GROWTH FACTORS & CHRONIC WOUND HEALING

Growth substances (cytokines and growth factors) are soluble signaling proteins affecting the process of normal wound healing. Cytokines govern the inflammatory phase that clears cellular and extracellular matrix debris. Wound repair is controlled by growth factors (platelet-derived growth factor [PDGF], keratinocyte growth factor, and transforming growth factor beta). Endogenous growth factors communicate across the dermal-epidermal interface. PDGF is important for most phases of wound healing. (Goldman R. Adv Skin Wound Care 2004;17:24-35.)

IMPACT OF PDGFs IN CHRONIC WOUND HEALING

Over the past several decades, the discovery of growth factors has led to much hope and speculation about the use of these potent peptides in the treatment of difficult to heal wounds, particularly chronic wounds. (Robson MC. Progress in Dermatology 1996:30:1-7.) Additionally, in the last 10 to 15 years, a large number of trials have been performed to evaluate the safety and effectiveness of growth factors in the healing of chronic wounds due to pressure (decubitus ulcers), diabetic neuropathy, and venous insufficiency. (Robson MC.)

As greater understanding of the growth factors involved in wound healing emerges, future patient care may include scarless wound healing and transplant of tissues engineered from stem cell progenitors. (http://www.bu.edu/woundbiotech/growthfactors/gfdevtest.html)

As soon as blood vessels are disrupted, platelets enter the wound in great numbers and release several growth factors, including platelet-derived growth factor (PDGF) and transforming growth factor-$\beta 1$ (TGF-$\beta 1$). These and other growth factors are chemotactic for a number of cell types critical to the repair process, such as macrophages, fibroblasts, and endothelial cells.

Later, during the proliferative phase of wound repair, several growth factors, including vascular endothelial growth factor (VEGF), fibroblast growth factors (FGFs) and PDGF and TGF-$\beta$ isoforms, provide a potent stimulus for angiogenesis and for fibroblasts to synthesize key extracellular components (i.e., collagens, proteoglycans, fibronectin, elastin). During the later stages of wound repair, growth factors are important in tissue remodeling, aided by the action of matrix-degrading metalloproteinases (MMPs). It is likely, however, that the action of growth factors does not end with wound closure and tissue remodeling, but that they are key players in the maintenance of tissue integrity and in cell-to-cell communication. (www.bu.edu)

Several studies have been performed that demonstrate growth factor deficiencies in chronic ulcers. The concentration of growth factors was shown to be markedly decreased compared with acute wounds, and the quantity and quality of cytokines and proteases and their inhibitors were different in acute and chronic ulcers.

Thus, it has been hypothesized that supplementing these missing factors may stimulate wound healing. The source of such growth factors is the thrombocyte or platelet. Platelets are described as nonnucleated cells arising from megakaryocytes that produce complex protein mitogens. These mitogens are stored in the platelet alpha-granules and are released on exposure to thrombin. As mitogenic proteins, growth factors participate in the regulation of normal cell proliferation (mitosis), differentiation, cell migration (chemotaxis), angiogenesis, production and degradation of the extracellular matrix, the production of growth factors by other cells, and organ growth. These factors are essential for wound repair. Therefore, the study authors proposed that adequate wound debridement with the application of concentrated autologous platelet-derived growth factors can stimulate healing and eliminate a portal for infection. (McAleer J, et al. J Am Podiatr Med Assoc 2006;96:482-8.)

OVERVIEW - DIABETIC FOOT ULCER

Approximately 24 million people in the United States have diabetes and 800,000 new cases are identified each year. Many diabetic patients develop diabetic peripheral neuropathy. Among all diabetic patients, 15% will eventually develop a Diabetic neuropathic Foot Ulcer (DFU), 25% of whom will have a foot amputation and subsequent 3-year survival rate of 50% despite currently available therapies. The current Standard of Care (SOC) for DFU includes surgical debridement, moist dressing changes, and off-loading. SOC treatment results in healing incidences of approximately 25% after 12 weeks and 30% after 20 weeks. In chronic DFU, the healing process is impaired in part due to deficiency of growth factors.

AUTOLOGOUS PLATELET-RICH PLASMA GEL

There is a growing body of evidence that suggests that wound healing in chronic diabetic foot ulcers is growth factor dependent and that the therapeutic delivery of these growth factors to wounds topically, has the potential ability to accelerate wound healing in conjunction with conventional wound care.

