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Original Research Article

Effect of taspine hydrochloride on KGF signal pathway in skin wound healing in rats

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Abstract

Purpose: *To investigate the effect of taspine hydrochloride and the regulatory involvement of fibroblast growth factor (FGF) signal on skin wound healing in rats.*
Methods: *Wound model rats were assigned to 3 groups (n = 15 each): untreated control, taspine hydrochloride high-dose treatment, and taspine hydrochloride low-dose treatment groups. Fibroblast growth factor (FGF) and keratinocyte growth factor receptor (KGFR) were determined using qPCR, while immunoblot assay was used to assess protein levels of hepatocyte growth factor (HGF), epidermal growth factor (EGF), transforming growth factor-β1 (TGF-β1), vascular endothelial growth factor (VEGF), interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor (TNF-α) and other inflammatory factors.*
Results: *There were significantly down-regulated levels of KGF, KGFR, TGF-β1, VEGF, EGF, HGF, IL-6, IL-8 and TNF-α in control group on the first day, relative to high- and low-dose taspine hydrochloride treatment groups ($p < 0.05$). Wound repair took more time in control rats than in the 2 taspine HCL-treatment rats. However, healing time was significantly shorter in rats given higher level of taspine HCL ($p < 0.05$).*
Conclusion: *Taspine hydrochloride down-regulates the high expression of FGF and inhibits inflammatory response in rats with skin trauma. Moreover, it accelerates skin wound healing. These findings support the clinical application of taspine hydrochloride for skin wound healing.*

Keywords: *Taspine hydrochloride, Fibroblast growth factor signal, Skin wound healing, Inflammatory factors, Growth factors*

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INTRODUCTION

The mechanisms underlying skin wound occurrence and healing in mammalian skin are complex. The skin wound repair process is associated with the proliferation of inflammatory cells and tissue remodeling [1,2]. Inflammatory cells also reach the injured site together with platelets, providing key signals called growth factors [3]. Fibroblasts are connective tissue cells involved in depositing collagen needed for repair of damaged tissue [4].

Studies have shown that taspine hydrochloride, an alkaloid extracted from cohosh roots, has strong affinity for vascular endothelial cells and

fibroblasts [5,6]. It is known that KGF, which belongs to FGF family, is tied to the mechanism involved in regeneration of epidermal tissue when intrinsic skin epidermal signal transduction is damaged [7, 8]. The KGF seems to play a unique role as a mediator of the interaction between stroma and epithelium. It promotes the proliferation, migration and adhesion of skin keratinocytes by binding to keratinocyte growth factor receptor (KGFR), thereby activating downstream signal conduction [9-11]. The effect of exogenous KGF on different types of wounds indicates the importance of KGF in epithelial regeneration. In most cases, the rate of wound closure increases and the healing process leads to epithelial thickening. These results show that KGF is crucial to the repair process [12,13]. At present, there are no relevant research on the effect of taspine hydrochloride on KGF. It is unclear whether taspine hydrochloride has an effect on KGF when acting on cells.

The purpose of this research was to determine the influence of taspine HCl on wound repair in a rat wound model, and its effect on KGF signal, and hence provide a new reference for research on skin wound healing.

EXPERIMENTAL

Animals

Male Sprague-Dawley (SD) rats, with body mass range of 200 - 230 g, were provided by Shanghai Slack Laboratory Animal Co. Ltd (certificate no. 2007000539824). The animals were kept normally at room temperature of 22 - 24 ℃. Approval for this research was received from ethical authority of Hanchuan People's Hospital, Hanchuan, China, and study was conducted according to international guidelines for animal studies.

Grouping and treatment of animals

Under *i.p*. pentobarbital anesthesia, transection wound was produced in each rat 1.5 cm from each side of the spinal column. Then, two round wounds, each with a diameter of about 1.8 cm and an area of about 2.54 cm^2, were cut at the same position with a special punch to form a mechanical injury animal model.

The animals were assigned to 3 groups (n = 15): untreated control group, high-dose taspine hydrochloride treatment group, and low-dose taspine hydrochloride treatment groups. Taspine hydrochloride (2 mg/mL) was applied topically to each side wound in the high-dose taspine hydrochloride group. For rats treated with lower drug dose, the drug at a dose of 0.5 mg/mL was applied to each side of wound surface. Wounds in untreated controls were only washed with normal saline once a day.

