Accelerated Healing of Chronic Sickle-Cell Leg Ulcers Treated With RGD Peptide Matrix

By Doris L. Wethers, Gloria M. Ramirez, Mabel Koshy, Martin H. Steinberg, George Phillips Jr, Robert S. Siegel, James R. Eckman, Josef T. Prchal, and the RGD Study Group

Leg ulcers are a chronic manifestation of sickle-cell disease (SCD) and are often painful, disabling, and difficult to treat. RGD peptide matrix treatment is a novel therapy designed to provide a topical synthetic extracellular matrix that can act as a temporary substitute for the damaged natural matrix at the ulcer site. In this randomized, placebo-controlled, double-blind, prospective, multicenter investigation, SCD patients with full-thickness leg ulcers were treated with standard therapy plus RGD peptide matrix or saline placebo once weekly for up to 10 weeks. Healing in patients with chronic ulcers (2 months or greater in duration) was significantly accelerated (P = .0086) in RGD peptide matrix recipients compared with the placebo group. In these chronic ulcer cases, the average percent ulcer closure (decrease in ulcer surface area) in the RGD peptide matrix group (54.4% ± 8.9%) exceeded that in the placebo group (19.0% ± 24.3%) nearly threefold by study endpoint. Furthermore, RGD peptide matrix was equally effective in promoting healing of long persistent ulcers and ulcers of shorter duration. In contrast, standard therapy plus placebo was significantly less effective (P = .001) in promoting healing for ulcers of progressively greater duration. The results of this study provide preliminary evidence that RGD peptide matrix treatment may significantly accelerate healing of chronic sickle-cell leg ulcers.

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LEG ULCERS IN PATIENTS with sickle-cell disease (SCD) are typically painful, disabling, long in duration, refractory to treatment, and prone to recurrence.1-3 In a study of 2,075 SCD patients 10 years of age and older, 25% of the patients had a history of leg ulcers or presented with leg ulcers during the study.4 In other studies, the reported percentage of SCD patients who will experience leg ulceration during their lifetimes ranges as high as 63%.4-7

The rate of ulcer healing in SCD patients has been found to be from threefold to 16-fold slower than the rate in patients with other types of leg ulcers.8 The average duration of ulcers in SCD patients has been reported to exceed 3 years.2 According to one report, recurrence rates after various types of therapy range from 25% to 52%.5 In another study, leg ulcers were found to be a frequent complication in SCD patients admitted to the hospital.9 An average of 55 days of hospitalization was required to heal each ulcer.

Both ulcer size and duration are thought to be important determinants of healing potential.10 Small ulcers tend to heal more rapidly, as do acute lesions.11 Ulcers of longer duration appear to be more refractory, possibly caused by "greater cutaneous fibrosis and further impairment of blood supply."11,12

Current treatments for sickle-cell ulcers generally fail to yield satisfactory results. The mainstays of present therapy include simple hygienic measures, protection from injury, antibiotic treatment of associated cellulitis, and bed rest in resistant cases.12,13 Among the standard dressings used for ulcers in SCD patients is Unna's boot.14 Skin grafting may be used in particularly refractory cases.9,15

Providing a synthetic extracellular matrix could facilitate ulcer healing and obviate surgery. The extracellular matrix is an essential component of tissues and organs that plays a key role in the healing process. The extracellular matrix provides cells with physical support, serving as an essential macromolecular scaffold that facilitates the migration of cells such as fibroblasts, endothelial cells, and keratinocytes into a wound site and allows the cells to organize and anchor themselves as healing progresses. Failure to regenerate the matrix normally leads to tissue deficiency and scarring.16

