Practical Application of Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) in Patients with Wounds

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ABSTRACT

Rapidly evolving advances in wound-care technologies and treatment modalities, including locally injectable granulocyte-macrophage colony-stimulating factor (GM-CSF), are increasingly being used. Based on its role in the stimulation and recruitment of key contributors to wound healing, such as keratinocytes, macrophages, and fibroblasts, GM-CSF is considered to play an essential role in the wound-healing cascade. Synthetic GM-CSF has been shown to have a positive effect on the healing of chronic wounds when given as a local injection in a small number of patients. Subsequent randomized, controlled trials

demonstrated that GM-CSF accelerated the healing of chronic wounds. This paper reviews the proposed mechanism of action of GM-CSF in wound healing. We also describe its method of application in the operating room at a tertiary care center for patients with wounds.

Key Messages: Many types of chronic wounds have an altered keratinocyte and macrophage function that can be potentially assuaged by the addition of locally injected growth factor therapy to standard-of-care treatment. Granulocyte-macrophage colony-stimulating factor (GM-CSF) has been shown to be beneficial for the treatment of chronic, non-healing wounds. This article reviews the data on GM-CSF, reports a proposed mechanism of action, and describes its use by a team of wound surgeons.

INTRODUCTION

Rapidly evolving advances in wound-care biologics have presented healthcare providers with a plethora of viable options to use when treating wounds. Patients with chronic wounds require complex care, especially since most of them have numerous co-morbidities that contribute to their non-healing status. Chronic wounds have an altered cellular function that can be augmented through the use of exogenous growth factors.[1-3] In this article we discuss the use of locally injectable granulocyte-macrophage colony-stimulating factor (GM-CSF) in the treatment of chronic wounds.

Wound healing involves a complex interplay of many factors interacting over time. The four phases of wound healing, coagulation, inflammation, proliferation, and maturation/remodeling, can overlap and vary in duration. However, some wounds persist in the inflammatory phase rather than proceeding through the subsequent stages of healing and are deemed "chronic." Compared to acute wounds, chronic wounds have been shown to exhibit elevated levels of proinflammatory cytokines and proteases, a combination that may contribute to decreased levels of growth factors in the wound bed.[4]

When a wound is formed, the first cells to arrive are platelets, which form a hemostatic plug. Exposed collagen in the wound and chemokines secreted by platelets initiate the healing cascade and attract numerous other factors, including macrophages.[5] Macrophages are essential during both the inflammatory and resolution phases of healing to regulate tissue repair. At the wound site, macrophage activation leads to increased phagocytosis of pathogens and damaged tissue. Historically, classically activated M1 macrophages participate in the inflammatory phase of healing, while alternatively activated M2 macrophages are involved in the resolution phase.[6] Studies now support the existence of multiple subtypes of the M2 macrophage that release various cytokines in different phases.[7] Co-existence of both the pro- and anti-inflammatory phenotypes in the wound bed has been reported, as evidenced by both the cytokines that are secreted and the receptors that are expressed.[8,9] It has also been shown that macrophages can rapidly and reversibly polarize between the pro- and anti-inflammatory phenotypes depending on the signals received from their environment.[7,9] As the wound matures and progresses from the inflammatory phase to the remodeling phase, macrophages switch from the pro- to anti-inflammatory state.[10]

Macrophages secrete bioactive molecules, including granulocyte-macrophage colony-stimulating factor (GM-CSF).[11] GM-CSF is known to promote the

Table I
GM-CSF relative indications, contraindications, and risks of local injection.

Relative Indications for Use	Wound refractory to standard of care ≤ 10 cm diameter ≤ 50 cm2 area WBC ≤ 11 K/µL Ulcer types: ischemic, venous, diabetic, pressure, traumatic, radiation
Contraindications	Acute or chronic osteomyelitis Isolated toe ischemia with full-thickness skin loss Skin flap (traumatic or recent graft) History of adverse reaction WBC >11 K/µL, Active sepsis (≥2 of the following): WBC >12 K/µL, Temp > 100.4°F, HR >90, RR >20
Risks/Side Effects	Fever Chills Tachycardia Hypotension Headache Pulmonary edema

proliferation and differentiation of bone marrow-derived monocyte precursors into macrophages.[12] In addition, GM-CSF has been shown to induce myofibroblast and endothelial cell differentiation (facilitating wound contraction), keratinocyte proliferation, mononuclear phagocyte activation, epithelial cell migration, cytokine production, and recruitment of inflammatory cells.[11,13] GM-CSF is also secreted by keratinocytes, which results in autocrine-mediated epidermal cell proliferation.[14]

