CHAPTER **56**

The Use Of Platelet-Derived Growth Factors In Chronic Non-Healing Wounds

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INTRODUCTION

The management and treatment of patients with chronic non-healing cutaneous wounds has recently received a great deal of attention in the medical literature. The majority of these patients suffer from long-standing diabetes mellitus with inadequate control of serum glucose levels. These patients frequently have concomitant peripheral neuropathy, vascular disease, and immunologic compromise. There are approximately 14 million people in the United States with diabetes, and as many as 2 million (15%) of these individuals may develop a foot or leg ulcer sometime during their lives. In fact, 45 to 70% of patients undergoing lower extremity amputations have diabetes mellitus and peripheral vascular disease. The most prevalent indications for lower extremity amputation in the diabetic patient includes gangrene, infection, and ulceration. An average of $157.8 million per year is spent for the medical care of diabetic patients undergoing extremity amputation as a result of a chronic non-healing ulcer. It is understandable that a tremendous amount of research has been directed toward the development of products that may accelerate the repair of chronic non-healing cutaneous wounds. It has been suggested that preparations of platelet-derived growth factors, in conjunction with the control of infection, revascularization (if indicated), and aggressive wound debridement promote the formation of granulation tissue and accelerates epithelialization of chronic non-healing ulcerations. This article will discuss the proposed role of platelet-derived growth factors in the stepwise treatment approach to chronic non-healing wounds.

FACTORS AVAILABLE

Platelet-derived wound healing factors are polypeptides produced inside the platelet and stored in the intracellular alpha granules. These factors are released when the platelets are activated by thrombin. Platelet-derived growth factors act in a paracrine fashion, meaning they are produced by one cell type and used by another. The first prospective study using topically applied growth factors in the treatment of chronic non-healing cutaneous wounds in humans was conducted by Knighton in 1986. Forty-nine patients with a total of 95 cutaneous ulcers previously treated with standard wound care for an average of 198 weeks were studied. Complete healing of these wounds was reported to occur in an average of 10.6 weeks after beginning topical growth factor application. This study was based upon previous research using thrombin-released platelets on rabbit corneal tissue. This study demonstrated that platelets contain intracellular factors that, when applied topically, promote neovascularization, fibroblast proliferation and migration, and increased collagen synthesis.

Tissue macrophages derived from intravascular monocytes, along with neutrophils, platelets, and red blood cells occupy the wound space for the first two days in the normal healing process. It is currently accepted that platelets and macrophages are the predominant cells responsible for the regulation of wound repair. The primary actions of platelet-derived growth factors when applied topically are the acceleration of granulation tissue formation and wound epithelialization. There are five polypeptide factors produced by the platelet and released by the alpha granule which are known to possess these properties:

- Platelet-derived growth factor (PD-GF)
- Platelet-derived endothelial cell growth factor (PD-ECGF)
- Platelet-derived epidermal growth factor (PD-EGF)
- Transforming growth factor-beta (TGF-β)
- Platelet Factor 4 (PF-4)

PD-GF is an important regulator of the healing process and is thought to be the most potent mitogen for cells of mesenchymal origin found in human serum. It serves as a chemotactic factor for leukocytes, fibroblasts, and smooth muscle cells. The migration of fibroblasts occurs at lower concentrations of PD-GF (0.5-1.0 ng/ml), whereas higher concentrations (2-5 ng/ml) are required to induce mitosis.

PD-ECGG serves as a chemoattractant and promotes the growth of vascular endothelial cells in vitro, and angiogenesis in vivo. **PD-EGF** functions as a mitogenic chemoattractant for epidermal skin cells and has been shown to increase collagen synthesis in epithelioid cell lines of normal rat kidneys.

TGF-β has two important functions, including monocyte chemotaxis and the deposition of intracellular matrix. The latter is accomplished by increasing the production of collagen and glycosaminoglycans by tissue fibroblasts. TGF-β, however, inhibits the replication of endothelial cells and fibroblasts at higher concentrations. It is also speculated that TGF-β may be partly responsible for wound epithelialization, but this role is still under investigation.

