The Effect of GM-CSF on the Inhibition of Contraction of Excisional Wounds Caused by Bacterial Contamination

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ABSTRACT. Bacterial contamination of open wounds significantly inhibits the wound contraction required in the healing process. Substances that enhance inflammatory capacity may aid in eliminating bacterial burden in the wound, thus improving the contraction process. This study was designed to determine the effect of GM-CSF (granulocyte/macrophage-colony stimulating factor) on wound contraction inhibition in an area contaminated with bacterial overgrowth. The topically applied GM-CSF reversed inhibition to wound contraction that normally occurs with bacterial contamination. The treated wounds showed no increase in breaking strength. The use of GM-CSF decreased the number of days required for wound healing despite active bacterial invasion and can be of value in the treatment of human contaminated wounds.

Key words : GM-CSF — wound contraction — bacterial contamination — breaking strength

Open wounds heal by a bimodal process of wound edge contraction and epithelial cell migration.[1,2] Contraction diminishes the size of a full-thickness open wound by centripetal movement of the whole thickness of the surrounding skin.

Wound healing by contraction is influenced by a number of variables. Infection is one such factor. Bacteria have been reported to secrete exogenous plasminogen activators and proteases capable of contributing to the breakdown of protein such as fibrin.[3] The exudates from bacterial infection contain proteolytic enzymes which have been shown to eliminate fibrinous elements and stop the contracting process. Bacteria are also known to block the migration of fibroblasts and the formation of new capillaries.[4]

A substance which could prevent the inhibition of contraction caused by bacteria has important clinical relevance. GM-CSF is potentially one such substance which has many characteristics important to wound healing.

Granulocyte/macrophage colony stimulating factor (GM-CSF) is a 22-KDa glycoprotein which stimulates the proliferation of multipotential progenitor cells and induces the proliferation, differentiation and functional activation of granulocytes and macrophages.[5,6] GM-CSF is expressed by hemopoietic cell types[5-8] and also in several nonhemopoietic cell types such as osteoblasts,

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smooth muscle, endothelial and epithelial cells,[5,6] as well as in murine feto-placental tissue.[9,10]   The biological activity of GM-CSF is mediated through the presence of a specific cell surface receptor which consists of two interacting subunits, the alpha and beta, and is encoded by two independent genes.[6,11–14]

Wound contraction results from a complex interaction between tissue tension, effector cells, the extracellular matrix, humoral factors, and locally derived growth factors.[15]   The complexity of this relationship renders *in vitro* culturing an incomplete way for studying wound dynamics.   Several animal
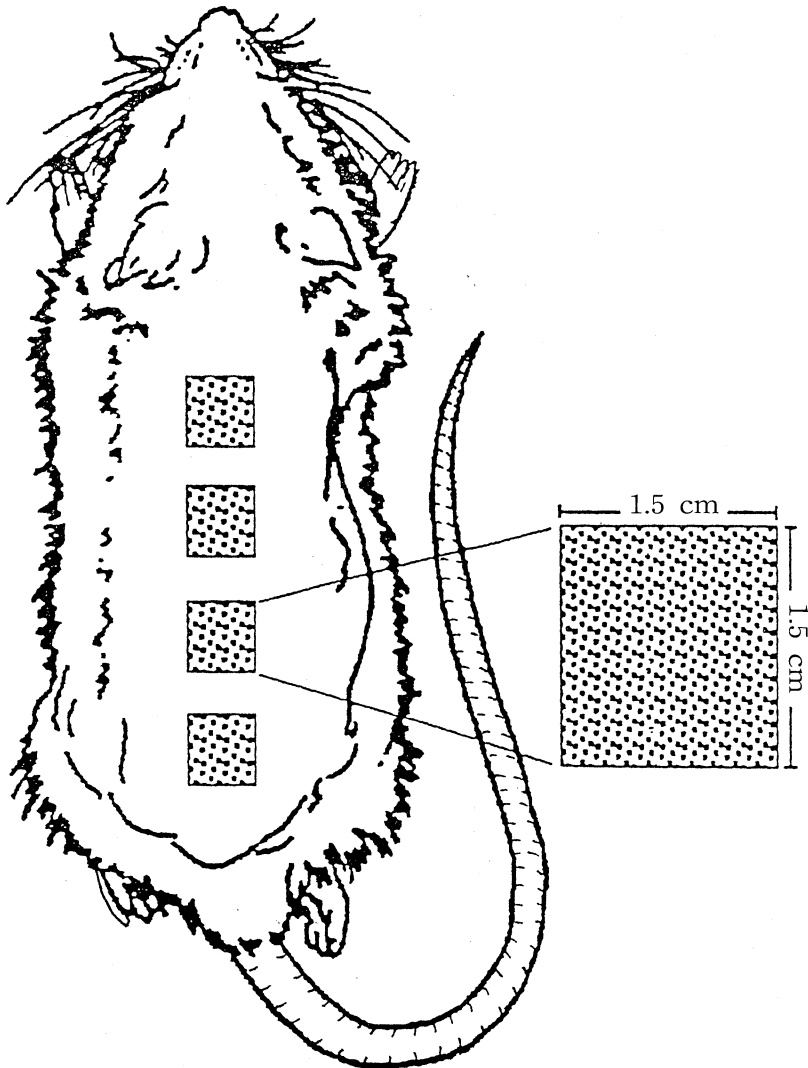

