Wound Healing: Experience With rHuGM-CSF

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Abstract: Skin wounds are exposed to a series of events that culminate in healing. This process is well known in relation to histological events, although mechanisms that underlie its regulation remain unclear, since not only are tissue growth factors involved, but also a number of cytokines. The present study focuses on treatment of chronic ulcers of lower limbs of two patients with recombinant human GM-CSF (rHuGM-CSF); the results were encouraging. Colony granulocyte/macrophage stimulating factor (GM-CSF) could be included between compounds promoting healing, in relation to direct or indirect effects of topical GM-CSF administration. Further study will be needed to determine dosage, method of application, and the type of recombinant material that will achieve the best results.

Wound healing proceeds through three phases:

Inflammatory phase. This phase creates a moist environment that helps to keep the wound cleansed and the temperature constant, thereby creating a barrier to bacteria that might otherwise develop. This phase lasts for a few days during which granulocytes clean and free the wound from cellular debris, foreign bodies, and possible bacterial contamination. Macrophages, which have bactericidal activity, also appear and stimulate endothelial cell and fibroblast migration. This migration is the impetus for two fundamental healing processes: angiogenesis and fibroplasias.

Proliferative phase. Production of granulation tissue occurs and begins with restoration of metabolic trade, input of nutrients and oxygen, expulsion of catabolites, and is characterized by the appearance of fibroblasts, which have a role in wound contraction and restructuring of the extracellular matrix.

Remodeling phase. This phase begins 20–30 days after injury and continues for 12–24 months thereafter. It is characterized by collagen degradation and synthesis of new repair matrix components. Fibroblasts transform into myofibroblasts, which have the contractile capacity necessary to reduce scarring.

Therefore, wound healing is essentially due to:
• generation of granulation tissue
• restoration of epithelial continuity due to migration and mitotic division of basal layer cells
• contraction of scar by myofibroblasts present on bottom and margins of lesion.

After healing, the wound continues to evolve and to mature. The remodeling phase can last several months to several years. At the beginning, scarring appears reddish, hyperemic, and even slightly hypertrophic because of the richness of vessels and cells in the newly formed tissue. The cellular components gradually reduce and new capillaries atrophy, which leads to a scar that is whitish, pearly, and smooth. Physiological healing initially produces a thin scar with little fibrosis until function and the architecture of the injured organ are restored.

If a wound does not heal within 3 weeks it becomes chronic. Chronic, nonhealing wounds impact the healthcare system considerably. The psychological and social implications of these wounds can impede physiological healing processes.1,2

Many studies have evaluated the effects of exogenous growth factors on cutaneous ulcer healing. One of the first studies1 to demonstrate the effectiveness of topical application of growth factors on wounds devised a “wound healing formula”: an autologous mixture of platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), platelet-derived angiogenesis factor (PDAF), platelet factor 4 (PF4), and other unknown factors were extracted from platelets of patients with diabetes and was applied locally on the ulcers. Healing rates increased as a result. Robson et al4 treated decubitus ulcers with PDGF-β and vascular endothelial growth factor (VEGF), and their results were encouraging. Experiments with interleukin 1 (IL-1) failed. Results obtained from experiments4-7 with colony stimulating factors (CSF) seem promising. Colony stimulating factors, among known growth factors, are a family of glycoproteins that regulate both production and differentiation of hemopoietic cells and multiple functions relating to the defense mechanisms of mature cells. Cellular source of GM-CSF (colony granulocyte/macrophage stimulating factor) includes T-lymphocytes, macrophages, endothelial cells, fibroblasts, and keratinocytes. Among the best known functions of GM-CSF are to activate synthesis and to increase expression of immunologically important surface molecules of monocytes-macrophages, and to accentuate primary immune response by regulator effects that amplify ability of monocytes-macrophages to present antigens. GM-CSF has been widely used in treatment of hematological recovery after high-dose chemotherapy or after bone marrow transplantation thanks to its ability to stimulate proliferation, migration, and the role of bone marrow granulocytes and monocytes-macrophage. The angiogenic and fibroblastic immunomodulatory properties on non-hematopoietic cells have generated considerable interest in healing. GM-CSF stimulates proliferation and differentiation of keratinocytes, increases collagen synthesis, and leads to endothelial cell proliferation. Researchers have placed emphasis on therapeutic products that could 1) increase contraction of wound margins; 2) determine keratinocytes migration and proliferation; 3) amplify neovascularization and create a local environment to stimulate specific immune events and inflammatory processes.

GM-CSF seems to meet all of these requirements, which has led to laboratory synthesis of this growth factor. Gene encoded human GM-CSF has been isolated by molecular cloning of complementary DNA (cDNA) obtained from a human inactivated T-cell line and expressed by a system of mammalian cells (monkey COS-1 cells); in this way, GM-CSF is especially known and used for its hematopoietic effects. Extra-hematopoietic capabilities, especially in tissue restoration, have also been attributed to it. Under homeostatic conditions, a low level of GM-CSF production occurs, but mechanical stress such as in-vitro exposure to IL-1 or tumor necrosis factor-α (TNF-α) would stimulate keratinocytes to accumulate mRNA GM-CSF, and consequently, excrete GM-CSF.10,11 Although not explicitly shown, it seems GM-CSF produced by cytokines and/or “stressed” keratinocytes acts as an autocrine and/or paracrine regulator of epidermal growth and differentiation. It is also possible that GM-CSF effects proliferation/differentiation of epidermal cells indirectly in relation to the release of additional secondary cytokines induced by GM-CSF.

