## Short Communication

# Effect of Isoliquiritin on Wound Healing in Scalded Rats

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## ABSTRACT

The aim of study was to investigate effect and possible mechanism of isoliquiritin on wound healing in scalded rats. The results show that at 24 h after the last administration, wound healing rate of isoliquiritin group was higher than that of model group ($p$=0.024). Compared with control group, thickness of epidermis and dermis in model group was thinner ($p<0.001$), and width of subdermal collagen fibers also decreased ($p<0.001$), inflammatory factors TNF-α, IL-6 and IL-1β in skin tissue increased (all $p<0.001$), and expression levels of Ang-1 and Tie-2 proteins in skin tissue decreased (both $p<0.001$). Compared with model group, thickness of epidermis, dermis and collagen fibers, and expression levels of Ang-1 and Tie-2 proteins in skin tissue in isoliquiritin group increased (all $p<0.001$), and inflammatory factors TNF-α, IL-6 and IL-1β contents and expression level of Ang-2 protein in skin tissue decreased (all $p<0.001$). Hence, this can be concluded that isoliquiritin can promote the healing of acute wounds in rats. The mechanism may be related to increasing skin thickness, inhibiting the release of inflammatory factors, up-regulating the expression of Ang-1 and Tie-2 proteins and down-regulating the expression of Ang-2 protein.

As a commonly used bulk medicinal materials, licorice comes from the dried roots and rhizomes of *Glycyrrhiza uralensis, Glycyrrhiza glabra* and *Glycyrrhiza inflata*. Its main active ingredients are triterpenoid saponins and flavonoids. Previous studies have suggested that licorice cures wounds (Rao, 1993). One study confirmed that licorice cream of 10% is a potent healing agent even better than phenytoin cream (Arzi *et al.*, 2003). However, a study found that Licorice extract in 10% concentration was shown not to be effective in healing of *P. aeruginosa* infected burn wounds (Tanideh *et al.*, 2014). Isoliquiritin is an important flavonoid in licorice. Isoliquiritin has anti-ulcer, anti-HIV, anti-bacteria, spasmolysis, analgesia, anti-oxidation, anti-inflammation, anti-depression and other effects. One study confirmed that the effects of isoliquiritin on granuloma angiogenesis was obvious (Kobayashi *et al.*, 1995). However, whether or not isoliquiritin has a promoting effect on skin wound healing has not been reported. After scald, wound tissue cells and inflammatory cells release a large number of inflammatory factors such as TNF-α, IL-6 and IL-1β, causing local or even systemic inflammatory reactions, which in turn induce wound bleeding, lysosomal enzyme release in cells and tissue ulceration and necrosis. As a key component of wound repair, angiogenesis is a dynamic process regulated by a variety of factors. The angiopoietin/receptor (Ang/Tie) system is an effective wound angiogenesis factor found in recent years, and it plays an important role in controlling neovascular sprouting, vascular remodeling and regulation of endothelial cell status. Both Ang-1 and Ang-2 are endogenous ligands of the tyrosine kinase receptor of vascular endothelial cells Tie-2, and can both bind to Tie-2. A study suggested a role for the angiopoietin/Tie-2 system in normal wound repair and scarring (Staton *et al.*, 2010). At present, the effect of isoliquiritin on the expression levels of wound inflammatory factors (TNF-α, IL-6 and IL-1β) and Ang/Tie system proteins (Tie-2, Ang-1 and Ang-2) in scalded rats is still unclear.

The aim of present study was to investigate the effect and possible mechanism of isoliquiritin on rats' skin wound repair, in order to provide reference for the application of isoliquiritin in wound repair.

*Materials and methods*

A total of 36 male SPF-class Wistar rats were selected as experimental subjects. The body weight was 200-256 g, with the average of 227.54±6.72 g. All rats were randomly divided into control group, deep II degree scald group (model group) and isoliquiritin group, with 12 rats in each group. The rat model of deep II degree scald wound was prepared as follows: rats were anesthetized by intraperitoneal injection of 10% chloral hydrate (30 mg/kg), depilated on the back, and prepared a scalded wound with a diameter of about 3.5 cm by 96 °C thermostatic

steam on the back, which was placed for 5 seconds; at 1 h after scald, 10 mL of lactic acid-Linger solution was intraperitoneally injected for resuscitation. One day after the preparation of the scalded rat model, the full-thickness skin of the wound was taken. The pathological section of HE staining confirmed the deep II degree scald.