PF-4 induces the chemotaxis of neutrophils and monocytes[1], and is thought by some investigators to play a role in the early induction of PMNs into the wound.

In general, endogenous growth factors regardless of origin are categorized as being competence or progression factors. They are categorized depending on which point during cell-cycle replication they regulate. PD-GF is an example of a competence factor because it brings the target cells to the first growth arrest point in cell-cycle replication. In contrast, progression factors such as TGF-β and fibroblast somatomedin C promote the commitment of a "competent" cell to begin DNA synthesis.

APPLICABILITY TO TYPE OF WOUND

Platelet derived growth factors can potentially be used on a variety of chronic non-healing wounds. They have been used to treat ulcerations and pressure sores present in diabetes mellitus, venous stasis, non-diabetic peripheral vascular disease, trauma, and rheumatoid arthritis. Immuno-suppressed patients, as well as individuals bedridden for long periods of time, are susceptible to chronic ulcerations and may also benefit from this type of therapy.

GROWTH FACTOR PREPARATION

To attain the platelet-derived growth factors, whole blood is centrifuged and the white and red blood cell components are removed leaving a platelet-rich plasma. The platelets are then removed by further centrifugation and treated with thrombin. This process activates the platelets and causes the alpha granules to release the PDWHFs into the supernatant. These wound healing factors can be attained from autologous or homologous blood sources. The former is prepared by obtaining platelets from the patient's own blood, whereas the latter is acquired from blood drawn from healthy volunteers confirmed to be sero-negative for the human immuno-deficiency and hepatitis viruses. Growth factors acquired from autologous sources carry no risk for disease transmission, and could therefore be considered advantageous over growth factors obtained from banked blood. Experimental in vivo studies have also been performed using PD-GF produced by recombinant DNA technology. Platelet-derived growth factors can be prepared as a solution, or as a salve by compounding micro crystalline collagen (Ativene®) with the solution.

At present, a commercially available source of PDGFs is manufactured by Curative Technologies, Inc. (Setauket, N.Y.) and sold under the trade name of Procuren. Other companies such as Genetech, Amgen, and Synergen are also in the process of developing various growth factor preparations.

PROTOCOL

It is important to emphasize that the application of platelet-derived growth factors is only one segment in the stepwise approach to patients with a chronic non-healing wound. Evaluation begins with a thorough history and physical examination to determine the etiology of the ulcer. It is important to determine how long the ulcer has been present, and if any local or systemic signs of infection are present. If the ulcer has been present for several months or probes to bone, the existence of osteomyelitis should be strongly suspected. Furthermore, it is common in the diabetic patient

because of decreased immunologic function, to have a superficially infected ulceration and remain afebrile with very little surrounding erythema, edema, or lymphangitis. However, deeply infected ulcerations will normally result in fever, elevated blood glucose levels, and the other cardinal signs of infection. The history should also include questions concerning intermittent claudication and distal rest pain, both of which are signs of arterial compromise.

Evaluation of the ulcer should assess the size and depth of the wound, as well as the presence of cutaneous undermining. Redness and swelling in the peri-ulcerative area, as well as the presence of purulent or serous discharge, necrotic tissue, or exposed bone should be noted. During the physical examination it is important to assess the vascular patency of the extremities. Pitting and/or brawny edema should also be noted if present. Signs of arterial insufficiency including non-palpable pedal pulses, cold feet, intermittent claudication, dependent rubor, absence of digital hair, and atrophic skin changes should be documented if present. If peripheral vascular disease is suspected, non-invasive vascular testing, including Doppler wave form analysis and laser Doppler capillary flow measurement, should be considered. The former test has been shown to accurately determine levels of potential arterial occlusion. A recently developed technique, based on arterial perfusion, measures transcutaneous oxygen tension (T_cPO_2) in the peri-ulcerative area. This technique has proven useful in predicting the healing potential of chronic wounds by measuring peri-ulcerative tissue perfusion. Although still commonly used, segmental pressures, if performed should be cautiously interpreted. Many of these patients have atherosclerosis and concomitant vessel calcification which falsely elevates occlusion pressures. If non-invasive tests suggest arterial occlusion and the T_cPO_2 is less than 30 mm Hg, then arteriography is indicated if the patient is a candidate for revascularization.