Many modifiable and non-modifiable risk factors can contribute to the development of a chronic wound. Diabetes is known to impair wound healing via decreased inflammatory cell infiltration, reduced neovascularization, increased collagen deposition, and decreased levels of the growth factors required for healing.[14-16] Through the use of a diabetic mouse model, exogenous GM-CSF was shown to substantially enhance wound healing by promoting re-epithelialization, reducing collagen deposition, recruiting leukocytes, increasing angiogenesis, upregulating the proinflammatory mediators interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1), and increasing the infiltration of neutrophils and macrophages.[14,17] In diabetes, there is an imbalance in the level of pro- versus anti-inflammatory cytokines [18] and the M1 subtype predominates, resulting in a chronically inflamed wound.[19] Correction of this imbalance by macrophage stimulation has been shown to increase vascular endothelial growth factor (VEGF) receptor expression,[16] which is a key contributor to angiogenesis.[11,20] We hypothesize that the addition of exogenous GM-CSF to the wound bed produces an environment that promotes the polarization of wound macrophages into the anti-inflammatory phenotype.[7-10] GM-CSF injection in combination with regular debridement of devitalized tissue and non-migratory cells allows the wound to convert from a chronic to an acute inflammatory state, with a subsequent increase in necessary local growth factors, such as VEGF,[7,13,16] platelet-derived growth factor (PDGF),[3] fibroblast growth factor (FGF),[10,21] hepatocyte growth factor (HGF),[17] and transforming growth factor beta (TGFβ),[7,10,22], and continue through the stages of healing.

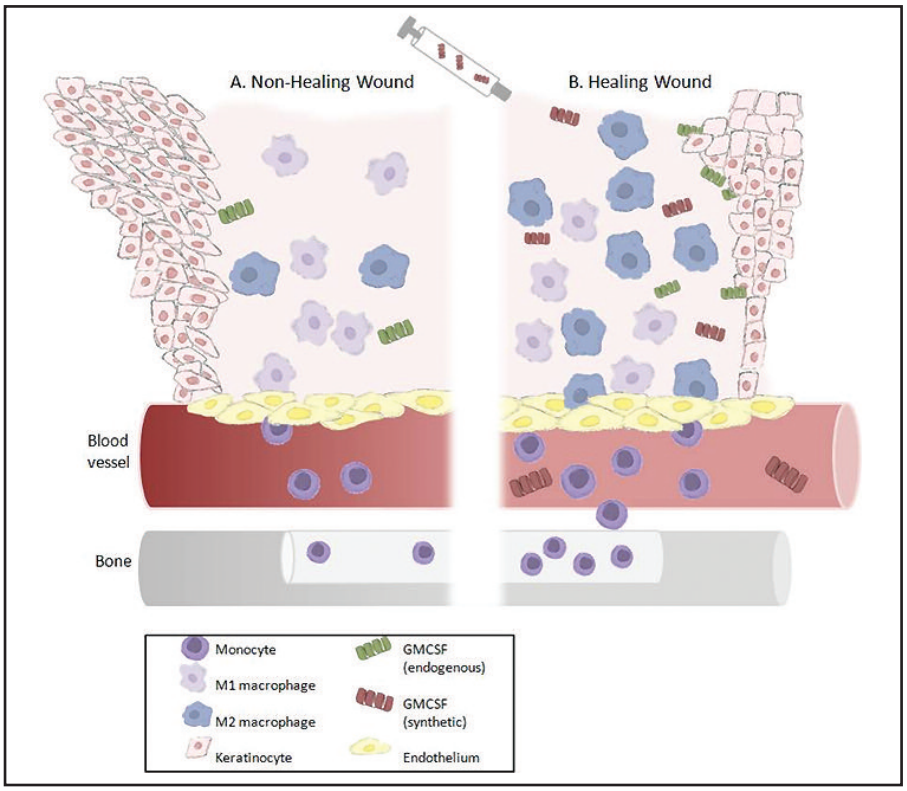

Figure 1A. The non-healing wound edge depicts heaped, non-migrating keratinocytes releasing very little endogenous GM-CSF and exhibiting both hyperkeratotic and parakeratotic features (abnormal increase in the number of layers and nucleated cells in the stratum corneum, respectively). The altered immune response to the chronic wound results in low amounts of monocytes being stimulated and released from the bone marrow into the circulation. The non-healing wound bed contains an unbalanced M1:M2 ratio, resulting in a pro-inflammatory environment and inactivated macrophages releasing little to no endogenous GM-CSF. B. After debridement of the wound and injection of synthetic GM-CSF, the healing wound edge exhibits proliferating and migrating keratinocytes releasing endogenous GM-CSF. The bone marrow is also stimulated to release monocytes that home to the wound bed and differentiate into macrophages. The wound bed environment then favors rapid polarization of the activated macrophages, resulting in an anti-inflammatory M1:M2 balance. In addition, GM-CSF stimulates endothelial cell differentiation, which facilitates wound contraction.