RGD peptide matrix is designed to act as a temporary, topical synthetic extracellular matrix that substitutes for the damaged natural matrix and provides support for cell ingrowth into the ulcer site. This synthetic matrix consists of an RGD-containing peptide complexed with sodium hyaluronate in a sterile, nonpreserved viscous gel. It is topically applied to the ulcer from a single-use syringe. At an ulcer wound site, the RGD peptide—like the natural matrix destroyed during ulcer formation—presents attachment sites for cells.17,18 The sodium hyaluronate provides a support scaffold, so that cell anchorage can occur. Cells attach themselves to the RGD sequences of the matrix via specific cell surface integrin receptors.19,20

In a number of in vivo and in vitro models, an RGD-containing peptide matrix has been shown to affect favorably various healing-related processes such as cell migration, granulation, keratinocyte layer formation, and wound strengthening.21,22 In a recent study, RGD peptide matrix was significantly more effective in promoting healing of diabetic foot ulcers than was standard therapy (Steed et al, submitted for publication). The present randomized, placebo-controlled, blinded, prospective, multicenter study was designed to determine the effectiveness and safety of RGD peptide matrix in the healing of sickle-cell leg ulcers.

MATERIALS AND METHODS

Of the 55 patients enrolled in the study, genotype was HbSS in 50 patients; HbSβ thalassemia in 2; and HbSC, HbSC Harlem, and

From St Lukes/Roosevelt Hospital, New York, NY; the University of Illinois Hospital, Chicago, IL; Veterans Administration Medical Center, Jackson, MS; Duke University Medical Center, Durham, NC; George Washington University Medical Center, Washington, DC; Emory University, Atlanta, GA; and University of Alabama at Birmingham, Birmingham, AL.

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Address reprint requests to Doris L. Wethers, MD, St Lukes/ Roosevelt Hospital, Comprehensive Sickle Cell Center, 411 W 114th St, New York, NY 10025.

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HBSD in 1 each. Patients were eligible for inclusion in the study if they presented with isolated full-thickness lower leg or ankle ulcers that did not involve bone or tendon and had persisted at least 1 month. The study ulcers ranged from ≥1.5 cm² to ≤100 cm² in surface area, as determined by computerized planimetry.

All patients were at least 18 years of age and had given their informed written consent according to Institutional Review Board requirements at the seven participating US institutions. All ulcers were free of both local infection and surrounding cellulitis, as judged by physical examination, and the use of topical antibiotics on the ulcer treatment site was not permitted during the study or within 2 days before study commencement.

Patients were excluded from the study if they presented with medical conditions that might retard healing, eg, immune system diseases, uncontrolled diabetes, bleeding disorders, neurological disorders, or cancer requiring chemotherapy or radiation treatment. Prospective study participants were also excluded if they were receiving medications that might adversely affect healing, such as systemic corticosteroids or anesthetic agents. A history of chronic transfusion therapy within the 3 months preceding study commencement was also a ground for exclusion.

A complete medical history was taken, and a physical examination was performed for all patients. Blood chemistry, hematology, and coagulation were evaluated and urinalysis was performed. Female study participants of child-bearing potential were not pregnant, as determined by laboratory assays, and were following a physician-supervised birth control regimen.

Randomization was performed with a 2:1 target ratio of RGD peptide matrix to normal saline placebo recipients to obtain a higher proportion of experimental data from RGD peptide matrix treatment than saline treatment. Patients were sequentially assigned to treatment groups based on a unique randomization number list established for each center before study commencement. On randomization, 32 patients were assigned to the RGD peptide matrix treatment group and 23 to the placebo group.

All patients received a standard therapeutic regimen to which applications of either RGD peptide matrix (Argidene gel; formerly Telio-Derm gel, Telios Pharmaceuticals, Inc, San Diego, CA) or saline placebo were added. Treatment applications and dressing changes were performed once per week at the clinic until the ulcer had healed or the patient had completed 10 weeks of therapy. Interim visits between the regularly scheduled visits were permitted in cases when the physician investigator deemed more frequent dressing changes to be appropriate for particular patients. At each visit, the ulcer was cleansed, debrided as needed, traced on acetate film for size determination, and photographed. Ulcer area was determined by computerized planimetry. Ulcer characteristics, patient complaints, and complications were recorded at each visit. To maintain blindling with respect to the investigator evaluating the ulcer, a member of the study support staff was responsible for cleansing the ulcer, applying the treatment, and dressing the treated site. Identical syringes were used to administer RGD peptide matrix and saline placebo, and no indication was given to patients as to the composition of the test preparations. The ulcer was then covered with a gauze dressing, and an Unna's boot was applied. Between treatment visits, the patients were allowed to remain ambulatory.

