Treatment of Chronic Leg Ulcers With Topical Activated Protein C

Kaley Whitmont, MBBS; Ian Reid, MPod; Sara Tritton, MBBS; Lyn March, MBBS, PhD; Meilang Xue, PhD; Michael Lee, MBBS; Greg Fulcher, MD; Phil Sambrook, MBBS, PhD; Eric Slobedman, MMed(Path); Alan Cooper, MBBS; Chris Jackson, PhD

Background: The treatment of skin ulcers frequently presents a management challenge. Nonhealing wounds with poor response to conventional wound management therapy represent a significant cause of disability, affecting approximately 1% of the global population. Activated protein C is a serine protease with anticoagulant, angiogenic, and anti-inflammatory properties that has shown efficacy in patients for the treatment of severe sepsis. We report 4 cases of nonhealing lower limb skin ulcers that were treated with activated protein C.

Observations: The study included 4 patients whose wounds were not improving despite standard wound treatment for 4 months or more. Activated protein C was applied topically to their wounds once weekly for 4 weeks. All 4 patients showed a rapid positive response to treatment that was maintained during a 4-month follow-up period. The treatment was well tolerated, with no remarkable adverse effects or complications.

Conclusions: Activated protein C can stimulate wound healing in patients with skin ulcers that are refractory to conventional wound-healing therapies. The likely mechanism of action is its recognized ability to stimulate angiogenesis and reepithelialization and to inhibit inflammation. Activated protein C has potential as a therapeutic option for patients with chronic skin ulcers.

Arch Dermatol. 2008;144(11):1479-1483

Chronic wounds or cutaneous ulcers are a common health problem, affecting approximately 0.2% of the general population and 2% of persons older than 80 years. They occur when the coordinated cellular and biochemical response to injury is disrupted. This disruption is usually due to a compromise in health from either local or systemic causes, such as trauma or diabetes, respectively. Some chronic wounds become locked in the inflammatory phase and are unable to progress to form granulation tissue. The treatment of chronic wounds is expensive and requires dedicated care, including regular dressings, frequent clinic appointments, and, when complications arise, hospital admission, which can potentially result in surgery or even amputation.

Strong evidence is now emerging that activated protein C (APC) contributes to wound repair. The mechanism of APC’s action is complex and involves a unique combination of inhibition of inflammation, stimulation of angiogenesis, and reepithelialization and antiapoptotic properties. By reducing inflammation, APC dampens excessive protease activity, yet it selectively increases matrix metalloproteinase 2 activity to promote angiogenesis and reepithelialization. In the chick chorioallantoic membrane assay, APC stimulates the formation of fine capillary blood vessels. In a rodent model, full-thickness wounds treated with APC show accelerated healing, have more blood vessels, and exhibit lower neutrophilic infiltration compared with controls. Many of these actions of APC are mediated through its receptor, endothelial PC receptor (EPCR), which not only is present on endothelial cells and leukocytes but also is strongly expressed by skin keratinocytes. Interestingly, APC signaling appears to be similar in both keratinocytes and endothelial cells, which helps explain the seemingly broad protective and regenerative effects of APC on angiogenesis and reepithelialization. To our knowledge, there have been no previous reports on the use of APC to treat chronic wounds in humans. We report 4 cases of chronic skin ulcers that were refractory to conventional wound-healing therapies but responsive to APC treatment.
Four patients (all men, aged 65-77 years) with nonhealing wounds or more with no improvement in the size of their skin wounds at Royal North Shore Hospital, St Leonards, Australia, for 4 months receiving standard wound care treatment in the high-risk foot clinic. The study selection criteria included a diagnosis of chronic lower limb skin ulcer with the condition that the patients had been re-

Table 1. Description of Cases

<table>
<thead>
<tr>
<th>Patient No./Sex/Age, y</th>
<th>Wound Origin</th>
<th>Wound Duration, mo</th>
<th>Previous Wound Care Management</th>
<th>% of Wound Area Healed at Visit 6 (Week 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/65</td>
<td>Venous</td>
<td>12</td>
<td>Venous valve replacement; routine debridement; silver sulfadiazine cream + foam dressing; short-stretch compression bandage; sclerotherapy considered as future management</td>
<td>52.7</td>
</tr>
<tr>
<td>2/M/75</td>
<td>Arteriovenous</td>
<td>8</td>
<td>Split-skin graft; nanocrystalline silver dressing (moistened with water); routine debridement; foam dressing and crepe dressing</td>
<td>88.5</td>
</tr>
<tr>
<td>3/M/71</td>
<td>Diabetic/neuropathic</td>
<td>4</td>
<td>Routine debridement; rayan dressing with 10% povidone iodine ointment, foam dressing, and medical adhesive tape; cephalaxin antibiotic</td>
<td>92.0</td>
</tr>
<tr>
<td>4/M/77</td>
<td>Diabetic</td>
<td>8</td>
<td>Routine debridement; cadexomer iodine dressing, foam dressing, and medical adhesive tape</td>
<td>100.0</td>
</tr>
</tbody>
</table>

The temporal sequence of events for the treatment protocol is detailed in Table 2. The average total cost of the APC to treat each patient over the course of 4 treatments was approximately $60.

