Thrombin peptide Chrysalin® stimulates healing of diabetic foot ulcers in a placebo-controlled phase I/II study

Caroline Fife, MD[1]; Jon T. Mader, MD[2]; Jeffery Stone, MD[3]; Leon Brill, DPM[3]; Kathleen Satterfield, DPM[4]; Andrea Norfleet, PhD[2,5]*; Amber Zwernemann, BS[2]; James T. Ryaby, PhD[5]; Darrell H. Carney, PhD[2]

1. Department of Anesthesiology, University of Texas Health Science Center, Houston, Texas,
2. Department of Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, Texas,
3. The Limb Salvage Center at the Brillstone Building, Dallas, Texas,
4. University of Texas Health Science Center, Texas Diabetes Institute, San Antonio, Texas, and
5. OrthoLogic Corp., Tempe, Arizona

Reprint requests:
Darrell H. Carney, PhD, Department of Biochemistry and Molecular Biology, The University of Texas Medical Branch, 301 University Blvd., Galveston, TX 77555-0645.
Tel: +409 772 3210;
Fax: 409-772-2880;
Email: dcarney@utmb.edu

*Dr. Norfleet's current address is: Genzyme Corporation, Cambridge, MA 02142.

Manuscript received: January 31, 2006
Accepted in final form: September 13, 2006

DOI:10.1111/j.1524-475X.2006.00181.x

ABSTRACT

Thrombin and thrombin peptides play a role in initiating tissue repair. The potential safety and efficacy of TP508 (Chrysalin®) treatment of diabetic foot ulcers was evaluated in a 60-subject, prospective, randomized, double-blind, placebo-controlled phase I/II clinical trial. Chrysalin® in saline or saline alone was applied topically, twice weekly, to diabetic ulcers with standardized care and off-loading. A dose-dependent effect was seen in the per-protocol population where 1 and 10 μg Chrysalin® treatment resulted in 45 and 72% more subjects with complete healing than placebo treatment. Chrysalin® treatment of foot ulcers more than doubled the incidence of complete healing ($p < 0.05$), increased mean closure rate ~80% ($p < 0.05$), and decreased the median time to 100% closure by ~40% ($p < 0.05$). Chrysalin® treatment of heel ulcers within this population resulted in mean closure rates 165% higher than placebos ($p < 0.02$) and complete healing in 86% (6/7) of ulcers compared with 0% (0/5) of placebo ulcers ($p < 0.03$). Local wound reactions and adverse events (AEs) were equal between groups with no reported drug-related changes in laboratory tests or serious AEs. These results indicate the potential safety and efficacy of Chrysalin® for treatment of diabetic foot ulcers.

Chronic diabetic ulcers of the lower extremities represent a major healthcare problem today, with over 850,000 diagnoses made in the United States each year.[1,2] Because of the increasing incidence of diabetes, the magnitude of the challenge presented to the healthcare system by chronic foot and leg ulcers is also expected to increase.[1,2] Chronic diabetic ulcers not only negatively impact the quality of life but can also lead to amputation and an increased likelihood of death.[3]

Healing a chronic diabetic ulcer is an expensive, time consuming, and complicated task. A significant fraction of these ulcers do not heal or do not remain healed, and become chronic wounds that endure for months or years. The cost to the healthcare system is estimated at greater than 10 billion dollars per year, in addition to untold losses in workplace productivity.[3,4] Diabetic ulcers are distinguished from acute wounds in healthy individuals by several factors that stem from the underlying pathology of diabetes including the aging of tissues, hypoxia, and infection.[5–7] In addition, diabetics exhibit various degrees of peripheral neuropathy. Diabetics also have a dysfunctional endothelium, which fails to respond to various growth factors and angiogenic stimuli,[8,9] and increased levels of metalloproteinases that degrade matrix molecules and decrease the half-life of growth factors in wound fluid.[10–12] The impaired wound environment characteristic of chronic, nonhealing ulcers has led to development of a standard regimen of chronic wound care that includes appropriate wound bed preparation, moist wound coverings, and pressure off-loading. For many larger ulcers, treatment in wound-care centers now often includes use of bioactive skin substitutes, delivery of autologous platelet concentrates, or application of a therapeutic agent such as becaplermin (Regranex® OMJ Pharmaceutical Inc., San Germán, Puerto Rico). Although these therapeutic options have shown potential efficacy in clinical trials and received FDA approval, they do not appear to have been widely accepted as standard of care.

The reasons why therapeutic alternatives approved by the FDA for diabetic foot ulcers have not been widely accepted include limited benefit over standard of care, treatment regimens that require extensive debridement, daily treatment, and/or repeated visits to specialized wound care facilities. Clinical trials with becaplermin (Regranex®), using a regimen that included daily treatment for 12 hours, rinsing with saline, and rebandaging, e.g., showed complete healing in ~50% of subjects relative to 35% who were treated with good clinical practice and placebo gel.[13,14] A subsequent post hoc analysis suggested that the efficacy of becaplermin was seen in subjects whose wounds were debrided almost every week.[15] In both the home health setting and in many wound clinics, treatment schedules and debridement fall short of the regimen that may be required for becaplermin efficacy. Therefore, a more ideal therapeutic for diabetic foot ulcers may be one that can be easily applied in the home care

environment, can be applied less often without reducing efficacy, and is less dependent upon surgical debridement for its efficacy.

Chrysalin® (TP508) is a 23-amino acid peptide representing the natural sequence of amino acids of human thrombin identified as the thrombin-binding domain for a specific class of thrombin receptors on fibroblasts and other cells.[16] Early studies showed that thrombin, the serine protease responsible for fibrin clot formation, initiated cell proliferation and other cellular postclotting events through a growth factor-like mechanism that involved its binding and activation of specific thrombin receptors on the surface of fibroblasts and other cells.[17–19] Although many of the cellular effects of thrombin appear to require proteolytic activity and activation of proteolytically activated receptors,[20] studies show that binding of thrombin or thrombin derivatives without proteolytic activity promotes a number of cellular events involved in tissue repair and wound healing.[21–24]. These observations have led to the hypothesis that nonproteolytic peptide fragments of thrombin released from a fibrin clot during early stages of wound repair may modulate inflammation and promote healing.

Unlike thrombin, which is activated at the site of injury, the Chrysalin® peptide has no enzymatic activity and does not promote or interfere with blood coagulation.[16] Preclinical safety studies have shown that the peptide can be injected intravenously or intraperitonealy at doses of up to 25 mg/kg with no adverse effects, that it is classified as a nonsensitizer based on hamster skin sensitivity testing, and that topical treatment of open porcine wounds (followed upon wound closure with dermal injection at the wound site) of 100 μg/day for 20 weeks had no apparent negative effects (OrthoLogic Corp., unpublished results).

In full-thickness incisional wounds in rats, a single topical application of Chrysalin® increased the breaking strength of wounds by approximately 80% over saline controls when measured at day-7 postincision.[25] Relative to control breaking strength, this single application of Chrysalin® shifted the healing curve forward by approximately 4 days. Significant effects of Chrysalin® were also seen on incisional wounds in rats with radiation-induced healing impairment.[26] In larger full-thickness excisional animal wounds in normal[27] and ischemic skin,[28] a single topical application of Chrysalin® also accelerated wound closure. In all of these model studies, Chrysalin® accelerated recruitment of inflammatory cells to the wound site, shortened the inflammatory phase, and promoted early revascularization of the tissues.[27–29] Chrysalin® also accelerated repair of rat fresh fractures[30] and promoted bone formation in rabbit critical size segmental bone defects[31] and in a rabbit model of distraction osteogenesis.[32] Thus, this molecule may serve as a natural initiator of tissue repair in a number of tissues.

Based on preclinical studies, we hypothesized that Chrysalin® may improve repair quality and accelerate the rate of tissue repair following surgical or traumatic acute tissue damage and reinitiate healing of chronic ulcers where normal repair processes are disrupted. We now report the results of the first human phase I/II pilot clinical trial designed to evaluate the safety and efficacy of Chrysalin® in the treatment of chronic diabetic lower extremity and foot ulcers. Modeled after other diabetic foot ulcer clinical trials, but with expanded entry criterion including inclusion of larger, more severe (Grade III) ulcers, ulcers on the leg and ankle, and those with O_2 tension ($TcPO_2$) levels of between 20 and 30 mmHg, this trial was designed to determine whether twice-weekly application of Chrysalin® could cause healing of diabetic ulcers and help us better define the role of thrombin peptides in tissue repair.

