Recombinant human granulocyte-macrophage colony-stimulating factor hydrogel promotes healing of deep partial thickness burn wounds

Hong Yan *, Jian Chen, Xi Peng
Institute of Burn Research, South-west Hospital, State Key Laboratory of Trauma, Burns and Combined Injury, The Third Military Medical University, Chongqing 400038, PR China

**Abstract**

Objective: To assess the effects of recombinant human granulocyte/macrophage colony-stimulating factor (rhGM-CSF) hydrogel on the healing of deep partial thickness burn wounds. Methods: Ninety three wounds of 65 burn patients who suffered from a deep partial thickness burn of <5% TBSA and could not heal over 3 weeks were included in this study. The patients were randomly assigned to use rhGM-CSF hydrogel (GC group, n = 32) or hydrogel without rhGM-CSF (control group, n = 33). rhGM-CSF hydrogel or hydrogel without rhGM-CSF was topically applied to the wounds, the dressing was changed once a day. Wound healing time and percentage, wound discharge, periwound inflammation, the positive wound swabs culture count, and adverse drug reactions were observed and compared between two groups. Results: Healing time was 12.2 ± 5.0 days after the application of rhGM-CSF hydrogel. This was significantly shorter than that of control wounds (15.5 ± 4.7 days). Healing percentage at 14 days in the rhGM-CSF-treated wounds was 97.5 ± 7.7%, which was markedly higher than the control (85.9 ± 6.8%). At 3, 6, 12, 14 day, the GC group was significantly superior to the control group with respect to the score of periwound inflammation, wound purulence and discharge. The positive wound swabs culture count of the GC group on the 7th and 14th day post-treatment was 14 and 4, respectively, which was significantly lower than the control. Conclusion: rhGM-CSF hydrogel promotes the healing process of deep partial thickness burns effectively. No adverse reaction of the drug was observed during the study.

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1. Introduction

Healing of deep partial thickness burns relies primarily on reepithelialization of skin appendages as well as proliferation and migration of epidermal basal cells from the wound edges in three to four weeks postburn. However, some wounds cannot heal timely.

Currently, the widely adopted options for deep partial thickness burn wounds were skin grafting when it failed to heal over 3 weeks after burn. But not all the patients or their relatives could be persuaded to receive operations, especially for those burned children's parents, which means wound dressing must be continued. Then, the effort to find some new drugs to promote the healing of those wounds is important and have practical significance.

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine capable of stimulating proliferation and differentiation of hematopoietic stem cells and subsequent formation of granulocyte, macrophage, and granulocyte-macrophage colonies. GM-CSF has been widely used in the clinic to stimulate bone marrow hematopoiesis for years.
Previous studies also found that GM-CSF induces the differentiation of myofibroblasts, thereby facilitating wound contraction [1]; causes local recruitment of inflammatory cells [2]; mediates epidermal cell proliferation [3] and induces keratinocyte proliferation [4]. Locally administered GM-CSF can enhance the wound healing in diabetic mice, adriamycin-treated or immuno-suppressed rats [5–7]. GM-CSF can also attenuate local inflammatory responses in the wounds and promote epithelial cell growth and wound healing. In the present study, we employed a recombinant human GM-CSF (rhGM-CSF) hydrogel to treat deep partial thickness burns and found satisfactory therapeutic effects.

2. Clinical data and study methods

2.1. Case selection

2.1.1. Inclusion criteria
Patients who were treated at the Burn Clinic of our hospital (Burns Institute, Southwest Hospital, and The Third Military Medical University) from August 2009 to March 2011 were enrolled according to the following criteria:

1. Burn depth was assessed according to the “Three degrees and four categories method”, and the size of the burn was determined according to the “rule of nine”. Patients with deep partial thickness burn wounds of TBSA ≤5%.
2. Patients whose wounds failed to heal after 3 weeks of routine dressing change and were in a stable general condition, had no serious systemic infection.
3. Patients met above conditions and refused to skin graft when operation suggested.
4. Patients with no associated cardiovascular, liver, kidney, nervous system or blood disease.

2.1.2. Exclusion criteria
Patients on immunosuppressive therapy or chemotherapy, patients with systemic or skin malignancy, patients with serious vascular disease that impaired microcirculation, pregnant or lactating women, and patients on steroids were excluded from the study.

