

Original Research Article

Effect of Paris polyphylla extract on second-degree burns in rats

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Abstract

Purpose: To investigate the healing effect of Paris polyphylla extract (PPE) on second-degree burns in rats.

Methods: Male Sprague Dawley (SD) 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 burn model (control) group, (2) burn model treated with 1 % silver sulfadiazine (SSD) group, and (3) burn model treated with 120 mg·mL⁻¹ PPE group. On days 3, 7 and 14 following the administration of the drug/extract, wound area and histopathological changes in rat epidermis were evaluated for the three groups. The minimum inhibitory concentration (MIC) of PPE on *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* were also assessed.

Results: On day 14, the mean wound area of PPE treatment group ($0.21 \pm 0.04 \text{ cm}^2$) was significantly smaller than that of the control rats ($2.78 \pm 0.18 \text{ cm}^2$, $p < 0.01$). Histological results indicate that inflammatory cells disappeared and were replaced by new granulation tissue in the group treated with 120 mg·mL⁻¹ PPE by day 14. Compared with SSD group rats, the inflammatory cells and fibroblast and granulation tissues of burnt rats with burns and treated with 120 mg·mL⁻¹ PPE decreased significantly. The antibacterial data revealed that the MIC of PPE against *S. aureus*, *P. aeruginosa* and *E. coli* was 2.35, 8.2 and 4.70 mg·mL⁻¹, respectively.

Conclusion: Paris polyphylla is an effective medicinal herb that holds promise for the treatment of second-degree burns.

Keywords: Paris polyphylla, Second-degree burns, Wound healing, Antibacterial, Inflammatory cells, Granulation tissues, Silver sulfadiazine

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INTRODUCTION

Every year, millions of people suffer major disabilities or even death from burns, caused by hot water, flame and boiling oil. People suffer from burns due to domestic and industrial accidents, which along with enormous cost of treatment, cause mortality and considerable morbidity [1]. According to the World Health Organization (WHO), there were 300,000 deaths worldwide due to burns in 2012, with 96 % of

these deaths occurring in developing countries [2]. Burns are one of the health problems in modern societies associated with irreparable damage to patients and family relationships [3].

Currently, SSD is the most used topical treatment for burns due to its potent antimicrobial efficacy. However, it has been reported that silver gets absorbed systemically, posing problems in prolonged use and systemic complications such as neutropenia, methemoglo-

binemia and renal toxicity [4]. Therefore, it was necessary to find more efficient agents with fewer side effects for treatment of burns.

Paris polyphylla, a traditional Chinese medicinal herb, is widely distributed in southern China. The root of *Paris polyphylla* has been used in treatment of inflammation, infection, jaundice, skin burns and hyperlipemia in China and Japan [5]. The antioxidant capacity of *Paris polyphylla* extract [6] and its anti-inflammatory activities, such as inhibition of NF- κ B [7] have been evaluated in several studies. In the present study, the aim of the study was to investigate the healing effect of *Paris polyphylla* extract on deep second-degree burn wounds in rats.

EXPERIMENTAL

Materials

Herbal samples of *Paris polyphylla* were collected from Shiyan City, Hubei Province in China in September 2015. Taxonomic identification of the plant was performed by Prof ShanLi, College of Pharmacy of Soochow University in China. A voucher specimen (no. PPE 201509016) was deposited in the College of Pharmacy, Soochow University, China for future reference.

Fifty grams of the powdered sample was placed in a round bottom flask with 70 % ethanol (1: 8, w/v) for reflux extraction at 70 °C. It was extracted twice, for 1.5 h each time. Thereafter, the ethanol which constituted the extracting solution was removed using a rotary evaporator, and the remaining solution was concentrated into 100 mL volume as the experimental drug. The working concentration of PPE was equivalent to 120 mg·mL⁻¹ (extract weight/final volume).