No treatment was done in the control group. The rats in the model group were injected with the same amount of normal saline as the isoliquiritin group, and the rats in the isoliquiritin group were treated with tail vein injection of 20 mg/kg isoliquiritin once a day for 10 consecutive days. Each group of rats was housed separately at room temperature with free diet, drinking water and defecation. No rats died in each group during the experiment.

At 24 h after the last administration, the wound area was determined by transparent film tracing and weighing method. The formula was: wound healing rate = (original wound area - unhealed wound area) / original wound area × 100%.

At 24 h after the last administration, the rats in the control group, the model group and the isoliquiritin group were anesthetized by intraperitoneal injection of chloral hydrate (3 mL/kg); the skin tissue in the hair removal area at the back of rats in the control group, wound tissue of rats in the model group and the isoliquiritin group were removed rapidly. The paraffin section was made with part of the tissue, and the remaining tissues were frozen and stored by liquid nitrogen for subsequent experimental use. Under the 100× microscope, 4 non-continuous paraffin sections were randomly selected from each group, and 5 non-overlapping fields/sections were randomly taken. The thickness of the epidermis, dermis and collagen fibers of each group was quantitatively determined by Image Pro Plus 6.0 software.

Appropriate amount of wound tissue of the model group and the isoliquiritin group as well as the skin tissue without the subcutaneous fat of the control group were taken, cut, ground with 1 mL of normal saline/100 mg tissue for homogenate, and centrifuged at 10,000 rpm for 15 min at 4 °C. The supernatant was assayed for the levels of inflammatory factors TNF-α, IL-6 and IL-1β in each group by ELISA.

The expression of Ang-1, Ang-2 and Tie-2 proteins in skin tissue of each group was detected by Western blotting. The specific methods were as follows: appropriate amount of the wound tissue of the model group and the isoliquiritin group as well as the skin tissue without the subcutaneous fat of the control group were taken and added appropriate amount of RIPA lysate containing protease inhibitor (protease inhibitor-RIPA lysate 1:100) to lyse the tissue. Total protein was extracted, protein concentration was measured by BCA, and protein was denatured. 12% sodium lauryl sulfate-polyacrylamide gel

(SDS-PAGE) was used for electrophoresis. Protein was transferred onto PVDF membrane by wet transfer, sealed with 5% skim milk powder for 2 h at room temperature, and incubated overnight at 4 °C with primary antibodies (Ang-1, Ang-2 and Tie-2); horseradish peroxidase was added to label secondary antibodies after TBST rinse, and the tissue was incubated for 1 h at room temperature; after TBST rinse, ECL chemiluminescence, development and photographic fixing were performed accordingly. A semi-quantitative analysis was conducted with GAPDH as an internal reference protein and gray ratio of the target protein to GAPDH as the relative expression abundance of the protein.

Data was analysed in SPSS version 25(SPSS Inc., Chicago, IL, USA). Mean value±SD was calculated for measurement data. Measurement data were compared between two groups using independent sample t test. Multiple comparisons of the measurement data were done by ANOVA with SNK test. The $p$-value less than 0.05 was regarded as significant.

*Results*

At 24 h after the last administration, the wound healing rate of the isoliquiritin group was (90.65±3.42) %, which was significantly lower than that of the model group (75.38±4.17) %. The difference between the two groups was statistically significant (p =0.024).

The difference in the thickness of epidermis, dermis and collagen fibers in the control group, the model group and the isoliquiritin group were statistically significant (all $p<0.001$, Table I). Compared with the control group, the thickness of epidermis and dermis of the model group was thinner ($p<0.001$, Table I), and the width of the subdermal collagen fibers also significantly decreased (p<0.001). Compared with the model group, the thickness of epidermis, dermis and collagen fibers of the isoliquiritin group increased significantly (all $p<0.001$, Table I).