MATERIALS AND METHODS

Overall study design

This study was a multicenter (four sites), prospective, randomized, double-blind, placebo-controlled pilot clinical trial to evaluate the safety and potential efficacy of Chrysalin® topically applied to diabetic ulcers. The study was designed as a three-arm, 60-subject trial including lower extremity (below the knee) ulcers ranging from 0.9 to 38.5 cm^2 ($\sim$1 to 7 cm in diameter) that had been present for more than 8 weeks and that were classified as Wagner Grades I, II, or early III[33] (Grade III ulcers included deep ischemic ulcers that exposed bone or tendon, but excluded those considered to have eroded into the bone or tendon). The exclusion criteria included the following: clinical infection of the ulcer, the presence of uncontrolled systemic infection or osteomyelitis; poor diabetes control, renal failure, abnormal liver function; treatment with steroids, chemotherapeutics, or radiation within 6 months before study enrollment; cancer; a history of drug or alcohol abuse; and wound oxygen tension ($TcPO_2$) of < 20 mmHg. Women who were pregnant, nursing, or of child-bearing potential and not using approved birth control were excluded.

The study included a preenrollment/screening visit, followed by twice-weekly office visits for up to 20 weeks or until the ulcer reached complete closure. Eligible patients, upon signing an informed consent, were randomized to one of three subject treatment groups: 1 μg Chrysalin®, 10 μg Chrysalin®, or placebo. The wound bed was prepared by sharp debridement as deemed necessary by the investigator physician, irrigated with saline, and blotted with gauze. Study treatment was administered topically in a volume of 0.1 cm^3 of saline solution. After approximately 1 minute, the wound was covered with Cutinova Foam® (Beiersdorf, AG, Germany) and bandaged. If the ulcer was on a pressure-bearing surface, the clinician prescribed offloading. In most cases, offloading was accomplished by using sponsor-provided D.H. Walker offloading boots (made by Royce Medical), although some subjects were prescribed crutches or wheel chairs without offloading boots. Bandages were removed during the twice-weekly visits for ulcer evaluation, debridement as needed to remove necrotic tissue, and retreatment for up to 20 weeks or complete wound closure. Subject compliance was monitored and treatment control was insured as study drug application and bandaging were conducted in the clinic by the attending clinician or nurse. Subjects were removed from the study if a clinical infection developed or if the wound condition significantly worsened. Any such removals were counted as closure failures.

Safety and efficacy endpoints

At each clinic visit, adverse events (AEs) were recorded, and local wound reactions were scored for erythema, edema, pain, and overall condition. At enrollment and at weeks 5, 10, 15, and 20, blood was drawn for chemical and hematological analyses, radiographs were obtained, and wound cultures were performed.

Before and after wound debridement, the ulcer perimeter was traced onto acetate and photographed with a digital camera. The acetates were preprinted with a standard ruler and 1 cm diameter circle. Each tracing was analyzed using digital morphometric analysis software (Image-Pro, Media Cybernetics, Silver Spring, MD) to determine both open ulcer area and the perimeter of the ulcer. The primary efficacy endpoint was the incidence of ulcers that progressed to complete closure during the 20-week study. Secondary endpoints included the time to 100% and to 80% closure of the study wounds.

A post hoc analysis was also performed to access the linear rate of wound closure (wound healing rate [WHR] expressed in mm of edge closure per day) using the reduction in area per day divided by the average wound perimeter using the following formula.[34,35]

$$\mathrm{WHR} = [(\mathrm{Area}\ T_0 - \mathrm{Area}\ T_X)/([\mathrm{Perimeter}\ T_0 + \mathrm{Perimeter}\ T_X]/2)]/\mathrm{days}(T_X)$$

This calculation of WHR (mm/day) provides an average vectoral rate of closure from the wound edge, which conceptually is the same as a decrease in wound radius. As debridement was performed as needed (in some cases, at almost every visit), the WHR reflects the average closure per day excluding any tissue growth that was removed by debridement.

Study drug

Chrysalin®, also known as TP508 (CAS #497221-38-2), is a synthetic peptide representing the native 23 amino acid sequence of human thrombin that appears to bind to high-affinity thrombin receptors on cells to activate a sequence of cellular events.[16] The peptide was chemically synthesized and purified to $> 95\%$ by HPLC under cGMP (Peninsula Laboratories, Belmont, CA), and then, filter-sterilized, sterile-filled, and lyophilized in 2 cc glass vials (Ben Venue, Bedford, OH) and stored at 4 °C. Upon subject enrollment, the peptide was dissolved and diluted in sterile, pyrogen-free, saline (Abbott Laboratories, Chicago, IL) by an unblinded pharmacist. To ensure blinding of the subject and clinician, treatment solution was delivered to the clinic in a vial identified with only the subject's ID number.

Study groups and statistical analyses

Two primary populations were defined for analysis before unblinding of subject treatments and were used in reporting this study to the FDA. All subjects with study ulcers who received at least one treatment were included in an intent-to-treat (ITT) population. Because a number of subjects were enrolled in this study with ulcers that should have been excluded based on ulcer size, chronicity of the ulcer (based on the length of time the ulcer had been present before treatment), or Wagner entry criteria, a per-protocol (PP) population was defined before unblinding and used for efficacy analysis. The PP population differed from the ITT population in that seven subjects were removed for protocol deviations, eight had ulcers $< 0.9\,\mathrm{cm}^2$ at baseline, four had been present for < 8 weeks, and one did not meet the Wagner entry criteria. This left a total of 40 subjects in the PP population: 15 placebos, 11 treated with 1 μg Chrysalin®, and 14 treated with 10 μg Chrysalin®. Subjects prematurely removed from the study for any reason, were counted as closure failures, but ulcer area measurements were used up to the time of discontinuation for healing rates and percent closure analysis.

Clinical monitoring of sites and statistical analysis was performed by Synergos Inc. (Woodlands, TX). Standard statistical methods were used to analyze all data. These included Fisher's exact test and Student's T test using two-tailed tests with an α of 0.05 and Kaplan–Meier analysis of time to 80 and 100% closure. No adjustment was made for multiple comparisons for either the efficacy analysis or the safety analysis.

As the ITT and PP populations included individuals with ulcers located below the kneecap on the leg, ankle, and foot, a subset analysis was performed post hoc to determine the potential effects of Chrysalin® on ulcers located on the foot. Ulcers located on the ankle and leg often have very different etiologies and may arise from vascular insufficiency and may not involve neuropathy or pressure. These lower limb ulcers are often distinguished from foot ulcers as they may require a different treatment regimen including pressure bandaging for optimal healing. Relative to the ITT population, the foot ulcer population for this analysis excluded: nine ulcers not on the foot; 10 that were

Table 1. Demographic data for intent-to-treat population

	Treatment group		
Demographic characteristic	Saline control (n=21)	1 μg (n=20)	10 μg (n=18)
Male [n (%)]	15 (71)	14 (70)	14 (78)
Race [n (%)]			
Caucasian	11 (52)	12 (60)	11 (61)
Black	6 (29)	4 (20)	2 (11)
Hispanic	3 (14)	4 (20)	5 (28)
Other	1 (5)	0 (0)	0 (0)
Age (years)			
Mean ± SD	55.7 ± 12.8	59.3 ± 6.4	53.4 ± 10.5
Median	54.7	59.6	53.7
Weight (lbs)			
Mean ± SD	196.3 ± 77.3	206.5 ± 41.8	229.5 ± 58.8
Median	203.5	211.0	220.0
Ulcer area (cm^2)			
Mean ± SD	4.11 ± 5.99 (20)	3.59 ± 5.31 (21)	3.15 ± 3.20 (18)
Median	1.63	1.21	2.02
Range	0.16–26.46	0.27–24.36	0.14–13.10

$< 0.9\,cm^2$; four that had been present for $<$ 8 weeks; and four that were removed from consideration due to unrelated SAEs (one—Chrysalin® 1 μg, two—Chrysalin® 10 μg, and one—saline placebo). This left a population of 35 subjects: 13 placebo subjects; 12 Chrysalin® 1 μg subjects; and 10 Chrysalin® 10 μg subjects. All subjects removed from the study for infection, osteomyelitis, or worsening of ulcer condition were included and counted as closure failures. Thus, this population is similar to, but not the same as the PP population. As described below, the demographics of this population showed no significant differences between the groups and group means for subject age and ulcer starting size were similar to values for the ITT populations (see Tables 1 and 4).

