TOPICAL Autologous Platelet-Derived Growth Factors in the Treatment of Chronic Diabetic Ulcers

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• Abstract

Background and Objectives—Diabetic foot is a major public health problem. None of the conventional treatments are anticipated to stimulate active wound healing. The aim of this study is to test the efficacy of topically applied autologous platelet-derived growth factors (PDGF) in active repair of chronic non-healing diabetic wounds.

Methods—Seven diabetic patients with 12 skin ulcers were studied; all of them had been receiving conventional wound care without evidence of healing for an average of 15 weeks. The wounds were randomized into control and treatment groups. Seven wounds were randomized to treatment with autologous platelet extract (APE) and silver sulfadiazine, and five were subjected to controlled wound care with saline solution and silver sulfadiazine dressings for eight weeks. At the end of the eight weeks, persistent non-healing ulcers in the control group were crossed-over to the treatment group. Ulcer parameters were recorded on the first day of each week during therapy until complete epithelialization was achieved. In the control group each ulcer acted as its own control.

Results—In the control group, only one ulcer achieved 50% healing in the stipulated eight-week period. However, when subjected to APE application, these ulcers healed completely; 100% healing occurred in 3.9±2.13 weeks (range 1.5-7 weeks, P<0.05). The difference between the extent of epithelialization in the treatment group at eight weeks of therapy and the control group at crossover point was highly significant (P<0.0001). The difference between healing rates with APE therapy (1.0±0.93 cm²/week) and controlled wound care (0.23±0.40 cm²/week) was highly significant (P<0.001). The overall mean 100% healing time for APE therapy was 5.4±1.35 weeks. There was no abnormal tissue formation, keloid or hypertrophic scarring.

Conclusion—This study clearly shows the efficacy of topically applied autologous PDGF in the repair of chronic non-healing diabetic ulcer.

Keywords: • Diabetes mellitus • wound healing • platelet-derived growth factor • diabetic foot • diabetic ulcer

Introduction

Patients suffering from diabetes mellitus, are seventeen times more likely to develop gangrene and five out of six major limb amputations occur in diabetic patients.1 In the United States, the diabetic foot problem accounts for 20% of all diabetic hospital admissions and 50% of all non-traumatic amputations. Ulcers occur in 15% of diabetics, and 6-20% of all hospitalized diabetic patients have foot ulcers.2,3 Nearly seven decades since the discovery of insulin and despite great advances in the understanding and treatment of diabetes, diabetic foot remains a cumbersome complication of the disease. Neuropathy and vascular changes in diabetes are risk factors for developing chronic foot ulcers. Minor trauma and pressure, or a breakdown of the integrity of the skin precipitate the effect.2 Numerous treatment modalities are currently used to control the associated infection and perfusion deficit in an attempt to foster an optimal environment for passive repair of the wound. None of these treatments, however, have been designed to stimulate active wound repair.4

Recently the role of growth factors in the healing process has received considerable attention, prompting the experimental use of autologous platelet-derived growth factors in the treatment of chronic non-healing ulcers.4 Polypeptide growth factors are a class of biologic mediators that promote cell proliferation, alone or in concert, by binding to specific cell surface receptors.5,6 The number of known growth factors are extensive and each has been reported to accelerate the
formation of various components in wound healing. Platelets are known to release certain factors from alpha granules, four of which have been identified. These include platelet-derived angiogenesis factor, which causes new capillary formation from the existing microvasculature; platelet-derived growth factor (PDGF), which is a potent fibroblast mitogen and chemotactiven; platelet-derived epidermal growth factor; and platelet factor 4, considered to be a chemo-attractant for neutrophils.

In this study, we test the efficacy of topically applied autologous platelet-derived growth factors (PDGF) in the repair of chronic non-healing diabetic wounds. It is our hope that the establishment of certain guidelines on the use of PDGF in the healing of chronic diabetic ulcers will reduce the number of amputations performed on diabetic patients.