Evaluation of outcome parameters

KGF and KGFR

Using fluorescence quantitative polymerase chain reaction (qPCR), levels of KGF and KGFR were measured on the 7^{th}, 14^{th} and 21^{st} days of drug administration.

Wound healing

Wound healing (W) was computed as in Eq 1.

$$W (\%) = (S1 – S2)/S1 \quad \ldots\ldots (1)$$

Where S1 and S2 are the initial and present surface areas of the wounds, respectively.

Statistical analysis

Statistical analysis was done with the SPSS 19.0 (Asia Analytics, formerly SPSS China). Comparison of counting data amongst groups was done with $\chi 2$ test. Measurement data are expressed as mean ± SD, and within-group comparison between pre- and post-treatment was done using paired *t*-test, while 2-group comparison was done with independent sample *t*-test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Effect of taspine hydrochloride on FGF and KGFR

On comparing the groups, it was found that on the 1^{st} day, the contents of FGF and KGFR in wound tissues of control rats were markedly lower, when compared to the two taspine hydrochloride treatment groups, and the difference was significant throughout the experiment *(*$p < 0.05$). On the 7^{th} day, the contents of KGF and KGFR in wound tissues of the two groups reached peak values and began to decrease, but they were markedly elevated in wound tissues of rats that received high-dose taspine HCl than in rats given lower dose of the drug. On day 14, differences in the contents of KGF and KGFR in both groups were not statistically marked, although their levels were down-regulated, relative to control values *(*$p < 0.05$, Figure 1).

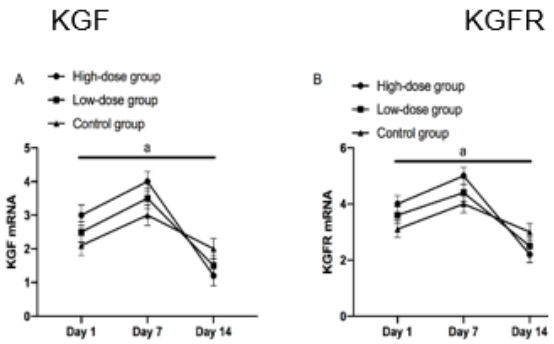

Figure 1: Comparison of the KGF and KGFR contents in rat wounds. A: Comparison of KGF contents in rat wounds; B: comparison of KGFR contents in rat wounds. [a]*P* < 0.05

Growth factors

On the 1st day, in comparison amongst groups, there were markedly lower wound tissue contents of TGF-β1, VEGF, EGF and HGF in model rats than in rats in high-dose taspine hydrochloride treatment group and low-dose taspine hydrochloride treatment group, and the difference was greater with duration of the experiment ($p < 0.05$). On the 7th day, the contents of these factors in the two groups reached peak values and began to decrease, but were still markedly raised in wound tissues in high-dose taspine HCl-treated rats than in low-dose taspine HCl-treated rats. On the 14th day, their contents in both groups were comparable, but lower when compared to control rat values ($p < 0.05$; Figure 2).

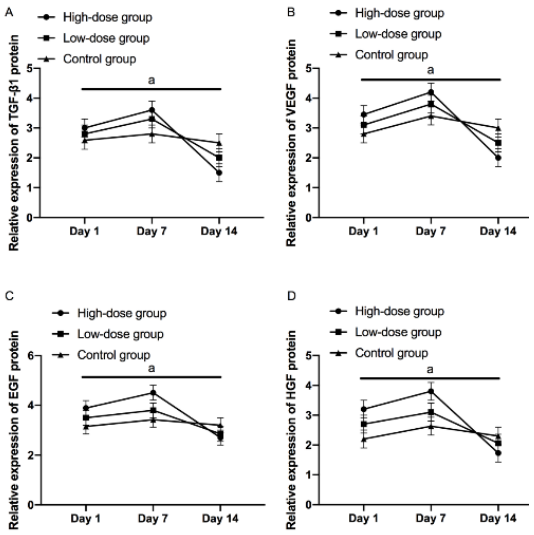

Figure 2: Levels of TGF-β1, VEGF, EGF and HGF in wound tissues of rats. A: comparison of TGF-β1 contents in wound tissues of rats; B: comparison of VEGF contents in wound tissues of rats; C: comparison of EGF contents in wound tissues of rats; D: comparison of HGF contents. [a]*P* < 0.05

