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Topical Administration of G-CSF Ameliorates Refractory Cutaneous Ulcers: A Clinical Case Series

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Abstract

Objective: Granulocyte colony-stimulation factor (G-CSF) is a chemokine that stimulates granulocyte proliferation and maturation and mobilizes bone marrow-derived stem cells into the bloodstream. G-CSF treatment has been shown to enhance tissue repair in various conditions characterized by chronic wounds; however, the mechanisms by which this cytokine promotes chronic wound healing remain unclear. The effect of recombinant G-CSF on wound healing was examined in six patients with intractable chronic cutaneous ulcers.

Methods

G-CSF was topically applied at 6 μg/cm2 over the ulcers daily for 14 days. The wound conditions were assessed using DESIGN-R, a comprehensive scoring system established by the Japanese Society of Pressure Ulcers that monitors ulcers' severity and healing states.

Results

The mean plasma concentration of G-CSF increased from 34.5 ± 14.1 μg/ml on day 0 to 70.8 ± 61.6 μg/ml on day 7; this increase was positively correlated with that of the WBC count. Total cutaneous ulcers graded with DESIGN-R scores significantly improved on day 7 ($p < 0.05$).

Conclusions

Topical administration of G-CSF induces amelioration of refractory cutaneous ulcers, probably via flare-up of inflammation without any damaging side effects.

Keywords: Topical administration, granulocyte colony-stimulation factor, refractory cutaneous ulcers, wound healing.

Introduction

Skin wound healing is a multi-stage process that involves the reestablishment of haemostasis, inflammation, cell proliferation, and tissue remodelling [1]. Inflammation is a crucial stage in this process. It is an essential initial reaction against injury and infection and is necessary for the removal of damaged tissues and pathogens. Inflammation is also required for the preliminary preparation for granulation, neovascularisation, and regeneration [2]. Monocytes, macrophages, and neutrophils recruited to the site of injury play a key role in not only phagocytosis but also remodelling in wound healing. Non-healed chronic wounds frequently stagnate the sequential healing cascade because of different exacerbating factors pre-existing in the patients [3]. Some of these factors include vascular insufficiency, diabetes, malnutrition, aging/senescence, and local factors including excessive compression, infection, and oedema. Such wounds are often stuck at the chronic inflammation stage and are precluded from entering the next stage—proliferation.

Tissue regeneration requires the proliferation of tissue-specific stem cells residing in not only individual tissues but also afield organs. Being rich in different types of stem cells, the bone marrow is also a potential source of mesenchymal stem/progenitor cells for local tissue repair. Some of the cells involved include endothelial progenitor cells (EPC) [4] and fibrocytes [5]. During inflammation, the granulocyte colony-stimulating factor (G-CSF) as an inducible robust chemokine that helps in mobilizing bone marrow-resided granulocytes and stem/progenitor cells into the bloodstream [6]. After an injury, G-CSF can be produced by damaged tissues to act as an initiating factor for the wound healing cascade [7]. In clinical settings, G-CSF has been successfully utilized to enhance wound healing in

neutropenic patients [8, 9], patients with dystrophic epidermolysis bullosa [10] and diabetic foot disorder [11]. However, the mechanisms of action through which G-CSF accelerates refractory wound healing are still unclear.

Here, study authors report the therapeutic and physiologic effects of topical G-CSF treatment in a preliminary clinical trial involving six patients with intractable chronic cutaneous ulcers.

Materials and Methods

Study design

Following approval by the Ethics Committee for Clinical Study (ECCS) at Fukuoka University Hospital (IRB#06-44), Fukuoka, Japan, G-CSF-chronic wound intervention trials (TRFU-CSF-01) were performed. Written informed consent was obtained from all participating patients or guardians in accordance with the Declaration of Helsinki.

The Phase 1 clinical trials aimed to determine the safety and efficacy of Filgrastim, or GRAN®, a recombinant human G-CSF (rhG-CSF) provided by Kyowa Hakko Kirin Co., Ltd., Tokyo, Japan. The drug was administered to patients with cutaneous ulcers in refractory stages. Assessments were performed by clinical research physicians from the Department of Plastic and Reconstructive Surgery at Fukuoka University Hospital. The DESIGN-R scoring system, established by the Japanese Society of Pressure, was utilized for the comprehensive evaluation of ulcer stages and its healing process. The categories in DESIGN-R are as follows: ulcers: depth (0-5 points); exudate (0-6 points); size (0-15 points), inflammation/infection (0-9 points), granulation (0-6 points), necrotic tissue (0-6 points), and pocket (0-24 points) [12]. In all categories except “Depth”, the higher the points mean the more serious the individual’s ulcer.