Statistical tests were performed by two-sided methods as applicable, and the criterion of significance adopted was P < .05. Differences between groups in patient age, clinical parameters, and baseline ulcer duration and area were evaluated by two-sample t-test or analysis of covariance. Either χ² or Fisher's exact tests were used for comparisons of sex, medical history, race, ulcer location, and frequency of complications. Differences in scar history, eschar, odor, erythema, fibrotic debris, maceration, necrosis, exudate, and severity of complications were assessed by Wilcoxon's nonparametric rank sum test, two-sample t-test, analysis of covariance, repeated-measures analysis of variance, χ², or Fisher's exact tests.

Changes in percent ulcer closure occurring between study commencement and endpoint were evaluated by t-test. Repeated measures analysis of variance was used to compare the rates of ulcer closure between the two groups, as well as between men and women within each of the two groups. The difference between the RGD peptide matrix and placebo groups in the proportion of patients with completely healed ulcers was assessed by χ² analysis. Changes in mean percent closure among ulcers of varying baseline durations, as well as baseline sizes, were tested for significance by regression analysis.

RESULTS

Of the 55 patients enrolled, 48 completed the study. Among the 7 patients—5 (16%) in the RGD peptide matrix group and 2 (9%) in the placebo group—failing to complete the study, the reasons for discontinuation were adverse events (3 RGD peptide matrix patients and 1 placebo patient) and noncompliance with the study protocol (2 RGD peptide matrix patients and 1 placebo patient). Comparisons of treatment effectiveness (extent and rate of ulcer closure and percent completely healed), except as otherwise noted, and safety (patient complaints, ulcer pathology, adverse events) encompassed the entire enrolled population of 55 patients through either study completion or time of discontinuation.

The RGD peptide matrix and placebo groups did not differ significantly with respect to gender, age, baseline ulcer duration, or baseline ulcer size (Table 1). The two groups were similar in baseline physical examination and laboratory results; ulcer characteristics such as granulation, eschar, and necrotic tissue; and patient complaints (itching, stinging/burning, edema, rash, cellulitis, folliculitis, and pain). There was no significant difference between the groups in number of interim visits for dressing changes.

In both the RGD peptide matrix and placebo groups, all the ulcers were situated at the ankle. In RGD peptide matrix patients, 68.8% of the ulcers were medial and 28.2% lateral. The remaining ulcers were situated either surrounding the ankle or at a posterior site, ie, over the Achilles tendon. In the placebo group, 69.5% of the ulcers were on the medial surface of the ankle, 26.1% on the lateral surface, and the remainder elsewhere. The distribution of ulcer locations did not differ significantly between the treatment groups.

Of the 55 patients enrolled in this study, 48 presented with

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<th>Table 1. Patient Data and Baseline Ulcer Characteristics</th>
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<td>Parameter</td>
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<td>No. of Patients</td>
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<td>Ratio of males to females</td>
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<td>Age* (yr)</td>
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<td>Baseline ulcer duration* (mo)</td>
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<td>Baseline ulcer area* (cm²)</td>
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* Mean ± SEM; ranges are shown in parentheses.
chronic ulcers (2 months or greater in duration). Healing of these chronic ulcers was significantly accelerated \( (P = .0085) \) in RGD peptide matrix recipients compared with the placebo group (Fig 1). In these chronic cases, the average percent ulcer closure in the RGD peptide matrix group \( (54.4\% \pm 8.9\%) \) exceeded that in the placebo group \( (19.0\% \pm 24.3\%) \) nearly threefold by study endpoint.

