

Original Research Article

Healing effect of Shaoshang Yuhe yihao on burns in rats

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Abstract

Purpose: To investigate the healing effect of Shaoshang Yuhe yihao (SYY) in a deep second-degree burns model in rats.

Methods: Female Wistar rats, weighing 200 - 220 g, were subjected to deep second-degree skin burns by electrical scald instrument. The animals were divided into three groups as follows: (1) second-degree burns model (control) group, (2) moist exposed burns ointment-treated group (positive control drug), and (3) SYY groups. The rats were treated with SYY for 21 consecutive days. The rate of healing, scabs dropped time and re-epithelialization time were observed every three days for 21 days after burns injury. Samples were collected from treated rats after sacrificing the animals on the 1st, 2nd, 5th, 9th, 14th, and 21st day post-burn creation, respectively. Plasma interleukin-1 (IL-1) levels were evaluated, and the tissue expressions of transforming growth factor-beta (TGF- β) were determined along with skin histopathology. The burned tissues were examined histologically to confirm the degree of burns and healing condition.

Results: The results showed that the scabs drop time decreased ($p < 0.05$), and healing accelerated after treatment with SYY in the burns injury rats (94.6 ± 2.6 %, $p < 0.05$). Furthermore, the expressions of TGF- β significantly increased in the tissue (329.4 ± 8.2 pg/mL, $p < 0.05$) at day 14; However, the levels of inflammatory factors in plasma decreased (11.3 ± 1.7 pg/mL, $p < 0.05$). On the 21st day after burns injury, the injured tissue treated with SYY was almost (> 90 %) covered with healthy epithelial tissue and new epidermis, and showed complete epithelialization of the burns injury area.

Conclusion: The results confirm the curative effects of SYY on burns injuries in rats. Thus, the herbal preparation has potentials to be developed for clinical application in burns cases.

Keywords: Shaoshang Yuhe yihao, Second-degree burns, Healing, Scabs drop time, Moist exposed burns ointment

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INTRODUCTION

Burn injuries, caused by heat, light, electricity, radiation and chemicals, are one of the most common and devastating forms of trauma, particularly partial- and full-thickness burns [1].

According to the report, about 5000 - 10,000 in one million people suffer from burns in China every year [2]. Depending on severity, burns are classified into three degrees [3]. Burns results in increase in free radical-mediated damage and reactive oxygen species. It can delay granulation

tissue formation, reduce angiogenesis and decrease collagen reorganization. Furthermore, due to its significantly prolonged period of rehabilitation, the healing of burn injuries is more difficult than that of ordinary wounds and is also associated with higher economic costs [4].

Current approaches to treat burns utilize antibiotics, antiphlogistics, and silver salt. They however, show major drawbacks and side effects [5]. Alternative and complementary medicines are increasingly being used because they are moderately beneficial to effective with little toxicity and are less costly than synthetic drugs. Many plants and plant-derived products have been found to possess potent wound-healing or burns healing activities [6].

Shaoshang Yuhe yihao, which is composed of *Polygonum cuspidatum* Sieb. et Zucc., *Aloe vera* var. *chinensis* (Haw.) and *Rheum palmatum* L., is a traditional Chinese medicine for treating burns. It has been used in treatment of inflammation, infection, jaundice, skin burns and hyperlipemia in China [7,8]. Several studies have evaluated the antioxidant capacity of SY Y and its anti-inflammatory activities, such as the inhibition of NF- κ B [9].

In the present study, a series of experiments were designed to investigate the effect of SY Y on burn injuries in rats.

EXPERIMENTAL

Materials

The herbal samples of Shaoshang Yuhe yihao were collected from Bozhou City, Anhui Province in China in May 2016. Taxonomic identification of the plant materials was performed by Prof Li Wu, College of Pharmacy of Hebei Chinese Medical University, China. A voucher specimen (no. SY Y 201605008) was deposited in the College of Pharmacy of Hebei Chinese Medical University, China for future reference.

Animals and model preparation

Female Wistar rats weighing 200 – 220 g were obtained from Hebei Center for Disease Control and Prevention, Shijiazhuang, Hebei. The rats had free access to feed and were allowed to acclimatize for at least one week before use. The animal experiment was approved by the Animal Care and Use Committee of Tangshan Gongren Hospital (approval ref no. 20070507) and was carried out in compliance with Directive 2010/63/EU on the handling of animals used for scientific purposes [10].