The difference in the content of inflammatory factors TNF-α, IL-6 and IL-1β in the skin tissue of rats in the control group, the model group and the isoliquiritin group were statistically significant (all p<0.001, Table II). Compared with the control group, inflammatory factors TNF-α, IL-6 and IL-1β significantly increased in the skin tissue of rats in the model group (all p<0.001, Table II). Compared with the model group, the content of inflammatory factors TNF-α, IL-6 and IL-1β in the skin tissue of rats in the isoliquiritin group significantly decreased (all p<0.001, Table II).

The difference in the expression levels of Ang-1, Ang-2 and Tie-2 in the skin tissue of rats in the control group, the model group and the isoliquiritin group were statistically significant (all p<0.001, Table III). Compared

**Table I. Comparison of the thickness of epidermis, dermis and collagen fibers.**

| Groups | Number of mice | Epidermal thickness (µm) | | Dermal thickness(µm) | | Collagen fiber thickness(µm) | |
|---|---|---|---|---|---|---|---|
| | | Mean±SD | p-value | Mean±SD | p-value | Mean±SD | p-value |
| Control group | 12 | 45.13±1.68 | <0.001 | 106.51±5.73 | <0.001 | 64.06±3.48 | <0.001 |
| Model group | 12 | 9.25±0.34 | | 31.86±2.92 | | 12.14±0.85 | |
| Isoliquiritin group | 12 | 32.07±1.55 | | 86.42±4.09 | | 53.57±2.64 | |

**Table II. Comparison of the content of inflammatory factors in skin tissue.**

| Groups | Number of mice | TNF-α(pg/ml) | | IL-6(pg/ml) | | IL-1β(pg/ml) | |
|---|---|---|---|---|---|---|---|
| | | Mean±SD | p-value | Mean±SD | p-value | Mean±SD | p-value |
| Control group | 12 | 15.36±0.91 | <0.001 | 14.78±0.46 | <0.001 | 10.63±0.52 | <0.001 |
| Model group | 12 | 58.44±1.53 | | 57.51±2.04 | | 45.49±1.67 | |
| Isoliquiritin group | 12 | 28.82±0.76 | | 23.21±1.55 | | 21.07±1.09 | |

**Table III. Comparison of the expression levels of Ang-1, Ang-2 and Tie-2 Proteins in the skin tissue.**

| Groups | Number of mice | Ang-1 /GAPDH | | Ang-2/GAPDH | | Tie-2 /GAPDH | |
|---|---|---|---|---|---|---|---|
| | | Mean±SD | p-value | Mean±SD | p-value | Mean±SD | p-value |
| Control group | 12 | 3.371±0.045 | <0.001 | 0.268±0.011 | <0.001 | 1.315±0.038 | <0.001 |
| Model group | 12 | 0.416±0.013 | | 1.605±0.214 | | 0.347±0.013 | |
| Isoliquiritin group | 12 | 1.957±0.021 | | 0.803±0.107 | | 0.682±0.019 | |

with the control group, the expression levels of Ang-1 and Tie-2 proteins in the skin tissue of rats in the model group significantly decreased (both p<0.001, Table III); and the expression level of Ang-2 protein significantly increased (p<0.001, Table III). Compared with the model group, the expression levels of Ang-1 and Tie-2 proteins in the skin tissues of rats in the isoliquiritin group significantly increased (both p<0.001, Table III); and the expression level of Ang-2 protein significantly decreased (p<0.001, Table III).

*Discussion*

Preliminary evaluation of this experiment showed that the wound healing rate of the isoliquiritin group was significantly higher than that of the model group 24 h after the last administration. Compared with the model group, the thickness of epidermis, dermis and collagen fibers in the isoliquiritin group obviously increased. It suggested that isoliquiritin can significantly promote wound healing and increase skin thickness. Wound repair of skin burns is an extremely complicated pathological process. Wound repaired tissues include blood vessels, epidermis, dermis, and skin appendages (such as hair, sebaceous glands, nerves, and subcutaneous tissues). Therefore, cells involved in wound repair include endothelial cells, macrophages, neutrophils and platelets. During the wound repair process, various cytokines and growth factors released by the above cells are directly involved in various stages of wound repair.