Although the ratio of men to women in the RGD peptide matrix group \((21:11)\) was greater than that in the placebo group \((12:11)\), as indicated in Table 1, this difference was not statistically significant \( (P = .147) \). The possibility of differences in response to treatment because of gender was tested by analysis of variance within and between treatment groups. No significant differences in the healing rate could be attributed to the difference in ratio of men to women between the two treatment groups.

Once weekly treatment with RGD peptide matrix was equally effective in promoting healing of long persistent ulcers and ulcers of shorter duration. For instance, the mean percent ulcer closure achieved with RGD peptide matrix treatment was \( 47.8\% \pm 13.0\% \) in ulcers of baseline duration greater than 24 months compared with \( 49.2\% \pm 8.1\% \) for ulcers 1 month or longer in baseline duration. As shown in Fig 2, there was no statistically significant diminution in RGD peptide matrix effectiveness with increasing ulcer duration. In contrast, percent ulcer closure in placebo patients progressively declined with increasing ulcer duration \( (P = .001) \), ie, ulcer surface area actually increased over the course of the study among placebo patients with long persistent ulcers.

Among all 55 patients enrolled, the average percent ulcer closure at study endpoint (either after 10 weeks of treatment or at last evaluation for patients healing in less than 10 weeks and discontinued patients) was \( 49.2\% \pm 8.1\% \) in RGD peptide matrix recipients compared with \( 23.5\% \pm 20.7\% \) in the placebo group. No statistically significant difference could be shown between the two groups in rate of ulcer closure. However, based on comparison within groups between ulcer surface area at study commencement versus study endpoint, a significant decrease in ulcer surface area \( (P < .001) \) was observed in patients receiving RGD peptide matrix, but not placebo. The change in mean ulcer closure between study commencement and endpoint was not found to be significantly related to baseline ulcer size in either treatment group.

Of the 32 RGD peptide matrix recipients, complete healing was achieved in 5 \( (15.6\%) \) compared with 9 \( (39.1\%) \) of the 23 patients in the placebo group. The difference in percent of patients completely healed was not statistically significant. The mean size of ulcers healed in the RGD peptide matrix group \( (10.2 \pm 2.8 \text{ cm}^2) \) was significantly greater \( (P = .03) \) than that in the placebo group \( (3.6 \pm 1.2 \text{ cm}^2) \). In addition, the mean baseline duration of healed ulcers in the RGD peptide matrix group \( (20.6 \pm 16.0 \text{ months}) \) was more than threefold greater than that in placebo recipients \( (5.9 \pm 2.6 \text{ months}) \), although this difference was not statistically significant. Because the healed placebo ulcers were substantially smaller in baseline size and shorter in baseline duration, they may have had an inherent capacity for more rapid spontaneous healing, as noted in published clinical reports previously discussed.

Among the 7 patients \( (4 \text{ in the RGD peptide matrix group and } 3 \text{ in the placebo group}) \) with acute ulcers \( (1 \text{ month baseline duration}) \), all 3 placebo patients and 1 RGD peptide matrix patient experienced complete healing. The difference in mean percent ulcer closure over the 10-week study period between the RGD peptide matrix \( (37.8\% \pm 26.9\%) \) and placebo groups \( (100.0\% \pm 0.0\%) \) was not statistically significant among this subset of patients with acute ulcers. The lower extent of ulcer closure in the 4 RGD peptide matrix patients with acute ulcers may have been at least in part attributable to the fact that the average baseline size of their ulcers \( (9.5 \pm 1.6 \text{ cm}^2) \) was \( 83\% \) greater than that of the 3 placebo patients \( (5.2 \pm 3.1 \text{ cm}^2) \), even though this difference was not statistically significant.