Ethyl carbamate solution was used for anesthetizing the rats after the rat hair was shaved. The top of electrical scald instrument was pressed on the skin at the back of the rats for 12 s at 80 °C. In this way, the deep second-degree burns rat model was prepared.

Experimental groups and treatments

All the rats were randomly divided into three groups of ten rats each: deep second-degree burn model group (control), moist exposed burn ointment-treated group (positive control drug) and SY Y-treated group. All the animals received a deep second-degree burns. Moist exposed burn ointment was used as the standard drug.

SY Y or moist exposed burn ointment was applied slowly with cotton bud to the burns wound area and extended slightly outside the wound area to ensure inclusion of the wound edges. The treatments were repeated twice daily for 21 days. The first application was done directly after the injury. The control group did not receive any treatment for 21 days. The wound remained exposed after the treatments.

Appearance changes

The healing rate of burned skin and scabs drop time were observed every 3 days for 21 days after burn. The rate of healing was assessed by digitized planimetry.

Sample collection

Blood samples (about 3 mL) were collected from rats by vein puncture in the mandibular region under anesthesia on the 1st, 2nd, 5th, 9th, 14th, 21st day post-burn creation, and centrifuged at 4000 rpm for 10 min at 15 °C for plasma. The blood samples were stored at -80 °C in aliquots until analysis. The full thickness of burn area was removed from different treated rats by sacrificing the animals on the 1st, 2nd, 5th, 9th, 14th, and 21st day post-burn creation, one aliquot (about 100 mg) was processed by standard histological techniques, and others samples were stored at -80 °C in aliquots until analysis.

Clinical chemistry

Plasma levels of IL-1 were analyzed with the IL-1 Assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The frozen full-thickness samples were processed by standard ELISA techniques. The tissue supernatant was analyzed with antibody magnetic beads in duplicate. TGF- β were assessed.

Statistical analysis

Values are expressed as mean ± SEM. Multiple group comparisons were performed by one-way analysis of variance (ANOVA) using SPSS 16.0 followed by Dunnett's test to detect intergroup differences. $p < 0.05$ was considered statistically significant.

RESULTS

Effect of SY Y on general observation in rats

The scab drop time was 12.2 days (range, 11 – 13 days) in control group; 10.4 days (range 9–11 days) in burn ointment (standard group); 7.1 days (range 6 – 8 days) in SY Y groups (Table 1). On the fourth day after burn, the rate of wound healing treated with SY Y was significantly greater than the control group. The burn wound was almost completely healed (> 90 %) on the 21st day, the re-epithelialization time correspondingly shortened (Table 2).

Table 1: Effect of SY Y on the scabs dropped time in rats (n = 10)

Group	Scabs dropped time(day)
Control	12.2 ± 1.2
MEBO	10.1 ± 1.4
SY Y	7.3 ± 0.8*

$P < 0.05$ and $**p < 0.01$ compared to the control group. MEBO: moist exposed burn ointment, SY Y: Shaoshang Yuhe yihao

Effect of SY Y on plasma IL-1 Level in rats

As shown in Table 3, a prominent decrease in IL-1 was observed with SY Y treatment five days

after burn. Compared with control group, plasma IL-1 level of SY Y treatment group was significantly decreased ($p < 0.05$).

Table 3: Effect of SY Y on plasma IL-1 level in rats (n = 10)

Group	Day 1(pg/mL)	Day 2(pg/mL)	Day 5(pg/mL)
Control	27.5±1.6	20.7±2.2	18.8±2.2
MEBO	18.3±2.4	16.5±2.4	14.5±2.4
SY Y	15.8±1.3	12.6±2.3	11.3±1.7

$P < 0.05$ and $**p < 0.01$ compared to the control group. MEBO: moist exposed burn ointment, SY Y: Shaoshang Yuhe yihao

Expressions of TGF-β in tissues after treatment with SY Y in rats

As shown in Table 4, the TGF-β expression of SY Y-treated group were significantly increased compared to those of the control groups. However, the levels of TGF-β reduced before the burn-skin dropped. With the beginning of re-epithelialization, the expressions of TGF-β were significantly higher than those of control groups, and reached the peak at 14th day.

