The Effect of Topically Applied Recombinant Human Growth Hormone on Wound Healing in Pigs

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Abstract: The beneficial wound healing effect of the systemic growth hormone (GH) mediated by insulin-like growth factor 1 (IGF-1) has been widely reported. Recent studies have suggested that GH facilitates wound healing not by circulating IGF-1, but by local IGF-1 produced in the wound itself. The aim of this study was to define whether the locally administered GH could accelerate the wound-healing rate. Full-thickness skin defects (diameter 4 cm) were made in the back of micropigs, and GH (2.5 IU/L) was applied every other day for 3 weeks (11 times total). Control wounds were given the vehicle only. The wound sizes were measured weekly by planimetry and biopsies were taken. The wound sizes were significantly reduced in the GH-treated groups as compared with the control group ($P < 0.05$) each week. Histological and immunohistochemical examination revealed that the production of IGF-1 and collagen 1 in the experimental group increased more than in the control group. The present results suggest that local treatment with GH effectively accelerates wound healing.

Recombinant growth hormone (GH) has been used as an anabolic treatment in burn and postoperative patients. More recently, growth hormone has been increasingly used in experiments and has shown promise in the acceleration of wound healing in several studies. Growth hormone stimulates granulation tissue formation, increases collagen deposition, and facilitates epithelialization. It can also accelerate donor site healing in patients with burns and bone healing.

Many studies have shown that insulin-like growth factor-1 (IGF-1) mediates the action of GH in wound healing. However, it has not yet been determined whether IGF-1 affects the wound through endocrine action or through paracrine and autocrine action. In the past, it was discovered that GH could have a positive effect on wound healing by stimulating the production of IGF-1 in the liver to increase circulating IGF-1 concentration. More recently, many evidences have been reported that circulating IGF-1 does not affect the wound, but that IGF-1 produced locally by fibroblasts, macrophages, and endothelial cells contribute to wound healing. And top-
ically applied GH increases the concentration of IGF-1 mRNA in the granulation tissue in vivo.12

If the effect of IGF-1 by GH on wound healing is produced locally within the wound, then topical administration of GH can facilitate wound healing. The present study tested the contribution of topically applied GH on wound healing.

Materials and Methods

Recombinant human growth hormone and other ingredients for cream formulation were obtained from LG Household & Health Care Ltd (Seoul, Korea). White petrolatum, cod-liver oil, glycerin, bisabolol, and propylparaben were used to formulate a GH cream base. To yield 2.5 IU/L of GH cream, GH powder was dissolved in warm water and then mixed with the other ingredients.

GH concentration. The concentration of GH was determined through a preliminary study. Growth hormone stimulates fibroblast proliferation, which is an integral step in the wound healing process.13 When GH was applied, the fibroblast proliferation increased significantly (P < 0.05; paired t-test). Cell proliferation was measured by tetrazolium-based colorimetric assay (MTT cell growth assay kit, Millipore, Temecula, Calif). The proliferation increase differed according to the concentration of GH (Figure 1). The concentration of GH applied to the skin defect in this study was 2.5 IU/L, which was the best amount for stimulating fibroblast proliferation.

Animal protocol. Five male micro-pigs weighing approximately 30 kg were used. Porcine skin is similar in structure to that of humans and does not have a panniculus carnosus.14 The Animal Ethics Committee of the Seoul National University Institute of Animal Sciences approved these experiments.

After an acclimatization period of 7 days, each pig was induced after an intramuscular (IM) injection of 10-mg/kg ketamine hydrochloride (Ketara, Yuhan Yanghang, Seoul, Korea) and 4-mg/kg xylazine hydrochloride (Rompun, Bayer Korea, Seoul, Korea). The skin in the back region was aseptically prepared after local infiltration with 5 mL of 1% lidocaine to each excision area. Six full-thickness, round skin defects 4 cm in diameter were created on the back of each pig with a No. 10 scalpel blade (Figure 2). The skin sample taken from each pig was used as a reference.

The three left-sided wounds were dressed with the GH-containing cream and foam dressing (Medifoam, Ildong Pharmaceutical Co, Seoul, Korea). The three right-sided wounds on each pig without GH administration

![Figure 1](image1.png)

**Figure 1.** The amount of fibroblast proliferation was represented by absorbance in the MTT assay. The proliferation increased in groups with concentrations of 0.5 and 2.5 IU/L after 3 and 5 days; proliferation decreased at levels above this concentration.

*P < 0.05 compared with control
†P < 0.05 compared with control and 0.5

![Figure 2](image2.png)

**Figure 2.** Six full-thickness skin defects were made on the back of the micropigs.
were used as controls and were dressed with a non-GH cream and the foam dressing. An elastic bandage was applied over the adhesive bandage (Hypafix, Smith & Nephew, London, UK) to prevent the pigs from removing the dressing. The dressings were changed every 2 days under brief intravenous (IV) ketamine narcosis. Wounds were cleansed with saline gauze and the GH- or non-GH cream was reapplied a total of 11 times. After the 3-week study period, the pigs were killed with IV potassium chloride.

**Healing time.** The wound sizes were measured by planimetry. Each week during the dressing change, wound outlines were drawn on clear film and measured using the computer program, AutoCAD 2005 (Autodesk, Inc, San Rafael, Calif). Wound sizes were then compared to the original wound sizes.