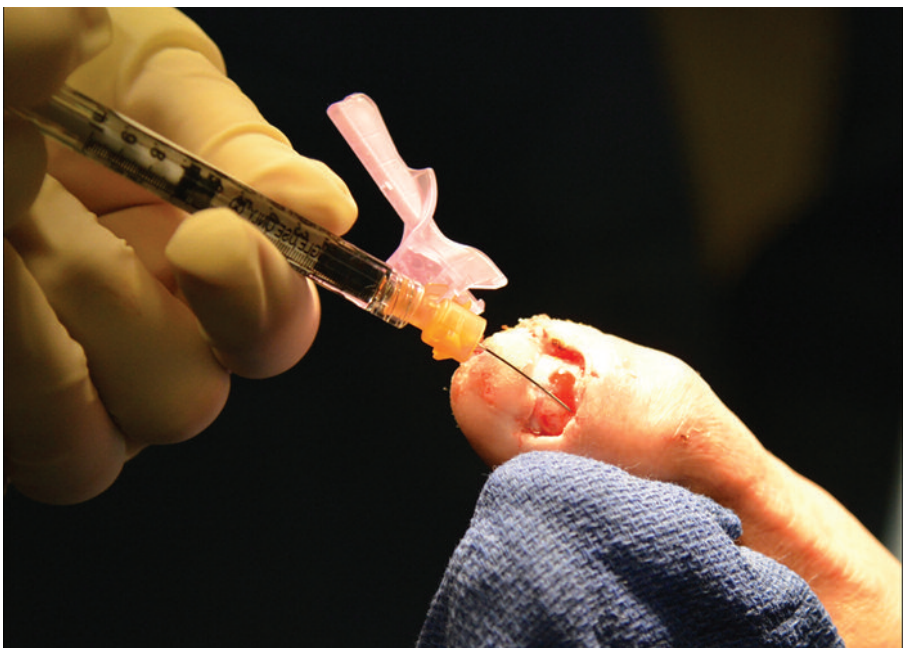

Figure 2. Injection of GM-CSF in a proximal nail bed wound that extended into the plantar aspect of the hallux following debridement.

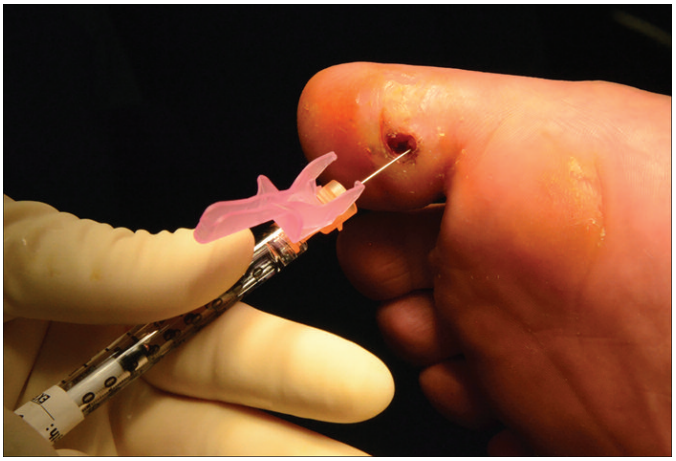

Figure 3. Injection of GM-CSF into a diabetic foot ulcer on the plantar aspect of the great toe.

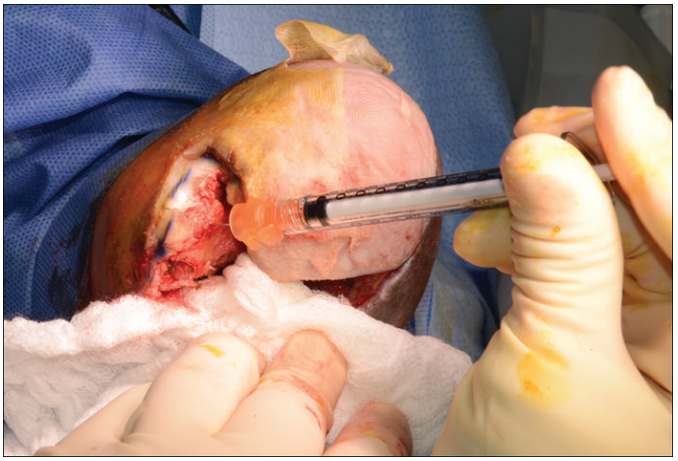

Figure 4. Injection of GM-CSF into a left forefoot amputation site wound.

