TREATMENT

Mean WSA was significantly decreased compared to baseline after 1 week (p=0.021), 2 weeks, 3 weeks, and 4 weeks (p=0.001) *(Table 3)*. The rate of WSA decrease is shown in Figure 1. Healing was apparent as soon as the first treatment week *(Figure 2)*. After 10 weeks, wound healing was complete in 6 patients, (patients 1 to 6) *(Figure 2)*. In these 6 patients, the mean time to wound healing was 5.4 weeks (ranging from 4 to 9 weeks). After 10 weeks, in three remaining patients (7, 9 and 10) WSA was decreased by 80% and in patient 8 by 50% compared to baseline. For patient 9 and 10, after an apparently complete closure of the wound at 8 weeks, a small but visible reopening of the wound was measured. None of the patients experienced an increase in WSA at any time after the study.

Figure 3 shows examples of wounds before treatment and at the last follow up after 10 weeks of CALCIPLIQ20®.

Wound healing or wound size reduction occurred in patients with both types of diabetes, in patients with and without severe vascular co-morbidities, in patients with various degrees of glucose control and was independent to baseline WSA.

OTHER EFFECTS OF THE STUDY TREATMENT

All patients consistently reported pain relief during the

Time	Mean area ± SD (cm^2)	P value [confidence intervals]
Baseline	7.46 ± 9.86	–
Week 1	5.59 ± 7.88 *	0.021 [0.352 – 3.385]
Week 2	4.96 ± 8.58 ***	0.001 [1.279 – 3.728]
Week 3	4.27 ± 8.46 ***	0.001 [1.682 – 4.697]
Week 4	3.92 ± 8.64 ***	0.001 [1.850 – 5.231]
Week 10	1.60 ± 4.71 **	0.01 [1.811 – 9.920]

*p<0.05 versus baseline; **p<0.01 versus baseline; ***p=0.001 versus baseline

Table 3. Changes in wound area during the treatment.

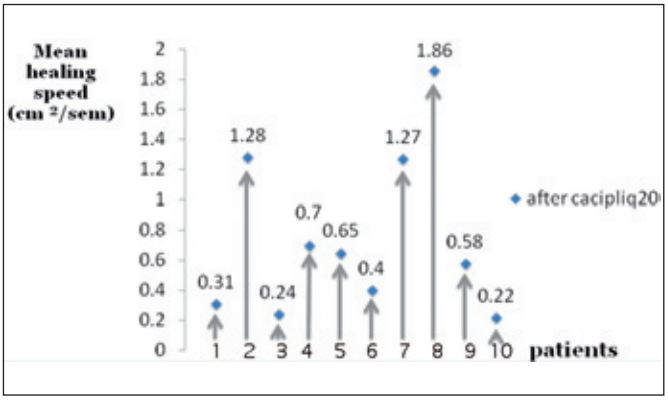

Figure 1. Mean wound healing speed in patients treated with CACIPLIQ20®. Healing speed is represented for each patient as the mean of the ratio of wound area reduction per each week.

