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The therapeutic effect of Recombinant Human Collagen Ⅲ on second-degree scald wounds in rats

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Abstract

Backgroud: Previous studies have shown that recombinant humanized collagen Ⅲ (rhCol Ⅲ) has a positive therapeutic effect on open wounds is a highly adhesive biomaterial comp. Here, we aimed to investigate the therapeutic effect of rhCol Ⅲ on type II scald wounds in rats.Method: Establish a second-degree burn model and continuously administer different concentrations of rhCol Ⅲ to the wound surface for 7 days (50 μg/mL,100 μg /mL,200 μg/mL). The effect of rhCol Ⅲ on scald wounds was assessed using gross and histological analyses.Result: rhCol Ⅲ can significantly accelerate scald wound healing wound healing and reduce scar formation, effectively promoting burn wound recovery.

Keywords

Recombinant humanized collagen Ⅲ, scald wound, wound healing.

1. Introduction

Burns are a common traumatic disease, ranking fourth in terms of trauma. Every year, a large number of people worldwide die from burns[1]. Therefore, searching for new effective and minimally adverse drugs or adjuvant drugs has become a research hotspot today.

Collagen is an important structural protein in organisms, characterized by high biocompatibility, high safety, and low immunogenicity[2]. Previous studies have shown that collagen is an indispensable substance for wound healing[3]. Therefore, collagen can serve as a potential therapeutic drug for repairing scald wounds.

At present, there are two main sources of collagen:1) extracting from animal tissues; 2) obtaining recombinant collagen through genetic engineering technology[4]. Collagen extracted from animal tissues has low purity, and may contain animal viruses. Its use in clinical practice may lead to epidemics, making it more dangerous[5]. The recombinant collagen obtained through genetic engineering technology is then produced in large quantities by microbial fermentation, resulting in high purity, good biocompatibility, low immunogenicity, less rejection reaction with the human body, and higher biological activity[6]. Therefore, compared to collagen extracted from animal tissues, recombinant collagen may be more suitable for the future demand for biological tissue engineering materials.

Recombinant humanized type Ⅲ collagen (rhCol Ⅲ) is an innovative biomaterial whose function is customized based on the sequence of human type Ⅲ collagen, an important structural protein that promotes tissue repair[7]. In full-thickness cutaneous wound model,

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rhCol Ⅲ accelerated wound closure and angiogenesis, increased the formation of dermal tissue, and reduced scar formation[8]. Injecting rhCol Ⅲ hydrogel is a promising method to enhance cardiac repair after myocardial infarction (MI) by reducing scar size and inflammation[9].

In this study, we established the second degree burn model to investigate the therapeutic effect of rhCol Ⅲ on scalded wounds in rats. Our findings showed that rhCol Ⅲ can accelerate scald wound healing. The rhCol Ⅲ may represent a novel clinical strategy for accelerating wound closure.

2. Material and methods

2.1. Animal and material

We purchased Sprague–Dawley (SD) rats (160±20 g) from the Experimental Animal Center of Guangdong Province, China (certificate No. SYXK (Guangdong) 2018–0002). All animal experiments were conducted in according with the National Institute of Health guidelines for the care and use of animals and approved by the Institutional Animal Care and Use Committee of Jinan University.

Recombinant humanized type Ⅲ collagen (rhCol Ⅲ) was purchased from Jy-RNA Biotechnology Co., Ltd.

2.2. Establishment and treatment of a second-degree burn model

Hair was removed from the back of each rat. On the day of modeling, the rats were fasted for 12 h and then anesthetized with 2 % pentobarbital sodium by intraperitoneal injection. To establish a second-degree scald wound, the back of the rat was the exposed to 100 ℃ aluminum cylindrical head (30 mm diameter) for 10 s. SD rats were randomly divided into four groups of six: model group (PBS); low-dose group (50 μg/ mL rhCol Ⅲ); medium dose group (100 μg/mL rhCol Ⅲ); high dose group (200 μg/mL rhCol Ⅲ). Add 2 mL of the medicinal solution onto a sterile dressing and apply it to the burned area.

2.3. Analysis of wound closure rate

The wound healing rate on days 0, 3, 7, 15, and 23 after the end of the modeling administration was calculated using ImageJ software. Wound healing rate=(Original wound area - Unhealed wound area)/Original wound area) × 100%. The rats were sacrificed, and the skin tissues were harvested for analysis.

2.4. Histological analysis

The skin tissues were harveated on day 7 and 23. All the tissues were fixed with 4 % paraformaldehyde at 4 °C. Hematoxylin and eosin staining (H&E), Masson's trichrome staining were performed by Wuhan Servicebio Technology (Wuhan, China). Images were captured using a Nikon DS RI orthographic microscope.