GM-CSF HISTORY

GM-CSF is a pleiotropic cytokine that is increasingly being used in the treatment of chronic wounds. Synthetic, recombinant human GM-CSF (Leukine®) was approved by the FDA in 1991 for use after bone marrow transplantation, before and after peripheral blood stem cell transplants, after the commencement of chemotherapy in elderly patients with acute myelogenous leukemia, and to prevent death following engraftment delay or failure.[23] Based on early findings that GM-CSF increased circulating myeloid cells and altered the function of macrophages, further clinical studies were suggested to fully elucidate the clinical role of GM-CSF in patients.[24,25] A randomized, placebo-controlled trial using GM-CSF in chronic ulcers was published in 1997.[26] Twenty-five of the planned 40 patients were enrolled before the study was terminated early due to a high percentage of healing patients in the treatment group compared to the controls (69% vs. 11% at week eight). In that study, 16 patients received GM-CSF, 9 received saline placebo, and all of the ulcers had either a varicose (13 GM-CSF and 7 placebo) or ischemic (3 GM-CSF and 2 placebo) etiology. Subsequent studies have suggested that GM-CSF is promising for the improvement of wound healing.[11,13,27,28] A study published in 2006 examined punch biopsies of eight patients with chronic venous leg ulcers prior to and five days following peri-lesional, intra-dermal injection of GM-CSF.[13] Immunohistochemical staining and histopathology of the day-five samples revealed significantly increased VEGF transcription by macrophages and an increase in blood vessel density in the wound bed, respectively. A review of GM-CSF in the treatment of poorly healing wounds suggested that injection of GM-CSF was superior to topical application because injection allowed for dosing standardization, optimal delivery and penetration of GM-CSF at the site, and was associated with a faster healing rate.[11] GM-CSF was also shown to be efficacious specifically in the treatment of venous ulcers.[27] In that double-blind, randomized, placebo-controlled trial, 60 patients were assigned to receive

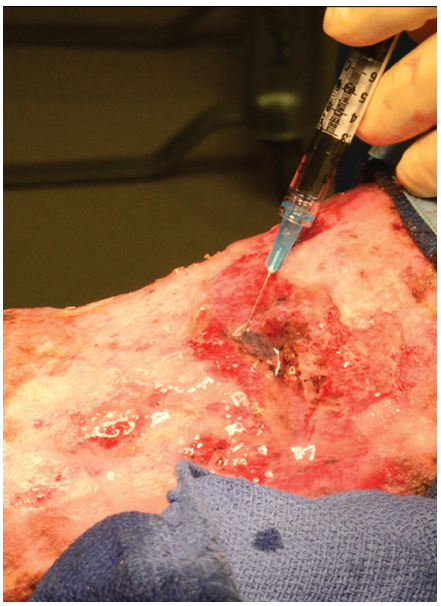

Figure 5. Injection of GM-CSF into a lower-extremity venous ulcer.

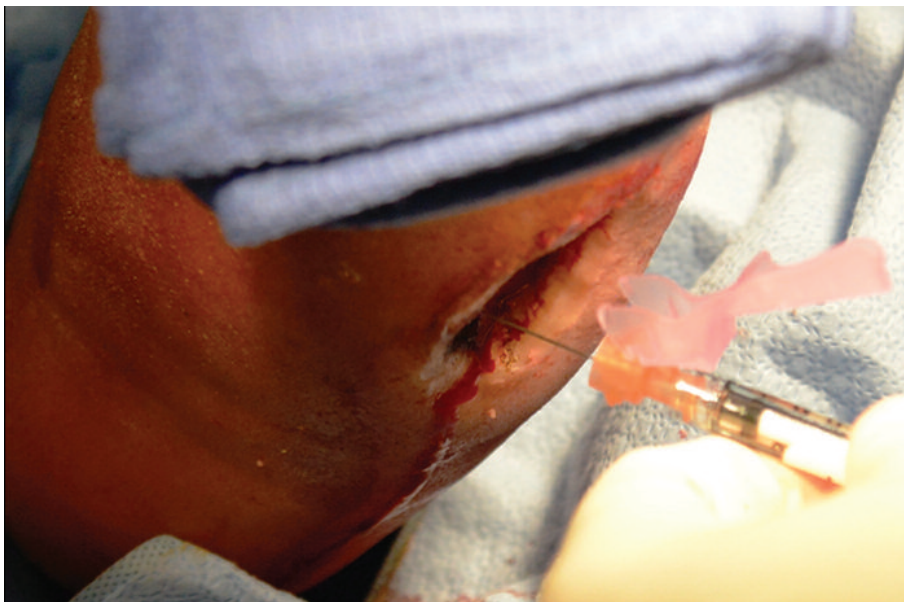

Figure 6. Injection of GM-CSF into a diabetic foot ulcer on the lateral plantar aspect of the left foot.