A further subset analysis was performed to ensure that the location of ulcers on the foot was not biasing data to favor groups treated with Chrysalin®. Foot ulcers located on the heel of the foot, e.g., are among the most difficult ulcers to heal.[36,37] As shown in Table 5, heel ulcers made up five of 13 (38%) of the placebo foot ulcers, three of 12 (25%) of those treated with 1 μg Chrysalin®, and four of 10 (40%) of those treated with 10 μg Chrysalin®. Analysis of Chrysalin® effects on this heel ulcer subpopulation was performed looking at the incidence of complete closure achieved by 20 weeks and the rate of healing as described above.

Human ethical considerations

This study was conducted as a part of US FDA IND # 56,811. Patient consent to become subjects in this trial was obtained before study treatment for each subject and the protocol conformed to ethical guidelines of the 1975 Declaration of Helsinki as reflected in approval by the human research review committee or appropriate Institutional Review Board at each clinical site.

RESULTS

Subject demographics

As shown in Table 1, randomization between ITT treatment groups resulted in three subject groups with similar demographic characteristics. In all groups, the number of males exceeded females (70–78% males, 22–30% females), with 52–62% being Caucasian. The median age for the saline placebo, 1, and 10 μg groups were 54.7, 59.6, and 53.7 years, respectively. The median weights for these groups were 204, 211, and 220 pounds, respectively. The average ulcer starting sizes for these groups were 4.11, 3.59, and $3.15\,cm^2$ with median sizes of 1.63, 1.21, and $2.02\,cm^2$, respectively. There were no significant differences between the randomized groups in this study.

Safety evaluation

A primary goal of this initial pilot clinical trial was to determine whether topical application of Chrysalin® caused local effects on the ulcer, the adjacent dermal tissue, or systemic effects that might be seen in hematology or blood chemistry analysis. For this analysis, all subjects receiving at least one treatment were included and are described as the ITT population. Laboratory values showed no statistically significant changes from baseline or significant transitions from value groupings (low, normal, or high) for any of the treatment groups at any of the time points

Table 2. Incidence of local ulcer reactions throughout study

Local ulcer reaction	Saline (*n*=21)	1 μg (*n*=20)	10 μg (*n*=18)
Well-defined to severe erythema	2 (10)	3 (15)	2 (11)
Well-defined to severe edema	3 (14)	3 (15)	4 (22)
Worsened pain	2 (10)	2 (10)	2 (11)

Values presented as: *n*, number of subjects experiencing a reaction during the study; (%), percent of subjects in the study group.

Table 3. Incidence of serious adverse events

Body system event	Saline (*n*=21)	1 μg (*n*=20)	10 μg (*n*=18)
Body as a whole			
Progressive disease	2 (10)	0 (0)	0 (0)
Infection	1 (5)	1 (5)	1 (6)
Fever	1 (5)	0 (0)	0 (0)
Chills	1 (5)	0 (0)	0 (0)
Pain	1 (5)	1 (5)	0 (0)
Sepsis	0 (0)	0 (0)	1 (6)
Metabolic/nutritional disorder			
Hypervolemia	0 (0)	1 (5)	0 (0)
Edema	0 (0)	0 (0)	1 (6)
Respiratory			
Dyspnea	0 (0)	1 (5)	1 (6)
Cardiovascular			
Myocardial Infarction	1 (5)	0 (0)	1 (6)
Peripheral gangrene	0 (0)	0 (0)	1 (6)
Coronary artery disorder	0 (0)	1 (5)	0 (0)
Urogenital			
Urinary tract infection	0 (0)	0 (0)	1 (6)
Oliguria	0 (0)	0 (0)	1 (6)
Acute kidney failure	0 (0)	1 (5)	0 (0)
Kidney failure	0 (0)	1 (5)	0 (0)
Hemic and lymphatic			
Ecchymosis	0 (0)	0 (0)	1 (6)
WBC abnormal	0 (0)	0 (0)	1 (6)
Digestive			
Gastrointestinal hemorrhage	1 (5)	0 (0)	0 (0)
Musculoskeletal			
Osteomyelitis	0 (0)	1 (5)	0 (0)

Table 4. Reasons for study discontinuations

Treatment	Placebo	1 μg	10 μg
Infections	3	0	2
Osteomyelitis	1	2	0
Amputation*	0	1	0
Myocardial complications	1	1	1
Nonmedical withdrawal	1	1	0

*Amputation occurred in limb contralateral to study ulcer.

(data not shown). Chrysalin® treatment did not result in any AEs that were probably or definitely drug related, although some subjects in all groups reported erythema, edema, and pain (Table 2). At least one non-drug–related AE was reported in 76% (16/21) of subjects in the saline treatment group, 78% (14/18) in the 10 μg treatment group, and 75% (15/20) in the 1 μg treatment group (not shown). Serious adverse events (SAEs) were reported in five subjects (24%) in the saline control group, four subjects (22%) in the 10 μg group, and four subjects (20%) in the 1 μg treatment group (Table 3).

Fourteen subjects discontinued treatment during the study due to SAEs, infections, or for nonmedical reasons. None of these discontinuations or AEs appeared to be drug related (Table 4). In the saline control group, three subjects discontinued because of infection, one subject discontinued due to osteomyelitis, one subject discontinued due to a fatal myocardial infarction, and one withdrew for nonmedical reasons. In the 1 μg treatment group, two subjects discontinued due to osteomyelitis, one subject discontinued due to an amputation of the contralateral (untreated) foot, one subject discontinued due to a coronary artery disorder, and one subject withdrew for nonmedical reasons. In the 10 μg treatment group, two subjects discontinued due to infection and a third discontinued due to a nonfatal myocardial infarction. No significant differences were found in the incidence of infection or other adverse effects among the groups.

Efficacy analysis

The primary efficacy variable in this study was the incidence of subjects achieving complete ulcer closure within 20 weeks, defined as complete reepithelialization of the wound. In the ITT population, 61% (11/18) of ulcers treated at the 10 μg dose achieved complete closure, compared with 52% (11/21) in the 1 μg treatment group and 48% (10/21) in the saline-treated group (Figure 1A). The ITT population included a number of ulcers that were smaller than 0.9 cm^2 and had existed for < 2 months. Therefore, we also examined efficacy in a PP population. As shown in Figure 1B, in the PP population the incidence of complete ulcer closure was 57% (8/14) in the 10 μg treatment group, 45% (5/11) in the 1 μg treatment group, and 33% (5/15) in the saline placebo control group. Thus, in this PP population, 1 and 10 μg treatments resulted in increasing the incidence of complete closure by 45 and 72%, respectively, over placebo treatment. These results suggest a dose-dependent trend, but in both ITT and PP

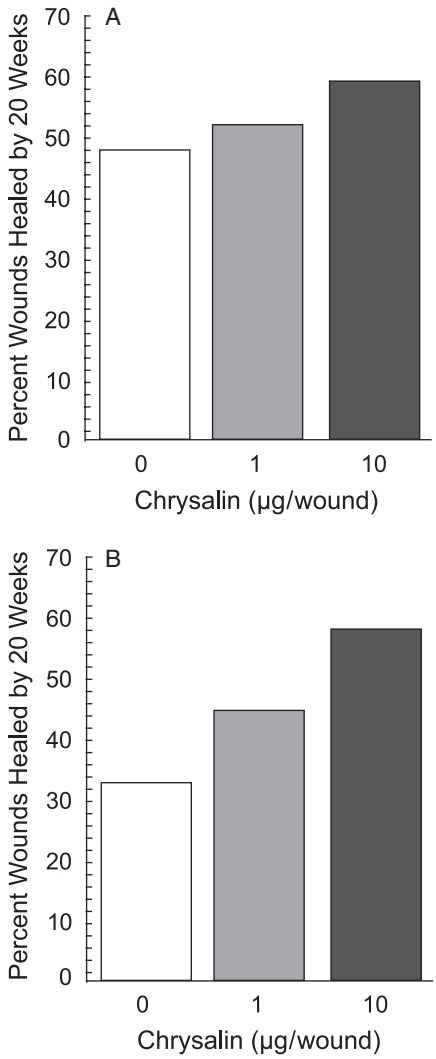

Figure 1. Effect of Chrysalin® on complete wound closure of lower extremity intent-to-treat (ITT) and per-protocol (PP) lower extremity (below the knee) ulcers. Data reflect the percentage of ulcers with complete healing within 20 weeks of twice-weekly treatment with 100 μL of saline (placebo, 0 μg) or saline plus 1 or 10 μg of Chrysalin® in combination with standardized care as described in ''Materials and methods.'' (A) ITT population. (B) PP population.

populations, there was no significant difference between the placebo control group and either of the treatment groups.