Fig 1.   Schematic diagram of model

models have been proposed for the *in vivo* study of wound contraction.[15,16]   A review of these models revealed a lack of standardization and an inability to compare studies meaningfully.[15]   Species differences, age, wound site, the presence of dressings, and the presence or absence of a panniculus carnosus are all variables that have been shown to be important. However, sex and time of day of wounding are not significant differences.[17]

The present study used the acutely contaminated wound model to evaluate the ability of GM-CSF to alter the inhibition of contraction by bacteria. The model allows high numbers of wounds (four per animal) to be generated. It also allows quantitative bacteriology to be performed weekly and by distributing the biopsies among the four wounds, any artifact due to wound biopsy can be managed.
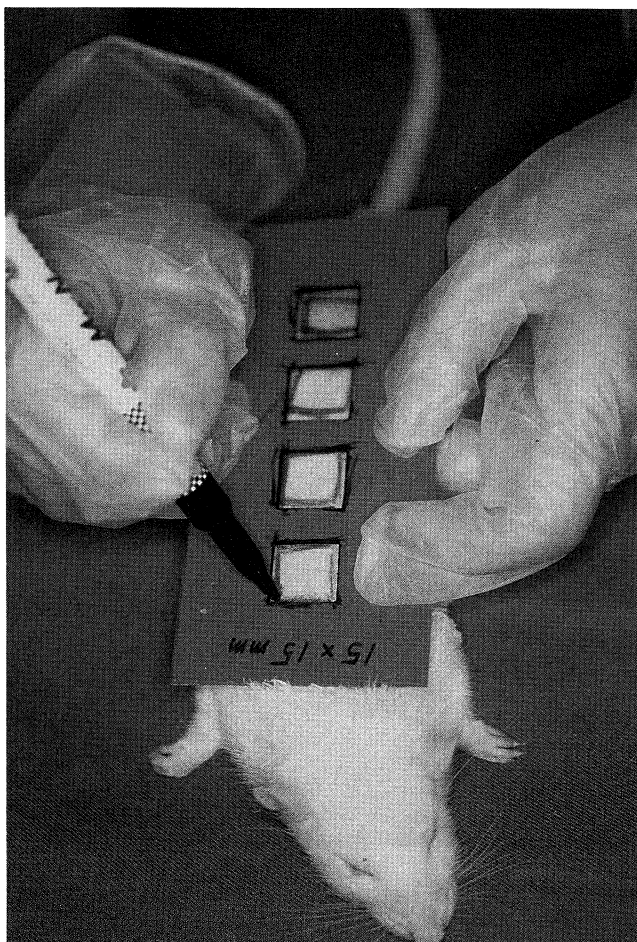