TNF-α is a pro-inflammatory cytokine secreted mainly by mononuclear-macrophages. It has obvious stimulating effects on cell proliferation, differentiation, granulation tissue formation, and new blood vessel growth. It plays an important role in mediating pathophysiological processes, such as shock, inflammation, fever, organ damage, etc (Tan et al., 2017). TNF-α is a second proinflammatory cytokine that contributes to a chronic wound state (Larouche et al., 2018). IL-6 is an important indicator that is sensitive to the severity of inflammation and tissue damage in the body (D'Arpa et al., 2017). Related research showed that an inflammatory response after cutaneous wounding was a prerequisite for healing, and inflammatory cytokines, such as IL-6, might be intimately involved in this process (Gallucci et al., 2000). IL-1β is mainly secreted by activated mast cells and mononuclear macrophages. It can induce cells to secrete inflammatory substances, such as various cytokines, chemokines and acute phase proteins to produce an inflammatory response. Karam et al. (2018) revealed IL-1βwas involved in the wound healing pathway. In the early stage, the inflammatory reaction of the wound will increase the levels of TNF-α, IL-6 and IL-1β. If the wound inflammation is improved, the values of TNF-α, IL-6 and IL-1β will decrease accordingly. The results of this study showed that TNF-α, IL-6 and IL-1β significantly increased in the skin tissue of rats in the model group compared with the control group. It suggested that inflammatory factors such as TNF-α, IL-6 and IL-1β are highly expressed in deep II degree scald wound. This is consistent with other research reports (Fan et al., 2006). Further studies showed that compared with the model group, the levels of TNF-α, IL-6 and IL-1β in the skin tissue of rats in the

isoliquiritin group are significantly lower. This suggested that isoliquiritin may reduce or improve wound infection by inhibiting the secretion of inflammatory factors such as TNF-α, IL-6 and IL-1β by inflammatory cells.

Studies have shown that Ang-1 can inhibit endothelial cell apoptosis and recruit vascular support cells such as pericytes to promote endothelial cell survival and maintain vascular integrity (Magkouta *et al*., 2018). As an antagonist of Ang-1, Ang-2 is mainly secreted by endothelial cells. It can increase the permeability of blood vessels, causing proteases, cytokines, inflammatory factors and the like to penetrate into local tissues and participate in inflammatory reactions (Zhang *et al*., 2015; Cha *et al*., 2018). Therefore, the angiopoietin/ receptor system plays an important role in maintaining vascular integrity and endothelial cell homeostasis. Ang-1 activates the Tie-2 receptor to maintain the resting state of endothelial cells. Ang-2 breaks the homeostasis of endothelial cells, increases the reactivity of endothelial cells to exogenous stimuli, and promotes the activity of inflammatory factors and angiogenesis-related factors. It can be said that the angiopoietin/receptor system is the link between angiogenesis and inflammation. Difficulties in healing of skin wounds may be related to the continuous increase of local Ang-2 expression level and the decrease of Ang-1 and Tie-2 expression level (Kämpfer *et al*., 2001). Elevated levels of Ang-2 not only lead to prolonged inflammatory processes, but also antagonize the effects of Ang-1, leading to disorders of new capillary maturation, increased permeability, and slow wound healing (Shyu, 2006). The results of this study showed that compared with the model group, the expression levels of Ang-1 and Tie-2 proteins in the skin tissue of rats in the isoliquiritin group significantly increased; and the expression level of Ang-2 protein significantly decreased. It suggested that isoliquiritin may, on the one hand, increase the expression level of Ang-1 and Tie-2 in skin wound tissue, promote vascular remodeling and new vessel maturation, prevent vascular leakage, inhibit endothelial cell apoptosis, and promote granulation tissue growth, thus promoting wound healing; on the other hand, it may reduce the expression of Ang-2, the inflammatory response caused by Ang-2 and the antagonism of Ang-2 on Ang-1, thereby promoting skin wound healing. However, the specific action mechanism of isoliquiritin on deep II degree scald wound healing in rats requires further experimental research.

## Conclusions

Isoliquiritin can promote the healing of acute wounds in rats. The mechanism may be related to increasing skin thickness, inhibiting the release of inflammatory factors, up-regulating the expression of Ang-1 and Tie-2 proteins and down-regulating the expression of Ang-2 protein.

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