Autologous-derived platelet concentrate is activated to release growth factors that are stored in the platelet granules. These secretory proteins include cytokines and growth factors such as transforming growth factor-beta, vascular endothelia growth factor, platelet derived growth factor, and so on. The enhancement of soft tissue healing by the application of autologous derived platelet rich plasma gel (APG) is supported by basic science and some clinical studies. (Akingboye AA, et al. J Extra Corporeal Technol 2010;42:20-9.)

In a 2006 study, investigators determined the safety and effectiveness of treating diabetic foot ulcers with platelet rich plasma gel versus a control treatment consisting of normal saline

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The main purpose of the 12-week prospective, randomized, controlled, double-blinded trial was to compare the safety and incidence of complete wound closure between PRP gel- and control-treated wounds. Secondary objectives were to compare the rate of wound healing during the 12-week study and incidence of wound recidivism among healed ulcers during a 3-month follow-up period. Safety variables included adverse events, serious adverse events, and clinical laboratory tests.

Eligible study patients were those with type 1 or type 2 diabetes with an ulcer of at least 4-weeks’ duration. They also had to meet additional inclusion/exclusion criteria: hemoglobin A1C <12; index foot ulcer located on the plantar, medial, or lateral aspect of the foot (including all toe surfaces); and wound area (length x width) measurement between 0.5 cm² and 20 cm², inclusive.

After meeting all initial inclusion criteria and signing the informed consent, all patients completed a 7-day screening-period. This included initial excision/debridement, baseline wound measurements and evaluation, and application of the control saline gel to the wound.

Each study participant was assigned to 1 of 2 treatment groups, PRP or control, and received the next available consecutive randomization number.

The PRP separation system utilized in the study was a point-of-care system for processing autologous platelets and plasma to be used for the treatment of nonhealing wounds. It comprised of 2 components: a small, portable centrifuge to separate whole blood into PRP and a convenience kit that includes items for the blood draw, processing, and PRP gel application.

The need for consistency in product application and maintenance of the blinding process dictated that dressings were applied only at the investigator’s site except for the provision of a 1-time dressing change at home should circumstances prevent clinic attendance. Patients returned twice weekly at 3- or 4-day intervals; procedures and processes described were performed at each visit for a maximum of 12 weeks. Subjects continued to receive treatment until the wound healed, the 12-week treatment phase ended, or study participation was terminated by the investigator, sponsor, or because the patient withdrew consent or failed to return for visits. To evaluate safety, clinical laboratory tests were conducted throughout the study to determine the impact of treatment interventions.

When the investigator pronounced the wound closed, the patient was scheduled for a visit 1 week later but was asked to continue wearing the offloading orthosis walker. At this visit, if the wound had reopened, the patient was re-entered into the study at the same timeline and continued until the wound either healed or until week 13, visit 1 without healing. If the wound stayed healed after the 1-week interval, the patient entered the follow-up phase and returned after 3, 7, and 11 weeks. In the 3-month follow-up phase, the healed wound was evaluated for breakdown and the patient was queried regarding adverse events.

At the end of the 12-week treatment period, unhealed wounds were treated per physician protocol. The main efficacy variable was the proportion of patients with a healed wound. Other efficacy variables were 1) percent change in wound area at end-of-study visit (EOSV) from baseline (BL); 2) percent change in wound volume at EOSV from BL; 3) area closure rate per day at EOSV; and 4) volume closure rate per day at EOSV.

Ultimately, 72 patients were enrolled. In the intent-to-treat (ITT) population, the mean and standard deviations (SD) for age, HgbA1C, wound area, and volume in the 2 treatments were not significantly different, but the wound volume in the PRP gel group was significantly more variable than in the control group.

In the ITT group, 13 out of 40 patients (32.5%) in the PRP gel and nine out of 32 patients (28.1%) in the control group had completely healed wounds after 12 weeks (P=0.79).

In the dataset for the per protocol (PP) analysis, 13 of 19 (68.4%) patients given PRP gel and 9 out of 21 (42.9%) patients in the control group healed. In the PP dataset, the average wound closure rate per day was 0.051 cm² for the PRP gel group versus 0.054 cm² for the control group. In the majority wound dataset, the wound area closure per day was 0.042 cm² for the PRP gel group and 0.043 cm² for the control group; these differences were not statistically significant.

In the PP dataset, wounds in the PRP gel group healed after a mean of 42.9 days (SD 18.3) compared to 47.4 days (SD 22.0) for wounds in the control group. While the number of days to healing was the same in the majority wound group (mean 42.9 and 42.8 days), 81.3% of PRP gel-treated wounds and 42.1% of control gel-treated wounds healed during that time.