In-vitro experiments demonstrate that GM-CSF induces fibroblast precursors and some mesenchymal cell line proliferation. Moreover, it would stimulate human endothelial cell migration and proliferation, and it would have an important role in neoangiogenesis.12,13 In-vivo studies on rodents have been performed to evaluate if topical and subcutaneous application of the growth factor could accelerate repair of wounds infected with E. coli, which is known to significantly inhibit wound margin contraction. Other studies have evaluated the bactericidal properties of GM-CSF. GM-CSF seem to stimulate both phagocytosis and intracellular bactericidal capacity.
of macrophages and neutrophils, although these conclusions have yet to be subjected to specific study. Other in-vivo studies demonstrated significant histological changes in epidermal and dermal compartments after subcutaneous or intradermal administration of GM-CSF, with accumulation and proliferation of fibroblasts and myofibroblasts, and an accentuated neovascularization. After intradermal injection, in particular, an increase in the number and layers of keratinocytes would appear. These considerations suggest intradermal GM-CSF injection in human skin causes keratinocyte proliferation and an epidermal “regenerative differentiation.” In relation to collateral effects, intradermal GM-CSF injection in human skin only slightly increases T-lymphocytes and even less so in white blood cells.

Case Reports

The following cases involved two anonymous patients who gave informed consent to participate. The local research ethics committee approved the study.

Case 1: A 65-year-old woman had a deep lesion in the right foot between the big toe and the first toe, which was resistant to common treatments, and had been present for 11 months (Figure 1A, B). The wound was neuropathic-vascular in etiology and the patient had type 2 diabetes mellitus.

Case 2: A 65-year-old woman was suffering of a chronic ulcerative lesion in the left leg, near the ankle, which was resistant to common treatments and had been present for 8 months (Figure 2A, B). The lesion had a phlebostatic etiology. This patient also had type 2 diabetes mellitus.

Neither patient took oral medication in relation to the authors’ treatment; they only took medication in relation to their pre-existing pathologies.

Methods

In both cases, the ulcers were clinically examined. Cultural examination, to value the possibility of a silent infection, was performed in relation to this point the presence of infective organisms was tolerable (≤ 10³); furthermore, it has been performed instrumental diagnostic tests for vascular assessing (ecocolordoppler, laser Doppler, transcutaneous oxygen pressure (TcPO₂) and capillaryscopy), hemocromocytometric examination with formula and platelets repeated during and at the end of treatment, and also measuring and photographic documentation of ulcers.

In the presented cases the patients were treated with recombinant human GM-CSF (rHuGM-CSF), not glycosylated glycoprotein, that was produced by fermentation with a strain of E coli transfected with a plasmidic vector in which the gene for human GM-CSF had been inserted. The molecular weight was 14,477 daltons (Da) and the degree of purity was ≥ 95%. The injection was prepared as a powder. Both infiltration and administration was done via drip using a solution that was made by diluting a vial of 150 mcg rHu GM-CSF in 10 mL saline solution to a final concentration of 15 mcg/mL. The wounds were cleaned with saline solution and the wounds were sharply debrided two times per week. Dressing changes and rHuGM-CSF treatments were also administered two times per week. The treatment period lasted 8 weeks. After the ulcers had been cleaned with
saline solution, intradermal infiltration of diluted rHuGM-CSF (15 mcg) was administered at a distance of 0.5 cm from the ulcer edge and away from each approximately 2 cm–2.5 cm. The needle was angled at 45 degrees. The rHuGM-CSF was administered by drip (7.5 mcg/15'). The total drug dosage used in each treatment was 22.5 mcg. Finally, elastic-compressive bandages, providing the correct level of graduated compression (30 mmHg–40 mmHg), were applied.

**Results**

One patient’s wound healed completely, which was encouraging. The deep lesion between the big toe and the first toe can be seen clearly (Figure 1A). After 8 weeks of treatment, the wound healed completely (Figure 1B). An excellent improvement is evident in the other patient; the chronic ulcerative lesion in the left leg near the ankle can be seen (Figure 2A). Significant improvement was achieved as a result of the treatment. A longer period of treatment is most likely necessary to obtain the complete healing (Figure 2B). These results concur with those obtained by other authors, albeit with different procedures, doses, and duration of treatment. During treatment, prominent collateral effects or changes in blood parameters have not been reported.

**Conclusion**

Technically advanced studies have shown that the use of growth factors in a wound is therapeutically successful, even if the outcome cannot be considered conclusive. More recent evidence has highlighted that in the wound healing process not only are tissue growth factors involved in the immune defense response, but multiple cytokines as well. Our research leads us to include GM-CSF among those compounds that promote healing in relation to direct or indirect effects of topical GM-CSF administration, namely:

- acceleration of wound contraction by probable in situ accumulation of contractile cellular elements and bacteria destruction
- neovascularization induction
- stimulus to keratinocyte proliferation and differentiation.

Further studies will be necessary to determine dosage, method of application, type of recombinant material, and the optimal dressing to achieve the best results (elastic-compressive bandage could have had an effect on wound healing, making it difficult to be certain that the effects seen were solely due to the use of rHuGM-CSF). Presently, three other patients are being similarly treated. The present results seem analogous to previous results. The literature suggests intradermal GM-CSF application would be better than subcutaneous in relation to reconstitution of keratinocytes. The goal of researchers will be to implement GM-CSF preparations that ensure sufficient penetration of cytokines in wound site and maintain GM-CSF activity in an environment rich in proteinases. Patients who would benefit most from application of growth factors are those with intrinsic alteration in scar formation and those with remarkable bacterial colonization in chronic, nonhealing wounds.
References