The progress of wound healing was monitored at each consultation by measuring the wound area with a portable digital device that provides accurate, reproducible data for tracking wound progress (Visitrak; Smith & Nephew, London, England). Briefly, the wound perimeter was traced with a thinned marker onto a sterile transparency. Tracings were then placed onto the wound measurement device and copied with an electronic stylus to obtain a digital reading of the wound area. Results were analyzed using a t test. Computer image analysis of digital photographs allowed confirmation of accuracy of the wound measurements. The primary outcome measure was change in wound area at 2 months after 4 treatments at weekly intervals and after 4 weeks of follow-up. Secondary outcome measures were (1) weekly alteration in wound area; (2) change in volume of APC injected into the wound space, which provided an approximate indication of wound volume; and (3) immunohistochemical analysis performed on 3-mm wound-edge punch biopsy specimens obtained before APC treatment (visit 1) and again at visit 4, using monoclonal antibodies to localize PC and EPCR. All immunohistochemical analysis was performed using a biotin-streptavidin amplification stain kit (LSAB + Systems; Dako Corp, Carpinteria, California) as previously described and processed simultaneously to allow comparisons between specimens treated with the same antibody.

Table 2. Outline of Treatment and Follow-up Protocol

<table>
<thead>
<tr>
<th>Visit No.</th>
<th>Week No.</th>
<th>Clinical Procedures a (in Order)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>Include or exclude according to criteria; obtain consent; physical examination; skin biopsy; photograph wound; wound analysis with digital measuring device; debride; apply APC</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>Photograph wound; wound analysis with digital measuring device; debride; apply APC</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>Photograph wound; skin biopsy; wound analysis with digital measuring device; debride; apply APC</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>Photograph wound; wound analysis with digital measuring device; debride; apply APC</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>Follow-up visits; photograph wound; wound analysis with digital measuring device; debride if necessary</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>Include or exclude according to criteria; obtain consent; physical examination; skin biopsy; photograph wound; wound analysis with digital measuring device; debride; apply APC</td>
</tr>
<tr>
<td>7</td>
<td>16</td>
<td>Debride if necessary</td>
</tr>
</tbody>
</table>

Abbreviation: APC, activated protein C. aSee “Methods” section for details.

METHODS

The study selection criteria included a diagnosis of chronic lower limb skin ulcer with the condition that the patients had been receiving standard wound care treatment in the high-risk foot clinic at Royal North Shore Hospital, St Leonards, Australia, for 4 months or more with no improvement in the size of their skin wound. Four patients (all men aged 65-77 years) with nonhealing wounds despite standard care, including debridement and dressing changes as detailed in Table 1, were selected for the study. This project was approved by the local Human Research Ethics Committee.

Wounds were inspected and any necrotic tissue was debrided, consistent with standard wound care management practice. Recombinant human APC (Xigris; Eli Lilly and Co, Indianapolis, Indiana) was then administered topically. The method of APC application was noninvasive and involved first sealing the wounds with a sterile, occlusive polyurethane adhesive film (Tegaderm; 3M, St Paul, Minnesota) through which a solution of APC (200 µg/mL in distilled water) was injected into the wound space until it was level with the skin surface. The volume of liquid required for each treatment was recorded as an estimation of wound volume. A maximum of 1.6 mL of APC (200 µg/mL) was sufficient to fill the space of the largest wound, and as the wounds reduced in size, the amount of APC was coordinately reduced. After the APC application, another occlusive polyurethane adhesive film dressing was applied to seal the needle puncture point. The APC was allowed to remain undisturbed in contact with the wound surface for 24 hours, after which the patients continued with the previous dressing or treatment that they had been receiving before the study began (Table 1). Therefore, the only modification to standard care was the treatment with APC. This protocol was designed so that any alterations in wound healing were likely to be attributable to the APC intervention and not to other dressing or treatment adjustments. Patients were treated with APC for 4 weeks at weekly intervals and then followed up at 2 fortnightly intervals, with a final follow-up visit after 4 months.