Secondary efficacy endpoints included Kaplan–Meier analysis of time to event determinations. In the PP population, Kaplan–Meier analysis indicated a median time to 80% closure of 32 days for the 10 μg treatment group, 47 days for the 1 μg treatment group, and 57 days for the saline control group (Figure 2A). The median time to 100% closure for this PP population was 87 days for the 10 μg treatment group, 122 days for the 1 μg group, and was not reached in the saline control group (indicating a median time to closure of > 140 days; Figure 2B). Although these

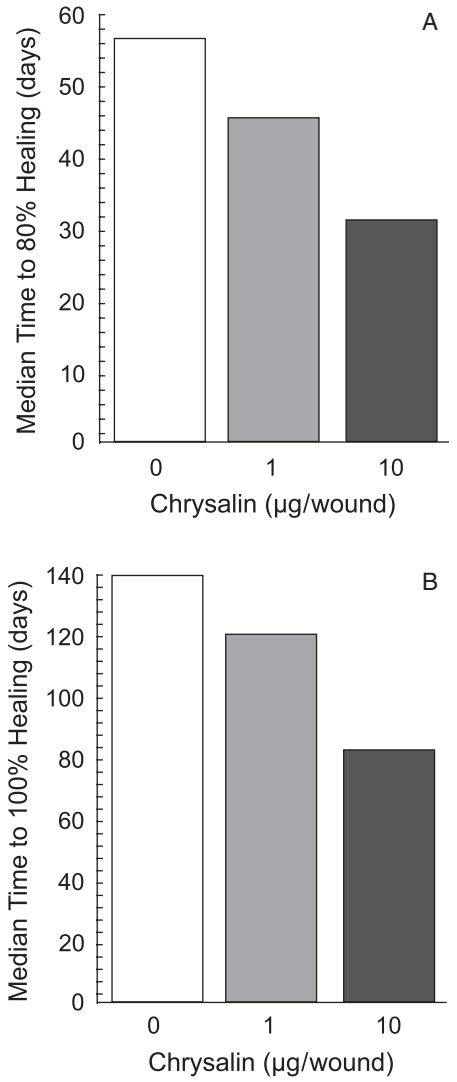

Figure 2. Effect of Chrysalin® on median time to 80 and 100% complete healing based on Kaplan–Meier analysis. (A) Median time for ulcers to reach 80% closure (based on their starting size). (B) Median time for ulcers to reach 100% closure.

differences did not reach significance with the number of ulcers examined, there appeared to be a dose-dependent trend for shortening of the time to both 80 and 100% closure. In all treatment groups, the median time to 80% closure appears relatively rapid compared with the median time to 100% healing, representing a slowing down in healing as the wounds approach complete closure. Interestingly, subject ulcers treated with Chrysalin® at 10 μg/ulcer reduced the median time to both 80 and 100% closure by approximately 40% compared with placebo controls.

Foot ulcer population

This initial trial with TP508 was designed to gain a broad view of the potential effects of TP508 and therefore included ulcers located below the knee. Several of the leg and ankle ulcers appeared to have vascular deficiencies or etiologies that could make them different in their responsiveness to treatment from ulcers on the foot. We therefore analyzed a separate subset of ulcers located on the foot (see "Materials and Methods"). Demographics for this foot ulcer population remained balanced among the groups with respect to age and gender (Table 5). There were no statistical differences between groups in any of the demographic areas analyzed. The mean and median starting sizes for wounds in the 1 μg group, however, were slightly smaller than either the 10 μg group or the placebo group.

Table 5. Demographic data for foot ulcer population

	Treatment group		
	Saline (n=13)	1 μg (n=12)	10 μg (n=10)
Gender			
Male	10 (77)	11 (91)	8 (80)
Female	3 (23)	1 (9)	2 (20)
Age (years)			
Mean ± SD	54.6 ± 11.1	59.4 ± 7.1	50.1 ± 10.7
Wound area (cm^2)			
Mean ± SD	3.7 ± 3.2	2.4 ± 2.5	3.6 ± 3.8
Median	2.2	1.2	2.0
Range	0.97–12.2	0.91–8.1	0.92–13.1

Examples of three similar-sized ulcers on the plantar surface of feet are shown in Figure 3. As shown, the placebo ulcer healed substantially, but did not close completely by 20 weeks (Figure 3A and B). In contrast, the depicted ulcers treated with 1 or 10 μg of Chrysalin® closed completely after 7 and 13 weeks, respectively (Figure 3C–F). Consistent with these photographs, complete healing of foot ulcers was achieved in 31% (4/13) of the placebo controls compared with 75% (9/12) and 70% (7/10) in the 1 and 10 μg groups, respectively (Figure 4). As shown, with this slightly smaller, but more uniform population, Chrysalin's® effect on incidence of complete healing achieved significance in the 1 μg group compared with placebo ($p < 0.05$) and in combined 1 and 10 μg groups compared with placebo ($p < 0.05$).

Kaplan–Meier analysis of the foot ulcer population showed significant effects of Chrysalin® on the length of time required for complete closure (Figure 5). This analysis predicts that by 60 days, twice as many ulcers treated with 10 μg of Chrysalin® would be 100% closed compared with those in the placebo group (Figure 5A). As shown in Figure 5B, the median time to closure for foot ulcers treated with 10 μg Chrysalin® was 71.5 days, compared with 94 days in the 1 μg group and a median closure time in the placebo group that was not reached by 140 days. The difference in median time to complete closure between the 10 μg and placebo groups was significant ($p < 0.05$), as was the difference between the combined treatment groups (1 and 10 μg) vs. placebo ($p < 0.05$). Thus, in these subjects, twice-weekly treatment with 10 μg Chrysalin® nearly doubled the rate of their diabetic foot ulcer healing.