2.1.3. Dropout/removal criteria
During the study period, patients who did not follow treatment requirements and used other drugs for wound treatment, such as antibiotics powder, Yunnan Baiyao (white medicinal powder), or epidermal growth factor hydrogel, were removed from the study. Besides, patients who were unable to take regular treatment were dropped out.

2.1.4. A total of 70 patients were recruited at the start of the study
Five patients dropped out during treatment due to inability to take regular treatment. The remaining 65 patients were included for data analysis consisted of 41 males and 24 females, with an average age of 20.6 ± 17.2 years. There were 26 cases of hot metal burns, 28 cases of hot liquid burns, and 11 cases of flame burns, involving a total of 93 wounds, with an average duration of 27.2 ± 7.1 days after burn and an average wound area of 48.9 ± 28.8 cm².

2.2. Study design

All enrolled patients aged 2–50 years. The patients’ consent was obtained after a full explanation of the treatment. Pediatric consent form had been obtained from their parents or guardians. The southwest hospital ethics committee approved the protocol. All the doctors in the trail were experienced in burn wounds treatment and trained for the standard protocol and unified observation aspects. The patients were randomized into rhGM-CSF treatment group (GC treatment group, n = 32) and control group (n = 33) using a randomized serial number. No significant differences in age, gender, severity of burn wounds, wound area, and complications were noted between two groups (P > 0.05, Table 1).

To minimize the pain of dressing exchange, dressings were fully soaked with saline and easily removed. After washing and rinsing the wounds with sterilized saline, rhGM-CSF hydrogel (Changchun GeneScience Pharmaceuticals Co., Ltd., Changchun, China) was topically applied to the wounds of treatment group with thickness up to 1 mm, covered with sterile paraffin gauze and bandaged. The dressing was changed daily and the wounds were observed and assessed when dress-exchanging at each set timepoint. In the control group, the same procedures were used except for using hydrogel without rhGM-CSF.

2.2.1. The wound healing percentage and the healing time
Wound healing time were observed and recorded by an experienced doctor. Healing percentage has been evaluated at day 7 and 14 after treatment. The contour of the wounds was recorded using a transparent medical film and scanned for area computation. Wound healing percentage = (area before treatment – area after treatment)/(area before treatment) × 100%.

2.2.2. Control of wound infection
Changes in wound infection before treatment and at day 3, 6, 12, and 14 after treatment were observed and evaluated. The following indicators were used to evaluate wound infection control. (1) Wound discharge (purulence, exudates) after treatment: no change was scored 0, reduction 1, significant

<table>
<thead>
<tr>
<th>Table 1 - Baseline data of the two groups.</th>
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<tbody>
<tr>
<td>Group</td>
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<tr>
<td>------------------------------</td>
</tr>
<tr>
<td>GC treatment group</td>
</tr>
<tr>
<td>Control group</td>
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</table>
reduction 2, dry wound 3, and increase –1. (2) Inflammation surrounding the wounds after treatment: no change in swelling around the wounds was scored 0, reduced swelling 1, subsided swelling 2, and exacerbated swelling –1. (3) Positive culture count of wound swabs: wound swabs were collected for bacterial culture before treatment and at day 6 and 12 after treatment.

2.2.3. Adverse drug reactions
Routine blood tests together with liver (serum enzymes, plasma albumin and serum bilirubin) and kidney function tests (blood urea nitrogen and serum creatinine levels) were performed before and after treatment. Local adverse reactions following administration were observed and recorded. High fever, liver and kidney dysfunction, local pain, aggravated wound infection, skin allergy, etc. that arose after application were defined as adverse drug reactions.

2.2.4. Statistics analysis
Statistical analysis was performed using SPSS version 18.0 software. Continuous data were analyzed using one-way analysis of variance. Logrank test for comparing the healing time of the two groups and Wilcoxon test for comparing the healing percentage of the two groups were used.

3. Results

3.1. Effect of rhGM-CSF hydrogel on wound healing rate and healing time
The healing time of the wounds in the GC group is shorter than that in the control group, on average 3.25 days shorter \( (P < 0.01) \). The GC treatment group also had significantly higher wound healing rates than the control group \( (P < 0.01) \) at day 14 after treatment, while at day 7 after treatment the difference of healing rate between the two groups was not significant (Table 2).