Effect of taspine hydrochloride on inflammatory response of rat wound

On the 1st day, the levels of proinflammatory cytokines in control wound tissues were significantly reduced, relative to the taspine hydrochloride high-dose treatment group and the taspine hydrochloride low-dose treatment, and the degree significance increased with duration of the experiment ($p < 0.05$). However, by the 7th day, the contents of IL-6, IL-8 and TNF-α in wound tissues of the two groups reached peak values and began to decrease. However, their contents in wound tissues of the high-dose group were still significantly higher than those in the low-dose group ($p < 0.05$). On the 14th day, differences in the contents of IL-6, IL-8 and TNF-α were not statistically significant, but the levels of these factors were markedly lower, relative to control rat values ($p < 0.05$).

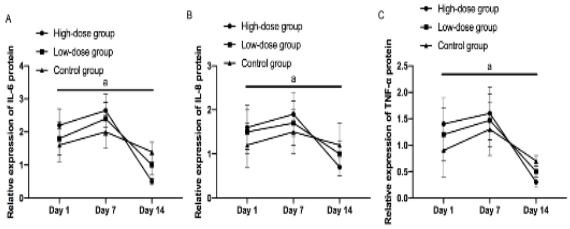

Figure 3: Comparison of IL-6, IL-8 and TNF-α contents in wound tissues of rats. A: comparison of IL-6 contents in wound tissues of rats; B: comparison of IL-8 contents in wound tissues of rats; C: comparison of tissue TNF-α contents in rat wounds; $p < 0.05$

Wound surface proteins of rats

Wound tissue protein levels were markedly lower in untreated control rats than in the two taspine hydrochloride dose treatment groups on the 1st day, and the difference between the two treatment groups became greater with time within duration of the experiment ($p < 0.05$). By the 7th day, the protein contents of wound tissues in the two groups reached peak values and began to decrease, but the protein contents of wound tissues were still higher in rats treated with high-dose taspine HCl than in rats given low-dose taspine HCl. On day 14, there was no statistically significant difference in protein content between the 2 groups, but their protein levels were decreased, relative to control rats. These data are presented in Figure 4.

Wound repair in rats

Wound healing values in rats in the high-dose taspine hydrochloride treatment group on the 1st, 7th and 14th days were 36.00 ± 3.20, 43.80 ± 3.80

and 82.20 ± 4.00 %, respectively. In the taspine hydrochloride low-dose treatment group, the wound healing values on 1st, 7th and 14th days were 23.50 ± 3.10, 34.40 ± 3.50 and 41.20 ± 3.00 %, respectively, while the corresponding control values were 14.50±3.20, 18.20 ± 3.20 and 27.30 ± 3.50 %, respectively. Comparison amongst groups revealed markedly lower wound healing rates in control rats than in high-dose taspine hydrochloride treatment group and low-dose taspine hydrochloride treatment group on the 1st day, and difference between the two treatment groups became greater with time during the experiment ($p < 0.05$). From the 7th day, there was significantly higher percentage wound repair in the high-dose taspine-treated rats than in low-dose taspine-treated rats. The largest difference in wound healing in rats in the three groups were seen on day 14.

Wound healing time of rats

The wound healing time of rats in high-dose taspine hydrochloride treatment group was 14.30 ± 1.00 days, while the corresponding time in the low-dose taspine hydrochloride treatment group was 17.00 ± 1.40 days, and that of the control group was 20.00 ± 2.30 days. While healing time was markedly longer in untreated control rats than in drug-treated rats, wound healing took markedly shorter time in rats given high-dose taspine HCl than in rats treated with the lower drug dose.

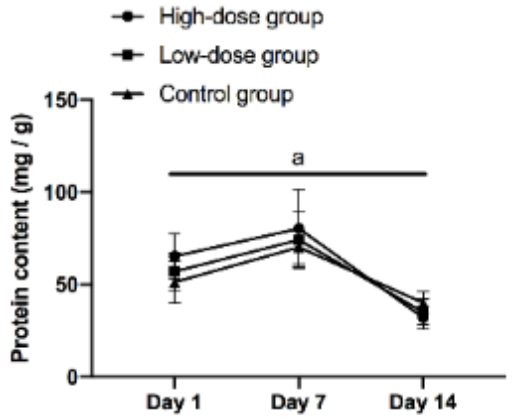

Figure 4: Comparison of protein contents in wound tissues

DISCUSSION

Taspine hydrochloride is non-toxic to fibroblasts, but it can increase the proliferation and migration of fibroblasts and accelerate the wound healing process [14]. The important pro-inflammatory cytokine IL-6 is required for effective healing of skin wounds [15]. In addition, studies have shown that IL-6-neutralizing antibody enhanced