Fig 2.   Designed in the dorsal midline with the use of a template

MATERIALS AND METHODS

The acutely contaminated excisional model of Robson *et al*[18] was used. Fifty male Wister rats weighing approximately 300 g were anesthetized with an intraperitoneal injection of pentobarbital sodium (Nembutal) (30 mg/kg). The dorsum of each animal was shaved and depilated. Four wounds (1.5 × 1.5 cm) were made in the dorsal midline with the use of a template. The skin and panniculus carnosus were removed, and the wounds were then ready for inoculation and/or treatment (Fig 1-4).
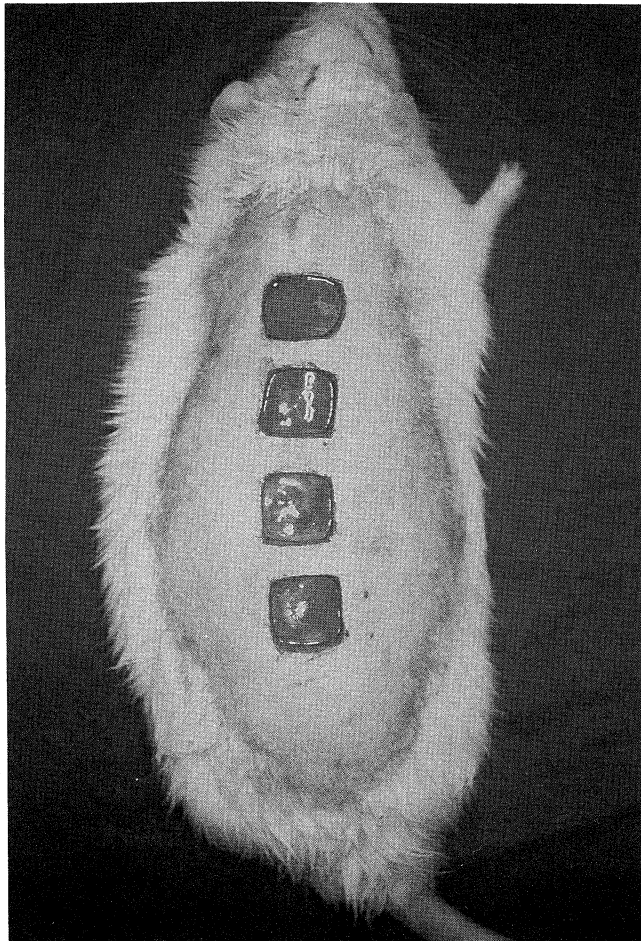

Fig 3.   Four wounds (1.5 × 1.5 cm²) excised through full-thickness of skin and underlying panniculus carnosus.
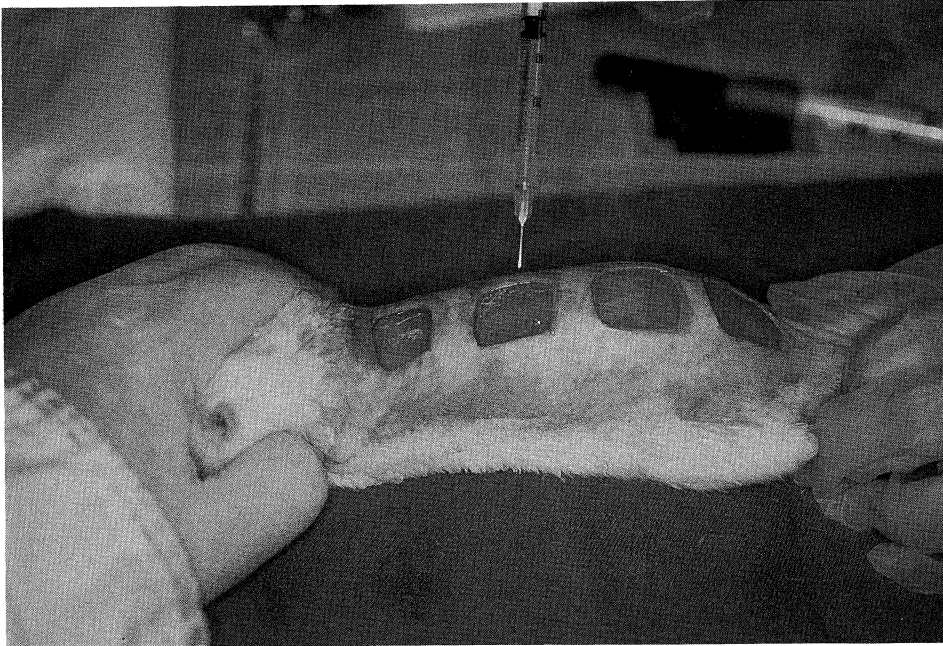
Fig 4.   Inoculation and/or treatment