3.2. Control of wound infection
Effects of rhGM-CSF hydrogel on wound infection were examined. No significant differences in the scores of wound discharge or periwound inflammation were noted between the two groups before treatment. At day 3, 6, 12, and 14, the scores of wound discharge and periwound inflammation were significantly higher in the GC treatment group than in the control group \( (P < 0.01) \), suggesting that the GC treatment group is far superior to the control group concerning the control of wound infection. In terms of wound bacterial culture, a total of 91 bacterial strains were isolated from wound swabs obtained from 93 wounds in the two groups, including 31 strains \( (34.1\%) \) of Staphylococcus aureus, 25 strains \( (27.5\%) \) of Pseudomonas aeruginosa, 7 strains \( (7.7\%) \) of methicillin-resistant S. aureus (MRSA), 4 strains \( (4.3\%) \) of Staphylococcus epidermidis, 17 strains \( (18.7\%) \) of Klebsiella pneumoniae, 4 strains \( (4.3\%) \) of Bacillus proteus, and 3 strains \( (3.3\%) \) of Escherichia coli. Most of the bacteria were resistant to a variety of antibiotics. The total rates of wound bacterial clearance before treatment, and at day 7 and day 14 after treatment were 69.5% and 91.3%, respectively, in the GC treatment group, and were 46.7% and 66.7%, respectively, in the control group (Table 4). This result indicates that the GC treatment group has markedly higher rates of bacterial clearance than those of the control group \( (P < 0.01) \).

3.3. Adverse drug reactions
During the study period, five cases of self-reported pain after dressing change were observed in the control group. In the GC treatment group, three patients complained of wound pain.

### Table 2 – Comparisons of wound healing rates after treatment between the two groups.

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>GC treatment group</th>
<th>Number of wounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healing rate at day 7 (%)</td>
<td>27.7 ± 7.6</td>
<td>31.8 ± 9.2</td>
<td>45</td>
</tr>
<tr>
<td>Healing rate at day 14 (%)</td>
<td>85.9 ± 6.8</td>
<td>97.5 ± 7.7</td>
<td>48</td>
</tr>
<tr>
<td>Healing time (days)</td>
<td>15.5 ± 4.7</td>
<td>12.2 ± 5.0&quot;</td>
<td>36</td>
</tr>
</tbody>
</table>

* Compared with control group \( P < 0.01 \).

### Table 3 – Comparisons of wound infection control between the two groups.

<table>
<thead>
<tr>
<th>Time course of treatment</th>
<th>Score of wound purulence and discharge</th>
<th>Score of periwound inflammation control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GC treatment group (n = 38)</td>
<td>Control group (n = 38)</td>
</tr>
<tr>
<td>Before treatment</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Day 3</td>
<td>70&quot;</td>
<td>48</td>
</tr>
<tr>
<td>Day 6</td>
<td>89&quot;</td>
<td>64</td>
</tr>
<tr>
<td>Day 12</td>
<td>98&quot;</td>
<td>73</td>
</tr>
<tr>
<td>Day 14</td>
<td>103&quot;</td>
<td>81</td>
</tr>
</tbody>
</table>

Note: \( n \) is the number of wounds included for data analysis.

* Compared with control group, \( P < 0.01 \).

* Compared with control group, \( P < 0.05 \).
during early application, but the pain resolved after the second use. No local allergy in the wounds occurred in the GC treatment group. Liver function test including serum enzymes, plasma albumin and serum bilirubin showed no abnormalities in both group. Kidney function tests (blood urea nitrogen and serum creatinine levels) and routine blood and urine tests showed no abnormalities in the two groups before and after treatment either. Besides, no general discomfort was noted in the two groups throughout the study period. These results suggest that topical application of the rhGM-CSF hydrogel has a good safety profile for burn wounds.

### 4. Discussion

The depth of deep partial thickness burn wounds is not entirely static. Both clinical observations and experimental studies have shown that those wounds will continue to deepen and are difficult to heal. This phenomenon results from factors like infection, edema, excessive inflammatory response in local area [8, 9], wound microcirculation dysfunction, hypoperfusion [10, 11], oxygen free radical injury [12], and metabolic and nutritional disorders [13]. Traditional conservative burn therapies depend primarily on the topical use of antimicrobial agents, such as silver sulfadiazine and iodophor, on the wounds in addition to simple debridement and dressing change. Despite the fact that such externally applied drugs can effectively inhibit and kill bacteria on the burn wounds, they also have cytotoxicity and thus will inhibit wound repair [14–16]. Unfortunately, because of the complexity and multifactorial nature of wound deepening, it remains difficult to avoid such phenomenon in the clinic, despite some mechanistic insights already gained. As a result, deep partial thickness burn wounds remain a challenging task for burn surgeons [17], especially when they face those patients who are unwilling to receive skin grafting and which is common phenomenon in China.

rhGM-CSF has a variety of functions, such as boosting vaccine immunogenicity and counteracting bone marrow suppression caused by radiotherapy, chemotherapy, stem cell transplantation, and severe infections [18]. In recent years, regulation of local inflammatory responses in the wounds and promotion of wound healing with topical application of rhGM-CSF have garnered an increasing interest.