REPORT OF CASES

Four patients underwent APC treatment for chronic lower limb skin wounds: 2 had diabetic wounds and 2 had ulcers due to vascular disease (Table 1). All 4 patients had experienced no improvement in the size of their skin wounds despite at least 4 months of conventional wound-healing therapy.

CASE 1

A 65-year-old man with a medical background of hepatitis C presented with a 12-month history of a nonheal-
ing, painful skin ulcer on the lateral aspect of the right lower leg area. He was initially found to have right common femoral vein reflux; however, even though he had undergone a peripheral venous valve replacement, his wound showed no improvement. He received twice-weekly home dressings by community nurses and attended the Outpatient High Risk Foot Clinic at weekly intervals for debridement and dressing application. He was treated for several months with antibiotics for recurrent localized infections. After 11 months, he was referred to another vascular surgeon, who discussed sclerotherapy for incompetent superficial veins; however, the patient was eager to avoid further intervention. His blood pressure and coagulation profile were normal, and he met the inclusion criteria, so he began APC treatment. One week after the first APC treatment, the wound area had reduced by 13% and continued to improve over the following 3 treatments by 18%, 26%, and 34%, respectively, compared with the pretreatment wound size (Figure 1A). By the sixth visit, 2 months after the APC treatment was initiated, the patient’s wound size had reduced by 52%, and the surrounding skin was visibly less inflamed (data not shown).

CASE 2

A 75-year-old man with a medical background of asthma, hypertension, gout, and depression presented with an 8-month history of a skin ulcer on the lateral aspect of his left lower leg area. He had a 20-year history of lower limb venous ulcers, mainly on the left side, which appeared to stem from venous thromboses in both legs dating back to 1970. A venogram obtained in 1993 showed popliteal and superficial femoral vein incompetence with no opportunity for surgical intervention. Conservative management over the years had entailed thrice-weekly ulcer debridement and dressing changes and full-time venous compression stockings. By 2005, however, the patient was experiencing increasing lower leg swelling and subsequent impediment of mobility. Most recently, a persistent Staphylococcus aureus infection of an ulcer on the lateral aspect of his left calf was being treated with oral antibiotics. After 8 months of conservative ulcer therapy, with no clinical wound improvement, the patient began treatment with APC. A dramatic reduction in wound area and depth was evident in the week after the first APC treatment, with a 73% reduction in wound size between week 1 and week 2. Subsequently, the patient described a worsening of his depression and reported that he had begun scratching and picking at his skin in general, as well as specifically at the wound. Healing progress slowed over the remaining 5 weeks; nonetheless, there was a further 15% reduction in wound size. By visit 6, the patient’s wound size had reduced overall by 88% (Figure 1A).

CASE 3

A 71-year-old man with type 1 diabetes, hypertension, and hyperlipidemia presented with a 4-month history of a neuropathic skin ulcer overlying a right dorsomedial bunion. Persistent wound infections had been treated by his general practitioner with long-term antibiotic therapy, and he had received twice-weekly outpatient wound dressings, debridement, and footwear modifications, with no clinical improvement in wound size or surrounding inflammation. At this time, the patient began treatment with APC. After the first APC treatment, there was a visible reduction in wound depth, with the formation of granulation tissue in the wound base. By visit 3, swelling and erythema surrounding the wound had reduced, and antibiotic treatment was discontinued. The patient was still not taking antibiotics at follow-up 4 months later. Over the trial course, his wound size had reduced by 92%, leaving a small (approximately 2 mm²) area that had not reepithelialized (Figure 1A).
A 77-year-old man with a medical background of type 1 diabetes, hypertension, and gastroesophageal reflux presented with an 8-month history of a nonhealing skin ulcer on the lateral aspect of the right lower leg area. Larvae were initially found within his ulcer, and pulses were absent in his right leg. Doppler ultrasound examination showed monophasic flow in the anterior tibial artery, triphasic flow in the posterior tibial artery, and biphasic flow in the dorsalis pedis artery. The patient received twice-weekly home dressings by community nurses and attended outpatient wound clinics at weekly intervals for debridement and dressing application. With no clinical wound improvement resulting from standard ulcer management, the patient began treatment with APC. Between visits 1 and 2, there was a 37% reduction in wound area (Figure 1A), with granulation tissue filling in much of the wound depth (Figure 2, visits 1 and 2). The wound area continued to reduce over the following 3 treatments by 62%, 68% and 95%, respectively, compared with the original wound size (Figure 1A). Two weeks after the completion of treatment, the wound had healed (Figure 2, visit 6), apart from remnants of the biopsy performed at visit 4, which completely healed after 2 more weeks. At 4 months, there
remained a flat scar, level with the surrounding skin's contour, with no palpable thickening (data not shown).