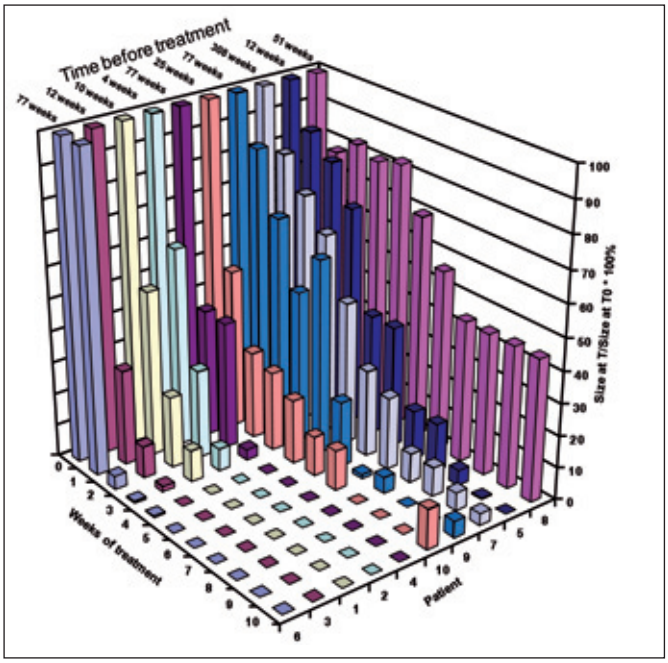

Figure 2. Standardized healing kinetics for each patient. At time of treatment wound surface area (WSA) was defined as 100% . Percentage of healing at a defined time is calculated for each patient from this origin value.

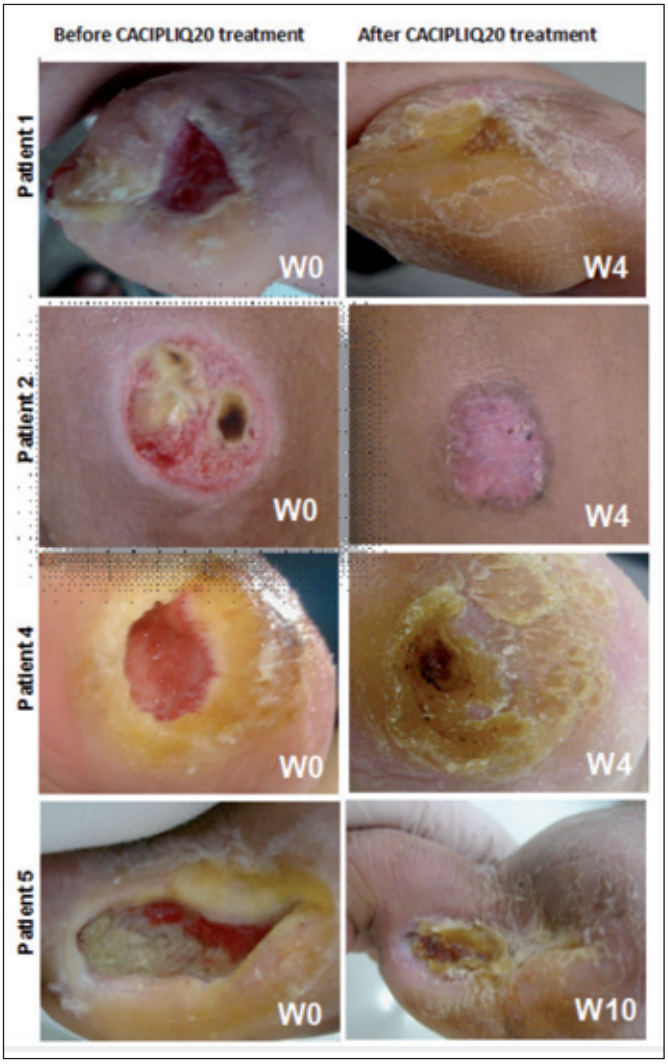

Figure 3: Wounds before and after CACIPLIQ20® treatment from patient 1, 2, 4 and 5

treatment. Odor and the amount of exudate and fibrin improved rapidly after starting the treatment. Also the wound aspect improved resulting in reduction of debridment duration and need.

No adverse effects were recorded. None of the patients required amputation or reamputation during and after the study period.

After the 10-week study, the patients received no further CACIPLIQ20® therapy leaving the 4 patients with persistent wounds at 10 weeks to standard care. After 6 months, in patient 7 the wound was fully healed. In the other 3 patients, no change in wound healing was noted compared to study completion. Moreover, no recurrence was observed after 6 and 9 months in the 6 patients with completely healed wounds. This in spite of their return to normal activity with well-tolerated weight bearing.

Among the three patients with non healed ulcer, treatment was reinitiated in two patients (the third, patient 10 was lost to follow-up). After 4 weeks of treatment, complete healing was obtained for patient 9. Patient 8 obtained a 50% reduction of the wound area. This patient 8 had the largest ulcer but also had the fastest healing rate (1.86 cm² per week). However, this patient was not compliant and the failure to eliminate weight bearing on the affected extremity likely also was associated with slower healing.