It should be pointed out that primary arterial ischemic ulcers in the diabetic patient are a rare finding. It is usually mixed peripheral neuropathy and altered skin structure that predispose the diabetic patient to ulceration. However, angiopathy resulting in diminished arterial perfusion is often a complicating factor, and may be responsible for delayed or chronic non-healing wounds. If an arteriogram confirms arterial occlusion, revascularization should be considered if the patient's vessels are amenable to reconstruction. Important advances in the treatment of chronic non-healing wounds have indirectly been made through the development of new revascularization procedures, including improved angioplasty techniques, profundoplasty, synthetic bypass grafts, and the in-situ distal bypass. The latter is a new technique utilizing the saphenous vein. The vein is converted to a vessel transporting arterial blood without removing it from the surrounding connective tissue bed. This procedure is carried out by first attaching the proximal end of the saphenous vein to either the common femoral or superficial femoral artery. It is then necessary to disrupt the valves and ligate the small venous branches. Finally, the distal end of the saphenous vein is directly attached to either the dorsalis pedis or posterior tibial artery utilizing microvascular anastomosis. This technique is advantageous over the reverse saphenous vein graft because the distal end of the vein closely matches the size of the anterior and posterior tibial arteries. The same principle holds true for the proximal end of the vessel. If the ulcer is secondary to venous stasis, then use of intermittent compression stockings should be considered.

The next step is the administration of antibiotics if infection is present. Oral antibiotics can be given on an out-patient basis if the wound is superficially infected and non-invasive. However, if a deep infection is present, the patient should be hospitalized and given intravenous antibiotic therapy.

After antibiotic therapy is instituted and revascularization is performed, the wound is aggressively debrided removing all nonviable osseous and soft tissue. If appropriate debridement is not conducted, the rate of granulation tissue formation and wound epithelialization will be significantly diminished. Protocol states that the PDGF's should only be applied after superficial purulence has disappeared and no necrotic tissue is present in the wound.

Once the wound is surgically debrided, the use of topical growth factors is employed. The topical growth factors are most often directly applied to the entire surface of the wound and covered with petrolatum impregnated gauze for a period of twelve hours. This is followed by removal of the dressing and cleansing of the area with sterile saline solution. The area is then treated with topical antibiotics (e.g.

Silvadene) for the next twelve hours. This sequence is repeated until healing occurs.

If the wound is on the plantar surface of the foot, the patient should remain non-weight-bearing until total healing has occurred. This may be accomplished with the use of crutches, a walker, or special contact casts. After healing has occurred, shoegear modification is important to redistribute excessive pressure in areas predisposed to ulceration.

No significant complications have been reported from the use of the PD-GFs, however, one study in animal models showed that TGF-β, when used by itself, produced an increase in inflammation, abnormal differentiation of epithelial cells, and decreased epithelial cell volume.

SUMMARY

Of paramount importance is the team approach in the total care of patients with neuropathic foot ulcerations. This includes the efforts of the primary care physician, endocrinologist, podiatrist, vascular surgeon, and infectious disease specialist. Sufficient vascularity, eradication of infection, and redistribution of pressure through shoegear modification are all required if these wounds are going to heal and not reoccur. The use of platelet-derived growth factors acts to accelerate the healing process, and should only be viewed as one of the many important steps in treating chronic non-healing ulcerations. It has not been conclusively answered why extracted platelet growth factors accelerate the healing process when applied topically to chronic wounds, but are ineffective while present in the systemic circulation.

An important consideration is the cost effectiveness of the topically applied growth factor treatment regimen. Mackey in 1986 estimated that in a two year period, an average of $40,563 is spent for the medical care of amputees. However, as costly as this may seem, the previous figure represents only a small portion of the total cost of extremity amputation. These patients lose enormous amounts of time from the workplace during surgery and rehabilitation, if the patient is able to return at all. A patient that can no longer work has a significant loss of future earning potential as well as an increased dependence on social programs. The reduction in a patient's quality of life after amputation should also be considered.

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