All 4 patients responded positively to APC treatment, showing a reduction in wound area over the course of treatment (Figure 1A). Cases 2, 3, and 4 showed early reduction in wound depth after the first treatment, with plump granulation tissue observed in the wound base. The volumes of APC injected beneath the dressing at each of the 4 treatment visits provided an estimation of wound volume. These volumes were consistent with the clinical response of diminishing wound areas (Figure 1B). There was an overall steady progress in healing over the 8-week period. When results from all 4 patients were pooled, there was a significant reduction in wound area between the pretreatment (visit 1) and the posttreatment (visit 6) visits (Figure 1C).

We have recently shown that EPCR and PC are produced by normal skin keratinocytes, especially those in the basal layer. This endogenous PC is activated by binding to EPCR on the cell surface, with the resulting APC stimulating a wound-healing phenotype in keratinocytes. Furthermore, the autocrine actions of APC are necessary for normal keratinocyte growth and function. In the current study, immunohistochemical analysis of biopsy specimens obtained immediately adjacent to the wound on visit 1 showed that EPCR was expressed throughout the epidermis, similar to normal skin (Figure 3). In contrast, PC and/or APC was faintly present in the upper layers of the epidermis but absent in the basal keratinocyte layer (Figure 3). Low PC levels were observed in all patients (data not shown), which raises the possibility that low epidermal PC/APC levels may predispose to chronic wounds. On visit 4, there was an increase in EPCR expression with no obvious change in the level of PC/APC (Figure 3). This increase in EPCR after APC treatment supports our previous finding that APC up-regulates this receptor in keratinocytes, while the low PC/APC levels indicate that the exogenous APC does not accumulate in the epidermis. Overall, our results suggest that exogenous APC acts through EPCR in the epidermis to mediate healing.

The patients felt a brief, mild tingling sensation at the wound site on application of APC. There were no adverse effects recorded. Because of APC’s known anticoagulant function, the patients were closely observed for bleeding, but this complication was not encountered. No relapses were recorded during follow-up, with wounds remaining stable 4 months after treatment was initiated.

To date, APC has been clinically used only for coagulation- and inflammation-related disorders. A large clinical trial has shown that APC administered by infusion in high doses leads to reduced mortality in a subset of patients with severe sepsis, although there is some debate regarding its safety and efficacy. To our knowledge, this is the first report to describe the use of APC to treat chronic wounds. Treatment is topical and conservative, being approximately 1000-fold less potent than the amount given to patients with sepsis. Our results back up the compelling experimental evidence that APC has potential as a topical treatment to accelerate wound healing. Activated protein C may provide a safe and efficacious therapeutic option for recalcitrant chronic wounds.

Accepted for Publication: February 2, 2008.
Correspondence: Chris Jackson, PhD, Sutton Research Laboratories, Kolling Institute, Royal North Shore Hospital, St Leonards, NSW 2065, Australia (cjackson@med.usyd.edu.au).
Author Contributions: Dr Whitmont had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Whitmont, Tritton, March, Xue, Lee, Fulcher, Sambrook, Cooper, and Jackson. Analysis and interpretation of data: Whitmont, Reid, Tritton, Slobedman, Cooper, and Jackson. Acquisition of data: Whitmont, Reid, Tritton, Slobedman, Cooper, and Jackson. Drafting of the manuscript: Whitmont and Jackson. Critical revision of the manuscript for important intellectual content: Whitmont, Reid, Tritton, March, Xue, Lee, Fulcher, Sambrook, Slobedman, Cooper, and Jackson. Statistical analysis: March. Obtained funding: March, Sambrook, and Jackson. Administrative, technical, and material support: Whitmont, Reid, Tritton, Xue, Sambrook, Slobedman, and Cooper. Study supervision: March, Lee, Fulcher, Sambrook, Cooper, and Jackson.

Financial Disclosure: The University of Sydney holds a patent application on the treatment and composition for wound healing, with Drs Sambrook and Jackson as inventors.
Funding/Support: This project was supported by a University of Sydney Vice Chancellors Seeding grant, an Isabel Millner and Henry Langley Research Fellowship, a National Health and Medical Research Council Development Grant, and a Northern Sydney and Central Coast Area Health Service Grant.
Role of the Sponsors: The sponsors had no role in the design and conduct of the study; in the collection, analysis, and interpretation of the data; or in the preparation, review, or approval of the manuscript.

REFERENCES