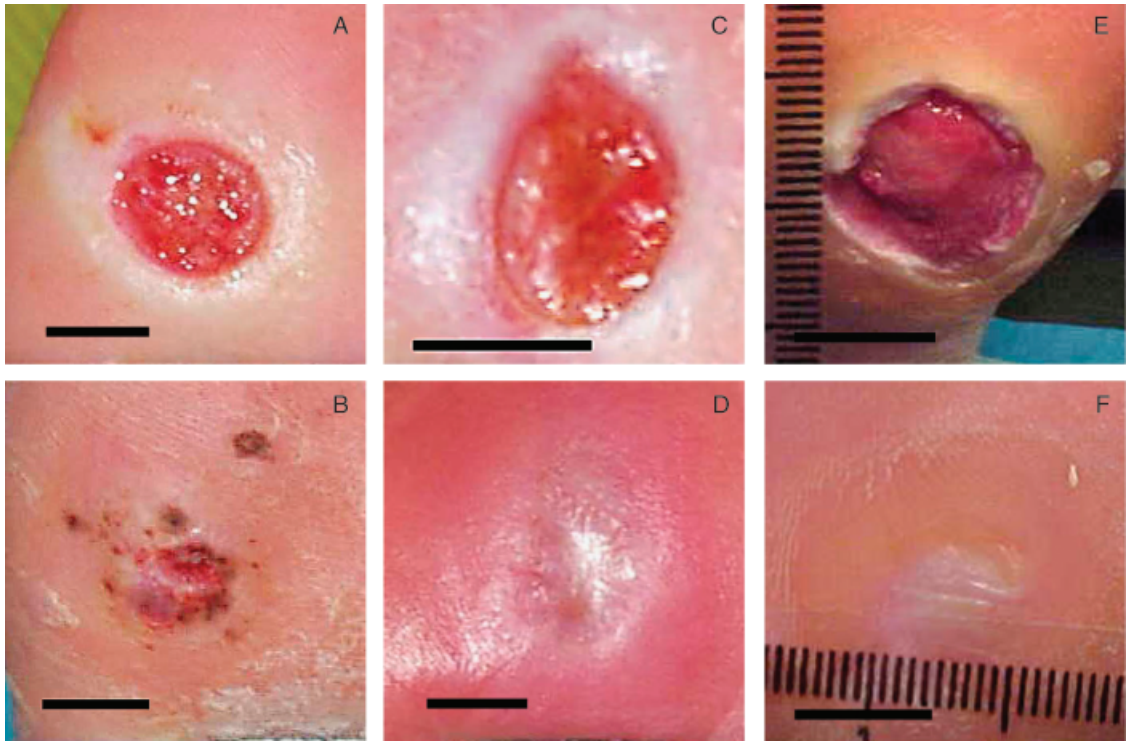

Figure 3. Effect of Chrysalin® on diabetic foot ulcers. Clinic photographs of diabetic foot ulcers located on the plantar surface of the foot are shown for the initial visit (A, C, and E) and at the end of treatment (B, D, and F). (A) Placebo ulcer, 63-year-old Caucasian male, 2-month duration, starting size 1.24 cm^2. (B) Same placebo ulcer at visit 40 after 20 weeks of treatment. (C) One microgram Chrysalin®-treated ulcer, 71-year-old Caucasian male, 14-month duration, starting size 1.21 cm^2. (D) Same 1 μg ulcer at visit 14 after 7 weeks of treatment. (E) Ten microgram Chrysalin®-treated ulcer, 44-year-old Caucasian male, 4-month duration, starting size 1.51 cm^2. (F) Same 10 μg ulcer at visit 26 after 13 weeks of treatment. The scale bar in each panel represents 1 cm.

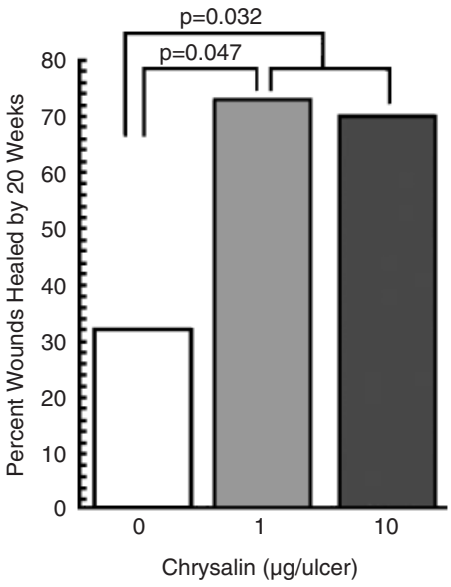

Figure 4. Effect of Chrysalin® on complete wound closure of ulcers located on the foot. Data reflect the percentage of ulcers with complete healing within 20 weeks of twice-weekly treatment with 100 μL of saline (placebo, 0 μg) or saline plus 1 or 10 μg of Chrysalin®.

To further evaluate the effect of Chrysalin® on diabetic foot ulcers, the linear rate of wound closure per day was determined.[34,35] This linear WHR (in mm/day) is calculated by determining the change in area per day divided by the average perimeter of the wound (Figure 6A) Thus, this analysis determines the amount of edge closure (or reduction in wound radius). As previously demonstrated, this analysis minimizes artifactual differences generated when comparing change in area or change in percent closure of different-sized wounds.[35] As shown in Figure 6B, the mean WHR for foot ulcers treated with saline, 1, and 10 μg was 0.058, 0.089, and 0.104 mm/day, respectively. The increased WHR for the 10 μg-treated ulcers represents an increase of ~80% over placebo ulcers ($p < 0.05$). This increase in WHR translates to an average decrease in the number of days required for the wound edge of ulcers to advance by 1 mm from 17.2 days in placebos to 9.6 days in those treated with 10 μg Chrysalin®.

Chrysalin® effects on ulcers located on the heel

A further subset analysis was performed to ensure that the location of ulcers on the foot was not biasing data to favor groups treated with Chrysalin®. It is well known, e.g., that heel ulcers are more difficult to treat effectively than ulcers located on other parts of the foot and that ulcers on the

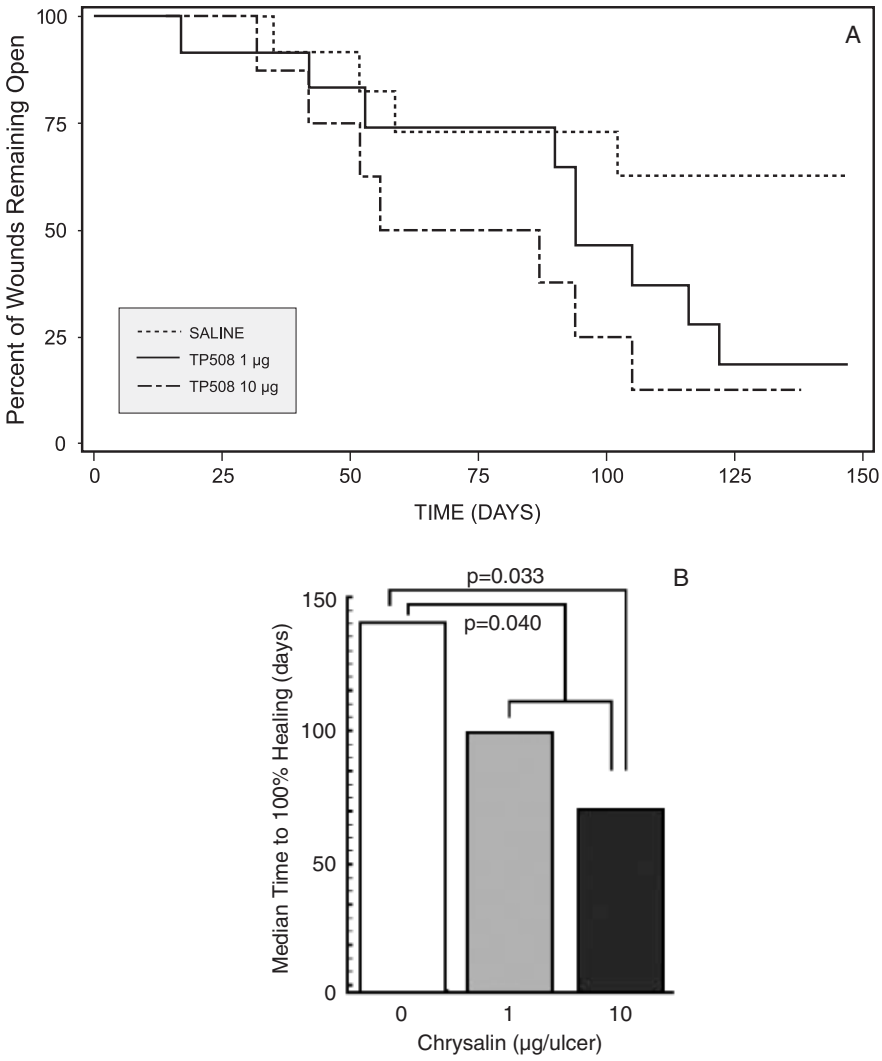

Figure 5. Kaplan–Meier analysis of time to 100% wound closure for foot ulcers. (A) Time to closure, based on percent of wounds remaining open; saline (-----); 1 µg Chrysalin® (—), and 10 µg Chrysalin® (– – –). (B) Median time of ulcers to reach 100% healing.

heel represent a higher probability of progression to amputation.[36,37] In the foot ulcer population, five of the 13 (38%) of placebo subjects and seven of 22 (32%) of subjects treated with either 1 or 10 µg of Chrysalin® had ulcers located on the heel of the foot (Table 6). Thus, the location of foot ulcers was not different between groups.

As depicted in Figure 7, heel ulcers included in this trial were larger and deeper than those located on the bottom of the foot, with mean starting size areas of 5.51 cm^2 in the placebo group and 5.39 cm^2 in those treated with Chrysalin® (Table 6). As shown in Figure 7A–C, placebo heel ulcers improved over the treatment period with good care and better offloading (compare Figure 7A and C), but did not show early week 5 (visit 10) improvement in granulation tissue within the wound bed (Figure 7B). In contrast, heel ulcers treated with 1 or 10 µg of Chrysalin® twice weekly (Figure 7D–H, respectively) showed early improvement in wound bed granulation tissue within the first 5 weeks of treatment (Figure 7E and H). These particular ulcers went to complete closure after 8 and 16 weeks with Chrysalin® treatment (Figure 7F and I), but the placebo ulcer (Figure 7C) did not close within 20 weeks.