Animals and model preparation

Male SD rats weighing 200-220 g were obtained from Jiangsu Center for Disease Control and Prevention, Nanjing, Jiangsu. 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 Soochow University (approval reference no. 20111008) and was carried out in compliance with Directive 2010/63/EU on the handling of animals used for scientific purposes [8].

After the rat hair was shaved, 20 % concentration of ethyl carbamate solution was used to anesthetize the rats. The top of electrical scald

instrument (Changhai Hospital of Second Military Medical University, China) was pressed onto the back skin of rats for 12 s at 80 °C to prepare second-degree burn model.

Groups and treatments

All rats were randomly divided into three groups of thirty-five rats each: second-degree burn model group, SSD-treated group and PPE-treated group. All the animals in the second-degree burn model, SSD-treated and PPE-treated groups received deep second-degree burn. SSD cream (1 %) was used as the standard drug.

In a preliminary study, the dose-response properties of PPE and silver sulfadiazine was examined to determine the optimal doses, and the most effective in the wound healing was 0.4 g SSD or 1 mL PPE per wound (data not shown). One mL of PPE or 0.4 g of SSD was applied slowly with cotton bud to the burn wound areas and extended slightly outside the wound areas to ensure inclusion of the wound edges. The treatments were repeated twice daily for 14 days. The first application was done directly after the injury. The control group did not receive any treatment for 14 days. The wound remained exposed after the treatment.

Wound area measurement

On days 3, 7 and 14, following treatment, mean wound area of the control, SSD and PPE group rats was measured. The wounds were photographed with a digital camera in order to calculate the wound surface areas (WSA) with Autocad software 2010 (made by Autodesk Co. Ltd). The change in wound surface area in a given day (WSA_{day-x}) was expressed as a percentage of the wound area on the second day (WSA_{day-2}) using Eq 1.

$$WSA = \{(WSA_{day-2} - WSA_{day-x})/WSA_{day-2}\}100 \dots\dots (1)$$

Histological study

Wound skin tissue samples were taken, using a scalpel, from the control, SSD and PPE groups on days 7 and 14 for histological observation. The skin tissues were fixed with 10 % formalin. After fixation, the samples were embedded in paraffin, cut into 3 mm frozen sections with a cryostat microtome, and then stained with hematoxylin eosin reagent. A collagen fiber, inflammatory cell, blood vessel and granulation tissue of the skin tissues were examined under a microscope.

Antibacterial test

Agar dilution was used to determine the minimum inhibitory concentrations (MIC) of PPE on *Escherichia coli* (ATCC23276), *Staphylococcus aureus* (ATCC26542) and *Pseudomonas aeruginosa* (ATCC25338). The three bacteria were all diluted to 1.5×10^5 CFU·mL⁻¹ with 0.9 % sodium chloride solution. The experiment was repeated three times to determine the MIC.

Statistical analysis

The data are expressed as mean \pm SEM. Multiple group comparisons were performed using one-way analysis of variance (ANOVA) with SPSS 16.0 software followed by Dunnett's test to detect intergroup differences. $P < 0.05$ was considered significant.

RESULTS

Wound healing

The wound area of burnt rats reduced progressively when applied with SSD or 120 mg·mL⁻¹ PPE treatment. The wounds treated with PPE healed more quickly than those of the control group. The average wound area of PPE and SSD treatment groups healed more quickly than the control groups by day 14 ($p < 0.01$, Figure 1), suggesting that both treatment probably accelerated the process of wound healing, hence, there was a significant reduction in the wound area. More, the average wound area of burned rats treated with SSD was significantly smaller than that of the model rats on day 14 ($p < 0.01$).

Histological features

After the initial burning and elimination of necrotic tissue, a second-degree burn showing muscular and adipose tissues appeared, and infection was absent. Histological findings of the wounded skin, treated with SSD and PPE on day 7 and 14 are investigated. On day 0, collagen fiber was necrotic, inflammatory cells infiltrated below the striated muscles and vascular engorgement and necrosis were seen in the burn skin of model rats. On day 7, there was severe infiltration of inflammatory cells and some fibroblast and granulation tissues appeared in the skin of control rats. The burned rats treated with SSD or PPE showed decreased inflammatory cells significantly and much fibroblast and granulation. By day 14, inflammatory cells were still seen and some fibroblast and granulation tissues grew in the burnt skin of the control rats. The inflammatory cells disappeared and new granulation tissue, collagen fibers and epithelialization progressed very quickly in rats treated with SSD or PPE.