Patients and treatments

Six patients were admitted to the study using the following criteria: 20-75 years old with an ulcer that never improved two weeks before the study; without wound infection; wound less than 12 cm in diameter at its longest end; not invasive into muscles, tendons, or bones, but remained subcutaneously.

Filgrastim at a dose of 6 µg/cm2 ulcer area was topically and administered daily for 14 days. The following agents were not allowed: antibiotics; reagents for granulation tissue development; basic-fibroblast growth factor (bFGF); heparin-like compounds; corticosteroids for topical use; and wound healing systemic administration agents, including prostaglandin-drug formulations, anti-platelet agents, corticosteroids at a dose of more than 20 mg prednisolone equivalent/day, and anti-thrombin agents. The study was ruled to be discontinued by the ECCS if patients developed bacterial infections, an ulcer reaching muscle, tendon, or bone, aggravated colour change, or the appearance of serious adverse effects.

Patient	Sex	Age	Ulcer lesion	Pre-treatments	Diseases	Comorbidities	DESIGN-R (Day 0)
#1	Male	63	Sacral	Debridement NPWT	CI	Type 2 DM Bronchial asthma	12
#2	Male	41	Dorsal	Povidone-iodine	SCI	None	15
#3	Male	74	Sacral	Iodine-coat Ointment	CI SCI	Type 2 DM	14
#4	Female	76	Sacral	Povidone-iodine	SCI	Autoimmune hepatitis	10
#5	Female	72	Hip (right)	Debridement Proteolytic-ointment	CH	None	14
#6	Male	54	Ischial	Povidone-iodine	SCI	Ossification of posterior longitudinal ligament	15

Table 1: Patient data.

CH: cerebral haemorrhage, CI: cerebral infarction, DM: diabetes mellitus, NPWT: Negative pressure wound therapy, SCI: spinal cord injury.

Patient profile and ulcer assessment

The patients’ profiles are listed in Table 1. All patients received the same standard wound care, and debridement was performed on patients #1 and #5 before Filgrastim administration. None of the patients had wound infections. The DESIGN-R score evaluation was done just before the study and on days 7 and 14 of the study. The total score, excluding the depth score, were calculated (maximum 66 points). Lesion severity is categorized into three levels – 9 or lower: mild; 10-18: moderate; 19 or higher: severe. The initial total DESIGN-R scores for the patients were 12, 15, 14, 10, 14, and 15 points, indicating that they were all at the moderate level (Table 1).

Laboratory analysis

Laboratory analysis was carried out just before the study (day 0) and on days 7 and 14. G-CSF plasma levels were measured using a sandwich ELISA with a human G-CSF antibody (R&D Systems, Inc., MN, USA). Haematological examinations were performed to determine the number of red blood cells (RBC), white blood cells (WBC), platelets, neutrophils, and CD34+ cells in the blood, and the levels of haemoglobin, and haematocrit. Blood levels of AST, ALT, LDH, Al-p, γ-GTP, CRP, total protein, total

cholesterol, urea nitrogen, blood glucose, creatinine, and uric acid were also measured. Urinalysis was performed to determine protein, sugar, and urobilinogen levels.

Statistical analysis

Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using ANOVA and regression analysis. A p-value of less than 0.05 was considered to be statistically significant.

Results

Changes in plasma G-CSF level

Table 2 shows the summarized laboratory data. The initial G-CSF plasma level in the patients ranged from 13.8 to 50.5 μg/ml (34.5 ± 14.1 μg /ml). With daily administration of Filgrastim, plasma G-CSF level on day 7 was 70.8 ± 61.6 μg /mL, and 36.1 ± 17.3 μg/mL on day 14. Plasma G-CSF level increased in all patients on day 7, except for patient #5, but mostly returned to the initial level on day 14.