Analysis of the incidence of closure and WHR rate for PP population ulcers located on the heel are shown in Table 6. It should be noted that the mean starting size of these ulcers was approximately equivalent (Table 6) as was the mean age of the subjects (saline 53.6 ± 14.3; 1 µg 55.7 ± 10.3; and 10 µg 51.48 ± 10.26). As shown, WHR rates of these heel ulcers more than doubled (165% increase) in the 10 µg group relative to placebo ($p < 0.02$). This increase in WHR translates to a decrease in the number of days on average required for the wound edge to advance by 1 mm from 25 days in placebos to 9.4 days in those treated with 10 µg Chrysalin®. Moreover, the incidence of complete closure of these heel ulcers by 20 weeks was observed in six of seven ulcers (86%) treated with either 1 or 10 µg compared with zero of five (0%) of the placebo-treated ulcers ($p < 0.03$). Interestingly, the one subject in the 10 µg group who failed to show healing had been removed from the study after one treatment due to an

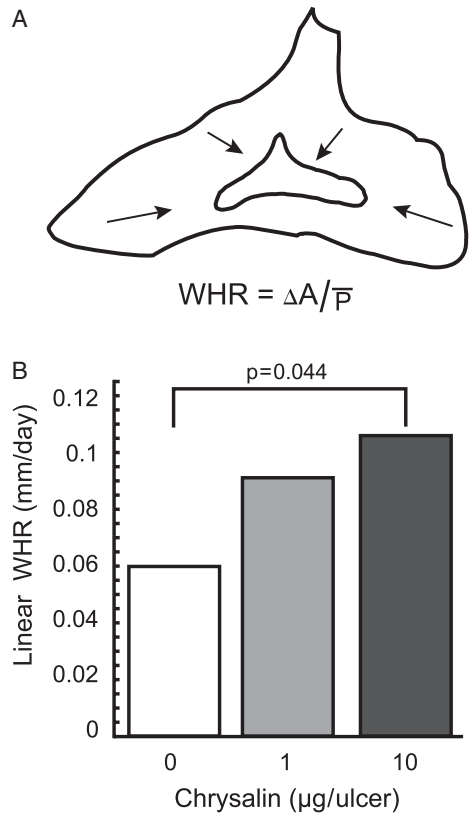

Figure 6. Effect of Chrysalin® on rate of linear wound closure of diabetic foot ulcers. (A) Linear wound healing rate (WHR) was calculated based on the change in the surface area of each ulcer per day relative to the perimeter of the wound.[35,36] In formula ΔA=change in area per day and P=average perimeter length [(P day 0+P last day)/2]. (B) Mean WHR for foot ulcers treated with indicated concentration of Chrysalin®. Data are calculated using all ulcers in the population from initial visit to time of treatment termination due to withdrawal from study, healing, or completion of 20 weeks of treatment.

unrelated infection and sepsis. Thus, in the subpopulation of heel ulcers that received repeated treatment, Chrysalin® treatment was 100% effective.

DISCUSSION

In spite of numerous efforts to develop cost-effective small molecule therapies and recombinant growth factor treatments for chronic wounds, there has been little success in changing standard clinical practice or reducing the cost and devastating effects of chronic wounds worldwide. The present phase I/II pilot study was undertaken to determine the potential safety and efficacy of treating diabetic ulcers with the synthetic thrombin peptide, Chrysalin®, which is also known as TP508 (CAS #497221-38-2).

Preclinical studies have shown that a single application of Chrysalin® accelerated the repair of dermal tissue and bone through mechanisms involving modulation of inflammatory cells and tissue revascularization.[25–32] These studies support the hypothesis that thrombin, and peptide fragments of thrombin released at the site of injury, initiate a cascade of events leading to tissue repair. All of the preclinical studies to date, however, utilized animal models with acute injury. As previously described, chronic wounds such as diabetic ulcers have distinct etiologies and different wound environments that make them more difficult to heal than acute wounds. The present pilot study was therefore undertaken to determine whether the cascade of events initiated by Chrysalin® could overcome chronic impairments to healing and effectively promote the healing of diabetic foot ulcers.

The data from this pilot study indicate that Chrysalin® is safe and is well tolerated with repeated topical application to ulcers. No significant differences were observed with respect to edema, erythema, pain, or adverse wound reaction between treated and placebo groups. Furthermore, there were no changes in laboratory values in Chrysalin®-treated subjects that would suggest adverse systemic effects. Chrysalin® treatment was not associated with any mild, moderate, or severe adverse effects. The percentage of subjects with SAEs and the number of SAEs for each treatment group were similar, and none of the SAEs were recorded as being drug related. Although safety must continue to be demonstrated in subsequent larger studies, these pilot data clearly support the potential safety of Chrysalin® as a topical therapeutic for diabetic ulcers.

The efficacy data for both the ITT and PP populations showed that Chrysalin® treatment showed a dose-dependent trend for increased incidence of complete wound closure and a reduction in median time to heal for all lower extremity leg ulcers. In the PP population, twice-weekly treatment with 1 or 10 μg of Chrysalin® increased the 20-week incidence of complete ulcer closure by approximately 45 and 72%, respectively, over that observed in placebo ulcers and cut the median time required for 100% closure by approximately 15% and over 42%, respectively. No significant differences were observed between groups in age, sex, race, starting ulcer size, or how long the ulcer had been present without healing. Therefore, the effects of Chrysalin® within these populations cannot be attributed to standard demographic differences.

In the PP group, the placebo rate of complete closure was 33%. This is consistent with historical values for diabetic ulcer trials where approximately 30–35% of all ulcers are expected to heal in response to good wound care alone. Treatment with 10 μg of Chrysalin® resulted in 57% complete closure in this population. All subjects who withdrew from the study after receiving even a single treatment for medical, personal, or noncompliance reasons were counted as closure failures. Thus, an ideal goal of close to 100% closure of all treated ulcers may never be achievable with this type of trial design.

This study included diabetic foot ulcers and ulcers on the leg and ankle. Leg and ankle ulcers have distinct etiologies and preferred treatment paradigms that may include pressure bandaging, etc., that were not used in this study. In light of this, a post hoc analysis of data obtained from the foot ulcer population alone was also completed. The demographic distribution in this population was similar to that of the ITT and PP population. In ulcers on the foot,