Discussion

In this pilot study on the wound healing efficacy of an HS mimetic, all 10 patients experienced improvements in their previously refractory diabetes-related ulcers. Improvements were noticeable as early as the first treatment week. This effect is remarkable, as mean ulcer duration was 38 weeks, despite optimal standard care. In addition, 6 of the 10 patients achieved complete wound healing within 10 weeks. The remaining 4 patients had substantial reductions in wound size. Finally, the patients reported relief of pain with the study treatment. 9 months follow-up data, on the patients who experienced complete wound healing during the study period, indicated no recurrence of the healed ulcer. Interestingly, even in non-healed ulcers, there was no increase in the wound area after treatment withdrawal. Thus, this treatment may hold promise as an efficient solution to heal ulcers and as a consequence a means of diminishing the amputation rate and improving quality of life in diabetic patients with foot ulcers.

In a parallel pilot study performed on a population of 16 patients (22 wounds) from patients with long standing venous and pressure ulcers in therapeutic failure (average 2.5 years) showed that all patient responded to CACIPLIQ20® which induced 15 to 18% ($p< 0.01$) WSA reduction at one month and another additive reduction of 18%-26% at 2 months[14] and 60-70% pain reduction ($p<0.001$). CACIPLIQ20® was well tolerated by all patient with a general satisfaction both patients and clinicians. Similar proves of efficacy and satisfaction were given in another pilot none-controlled study on long term (7 months) 14 none healing ulcers from 12 patients with critical ischemia which could not or no more benefit from vascular surgery. 10 patients responded with 37% WSA reduction in one month and complete ulcer closure in 50% of patients in 8 weeks. Furthermore a two years follow up indicated no recurrence of the healed ulcer (prof Desgranges, personal communication). These clinical data are consistent with this study and the preclinical studies of RGTAs. In a rat doxorubicin induced ulcer model in rats, mimicking a chronic ulcer, topical RGTA administration →

every 3 days for 5 minutes was sufficient to induce a dramatic increase in wound healing and quality of ulcer closure over saline controls.[15] Other skin injury experimental models were also demonstrated that RGTA reduced skin ulcer inflammation, modulated angiogenesis, and enhanced the speed and quality of tissue repair.[16-20] RGTA therapy is considerably easier to use than cell or gene therapy. RGTAs have no direct effects. Instead, they protect the cell microenvironment, allowing the normal local signaling cascade to unfold, thus enabling tissue regeneration.[15]

Most of clinical studies with various treatments assessed healing over a longer period (12-24 weeks) and included patients with diabetes-related wounds and other types of wounds.[21, 22] In recent years, several innovative local treatments for neuropathic foot ulcers have been evaluated including matrix elements, matrix scaffold alone or associated with living cells or growth factors to favor cellular migration and coverage of the wound.[23,24] Although use of negative pressure therapy[25] or oxygen therapy have provided some solution, there is a great need for a product to improve healing over standard care of the remaining 20-30% of none healing diabetic foot ulcers.[26, 27]

RGTA based matrix therapy is a unique approach and doesn't easily compare to other approaches by its mode of action and there are still too few clinical data to make comparisons.

Our study has several limitations. First, we had no control group. However, the rapid onset of wound healing after study treatment initiation was a marked departure from the previous absence of healing over many weeks despite optimal standard care. Our patients were under standard hospital care every 3-4 days. Despite this high frequency of care, that only rarely is met in clinics, they had not improved before RGTA matrix therapy treatment for an average of 38 weeks length, a delay placing our trial patient population among the 20-30% none healing diabetic foot ulcer.[26, 27]

Conclusion

The data reported here indicate that the topical application of CACIPLIQ20®, a member of the RGTA family, to ensure matrix protection, promotes the healing of refractory diabetes-related lower-extremity wounds when used in combination with appropriate wound care. However, a randomized controlled trial is warranted to further assess this treatment as a solution for the unsolved problem of chronic wounds. ■

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