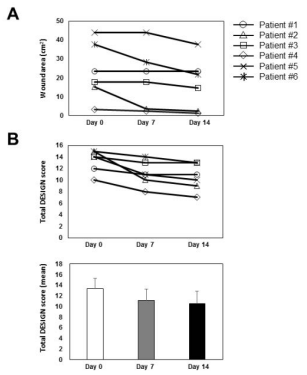

Figure 1: Wound healing process during the study.

Changes in wounds during the 14 days of G-CSF administration in representative cases (patient #1 and #2) are shown. Both cases healed progressively.

Patient	Analysis	G-CSF	CD34+ cells	WBC	Haemoglobin	RBC	Platelet	Total protein	CRP
		(ng/mL)	(/μL)	(/μL)	(g/dL)	(×104/μL)	(×104/μL)	(g/dL)	(mg/dL)
#1	Day 0	29.7	7	6900	12.2	425	34.2	6.4	1.11
	Day 7	45.8	9	10500	13.0	439	34.9	6.8	2.89
	Day 14	31.0	7	9300	13.1	454	43.1	7.2	3.89
#2	Day 0	13.8	3	5500	10.8	443	33.9	7.6	0.44
	Day 7	18.6	3	8000	11.2	469	40.3	7.3	0.05
	Day 14	17.7	3	5700	10.9	459	36.3	7.1	0.12
#3	Day 0	25.8	3	6800	10.4	334	18.5	7.1	0.21
	Day 7	71.0	3	6800	10.4	332	16.4	7.3	0.26
	Day 14	24.8	7	7400	10.7	342	19.1	7.3	0.19
#4	Day 0	38.8	5	5900	11.6	350	14.3	6.3	0.41
	Day 7	60.2	9	7000	12.3	376	14.3	6.4	0.37
	Day 14	53.9	4	7700	11.8	358	14.1	6.2	0.30
#5	Day 0	50.5	11	9200	12.7	546	34.4	7.0	0.53
	Day 7	38.0	6	6900	13.2	566	28.2	7.1	0.23
	Day 14	28.2	5	8700	13.4	566	24.2	7.3	0.12
#6	Day 0	48.8	2	3700	12.0	392	20.4	7.4	4.66
	Day 7	191.0	4	10200	13.0	416	26.4	8.1	1.75
	Day 14	61.0	3	3100	13.0	425	17.8	7.8	3.11

Table 2: Haematological data.

WBC count

WBC count on day 7 increased up to 276% in four cases (patients #1, #2, #4, and #6). In two cases (patients #2 and #6), WBC count returned to around the initial level on day 14 (Table 2 and Figure 1A).

Regression analysis indicates a significant correlation between the increase in plasma G-CSF level and the percentage increase in WBC level from day 0 to day 7 ($p < 0.05$)(Figure 1B). No significant changes in CD34+ cell count and other haematological parameters were observed during the study.

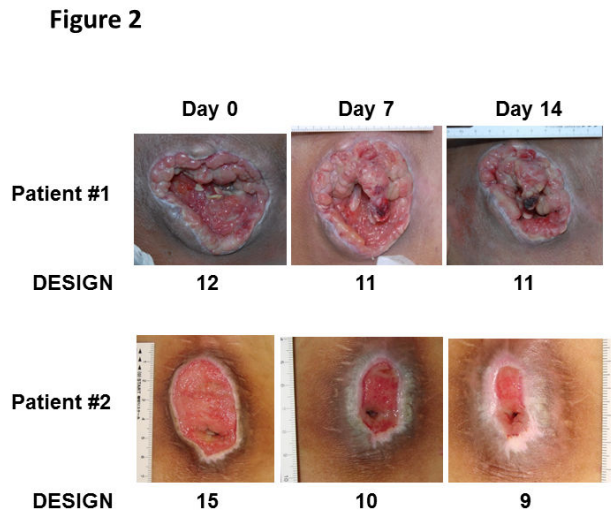

Figure 2: Wound area and DESIGN-R score.

Changes in wound area (A) and total DESIGN-R score (B upper) in all six patients are shown. A significant reduction in the DESIGN-R score was noted from day 0 to day 7 ($p < 0.05$ by regression analysis), but not from day 7 to 14.