RGD peptide matrix was well-tolerated. Patient complaints and observed pathology at the ulcer site during treatment were comparable between the two study groups. There was no statistically significant association between RGD peptide matrix treatment and the occurrence of adverse events. The adverse event incidence rate among the RGD peptide matrix recipients was 0.53 events per patient \( (17 \text{ events in } 32 \text{ patients}) \) compared with 0.70 \( (16 \text{ events in } 23 \text{ patients}) \) in the placebo group. No adverse events were classified as either probably or definitely related to the study treatment. Three of the adverse events in the RGD peptide matrix patients (mild blistering; erythema, itching, and rash; sweating and itching) were judged to be possibly related to the study treatment compared with two such adverse events in the placebo group (swelling and impaired function of the hands; pain, tenderness, and skin discoloration).
INTERACTIONS between cells and the extracellular matrix are important in the complex process of wound healing. Failure to epithelialize, despite the appearance of apparently healthy granulation tissue, is commonly observed in chronic wounds treated with occlusive dressings. Epithelialization is critically dependent on epidermal cell movement. RGD peptide matrix treatment is designed to facilitate such cellular movement and thereby to promote healing.

The results of this study provide evidence that RGD peptide matrix treatment significantly accelerates healing of chronic sickle-cell leg ulcers. Chronic ulcers have, on the basis of clinical experience, long been considered highly refractory to treatment. The present study provides quantitative data in support of this clinical impression. Standard therapy plus placebo proved progressively less effective in promoting healing of ulcers of increasing baseline duration. No such diminution in healing of more persistent ulcers was demonstrable with RGD peptide matrix. This observation suggests that RGD peptide matrix may prove particularly useful for established sickle-cell ulcers, a category of lesions associated with treatment failure, complications, and relatively high costs of care.

In RGD peptide matrix recipients ulcer closure increased continuously over the course of the comparatively short 10-week regimen of once weekly treatment (Fig 1) with no evidence of having plateaued by week 10. This pattern of progressively increasing closure suggests the possibility that further clinical benefits—such as an increase in the proportion of patients achieving total healing—might be derived from a longer treatment course with RGD peptide matrix. It is also possible that more frequent applications of RGD peptide matrix, eg, twice weekly, would further enhance efficacy. In a recent randomized, placebo-controlled, blinded, prospective, multicenter study of 65 diabetic patients with foot ulcers, the percentage of patients whose ulcers healed completely with twice weekly RGD peptide matrix treatment was more than fourfold greater than that in the placebo group over the 10-week course of the study (Steed et al, submitted for publication).

Topical treatment with RGD peptide matrix—a synthetic, chemically well-defined gel—proved in this study to be capable of significantly accelerating healing with once weekly application over a relatively short 10-week period in ambulatory patients with chronic lesions. Ulcers of long duration were found to be responsive to RGD peptide matrix. Further clinical experience will be needed to assess the possible incremental benefits of longer and/or more frequent treatment with RGD peptide matrix than was used in this study. Nevertheless, the present study documents a significant clinical benefit of RGD peptide matrix treatment for chronic leg ulcers in SCD patients.

APPENDIX

The members of the RGD Study Group were Viola Knors at St. Luke's/Roosevelt Hospital, New York, NY; Nasrin Talischy, MD, Glenda Pendarvis, Eliza Gallo, and J. Louise Dorn at the University of Illinois Hospital, Chicago, IL; Arleen Anderson at the Veteran's Administration Medical Center, Jackson, MS; Adrena Johnson at Duke University Medical Center, Durham, NC; Helen D. Howse, Dao Mai, and M.K. Holohan at George Washington University Medical Center, Washington, DC; Allan Plat and Joyce Howard at Emory University, Atlanta, GA; Brian Addler, MD, and Jennifer Braddock at the University of Alabama at Birmingham, Birmingham, AL; and Thomas O. Thayer, Lori S. Mann, and Peter R. Nicholson at Telios Pharmaceuticals, Inc, San Diego, CA.

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DL Wethers, GM Ramirez, M Koshy, MH Steinberg, G Jr Phillips, RS Siegel, JR Eckman and JT Prchal

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