subcutaneous, peri-lesional injections of either 200 µg GM-CSF, 400 µg GM-CSF, or placebo once per week for four weeks or until the ulcer had healed. At 12-14 weeks, complete healing was observed in 19%, 57%, and 61% of the patients for the placebo, 200 µg GM-CSF, and 400 µg GM-CSF groups, respectively. The difference in healing was significant between the placebo and treatment groups, but not between the 200 µg and 400 µg treatment groups. Minimal adverse events, including pain, malaise, vagal reaction, and chills, were reported, and none of these were life-threatening. At a six-month follow-up, none of the healed patients who received GM-CSF showed ulcer recurrence.

PROPOSED USE OF GM-CSF

GM-CSF is routinely injected as part of the treatment regimen for patients seen in our practice with non-healing wounds: 500 µg of lyophilized GM-CSF is reconstituted in 1 mL of sterile H_20 and injected using a 25-gauge needle on a 1 mL syringe to reduce the amount of GM-CSF remaining in the needle. Dosing is based on prior work which showed that 250 µg/m^2/day for up to 21 consecutive days had no adverse effect on patient survival.[29,30] The GM-CSF is injected into the peri-wound subcutaneous tissue and into the base of the wound, which, depending on the wound depth, includes the periosteum. Injections are given during intra-operative debridement under intravenous sedation to ease or ameliorate possible side effects, such as chills and headache. Table 1 presents our proposed indications, contraindications, and risks/side effects of GM-CSF injection in patients with chronic wounds. Relative indications for use include wounds in elderly patients and patients with diabetes, both of whom have been shown to have poorly functioning cells.[31,32] In our experience, GM-CSF appears to work better on wounds < 10 cm in diameter or ≤ 50 cm^2 in area, and works very well for ischemic wounds. To date, among more than 400 patients with wounds who have received GM-CSF at our institution, there has been only one major adverse event of acute lung injury.[33] The following case presents a typical use of GM-CSF for a non-healing diabetic foot ulcer.

CASE REPORT

A 61-year-old male presented with a non-painful, left great plantar toe ulcer that had been present for almost a year. His past medical history was significant for atrial fibrillation, for which he was taking an anticoagulant, type II diabetes mellitus, and a cerebrovascular accident with residual weakness in the left leg. The patient received local wound care with topical antimicrobials, offloading, and systemic antibiotics as part of the standard-of-care therapy, while ruling out other causes of ulceration and optimizing the patient for surgical debridement. Ankle-brachial indices were within normal limits, indicating adequate blood flow to the left foot and allowing us to rule out ischemia. The patient was seen in the operating room for weekly debridement and GM-CSF injection at the ulcer site (Fig. 1). Pathology and microbiology results obtained from the first debridement revealed a fungal infection of the left great toenail, which was subsequently removed during the second debridement to eliminate the source of infection and improve healing. The wound was completely healed after five weeks of debridement and GM-CSF injection. Figures 2 through 6 illustrate the injection of GM-CSF into wounds of various etiologies following operative debridement by our team of four wound surgeons.

CONCLUSION

Wound healing requires a combination of complex intracellular and extracellular actions. The use of GM-CSF may assist this sequence in patients who have compromised healing due to disease processes, such as diabetes. Exogenous GM-CSF can promote healing progression in both an autocrine and paracrine fashion. Local inflammatory cells in the wound bed may be stimulated by GM-CSF, favoring a transition to a wound-healing environment. GM-CSF also works to increase the amount of local growth factors in the wound bed [11,13,17,21] and promote wound healing. In our anecdotal experience, over 400 patients were injected with GM-CSF. These patients were specifically chosen due to the refractory nature of their wounds. The retrospective nature of this study limits the conclusions that can be made; therefore, further randomized controlled trials will be needed to elucidate the efficacy of GM-CSF as an adjunct to standard-of-care therapy compared to standard-of-care alone, further usage indications, and optimal dosing and injection techniques. STI

AUTHORS' DISCLOSURES

The authors have no funding sources, commercial associations, or conflicts of interest to disclose.

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