The animals were divided into five groups as follows:
1. Control (no bacteria, no GM-CSF) n=10
2. Infected Control (inoculation with 10^5 E. coli ATCC #25922, no GM-CSF) n=10
3. GM-CSF 10 μg/wound (no bacteria) n=10
4. Infection+GM-CSF 1 μg/wound (inoculation with 10^5 E. coli) n=10
5. Infection+GM-CSF 10 μg/wound (inocuiation with 10^5 E. coli) n=10

Two groups served as infected and non-infected controls. Infected groups were inoculated with 10^5 Escherichia coli. ATCC strain #25922. The bacteria was applied to each wound once at the time of excision in Groups 2, 4 and 5. Mouse recombinant GM-CSF (mGM-CSF, Genzyme Corporation, Cambridge, MA, USA) was administered to control Groups 1 and 2.   No dressings were applied to the wounds since dressings can act as splints in contracting wounds and allow accumulation of exudate which may contain proteolytic enzymes, prostaglandins, or endogenous growth factors.[15,19]

Serial photographs and wound area measurement of acetate sheets were performed on Days 0. 3, 5, 7, 14 and 21 using computerized digital planimetry (Fig 5).   Quantitative and qualitive bacterial analyses were performed on a subset of each group.

Breaking strength
Animals were sacrificed by pentobarbital sodium overdose when the wound had healed or when contraction had ceased.   Representative areas of
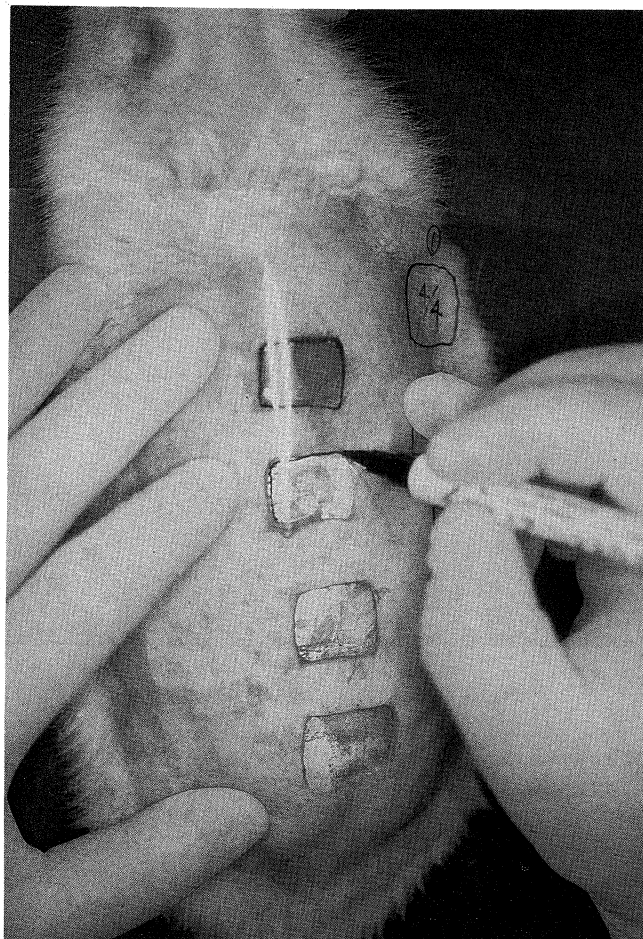
Fig 5.   Wounds were traced on acetate sheets.

healed scar were excised as 1 cm strips with the panniculus carnosus included.
These were disrupted with a tensiometer (Instron 4201 : Instron Corp, Canton,
Mass USA), and the values for breaking strength were determined. For this
experiment, only the dermal breaking strength was compared.   Breaking
strength was defined for this experiment as the force required to rupture the
scar reported in kilograms.