2.5. Statistical analysis

The results were presented as means ± SD or mean ± standard error of the mean (means ± SEM) as indicated. The data were compared by Student's t test between two groups and ordinary one-way analysis of variance (ANOVA) for three or more groups. All statistical analyses were conducted by the GraphPad Prism software. Statistical significance was established as indicated by asterisks $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ and $****p < 0.0001$.

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3. RESULTS

3.1. RhCol Ⅲ accelerated wound healing processes

To determine the therapeutic effect of rhCol Ⅲ on scald wounds, we observed the healing of the wounds on 0-23 days after surgery. On the 3rd day after surgery, there was no significant change in the wound area in each group; On the 7th day, compared to the model group, wounds had significantly reduced in the rhCol Ⅲ groups, with relatively moist and no ulceration; On the 15th day, compared to the model group, the rhCol Ⅲ groups had a more significant reduction in wound size, and the scabs on the wound site in the 100 μg/mL rhCol Ⅲ and 200 μg/mL rhCol Ⅲ groups were basically detached; On the 23rd day, compared to the model group, the collagen group had more significant wound shrinkage. The wounds in the 200 μg/mL rhCol Ⅲ group were completely covered by newly formed epithelium, and the newly formed epithelium was pink and shiny, with little difference from the original skin, see Fig. 1A.

To evaluate the effect of the different treatments macroscopically, we have calculated the wound healing rate and healing time. After 3 days of treatment, the wound healing rate in the 50 μg/ mL rhCol Ⅲ, 100 μg/mL rhCol Ⅲ and 200 μg/mL rhCol Ⅲ group was significantly higher than that in the PBS group (****p<0.0001); On the 7th day, the wound healing rate in the treatment group was significantly higher than that in the PBS group (**p<0.01 or ***p<0.001); On the 15th day, the wound healing rate of the medication group was still higher than that of the PBS group (*p<0.05 or ***p<0.001); On the 23rd day, there was no significant difference in wound healing rate among the groups (p>0.05). In terms of wound healing time, the 50 μg/mL rhCol Ⅲ, 100 μg/mL rhCol Ⅲ and 200 μg/mL rhCol Ⅲ group had significantly shorter wound healing time than the PBS group (*p<0.05,**p<0.01), see Fig. 1B,C. Collectively, these data demonstrated that the rhCol Ⅲ accelerated scald wound healing.

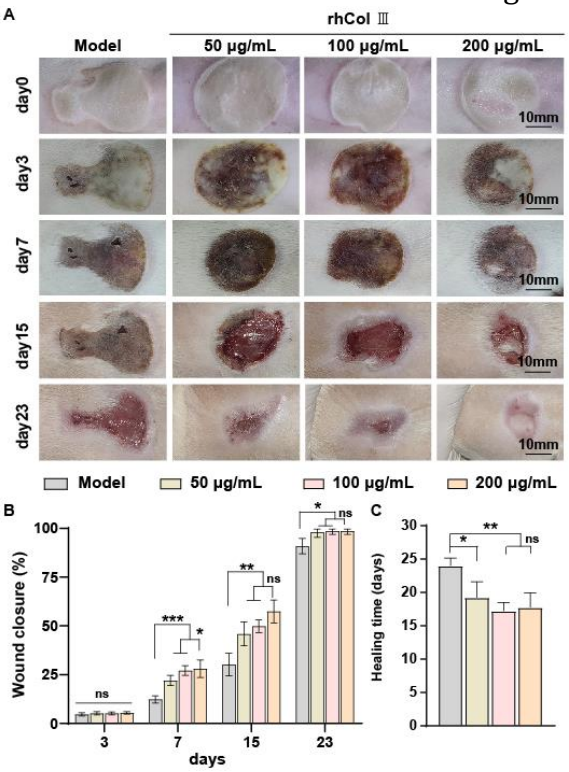

Fig. 1 RhCol Ⅲ accelerated wound healing processes

3.2. RhCol Ⅲ promoted re-epithealization and angiopoiesis

To evaluate the effect of the different treatments histologically, full–thickness traumatic tissue samples were collected on day 7 and 23 for H&E and MASSON staining. On the 7th day, the

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damaged epidermal layer in the PBS group showed no significant repair, and the structure of the dermis was scattered, with a large number of inflammatory cell infiltration; There is no scatter within the organization in 50 μg/mL rhCol Ⅲ group; There is already epithelial regeneration in the wound of 100 μg/mL rhCol Ⅲ group and 200 μg/mL rhCol Ⅲ group (marked by red arrows); On the 23rd day, compared to the regenerated skin tissue in the PBS group, the epidermis in the different dosages of rhCol Ⅲ treatment group was smoother and more uniform, with a thickness similar to that of normal skin epidermis, and a large number of hair follicle and blood vessels regenerated in the dermis. Among them, the high-dose (200 μg/mL rhCol Ⅲ) group showed the most significant regeneration of blood vessels and hair follicles in the regenerated skin , see Fig. 2.

The H&E staining results indicated that rhCol Ⅲ can accelerate wound healing by promoting epithelial.