DISCUSSION

Burns is a common injury in daily clinical practice. Despite the advanced clinical care and reduced mortality of scalded patients [13,14], some problems still exist such as burn wound infection, delayed wound healing and hypertrophic scarring [15]. Patients were also faced with high economic costs as well. Alternative or complementary medicines, such as traditional Chinese medicine or ethnic drugs, have been used to improve this situation [16].

Table 2: Effect of SY Y on the rate of wound healing in rats (n = 10)

Group	Day 3 (%)	Day 6 (%)	Day 9 (%)	Day 12 (%)	Day 15 (%)	Day 18 (%)	Day 21 (%)
Control	23.6±1.4	38.2±2.6	42.2±2.2	58.4±2.6	63.5±2.3	71.4±2.5	76.6±2.4
MEBO	28.6±2.2	51.3±2.4	59.2±2.8	67.2±2.2*	74.2±2.2*	88.6±2.7*	88.6±3.0*
SY Y	26.5±1.7	56.7±2.3*	63.8±1.7*	73.4±2.4*	83.5±2.5*	86.9±2.1*	94.6±2.6*

$P < 0.05$ and $**p < 0.01$ compared to the control group. MEBO: moist exposed burn ointment, SY Y: Shaoshang Yuhe yihao

Table 4: Expressions of TGF-β in tissues following treatment with SY Y in rats (n = 10)

Group	Day 1 (pg/mL)	Day 2 (pg/mL)	Day 5 (pg/mL)	Day 9 (pg/mL)	Day 14 (pg/mL)	Day 21 (pg/mL)
Control	243.7±8.1	255.2±8.5	258.6±7.5	257.4±8.1	276.2±8.1	327.3±8.7
MEBO	256.4±7.4	257.1±7.3	262.1±8.0	277.1±7.5*	288.4±7.6*	353.4±8.3*
SY Y	308.3±9.1*	303.2±9.5*	308.4±8.4*	318.6±8.3*	329.4±8.2*	282.2±8.4

$P < 0.05$ and $**p < 0.01$ compared to the control group. MEBO: moist exposed burn ointment, SY Y: Shaoshang Yuhe yihao

In this study, different concentrations of SY Y have been shown to significantly accelerate the burn-wound healing in rats. The anti-inflammatory effect was a main response in the body after burn injuries. The tissue water content was a key index in anti-inflammatory, and its decrease displayed inflammatory was weakened. The levels of inflammatory factors such as IL-1 on plasma were analyzed at the early stage of burn.

Previous studies suggested that IL-1 could accelerate the anti-inflammatory activity at low concentration at the early stage of burn, and high concentration could cause systemic inflammatory response syndrome [17].

These results confirmed significant decrease in the levels of IL-1 of rats after treatment with SY Y on plasma ($p < 0.05$). In the present study, SY Y were found to promote anti-inflammatory activity by reducing the levels of inflammatory factors.

Many types of cytokines and growth factors are responsible for inflammation, re-epithelialization, wound contraction, formation of granulation tissue and neovascularization during the burn-wound healing process [18]. TGF- β plays a key role through regulating different cellular functions, and enhancing granulation tissue formation and collagen formation [19]. In addition, TGF- β has also been reported to be able to promote wound contraction through its direct induction of alpha smooth muscle act in expression in fibroblasts [20].

These results suggest that TGF- β expressions were observed at the beginning of re-epithelialization, and significantly increased in SY Y-treated groups on the 14th day. Compared with some previously published papers on the use of traditional Chinese medicines for burn healing [11,16,17], Shaoshang Yuhe yihao showed complete epithelialization of the burns area on the 21st day after infliction of burns by regulating the expressions of TGF- β and plasma inflammatory factors levels.

CONCLUSION

The findings of this study show that SY Y facilitates wound healing in an experimental animal model by influencing anti-inflammatory response and expressions of cytokines and growth factors in burns repair process.

Thus, the plant material can potentially be developed into a suitable therapeutic product for burns management.

DECLARATIONS

Conflict of Interest

The authors declare that there is no conflict of interest with regard to this study.

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. Gang Xu designed all the experiment and revised the paper. Hai Jiang and Yu Liu performed the experiment, and Xuan Liu wrote the paper.

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