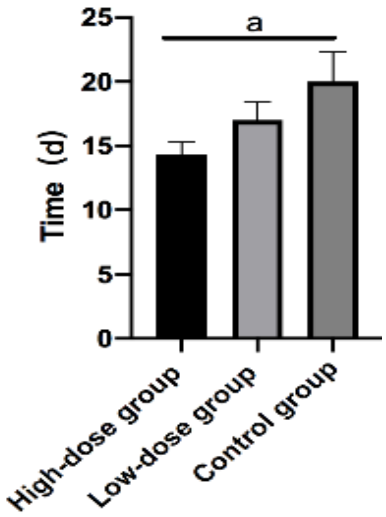

Figure 6: Wound healing time of rats in each group; [a]$p < 0.05$

wound healing, but excessive IL-6 antibody led to poor skin wound closure [16]. The contradictory roles of inflammatory factors in skin wound healing are crucial in the induction and propagation of inflammatory reactions, and changes in these roles are crucial for effective wound healing [17]. The present research first analyzed the effects of taspine hydrochloride on FGF and receptor KGFR. Through fluorescence quantitative PCR test, it was found that the FGF and KGFR contents in wound tissues of rats were markedly up-regulated in the untreated control rats, relative to the corresponding levels in high-dose taspine hydrochloride and low-dose taspine hydrochloride treatment groups, while their contents in wound tissues were markedly reduced in high-dose drug-treated rats, when compared with rats treated with low-dose of drug. However, FGF and receptor KGFR of rats in the three groups reached their peaks at the 2nd week of the study, and decreased at the 3rd week.

It is known that FGF and KGFR exist in cancer epithelial cells and stromal cells, and act on epithelial cells through their receptor KGFR [18]. Moreover, FGF plays a unique part in the mediation of stroma and epithelial cells, stimulates the proliferation of wound tissue cells, and enhances the repair process. It is a candidate drug involved in treatment using taspine hydrochloride [19]. Therefore, taspine hydrochloride had good regulatory effect on KGF and its receptor KGFR.

Next, inflammatory response and growth factor were determined in wounds of rats. It was found that the contents of IL-6, IL-8 and TNF-α in rats in the control group were significantly higher than

those in the taspine hydrochloride-treated groups. The downward adjustment was greatest in the high-dose group. It has been reported that EGF promotes metabolism of various skin cells and enhances cell absorption of nutrients [20]. Animal experiments show that HGF has the potential to accelerate wound healing and enhance wound healing effects [21].

Some studies have demonstrated that TGF-β1 and VEGF are key regulators of wound angiogenesis. For instance, GF-β1 binds to VEGF receptor and finally regulates neovascularization [22]. Decreased expressions and functions of TGF-β1 and VEGF may hinder wound healing [23]. The present research has demonstrated that taspine hydrochloride produced significant regulatory effect on wound inflammatory response and growth factor in rats.

Finally, wound healing in rats and protein contents in wound tissues of rats were determined. Several investigations have revealed that collagen is the major component of extracellular tissue, and is crucial for repairing damaged tissue cells [24]. This study showed that taspine hydrochloride was effective in promoting wound repair in rats and shortening wound healing time. Studies by other workers have confirmed the potential of taspine hydrochloride to promote skin repair in experimental rats.

In this study, the effects of taspine hydrochloride on skin wound in rats and KGF were investigated to monitor levels of inflammatory and growth factors during wound healing. However, wound healing is a complex biological process, and there may still be deficiencies in the experimental design. For instance, the use of different time points should be further modified, and relevant impact indicators should be appropriately supplemented.

CONCLUSION

Taspine hydrochloride down-regulates the high expression of KGF on wound surface in rats, inhibits inflammatory reactions, and accelerates skin wound healing. Thus, the findings confirm the effectiveness of the compound in the healing of skin wounds.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Xiaojun Zhang and Jiadong Liu conceived and designed the study, collected, analyzed and interpreted the experimental data, drafted the manuscript and revised the manuscript for important intellectual contents. All authors read and approved the final manuscript.

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