Materials and Methods

Research design and patient selection:

This study is a controlled one-way cross-over therapeutic clinical trial with simple random sampling. After obtaining clearance from the ethical committee, patients who attended our diabetic clinic at the Namazi Hospital (Shiraz University of Medical Sciences) from September 1, 1996 to April 1, 1997 were enrolled for the study. The inclusion criteria were: (1) The presence of a chronic non-healing diabetic ulcer of at least eight weeks duration; (2) Controlled blood sugar; (3) Normal peripheral blood platelet count (>150,000/cu mm); (4) Negative history of malignancy; (5) Patient’s cooperation and reliability. In the case of infection, the ulcer would be treated before inclusion in the study. The age and sex of the patients, the size and location of the ulcers, and the duration of diabetes had no role in patient selection. The randomization of the patients was based on the sequence of referral to the clinic. If the patients had two ulcers, one was randomized to treatment and one to the control group. In the patients who had four ulcers, two were randomized to the treatment and two to the control group. Patients randomized to the treatment group received autologous platelet extract (APE) and silver sulfadiazine for application therapy until complete epithelialization of the wounds, but those randomized to the control group received only silver sulfadiazine; the ulcers with incomplete or no healing were crossed over to the treatment group.

Preparation of APE:

After obtaining an informed consent from all patients, 60 ml of blood was drawn into a syringe containing 5 ml of the anticoagulant citrate dextrose. This blood was centrifuged (140gr for 20 minutes at 4°C) to remove red and white blood cells, leaving platelet-rich plasma. Platelets were removed from this plasma by further centrifugation (800g for 10 minutes at 4°C), and then resuspended in normal saline solution at a concentration of $10^9$ platelets/ml. They were then treated with 1 unit/ml thrombin (T-4648; Sigma. St. Louis, Mo) to create a supernatant containing released APE. The APE-containing suspension was then added to 1 gr of collagen (C-9879; Sigma) to produce a sterile topical salve. Each 10-ml of salve was used for 1 week and subsequently discarded.

Procedure:

At the first visit, a record was made of the patient’s complete history, physical examination, and ulcer parameters, and the wounds were debrided of all necrotic tissue if indicated. Initial paraclinical work-up included CBC, platelet count, FBS, and X-ray (antero-posterior and lateral) of the ulcer site. A surgeon debribed wounds with a significant amount of infection and callosity. Patients were treated as outpatients, visiting the clinic weekly, and used a twice daily wound dressing protocol as follows:

Treatment group (APE+silver sulfadiazine):
A thin layer of APE salve was applied to the entire surface of the ulcer, and then completely covered with paraffin-impregnated gauze and sterile gauze dressing; this remained in place for 12 hours. The dressing and salve were then completely removed by washing with normal saline solution, and silver sulfadiazine ointment was applied to the ulcer and covered with paraffin-impregnated gauze dressing for another 12 hours. The ulcer was again washed with normal saline solution before the APE salve was re-applied. These applications were continued until complete epithelialization of the wounds occurred.

**Control group (Silver sulfadiazine):**

A layer of silver sulfadiazine ointment was applied to the entire surface of the ulcer and covered with paraffin-impregnated gauze dressing; this remained in place for 12 hours. The dressing and salve were then completely washed with normal saline solution and the ulcer was covered with only paraffin-impregnated gauze and sterile gauze dressing for the next 12 hours. The ulcer was again washed with normal saline solution before application of silver sulfadiazine. These applications continued for eight weeks. At this time, the ulcers with incomplete or no healing were crossed over to the treatment group.

**Standard patient-care protocol:**

Each patient received supportive, conventional care of the wound throughout the trial. Wounds were sharply and extensively debrided of all necrotic tissues. Oral antibiotics were prescribed if deemed necessary. Blood sugar was accurately controlled by a weekly check. If the wound was on the plantar surface of the foot, the patient was instructed not to bear weight upon the area until completion of the study.

**Statistical analysis:**

Statistical analysis was performed using unpaired student’s *t*-test and McNemar’s test, and a *p*-value less than 0.05 was considered statistically significant.

**Results**

Nine male patients with 14 wounds were entered into the study. One patient was excluded for non-compliance. One patient was further excluded because his ulcer was found to be due to venous stasis rather than a diabetic ulcer. Thus, 7 patients with 12 ulcers remained in this study; one patient with 4 ulcers, two patient with 2 ulcers each, and four patient with one ulcer each. Seven wounds were in the treatment group and 5 in the control group. Table 1 shows the initial patient data in each group. As shown in Table 1, the two groups are partially matched in various parameters. Healing rates were determined by the number of weeks taken to achieve 50%, 80%, and 100% epithelialization. During the initial eight weeks after randomization, 4 of 7 wounds in the treatment group achieved 100% epithelialization, and 3 of 7 decreased in size to an average of 90±8.7 percent epithelialization. In the control group during the initial eight weeks after randomization, no wound achieved 100% epithelialization, 2 of 5 wounds decreased in size to an average of 45±7.07 percent epithelialization, 1 of 5 had no change in size and 2 of 5 increased in size. Epithelialization of the wounds in the treatment group after eight weeks of treatment was 95.71±7.31 percent. In the control group at the cross-over point, epithelialization was 18±24.89 percent (*t*=7.98; *P*<0.0001). The progression of healing based on controlled wound care over an 8-week period gave a baseline for the predictability of healing, and as a result, every ulcer acted as its own control. In the control group, only one ulcer achieved 50% healing in the stipulated 8-week period; these patients had a highly significant improvement in healing when treated with APE (*P*<0.05, Table 2). Healing rates were determined as the decrease in ulcer size (cm²) per week. In the treatment group and in the control group after cross-over point, the healing rate was 1.0±0.93 cm²/week compared to 0.23±0.40 cm²/week in the control group. The difference between healing rates in the APE therapy and control group is highly
Discussion