Of the 40 patients in the PP dataset, 22 with healed wounds participated in the 12-week follow-up phase: of those, one in the PRP gel group had a wound that reopened. None of the control-treated patients’ wounds re-opened; this difference was not statistically significant. Of the 122 adverse events that occurred after randomization, 60 (49%) were in the PRP gel group and 62 (51%) in the control group.

“The results of this study show that PRP gel is safe for use in the treatment of nonhealing diabetic foot ulcers,” the authors stated. “Using PRP gel to treat diabetic foot ulcers may not only enhance healing, but it also may prevent lower extremity amputations caused by nonhealing wounds.” (Driver VR, et al.)

FORMULATED COLLAGEN GEL

Meanwhile, in another study, researchers recently assessed the safety and efficacy of highly refined collagen-based topical gel wound care dressing for the treatment of chronic non-healing diabetic lower extremity ulcers. In particular, the controlled, double-blind randomized Matrix Phase IIb clinical study evaluated formulated collagen gel (FCG) alone and with adenovirus encoding human platelet-derived growth factor (PDGF)-B formulated in a bovine collagen (GAM501) in comparison to Standard of Care (SOC). The analysis included patients with a Wagner Classification Grade 1 cutaneous lower extremity 1.5–10.0 cm² chronic diabetic neuropathic foot ulcer that healed <30% during Run-in. Patients had peripheral neuropathy and adequate blood flow. Patients were randomized to treatment at one of 22 clinical sites. (Blume P, et al. Wound Repair Regen 2011;19:302-308.)

Following qualification and informed consent, patients underwent surgical debridement of the ulcer, biopsy for culture, clinical ulcer assessment, ulcer photograph, and ulcer size measurement (acetate tracing for planimetry) on day –14 to start a screening 2-week run-in period with SOC treatment. The primary data was the site-generated weekly acetate tracing of the wound edge faxed to a central laboratory for area measurement; weekly ulcer photographs were archived at the same central laboratory as a back up if confirmation was needed.

During the run-in period and throughout the trial, all patients wore a special off-loading orthopedic shoe. On day –3, repeat clinical ulcer assessment was performed and qualified patients were randomized into 1 of 5 treatment groups: (1) SOC; (2) FCG one application on day 1; (3) FCG 2 applications on days 1 and 29 (4 weeks); (4) GAM501 one application on day 1; (5) GAM501 2 applications on days 1 and 29 (4 weeks).
The day 1 visit consisted of surgical debridement of the ulcer if medically necessary, clinical assessment of the ulcer site, ulcer photograph, and ulcer size measurement by acetate tracing to confirm that the patient qualified.

Patients randomized to the SOC group continued with daily dressing changes. All patients were seen and assessed weekly until ulcer closure or week 12. Patients whose ulcer closed entered a 12-week follow-up phase to assess durability.

Taking certain factors into consideration, a decision was made to modify the trial design to examine initial healing rates as an additional primary endpoint, to combine the 1- and 2-dose treatment arms and reduce the combined study group sizes (thus overall patient enrollment). Following this determination and before unblinding the trial, the Statistical Analysis Plan (SAP1) was written to combine the 1- and 2-dose treatment arms of both GAM501 and FCG, making the trial exploratory, since the initial healing rate would not be affected by the second treatment and the power to detect a treatment effect on initial healing rate would thus be increased. SAP1 also specified that the rate of change of wound radius (wound-healing rate) during the first 4 weeks and the incidence of complete wound closure in the combined 1 and 2 dose groups were co-primary endpoints, and that the analysis would be per-protocol. The weekly healing rates were secondary endpoints. Early wound healing rates have been shown to predict eventual wound closure and are a variable that likely would be affected by an effective treatment compared with SOC. Note that the primary interest of the trial was to compare GAM501 to SOC and FCG as it had been assumed based on a number of prior animal studies that FCG alone would not have an effect and be a negative control.

As enrolled, 124 treated patients constituted the intention to treat (ITT) population included in the demographic and safety analyses. After database lock, a blinded data review identified 11 patients with important protocol deviations who were not included in the per-protocol analysis (PP). Since the trial was exploratory the statistical analyses were performed PP on the resulting 113 patients.

Review of the data by the DSMB found GAM501 and FCG to be safe in the ITT population.

Of the 124 patients treated, 116 completed the study and 8 were withdrawn. For SAP1, the blinded data review identified 11 patients with protocol deviations for inclusion/exclusion criteria not met and treatment or visit non-compliance leaving 113 patients in the PP population. A 10% variation was allowed so that minimum wound size for inclusion was 1.35 cm² and maximum allowable decrease from day −14 to day 1 was 33%.