**Histological and Immunohistochemical Examination**

Histological and immunohistochemical studies were conducted to confirm that local GH increased IGF-1 protein expression and granulation tissue formation in the wound. Each week, a 3-mm diameter skin punch biopsy was made just beside each wound margin, since the wound margin was newly healed tissue. The biopsies were examined histologically (hematoxylin and eosin stain) and immunohistochemically. Biopsies were fixed in 10% buffered formalin overnight. Paraffin-embedded samples were cut (4-µm thick) and placed in increasing alcohol concentrations and finally in phosphate-buffered saline (PBS). The samples were then washed with PBS with endogenous proteases blocked with 0.3% H$_2$O$_2$ for 15 minutes. Protease K solution (EnVision, Dako, Glostrup, Denmark) was applied at 37˚ for 10 minutes. The sections were incubated with primary antibody (rabbit polyclonal anti-human IGF-1 antibody 1:25 [Santa Cruz Biotechnology, Santa Cruz, Calif]) for 2 hours at room temperature followed by biotinylated anti-rabbit IgG 1:200 at 1:1000 dilution for 2 hours at room temperature. Diaminobenzidine/H$_2$O$_2$ was used to detect bound antibodies. The same procedures were done for collagens 1 and 3. The primary antibodies were mouse polyclonal anti-collagen 1 (1:50, Abcam, Cambridge, UK) and rabbit polyclonal anti-collagen 3 (1:50).

Data were analyzed semiquantitatively. Two independent, blinded observers counted the staining intensity of each biopsy at three different sites. A score of 1 to 5 was assigned to each parameter (1 = weakly stained, 5 = strongly stained).

**Figure 3.** The percentage of wound size as compared to the original wound. Data presented as mean ± SEM; n = 15 for each group.

*P < 0.05 compared with control group

**Figure 4.** Staining intensities of immunostaining for IGF-1, collagen 1, and 3 were compared in two groups by two blinded observers. Staining for IGF-1 and collagen 1 was significantly stronger in the GH-treated group than control. There was no significant difference in staining intensity in collagen 3. Data presented as mean ± SEM; n = 30 for each group.

*P < 0.05 compared with control group
Figure 5. Micrographs for control (left) versus GH-treated (right) wounds showing hematoxylin and eosin (H&E) staining and immunostaining for IGF-1 and collagen 1 and 3. The biopsy was taken at the healed wound margin after 3 weeks (magnification x200).
**Statistical evaluation.** Statistical assessment of the changes in wound size and staining intensity in both groups was performed using the Wilcoxon test; \( P < 0.05 \) was considered significant.

**Results**

**Healing time.** A significant reduction in the wound sizes of the GH-treated group was observed as compared with the control group (\( P < 0.05 \)). The wound size of the experimental group decreased significantly more than the control group each week. In the later weeks, the ratio of wound area reduction between the two groups increased. The healing rate in the GH-treated group was faster than that of the control group (Figure 3).

**Histological and immunohistochemical examination.** To compare IGF-1 levels, the initial (week 1) biopsies of both groups were reviewed, because the healing might be most actively processed. To examine collagen production, the third week biopsies were compared because the healing process was almost finished and the collagen deposition would be most abundant.

Immunostaining for IGF-1 revealed that in GH-treated group, production of IGF-1 increased significantly more than that of the control group, meaning that locally administered GH promoted IGF-1 production within the wound.

Immunostaining for collagen 1 revealed that collagen production in the GH-treated group was greater than that of the control group. This means that locally administered GH can produce IGF-1 in the wound area, and IGF-1 can stimulate granulation tissue growth. But immunohistochemical analysis in collagen 3 showed no significant difference in staining intensity (Figures 4, 5).

**Discussion**

The foremost role of IGF-1 is its insulin-like metabolic effects; it can stimulate the growth of target tissues such as the liver, adipose tissue, muscle, cartilage, and fibroblasts.\(^8,9\) IGF-1 can enhance protein production, cell proliferation, and migration, which is essential in the wound-healing process.\(^15,16\) IGF-1 expression is increased in subcutaneous\(^7\) and incisional\(^8\) wounds, and in post-burn injuries.\(^9\) Some studies have shown that the administration of exogenous IGF-1 enhanced protein synthesis in severely burned experimental animals.\(^20\) However, recent data have demonstrated that administered IGF-1 did not improve wound healing, and only the IGF-1 produced locally by fibroblasts and macrophages contributed to the regulation of wound healing.\(^10,11\)

To enhance the wound healing process, especially in chronic wounds, many growth factors were tested and are now clinically applied. If the systemic IGF-1 was ineffective in wound healing, topical administration of IGF-1 can be considered as other growth factors such as EGF, TGF-β, etc. Unfortunately, topical administration of growth factors has some shortcomings. The cost to produce growth factors is significantly high as it is difficult to keep and transport; hence, increasing IGF-1 production locally by GH administration is superior to administration of IGF-1 itself.

It has been reported that systemic application of GH has a positive effect on wound healing. Systemic GH can increase the collagen production and mechanical strength of wounds.\(^21\) Ghofrani et al\(^*\) reported that systemic GH could accelerate the split-thickness skin defect in pigs. However, systemic GH has some side effects such as hypoglycemia, changes in mental status, edema, fatigue, and headache. Therefore, if topical GH application has a positive effect on wound healing, then topical administration of GH is the superior delivery method over systemic application regarding wound-healing efficacy.

**Conclusion**

The results of this study suggest that topical application of GH can be used to facilitate wound healing.

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**References**