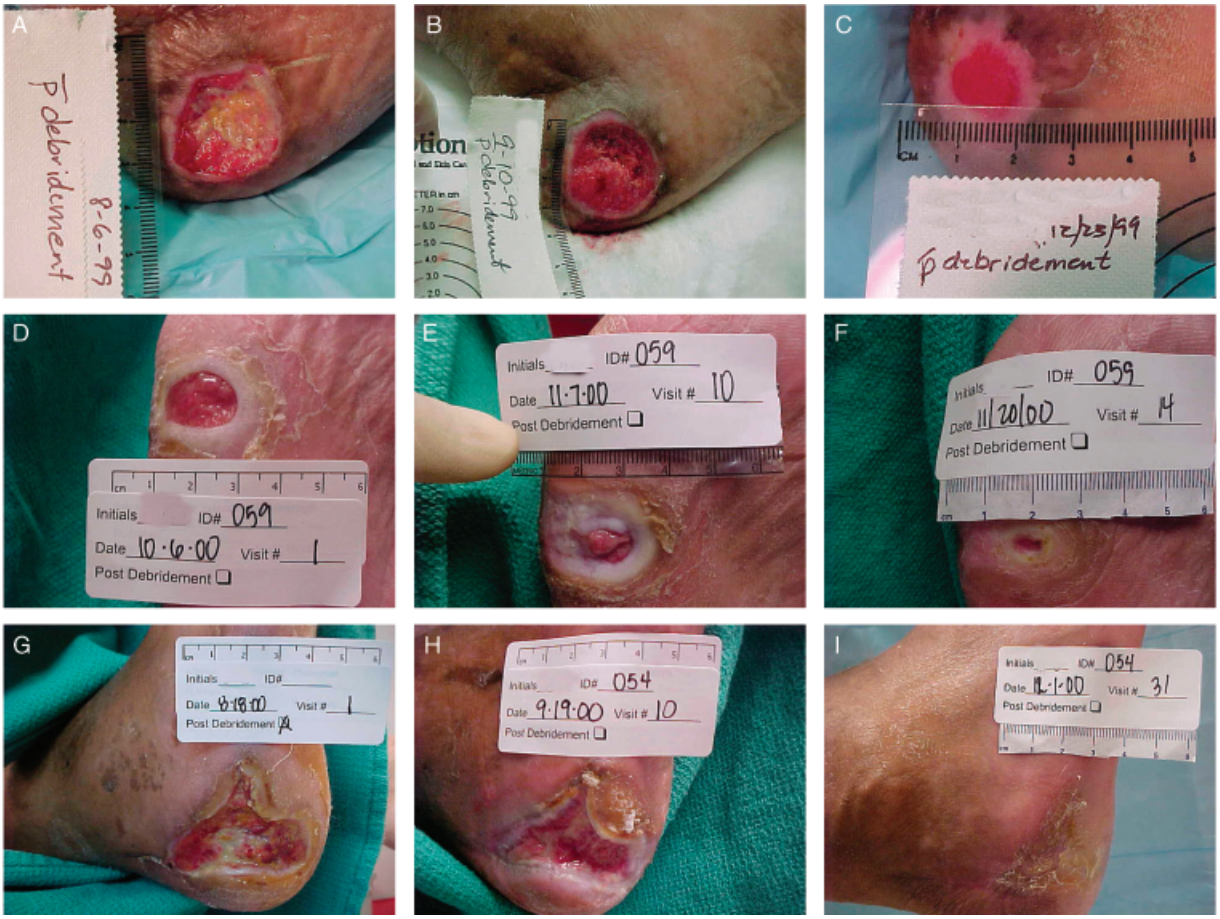

Figure 7. Effect of Chrysalin® on diabetic foot ulcers located on the heel of the foot. Clinic photographs of diabetic foot ulcers located on the heel of the foot are shown from their initial visit, at visit 10 (5 weeks), and at the end of treatment for placebo (A–C), 1 μg Chrysalin® (D–F), and 10 μg Chrysalin® (G–I). (A) Placebo ulcer, 79-year-old Black female, 2+ months duration, starting size 5.23 cm^2. (B) Same placebo ulcer at visit 10 after 5 weeks of treatment. (C) Same placebo ulcer at visit 40 after ~4.5 months of treatment. (D) One microgram Chrysalin®-treated ulcer, 47-year-old Hispanic male, 2-month duration, starting size 1.8 cm^2. (E) Same 1 μg ulcer at visit 10 after 5 weeks of treatment. (F) Same 1 μg ulcer visit 14 after ~7 weeks of treatment (wound healed at visit 16 after ~8 weeks of treatment). (G) Ten microgram Chrysalin®-treated ulcer, 47-year-old Hispanic male, 2+ months duration, starting size 13.1 cm^2. (H) Same 10 μg ulcer at visit 10 after 5 weeks of treatment. (I) Same 10 μg ulcer at visit 31 after ~14.5 weeks of treatment. The scale bar in each panel represents 1 cm.

Table 6. TP508 effect on healing of foot ulcers located on the heel

Treatment	Total subjects	Mean area (day 0, cm^2)	Total healed	Percent healed	Mean WHR (mm/day)	WHR (days per mm edge closure)
Saline	Z5	5.32	0	0	0.040	25
1 μg Chrysalin®	3	3.62	3	100*	0.081	12.3
10 μg Chrysalin®	4	6.19	3	75†	0.106‡	9.43‡
1 or 10 μg	7	5.09	6	85.7*	0.095§	10.52§

*Fisher's exact test: $p < 0.02$ relative to saline.
†Fisher's exact test: $p < 0.05$ relative to saline.
‡Student's T test: $p < 0.02$ relative to saline.
§Student's T test: $p < 0.03$ relative to saline.
WHR, wound healing rate.

complete closure was achieved in 75 and 70% in ulcers treated with 1 and 10 μg of Chrysalin®, respectively, compared with 31% of the placebo controls. These differences between Chrysalin®-treatment and placebo were significant (placebo vs. 1 μg $p < 0.05$; and placebo vs. 1 or 10 μg $p < 0.05$). Of note, this effect of Chrysalin® represents an increase in the incidence of complete closure of greater than 125% over placebo controls with good standard wound care. Based on Kaplan–Meier analysis, treatment of foot ulcers with 10 μg of Chrysalin® produced a median time to closure of 71.5 days while in the control group the median was not reached by 140 days ($p < 0.05$). Thus, Chrysalin®-treated ulcers appeared to heal approximately twice as fast as the placebo ulcers. Consistent with the Kaplan–Meier analysis, the linear WHR in the 10 μg group was ~80% greater than ulcers treated with saline ($p < 0.05$).

A number of other clinical trials examining potential treatment modalities have only examined relatively well-vascularized Wagner Grades I and II diabetic foot ulcers and have not included ulcers on the leg or ankle. Therefore, having determined the effects of Chrysalin® on the foot ulcer subpopulation allows a better comparison with data obtained in previous trials. For example, in the four major becaplermin (Regranex®) trials that included a total of 922 subjects, placebo healing was ~35% compared with ~50% healing in diabetic foot ulcers treated daily with becaplermin.[13,14] This represents an increase of approximately 40% in complete healing over placebo controls. In the current study, the incidence of placebo healing was comparable with that seen in the becaplermin studies, but Chrysalin® treatment twice weekly with 1 μg/ulcer resulted in a 75% incidence of complete closure by 20 weeks. This represents a 140% increase over the incidence of healing in placebos. The linear WHR also nearly doubled with Chrysalin® treatment. These data suggest that the twice-weekly treatment with Chrysalin® may be at least as effective in closing diabetic foot ulcers as daily application of becaplermin.

The data from this study are especially encouraging, considering that the foot ulcer population in this study included a number of Wagner category III (deeper) ulcers, many of which were located on the heel of the foot. It is recognized that ulcers located on the heel of the foot require special treatment, are among the most difficult of foot ulcers to heal, and are the ones most likely to result in amputation of the foot.[36,37] Subset analysis of heel ulcers in a recently published clinical trial with over 250 diabetic foot ulcers, e.g., showed that only 8% (1/13) control heel ulcers achieved complete closure compared with 33% (6/18) of heel ulcers treated with Dermagraft.[38] In our foot ulcer population, wound closure rates of heel ulcers more than doubled (165% increase) in the 10 μg Chrysalin® group relative to placebo ($p < 0.02$). This increase in WHR translates into a decrease in the average time required for 1 mm of edge closure from 25 days in placebos to 9.4 days in ulcers treated with 10 μg of Chrysalin®. Moreover, complete closure of these heel ulcers was observed in six of seven ulcers (86%) treated with either 1 or 10 μg compared with zero of five (0%) of the placebo-treated ulcers ($p < 0.03$). These data suggest that Chrysalin® treatment may be especially effective in larger and more difficult chronic ulcers.

In this study, twice-weekly treatments with Chrysalin® or placebo were combined with a standardized regimen of good wound care, including a moisture-retentive primary dressing, initial and ongoing sharp debridement as deemed necessary by the attending physician, and pressure off-loading. Subjects were treated during office visits to ensure treatment compliance and limit treatment variability; yet, with only twice-weekly application and inclusion of both orthopedic wound centers and podiatry centers, the data may provide a realistic prediction of real-world use. Therefore, the significant data obtained in this pilot study suggest that topical application of Chrysalin® may have considerable therapeutic value for diabetic foot ulcers.

ACKNOWLEDGMENTS

The authors wish to thank Dennis McWilliams, Dr. Roger Crowther, and all of the former employees of Chrysalis BioTechnology Inc. for their support and early management of this study, Dr. Jon Mader, who died in 2003, and his group at the University of Texas Medical Branch (UTMB) who treated the majority of the subjects in this study, the research coordinators and pharmacists without whom clinical trials would not be possible, Jaye Thompson, Paul Lewis, and the staff at Synergos Inc. in Woodlands, TX, for monitoring, data analysis, and statistical support, Dr. Thomas A. Mustoe for his advice and critical review of this manuscript, and Drs. Gerald M. Fuller and Gordon H. Otto, who provided outside reviewers of the manuscript. This phase I/II clinical trial was sponsored by Chrysalis BioTechnology Inc., Galveston, TX, a spinout of UTMB, Galveston. Chrysalis was acquired in 2004, by OrthoLogic Corp., Tempe, AZ (NASDAQ: OLGC), who now owns the rights to the Chrysalin® Technology for all applications worldwide. Dr. J.T. Ryaby is currently employed by and is an OrthoLogic Corp shareholder. Dr. D.H. Carney (the founder of Chrysalis BioTechnology Inc.) is an OrthoLogic shareholder and receives monetary compensation as a consultant to OrthoLogic. Potential conflicts of interest and oversight of Dr. Carney's research related to this technology are monitored and managed by the UTMB Conflicts of Interest Committee.