Previous research has shown that GM-CSF is synthesized and secreted by a host of cells involved in wound repair, such as activated T cells, dendritic cells, macrophages, keratinocytes, endothelial cells, and fibroblasts [3]. Besides, GM-CSF is also shown to enhance the functions of various cells necessary for wound healing, such as activating neutrophils and monocytes/macrophages, promoting keratinocyte migration and proliferation, and regulating fibroblast phenotype [19]. Over the past decade, rhGM-CSF has been applied to a plethora of clinical wounds and yielded promising effects. Several evidences have proved that topical application of rhGM-CSF are safe and effective for promoting the healing of acute and chronic wounds of various causes, such as burn wounds, venous ulcers, diabetic ulcers, pressure ulcers, residual burn wounds and other refractory wounds caused by cancer chemotherapy [20]. In the present study, wound healing percentages in the GC treatment group were significantly higher than those in the control group at 14 day time points ($P < 0.05$) and the wound healing time was significantly shorter ($P < 0.01$). The healing of deep second-degree wounds depends primarily on the reepithelialization of residual skin appendages as well as proliferation and migration of epidermal basal cells at the wound margins. In this study, we found that the rhGM-CSF hydrogel dramatically promoted reepithelialization from residual skin appendages in the burn wound bed. During the treatment process, particularly at day 7–14 after application, marked proliferation and migration of epidermis were noted in wound margins of most wounds. Mann et al. proposed that GM-CSF can not only directly stimulate proliferation and migration of human keratinocytes and fibroblasts, but also induce proliferation and migration of vascular endothelial cells and accelerate neovascularization, thereby facilitating wound reepithelialization [3].

Infection is often present in burn wounds. Wound infection and excessive local inflammation responses could hinder the proliferation and migration of skin appendages [21]. Bacteria are often detected in the granulation tissue of burn wounds, manifested clinically as persistent purulent discharge despite repeated dressing changes and even cause severe inflammation reaction of adjacent normal tissues. In the current study, purulent discharge from the wounds in the GC treatment group occurred much less frequently than that from the control group, and the rates of periwound swelling resolution and bacterial clearance in the GC treatment group were prominently higher than those in the control group, indicating that the rhGM-CSF hydrogel can more effectively control the infection of burn wounds. A recent study also demonstrated that rhGM-CSF could regulate cells involved in acute and

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### Table 4 – Comparisons of wound bacterial swab cultures between the two groups.

<table>
<thead>
<tr>
<th>Species of bacteria</th>
<th>GC treatment group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before treatment</td>
<td>Day 7</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td>MRSA</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Bacillus proteus</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Accumulative bacterial count</td>
<td>46</td>
<td>14$^a$</td>
</tr>
</tbody>
</table>

* Compared with control group, $P < 0.01$. 

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chronic inflammatory responses, modulate inflammatory responses, and potentiate chemotactic, adhesive, phagocytic and bactericidal capacities of neutrophils and macrophages [22]. Topical application of rhGM-CSF can attract inflammatory cells and endothelial cells and prevent their escape from the wounds, allow Langerhans cells to enter the dermis, and activate neutrophils and monocytes/macrophages, thereby promoting wound healing [23,24].

Taken together, when skin grafting was rejected, more effective drugs to promote healing of deep partial thickness burn wounds were needed. Dressing change aimed at infection control alone is not enough for stimulating the residual epidermal growth potency. Our present study showed that rhGM-CSF hydrogel could promote reepithelialization and control infection of burn wounds. Apart from regulating proliferation and activation of macrophages, neutrophils, endothelial cells, epithelial cells and fibroblasts that are crucial in wound repair, rhGM-CSF can also chemotactically pro-inflammatory cells and endothelial cells, induce proliferation and migration of keratinocytes, trigger the cascade of wound repair. Our observation showed that rhGM-CSF could promote burn wound healing without obvious adverse effects and provides a novel therapeutic option for management of deep partial thickness burn wounds.

Conflict of interest

The authors declare that they have no conflict of interest.

References

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