Ulc er closure incidences were 5/16 (31%) in SOC, 14/31 (45%) in FCG, and 27/66 (41%) in GAM501, a non-significant trend. Using acetate data, there were no significant differences in wound radius healing rates from day 1 to week 4 between groups. The finding that GAM501 and FCG had nearly identical effects was surprising as several non-diabetic and diabetic preclinical models had found GAM501 to be superior. To investigate this observation the wound photographs and acetate tracings were printed for visual comparison; the striking differences noted between the acetate tracing and corresponding photograph on day 1 from some sites led to blinded wound photograph analysis as primary data using SAP2.

The results from the blinded photographic data review were unexpected. Wound area by photograph on day 1 was less than 1.35 cm² in 33 out of 133 patients (29%) and 10 patients had wound size decreases of greater than 33% during run-in; 8 patients met both exclusion criteria, making a total of 35 patients (31%) that likely should have been excluded from enrollment on day 1. An additional patient was excluded because the wound covered a curved surface and area by photograph could not be accurately determined. Five patients excluded from SAP1 were found to qualify for SAP2, leaving a total of 82 patients for analysis in SAP2.

On day 1, the difference between acetate and photographic areas was 163% for excluded patients and 20% for included patients (P<0.001). The data revealed systematically greater area measurements with acetate tracings compared with photographs with differences that were very large on day 1 and much less thereafter. Exclusions were clustered among a few sites: 6 sites had 26 of 43 (60%) patients excluded in SAP2; the remaining 14 sites had 8 of 74 patients (11%) excluded in SAP2.

The 12-week complete closure incidences in SAP2 were: SOC: 4/13 (31%), FCG: 6/17 (35%), and GAM501: 21/51 (41%). Note that the complete closure incidence for patients excluded using photographs as primary data in SAP2 was 17 of 35 (49%) suggesting that these small and/or rapidly closing wounds were likely to close without intervention as reported in published literature.

SAP2 showed the cumulative wound healing rates for run-in and for day 1 through week 4. Pair-wise comparisons between the 3 groups by ANOVA found that the only significant difference was between FCG and SOC for day 1 to week 1 and day 1 to week 2. Within each group, all subsequent healing rates were compared with run-in by paired t-tests. In the SOC group, there was no significant change in healing rate from run-in until day 1 to week 2. In the FCG and GAM501 groups, all cumulative healing rates were significantly different than run-in.

The authors concluded from the exploratory trial that a single application of GAM501 or FCG increases the healing rate of neuropathic DFUs for the first 2 weeks after treatment; whereas SOC with weekly visits seems to have a much smaller and delayed effect on wound healing rate.

“Whether one or more administrations of GAM501 has advantages over FCG alone in certain circumstances, such as larger or more difficult to treat wounds, will require testing in future trials,” the trial investigators reported.

Despite the findings, there are currently no studies of GAM501 being conducted. FCG is approved by the FDA to support advanced wound care in a broad range of dermal wounds.

AUTOLOGOUS PLATELET-DERIVED GROWTH FACTORS

In other research, investigators studied the efficacy of concentrated autologous PDGFs in the healing and closure of chronic lower-extremity wounds in 24 patients (aged 25 to 91 years) with 33 lower-extremity wounds. In order to be enrolled in the trial, patients had to have chronic nonhealing lower-extremity wounds that were treated for at least 6 months with traditional wound-healing methods and exhibited no reduction in total surface area. (McAleer J, et al.)

Surgical wound debridement was performed to convert chronic ulcers into acute wounds. Concentrated autologous PDGFs and thrombin were applied to the wound bases and protected with a nonadhering compression dressing that remained intact for 7 days. Wounds were evaluated and the concentrate was reapplied every 2 weeks. The patients were treated over 10 months.

In 20 wounds, wound closure and complete epithelialization was achieved. Meanwhile, 75% percent or greater wound closure was obtained in 3 wounds, 50% to 74% closure in 3 wounds, and 25% to 49% closure in 2 wounds. No improvement was observed in 5 wounds. The average time for wounds to completely close was 11.15 weeks.
These findings are significant considering the failure of past treatment methods in this patient group,” the study authors said. Overall, the treatment of the patient population using the studied modality resulted in a 50% or greater decrease in surface area in 26 wounds, which involved approximately three-quarters of the treated patients. With regard to time, the investigators noted the total time required from initial patient contact to patient discharge was about 25 minutes.

“The application of concentrated autologous platelet-derived growth factors and thrombin resulted in substantial wound healing and wound-diameter reduction,” the study authors concluded. “This technique constitutes a safe and effective treatment option and avoids lengthy treatment periods that increase the potential for infection.” (McAleer J, et al.)