Antibacterial activity

MIC values of PPE for *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* were 2.35, 8.2 and 4.70 mg·mL⁻¹, respectively.

DISCUSSION

Thermal burn injury is still a major cause of death and disability in the world and its healing process is a challenge in modern medicine. Burns on human body may be treated by different methods depending on the extent and severity of the burn.

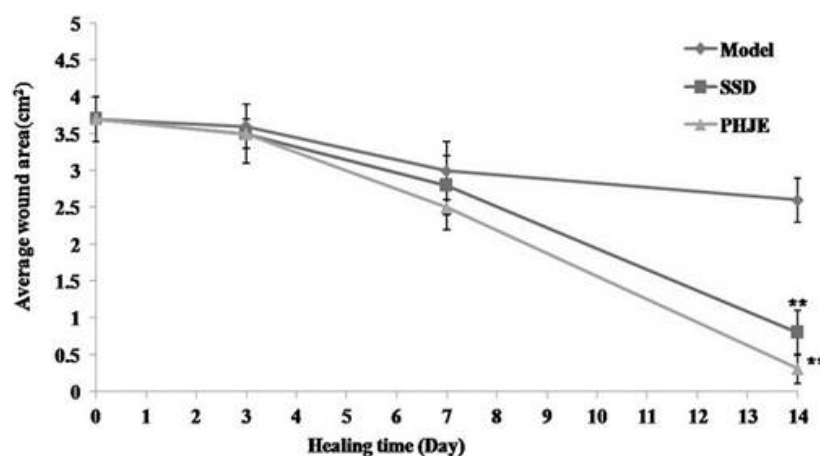


Figure 1: Evaluation of the wound area as a function of time. Data are expressed as means \pm SEM ($n = 10$). Differences between the model and the treated groups were significant ($*p < 0.05$ and $**p < 0.01$) compared to the model

SSD is bactericidal on a wide variety of bacteria, so it is commonly used to prevent and treat infections of second and third degree burns. Recent studies revealed that SSD ointment has positive effects on the proliferation of fibroblasts which are the main source of collagen and fibronectin [9]. However, current reports suggest that silver-based products show side effects and researchers are making efforts to seek for better topical antimicrobial products [10].

For the treatment of burn, the important method is to control bacterial infection. The common bacteria isolated from burned patients was *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. SSD is the most used topical treatment for burn injury due to its anti-microbial efficacy. However, it has systemic complications such as neutropenia, methaemoglobinemia and renal toxicity. Traditional Chinese medicine has the advantage of inhibiting bacterial growth with few side effects. It is reported that gallic acid was one constituent of *Paris polyphylla*, and showed strong antibacterial activity [11]. In recent years, there has been a growing interest in alternative medicines and natural medicinal products for the local treatment of wounds due to the high costs of traditional drug treatments [12]. Skin integrity is restored by a physiological process aimed at repairing the damaged tissues. The healing process proceeds in four phases: hemostasis, inflammation, proliferation and remodeling [13]. The time required for healing of deep second-degree burns would be three to six weeks or more, and there will be a scar tissue that may undergo hypertrophy and contract itself [14]. In our study, PPE accelerated the healing of deep-degree burn wound and prevented infection effectively. Burn wounds treated with PPE recovered well by day 14. However, the wounds of the control group still showed severe inflammatory cell infiltration and the epidermis of burn was healed.

CONCLUSION

The findings from this study indicate that could accelerate the healing of second-degree burns and has a strong antibacterial activity. Thus, PPE has the potential to be developed into a therapeutic product for burn treatment.

DECLARATIONS

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors 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 them.

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