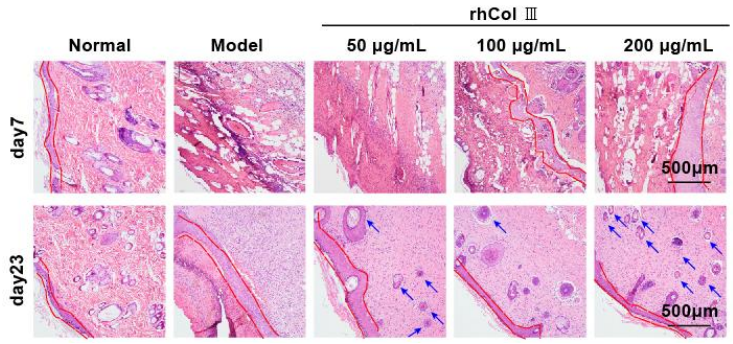

Fig.2 RhCol Ⅲ promoted re-epithealization and angiopoiesis

3.3. RhCol Ⅲ promoted collagen deposition

To evaluate the effect of the different treatments on collagen regeneration, full–thickness traumatic tissue samples were collected on day 7 and 23 for MASSON staining (Collagen stained blue). On the 7th and 23rd day, compared to PBS group, the 50 μg/mL rhCol Ⅲ, 100 μg/mL rhCol Ⅲ and 200 μg/mL rhCol Ⅲ group showed significant regeneration of collagen on the wound surface, see Fig. 3. This result indicated that rhCol Ⅲ can accelerate wound healing by promoting collagen regeneration.

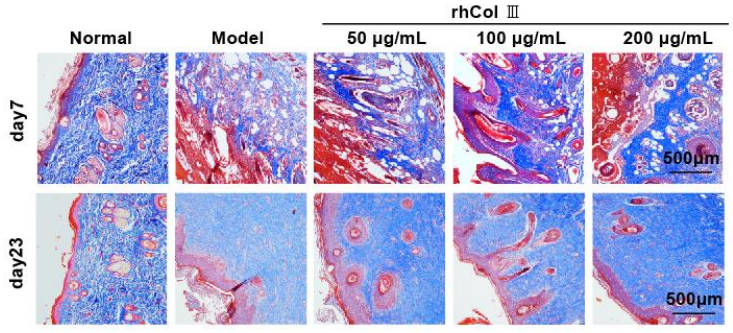

Fig. 3 RhCol Ⅲ promoted collagen deposition

4. Conclusion

Therapies to deep burn injuries remain a global challenge. In this experiment, we established a second-degree burn model in rats, using recombinant human collagen III (rhCol Ⅲ) as a therapeutic drug. Based on the appearance of wound healing and the results of histopathological examination, we found that the experimental group significantly promoted the healing of scalded wounds compared to the model group.

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Macroscopical analysis showed that, compared with PBS treatment, the surface shrinkage of the wound treated with rhCol Ⅲ was significantly accelerated, and the degree of wound repair was significantly improved with increasing rhCol Ⅲ concentration. In addition, the newly formed epithelium in the 200 μg/mL rhCol Ⅲ group was smooth, pink, and had no obvious scar residue. In addition, as the concentration of collagen increased, the wound recovery improved. Histologically, whether burn tissue returns to normal structure mainly is determined by observing the regeneration of epithelium, collagen, hair follicles, and blood vessels within the wound surface[10]. In many studies, hydrogels and microneedles containing rhCol III were applied to infected chronic diabetes skin wounds and acute skin wounds to promote the healing process by promoting re-epithealization, collagen regeneration and angiogenesis[6]. Compared to PBS treatment, rhCol III treated wounds have faster internal epithelial regeneration rate, and the regenerated epithelium is smoother, with a thickness close to normal skin; And the amount of regenerated collagen, hair follicles, and blood vessels significantly increased in rhCol III treatment group. These results indicated that rhCol III can accelerate scald wound healing by promoting epithelial, collagen, and vascular regeneration.

In summary, We demonstrated that rhCol III improved scald wounds healing by promoting epithelial, collagen, and vascular regeneration. Our data suggested that a therapeutic potential of rhCol III for scald wounds.

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FIGURE LEGEND

Figure 1 RhCol III accelerated the wound healing process. (A) Morphological changes during wound healing. Scale bar, 10 mm. (B) Wound closure rate was measured in each group after wounding. (C) Quantification of wound healing time in each group. Data are presented as the mean ± SD. (*p<0.05, **p<0.01, ***P<0.001, ****p < 0.0001).

Figure 2 RhCol III can promote re-epithealization and angiopoiesis. Representative images of hematoxylin and eosin staining of wound healing. Black arrows: skin appendages; red dotted line: the boundary of the epithelium. Scale bar 500 μm.

Figure 3 RhCol III promoted collagen deposition. Representative images of Masson's trichrome staining of healing wounds at different time points. Scale bar, 500 μm.