RECOMBINANT HUMAN PDGF

Diabetic neuropathic foot ulcers represent a serious health care burden to patients and to society. While the management of chronic diabetic foot ulcers has improved in recent years, it remains a frustrating problem for clinicians. In the current review, researchers examined the scientific underpinnings supporting the use of becaplermin, or recombinant human platelet-derived growth factor (rhPDGF-BB), in diabetic forefoot wounds. An emphasis is placed upon proper medical and surgical care of diabetic foot wounds, as studies have shown that the success of this growth factor in accelerating healing is ultimately dependent on proper ulcer care.

The efficacy of becaplermin gel treatment was tested in 4 randomized control trials (RCTs), which involved a total of 922 patients. The first 2 studies were double-blinded, while the third and fourth studies were only evaluator-blinded. Each trial continued for 20 weeks to evaluate complete wound closure as the primary endpoint, but the only reported follow-up after the conclusion of each study was a 3-month period.

The first study was a double-blinded RCT that compared becaplermin gel (30 μg/g) against a placebo gel (NaCNC) for clinical efficacy and safety (Steed DL. J Vasc Surg 1995;21:71-8). The Phase II trial was the first to study PDGF in human diabetic ulcers and included 118 patients. The authors reported a statistically significant greater incidence of complete healing in the treated wounds (48% vs. 25%; P<0.02).

A subsequent Phase III trial assigned 382 patients to becaplermin gel (30 μg/g and 100 μg/g) and placebo gel treatment groups (Wieman et al. Diabetes Care 1998;21:822-7). The becaplermin-treated wounds had higher incidences of complete healing over the study period, but only the 100 μg/g becaplermin dose yielded statistically significant results when compared with placebo gel (50% vs. 35%; P=0.01).

The third, smaller study randomized 138 patients to placebo gel treatment and good ulcer care comparison groups, in addition to a smaller group of 34 patients who opted to receive active becaplermin gel treatment (100 μg/g) instead (d’Hemecourt, et al. Wounds 1998;10:69-75.). However, the becaplermin group was not powered for statistical comparison of wound healing because the primary goal of the trial was to compare the safety of the placebo gel group against good ulcer care. The complete wound healing incidences were reported to be 44% for becaplermin gel treatment, 36% for placebo gel treatment, and 22% for good ulcer care only. However, those outcomes were not statistically significant at the P<0.05 level.

A fourth trial of 250 patients was designed to assess resource utilization by comparing becaplermin gel treatment (100 μg/g) against good ulcer care alone. The study did not show a statistically significant difference in complete wound healing incidences between the two groups (36% vs 32%), although the authors noted that a trend in favor of becaplermin gel treatment was present (Smieil, et al. Wound Repair Regen 1999;7:335-46.)

NONDIADETRIC ULCERS

PDGFs have also been studied in nondiabetic ulcers. During a 2002 analysis, researchers evaluated the off-label use of topical recombinant human platelet-derived growth factor-BB (rhPDGF-BB or rhPDGF gel 0.01%) for refractory ulcers. (Harrison-Balestra C, et al. Dermatol Surg 2002;28:755-760.) They conducted a retrospective chart review on patients without diabetes mellitus-related ulcers treated with topical rhPDGF gel 0.01%. A total of 12 patients (mean age, 70 years) with 14 ulcers were treated.

The ulcers had a median size of 8.7 cm², and the median wound duration prior to the use of rhPDGF gel was 10.5 months. All 14 ulcers received wound care before rhPDGF gel was instituted. These therapies included occlusive dressings, compression therapy, debridement, skin grafting with autologous and tissue-engineered skin, oral medications such as stanozolol, pentoxifylline, or antibiotics, hyperbaric oxygen, topical antibiotics, antimicrobials, emollients, steroids, and electrical stimulation. RhPDGF gel was either added to or replaced some of the patients’ previous regimen of wound care. A thin layer of rhPDGF gel was applied to the ulcer bed. It was applied once a day and then covered with a moist saline dressing. A dressing change without additional treatment was performed daily, 12 hours later. Complete healing was achieved in 9 of 14 ulcers (64%) with a mean time to healing of 26 weeks. Seven of the ulcers remained healed while 2 (22%) reopened during a 15-month follow-up period. Five ulcers failed to heal with rhPDGF after a mean treatment period of 11 weeks. No adverse effects of rhPDGF gel were reported or observed.

The researchers concluded that rhPDGF gel was an effective and well-tolerated treatment for refractory chronic ulcers.

CONCLUSION

More evidence supporting the use of growth factors in chronic ulcers is still needed.

REFERENCES

c) http://www.bu.edu/woundbiotech/growthfactors/gdfdevtest.html