Animal care

Animals were individually caged, and infected animals were housed in a
separate facility.   All animals were given food and water ad libitum.   All
experiments were conducted in accordance with the Animal Care and Use
Committee guidelines, University of Texas Medical Branch, Galveston, Texas,
USA.

Statistical analysis

Serial area measurements were plotted against time. Comparison between groups was performed with Duncans' test. Breaking strengths were compared by use of the paired t-test.
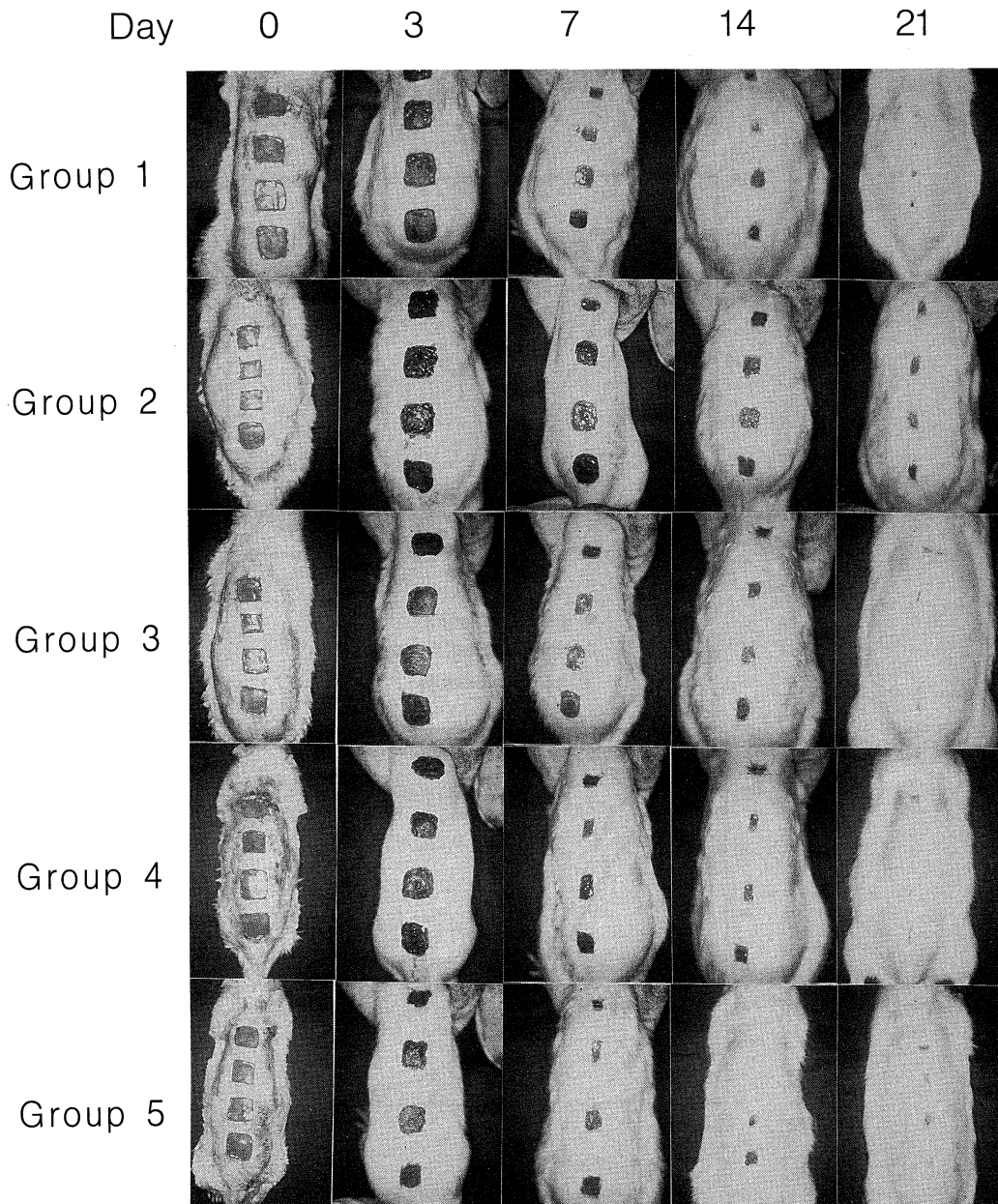