Wound healing and DESIGN-R score

Macroscopically, wounds in all the cases healed progressively (Figure 2). The initial wound area ranged from 3.1 to 44 cm2, with a mean of 23.5 ± 15.1 cm2 (Figure 3A). No significant change was observed in the wound area on day 7(19.9 ± 15.7 cm2; 20.3% reduction vs. day 0), but a significant decrease was observed on day 14 (16.9 ± 13.8 cm2; $p < 0.01$, 37.5% reduction vs. day 0) (Figure 3A). The mean of DESIGN-R score was initially 13.3 ± 2.0 points and decreased on day 7 (11.2 ± 2.1 points, $p < 0.01$) and day 14 (10.5 ± 2.3 points, $p < 0.01$) (Figure 3B). The reduction of 2.2 ± 1.6 points from day 0 to day 7 was statistically significant ($p < 0.05$ by regression analysis). However, that from day 7 to 14 was not significant (reduction of 0.7 ± 0.5 points). No adverse wounds or systemic conditions were found in all six patients.

Discussion

It is important for physicians to choose a regimen for patients with intractable wounds to allow the shift in tissue stages from inflammation to proliferation. Various types of growth factors [13], cytokines, and chemokines [14] are preferentially secreted in accordance with the changing circumstances during the progression in the wound healing cascade. Growth factors such as bFGF, epithelial growth factor, and hepatocyte growth factor have been clinically utilized and their effects are well-investigated [15, 16]. Growth factors applied topically on wound surfaces stimulate certain cell types to proliferate. Unlike growth factors, G-CSF is a chemokine that stimulates granulocytes and hematopoietic stem cells in the bone marrow. It also regulates neutrophil proliferation, maturation, and survival, and induces inflammatory reactions in wound tissues [17,18]. G-CSF is also known to initiate the mobilisation of not only hematopoietic stem cells but also circulating fibrocytes and EPC, which are involved in granulation tissue formation. However, no increase in circulating CD34+ fibrocytes and EPC was noted in the patients treated with rhG-CSF.

In animal models, recombinant G-CSF administered to haemorrhagic rats with skin wounds accelerates wound healing. This effect was accompanied by an increase in neovascularisation and a decrease in apoptosis [19]. The positive effects of G-CSF on wound healing have been also addressed in clinical studies [8-10, 20]. However, the mechanisms of action through which G-CSF accelerates refractory wound healing are still unclear. In the investigator's clinical trial, rhG-CSF was topically applied to decubitus skin ulcers of six patients with refractory wounds. Despite the small number of patients, study authors found a statistically significant improvement in wound healing. G-CSF may act by shifting chronic wounds to acute ones and this might involve the activation of indolent neutrophils [21], and neutrophils may play an initial role in the progression of wound healing. However, the involvement of G-CSF in the stimulation of stem cells or progenitor cells to allow regenerative healing could not be verified in this study.

In the present clinical trial, study authors used a unique regimen of drug administration – the direct administration of rhG-CSF on the wound surface. They expected the induction of cell mobilisation from the bone marrow and the stimulation of tissue-resident granulocytes in wound tissue. The former was achieved as evidenced by the increased plasma level of G-CSF and WBC count. On the other hand, the later as a major source of neutrophils could not be determined. However, indolent neutrophils may also pre-exist in chronic wounds and can allow the activation of wound healing reactions. In the investigator's previous study [22], G-CSF is produced at the site of injury, and G-CSF supplementation to the indolent wounds in diabetic mice accelerated wound healing. The local effects of G-CSF in peripheral tissues should be investigated in a future study.

Previous studies show that systemic G-CSF administration is effective for wounded patients with neutropenia [8-10, 20] and burn wound sepsis in rats [23]. These suggest that its efficacies are due to enhancing neutrophil numbers and/or functions. On the other hand, Iwamoto et al. showed that the systemic administration of G-CSF in mice is effective in the mobilisation of bone marrow stem cells into the circulation, and the cells home to the wound site [24]. In the case of a local administration, the excised wound is more significantly healed than with a systemic injection [25]. These pieces of evidence provide an idea of the dual functions of G-CSF in the body. The first is the classical mobilisation of bone marrow cells such as neutrophils. The

second is the local action of G-CSF in wound tissues. Both are highly possible because G-CSF functions as a signal molecule at the site of wounds and acts both locally and systemically. The present results thus suggest the complex mechanisms through which G-CSF can aid in wound healing.

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