REFERENCES

1. Gordois A, Scuffham P, Shearer A, Oglesby A, Tobian JA. The health care costs of diabetic peripheral neuropathy in the US. *Diabetes Care* 2003; 26: 1790–5.
2. Harrington C, Zagari MJ, Corea J, Klitenic J. A cost analysis of diabetic lower-extremity ulcers. *Diabetes Care* 2000; 23: 1333–8.
3. Girod I, Valensi P, Laforet C, Moreau-Defarges T, Guillon P, Baron F. An economic evaluation of the cost of diabetic foot ulcers: results of a retrospective study on 239 patients. *Diabetes Metab* 2003; 293: 269–77.
4. Redekop WK, Stolk EA, Kok E, Lovas K, Kalo Z, Busschbach JJ. Diabetic foot ulcers and amputations: estimates of health utility for use in cost-effectiveness analyses of new treatments. *Diabetes Metab* 2004; 30: 549–56.

5. Mustoe T. Understanding chronic wounds: a unifying hypothesis on their pathogenesis and implications for therapy. *Am J Surg* 2004; 187: 65S–70S.
6. Jeffcoate WJ, Harding KG. Diabetic foot ulcers. *Lancet* 2003; 361: 1545–51.
7. Greenhalgh DG. Wound healing and diabetes mellitus. *Clin Plast Surg* 2003; 30: 37–45.
8. Cosentino F, Luscher T. Endothelial dysfunction in diabetes mellitus. *J Cardiovasc Pharmacol* 1998; 32: 54–61.
9. Jude EB, Tentolouris N, Appleton I, Anderson S, Boulton AJ. Role of neuropathy and plasma nitric oxide in recurrent neuropathic and neuroischemic diabetic foot ulcers. *Wound Repair Regen* 2001; 9: 353–9.
10. Trengove NJ, Stacey MC, MacAuley S, Bennett N, Gibson J, Burslem F, Murphy G, Schultz G. Analysis of the acute and chronic wound environments: the role of proteases and their inhibitors. *Wound Repair Regen* 1999; 7: 442–52.
11. Lobmann R, Ambrosch A, Schultz G, Waldmann K, Schiweck S, Lehnert H. Expression of matrix-metalloproteinases and their inhibitors in the wounds of diabetic and non-diabetic patients. *Diabetologia* 2002; 45: 1011–6.
12. Wall SJ, Sampson MJ, Levell N, Murphy G. Elevated matrix metalloproteinase-2 and -3 production from human diabetic dermal fibroblasts. *Br J Dermatol* 2003; 149: 13–6.
13. Wieman TJ, Smiell JM, Su Y. Efficacy and safety of a topical gel formulation of recombinant human platelet-derived growth factor-BB (becaplermin) in patients with chronic neuropathic diabetic ulcers. A phase III randomized placebo-controlled double-blind study. *Diabetes Care* 1998; 21: 822–7.
14. Smiell JM, Wieman TJ, Steed DL, Perry BH, Sampson AR, Schwab BH. Efficacy and safety of becaplermin (recombinant human platelet-derived growth factor-BB) in patients with nonhealing, lower extremity diabetic ulcers: a combined analysis of four randomized studies. *Wound Repair Regen* 1999; 7: 335–46.
15. Steed DL, Donohoe D, Webster MW, Lindsley L. Effect of extensive debridement and treatment on the healing of diabetic foot ulcers. Diabetic Ulcer Study Group. *J Am Coll Surg* 1996; 183: 61–4.
16. Glenn KC, Frost GH, Bergmann JS, Carney DH. Synthetic peptides bind to high-affinity thrombin receptors and modulate thrombin mitogenesis. *Peptide Res* 1988; 1: 65–73.
17. Carney DH, Cunningham DD. Cell surface action of thrombin is sufficient to initiate division of chick cells. *Cell* 1978; 14: 811–23.
18. Carney DH, Cunningham DD. Role of specific cell surface receptors in thrombin-stimulated cell division. *Cell* 1978; 15: 1341–9.
19. Carney DH, Bergmann JS. 125I-thrombin binds to clustered receptors on noncoated regions of mouse embryo cell surfaces. *J Cell Biol* 1982; 95: 697–703.
20. Coughlin SR. Thrombin signalling and protease-activated receptors. *Nature* 2000; 407: 258–64.
21. Carney DH, Stiernberg J, Fenton JW II. Initiation of proliferative events by human alpha-thrombin requires both receptor binding and enzymic activity. *J Cell Biochem* 1984; 26: 181–95.
22. Carney DH, Herbosa GJ, Stiernberg J, Bergmann JS, Gordon EA, Scott D, Fenton JW II. Double-signal hypothesis for thrombin initiation of cell proliferation. *Semin Thromb Hemost* 1986; 12: 231–40.
23. Carney DH. Postclotting cellular effects of thrombin mediated by interaction with high-affinity thrombin receptors. In: Berliner L, editor. *Thrombin Structure and Function*. New York: Plenum, 1992: 351–96.
24. Carney DH, Redin W, McCroskey L. Role of high-affinity thrombin receptors in postclotting cellular effects of thrombin. *Semin Thromb Hemost* 1992; 18: 91–103.
25. Carney DH, Mann R, Redin WR, Pernia SD, Berry D, Heggers JP, Hayward PG, Robson MC, Christie H, Annable C, Fenton JW II, Glenn KC. Enhancement of incisional wound healing and neovascularization in normal rats by thrombin and synthetic thrombin receptor-activating peptides. *J Clin Invest* 1992; 89: 1469–77.
26. Cromack DT, Porras RBH, Wee SS, Glenn KC, Purdy JA, Carney DH, Mustoe TA. Acceleration of soft tissue repair by a thrombin-derived oligopeptide. *J Surg Res* 1992; 53: 117–22.
27. Stiernberg J, Norfleet AM, Redin WR, Warner WS, Fritz RR, Carney DH. Acceleration of full-thickness wound healing in normal rats by the synthetic thrombin peptide, TP508. *Wound Repair Regen* 2000; 8: 204–15.
28. Norfleet AM, Huang Y, Sower LE, Redin WR, Fritz RR, Carney DH. Thrombin peptide TP508 accelerates closure of dermal excisions in animal tissue with surgically induced ischemia. *Wound Repair Regen* 2000; 8: 517–29.
29. Norfleet AM, Bergmann JS, Carney DH. Thrombin peptide, TP508, stimulates angiogenic responses in animal models of dermal wound healing, in chick chorioallantoic membranes, and in cultured human aortic and microvascular endothelial cells. *Gen Pharmacol* 2000; 35: 249–54.
30. Wang H, Li X, Tomin E, Doty SB, Lane JM, Carney DH, Ryaby JT. Thrombin peptide (TP508) promotes fracture repair by up-regulating inflammatory mediators, early growth factors, and increasing angiogenesis. *J Orthop Res* 2005; 23: 671–79.
31. Sheller MR, Crowther RS, Kinney JH, Yang J, Di Jorio S, Breunig T, Carney DH, Ryaby JT. Repair of rabbit segmental defects with the thrombin peptide, TP508. *J Orthop Res* 2004; 22: 1094–9.
32. Li G, Ryaby JT, Carney DH, Wang H. Bone formation is enhanced by thrombin-related peptide TP508 during distraction osteogenesis. *J Orthop Res* 2005; 23: 196–202.
33. Wagner FW. The dysvascular foot: a system of diagnosis and treatment. *Foot Ankle* 1981; 2: 64–122.
34. Gilman TH. Parameter for measurement of wound closure. *Wounds* 1990; 3: 95–101.
35. Gorin DR, Cordts PR, LaMorte WW, Menzoian JO. The influence of wound geometry on the measurement of wound healing rates in clinical trials. *J Vasc Surg* 1996; 23: 524–8.
36. Younes NA, Albsoul AM, Awad H. Diabetic heel ulcers: a major risk factor for lower extremity amputation. *Ostomy Wound Manage* 2004; 50: 50–60.
37. Hampton S. The complexities of heel ulcers. *Nurs Stand* 2003; 17: 68–70, 72, 74.
38. Marston WA, Hanft J, Norwood P, Pollak R. The efficacy and safety of Dermagraft in improving the healing of chronic diabetic foot ulcers: results of a prospective randomized trial. *Diabetes Care* 2003; 26: 1701–5.