Fig 6. Serial photographs of each group

RESULTS

Fig 6 shows the serial photographs of each group. Infection significantly delayed wound contraction as compared with non-infected controls (Fig 7). No significant differences were noted between Groups 1 and 3, which all healed within the same time frame (Fig 8). GM-CSF-treated wounds (Groups 4 and 5) overcame this bacterial inhibition and healed significantly faster than the non-treated infected control wounds (Group 2) ($p < 0.05$ Duncans' test) (Fig 9). Group 5 (GM-CSF 10 ug/wound) healed faster than Group 4 (GM-CSF 1 μg/ wound) until Day 7, but the difference did not reach statistical significance (Fig 9).
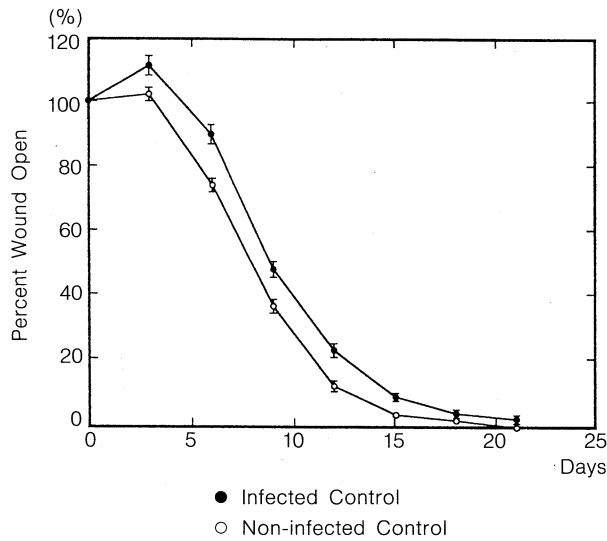

● Infected Control
○ Non-infected Control

Fig 7.   Controls

● Infected Control
○ Non-infected Control
□ Non-infected Model
    treated mGM-CSF (10ug/wound)

Fig 8.   Non-infected Model

(%)

Percent Wound Open

Days

● Infected Control
○ Non-infected Control

Infected Models

▽ treated mGM-CSF (1 ug/wound)
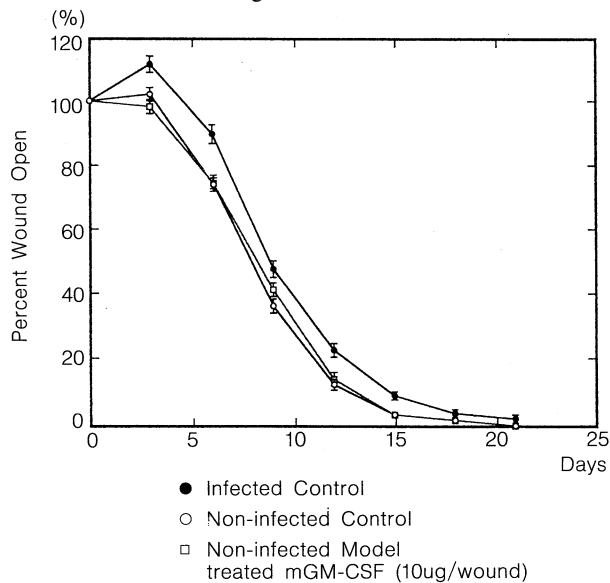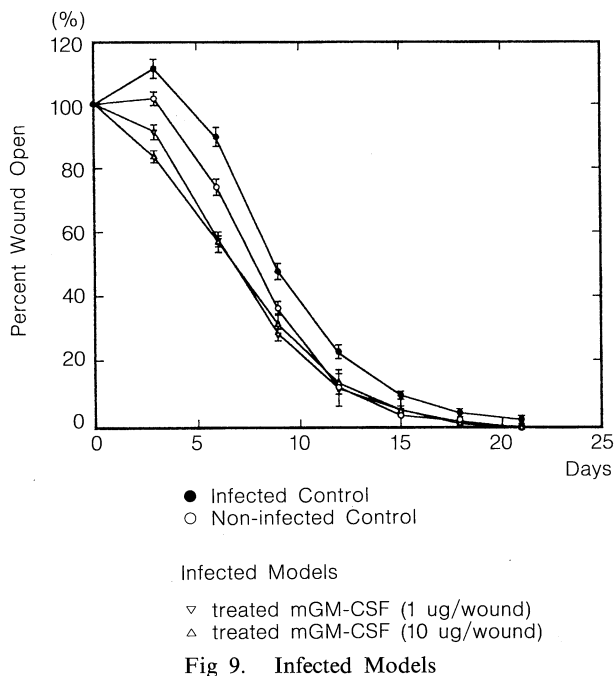△ treated mGM-CSF (10 ug/wound)