The first indication that platelet contained a mitogen, came from comparisons of serum and plasma-induced stimulation of fibroblast growth in culture. Rutherford and Ross showed that the growth stimulating activity in serum for smooth muscle cells and fibroblasts, was abolished by completely removing the platelet-poor plasma. \(^{13}\) These \textit{in vivo} properties suggest that PDGF, derived from platelets at the site of injury, may play an important role in the initiation of the repair process of wounds. Locally acting growth factors from thrombin-activated platelets initiate the connective tissue response by causing fibroblast division and migration as well as capillary formation. The end result of this complex interaction is the transformation of a resting connective tissue into an area of intense cellular movement, division, and biosynthesis, which results in closure of the wound space with a neovascularized collagen mesh. Granulation tissue formation is followed by epidermal division and migration, which covers the collagen-vascular mesh with new skin.

In 1982, Knighton et al. utilized the rabbit corneal assay to demonstrate that thrombin-activated platelets have the capacity to stimulate angiogenesis and increased collagen synthesis, but implantation of collagen and albumin into the cornea demonstrated that native proteins other than fibrin produce no corneal reaction. \(^{7}\) In 1986, Knighton et al. showed that the accelerated epithelialization of granulation tissue leading to complete repair of chronic non-healing ulcers is attainable by the use of autologous platelet factors. \(^{14}\) This was the first clinical demonstration that the locally acting factors derived from autologous blood promotes healing of chronic cutaneous ulcers. In this study, the time to 100\% healing after initiation of platelet-derived wound-healing factors (PDWHF) was \(7.5\pm6.5\) weeks. There was a direct correlation between initiation of PDWHF therapy and 100\% healing. The age of the patients and the location of the ulcers had no statistically significant effect on PDWHF stimulated wound repair. \(^{14}\) In 1990, Atri et al. confirmed that recalcitrant skin ulcers of any cause could be stimulated by homogenous platelet-derived growth factors to produce a reparative cellular response. In this study, the time taken for 100\% healing were respectively \(9.67\pm4.9\) weeks. Only the ulcer type determined the healing rates; the shortest and the longest time to achieve 100\% healing was \(6.88\pm2.97\) weeks in diabetic patients versus \(14.00\pm7.07\) weeks in the venous stasis group. Variables such as age, sex, location of the ulcer, ulcer duration and ulcer measurements had no influence on the homologous PDWHF-stimulated healing rates. This was the earliest report of homologous platelet extract-stimulated repair in chronic non-healing skin ulcers. \(^{15}\)

In this study, we used APE to heal chronic diabetic ulcers. There was significant healing improvement in control wounds after cross-over point; in comparison to the same group before cross-over point, in which only one wound epithelialized by 50\% in an 8-week period. We used every ulcer as its own control using objective ulcer observation over weekly intervals during controlled therapy. We then applied APE to persistently non-healing ulcers. As an outcome of 50\% or 80\% healing could have been subject to observer variations, but we used a single observer and adhering to the one hard fact of 100\% epitheli-alization to minimize the variations. The data presented in this study demonstrates that topical application of APE stimulates repair of chronically non-healing diabetic wounds and leads to accelerated epithelialization.

This study, however, is not without flaws. Firstly, the study sample is small and secondly, the two groups are not completely similar (probably because of the small sample size). There was no evidence of overhealing, such as hypertrophic scars or keloid formation. The patients applied the APE at home without difficulty, requiring only periodic outpatient examination. One emerging hypothesis from this study is the future possibility that these factors, gleaned either from autologous pools or synthesized, may become therapeutic tools in the management of recalcitrant diabetic ulcer.
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References