Fig 9.   Infected Models

Bacteriology counts in biopsy specimens remained at 10^4- 10^5 CFU/gm tissue in animals from Groups 2, 4 and 5 throughout the experiments.

Table 1 shows the mean breaking strength of all groups. The addition of GM-CSF to clean and contaminated wounds decreased the breaking strength of the healed wounds as compared with controls. Histologically, the differences between groups were not marked. All the groups appeared to be at a similar stage of healing.

TABLE 1.   The effect of GM-CSF on breaking strength of full thickness excisional wound healing by contraction

Group	Treatment	Means (kg)	Standard error
1	Control	1.108	0.04
2	Infection	0.930	0.045
3	GM-CSF(10 μg/w)	0.895*	0.031
4	Inf+GM-CSF(1 μg/w)	0.960	0.037
5	Inf+GM-CSF(10 μg/w)	0.925	0.048

μg/w=μg/wound, Inf=Infection
*$p<0.05$ compared to control

DISCUSSION AND CONCLUSIONS

Despite the fact that in wound healing research much of the current emphasis has been focused on unlocking the mechanisms and roles that various growth factors play in the normal wound healing process,[20-25] the sequence of events is still unclear. Although from a clinical perspective, healthy patients with intact healing abilities are important models. it is the debilitated patient with impaired wound healing abilities and the acutely injured patient with a wound environment out of bacterial balance[26] that present the major challenges. It is these patients who can benefit most from therapeutic assistance such as a growth factor.

Wound healing by contraction is influenced by a number of different factors. Infection, or the numbers of organisms present in the wound, is one critical factor.[27] Bacteria are known to block the migration of fibroblasts and the formation of new capillaries.[19] Wounds that contain bacteria $> 10^5$ CFU/gram tissue fail to contract or heal. Additionally, these bacteria secrete exogenous plasminogen activators and proteases capable of breaking down such proteins as fibrin.[3] Such exudates from these bacterial infections contain proteolytic enzymes, which have the ability to eliminate fibrinous elements and to stop the contracting process. Consequently, the number of bacteria present may not be the only factor influencing the healing process.

The fact that GM-CSF did not significantly effect the wound contraction rate in non-infected animals is not surprising. The inability of many putative healing factors to affect normal wound contraction has led to the use of "retarded" models such as steroids, radiotherapy, or malnutrition.[15] This study uses infection to retard contraction rather than a chemically impaired model.

The study confirms that bacteria can significantly inhibit wound contraction and demonstrates that the inhibition can be overcome by application of GM-CSF. Topical application of GM-CSF directly to the wound site presumably augments the endogenous supply of GM-CSF, causing the recruitment and division of cells required for granulation tissue and subsequent contraction of the wound.

Quantitative biopsies of the wounds treated with GM-CSF failed to show a significant decrease in bacterial counts/gram of tissue despite contraction of the wound.

It does not appear that the faster wound closure in the GM-CSF groups was due to increased collagen activity since breaking strength was not increased. The breaking strength data in these experiments were generated at the time of closure and are difficult to correlate with data generated at 7 or 14 days after primary approximation of a linear incision.[17]

Although the mechanism needs to be elucidated, it is suggested that GM-CSF can be beneficial in treating contaminated wounds.

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