Hemostatic, anti-inflammatory and antibacterial effects of Sanqixiantao dressing in vivo and in vitro

Xiaohua Qin¹, Fang Hu¹, Sudan Wu¹ and Jie Yun²*

¹Department of General Surgery, ²Department of Nursing, Teaching Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu 610072, PR China

*For correspondence: Email: yunjiecdtcm@163.com; Tel: 028-87783502; Fax: 028-87765097

Sent for review: 24 April 2017 Revised accepted: 21 August 2017

Abstract

Purpose: To study the hemostatic, anti-inflammatory and antibacterial effects of Sanqixiantao dressing.

Methods: Sanqixiantao dressing was prepared by mixing with sanqixiantao extract (8%) with membrane-forming matrix (5:4:9:2 volume ratio of polyvinyl alcohol: Na CMC: gelatin: glycerol). Rats with local surface wounds were used to evaluate the effects of Sanqixiantao dressing on hemostatic time, wound healing time and infection rate. Serum levels of tumor necrosis factor (TNF)-α and interleukin (IL) 6 were determined. The anti-inflammatory and analgesic effects of Sanqixiantao extracts were assessed by dimethylbenzene-induced ear edema and acetic acid-induced abdominal writhing tests. In in vitro studies, the effect of the extract on blood clotting time, and its antibacterial activities against six pathogenic bacteria (Escherichia coli, Staphylococous aureus, Pseudomonas aeruginosa, Staphylococcus epidermidis, Clostridium perfringens and Clostridium tetani) were evaluated.

Results: Sanqixiantao dressing significantly decreased hemostatic time (p < 0.01), wound healing time (p < 0.01) and infection rate (10 vs 100 %), when compared to control rats. Sanqixiantao extract significantly shortened blood clotting time in vitro (p < 0.01), and showed antibacterial activities against E. coli (minimum inhibitory concentration, MIC: 0.4 mg/mL, MBC: 1.6 mg/mL), S. aureus (MIC: 0.8 mg/mL, minimum bacterial concentration, MBC: 3.2 mg/mL), P. aeruginosa (MIC: 0.8 mg/mL, MBC: 3.2 mg/mL), S. epidermidis (MIC: 1.6 mg/mL, MBC: 3.2 mg/mL). Besides, Sanqixiantao extracts (100, 200, 400 and 600 mg/kg) dose-dependently decreased dimethyl-benzene-induced ear edema and acetic acid-induced abdominal writhes in mice (p < 0.05, p < 0.01, p < 0.01, p < 0.01).

Conclusion: The results demonstrate that Sanqixiantao dressing has significant hemostatic, anti-inflammatory and antibacterial effects in vivo and in vitro, and thus provide some support for the therapeutic application of Sanqixiantao dressing for treating skin wounds.

Keywords: Sanqixiantao dressing, Acute skin wound, Hemostatic, Anti-inflammatory activity, Antibacterial effect, Herbal medicine

INTRODUCTION

The skin is the largest organ of human body and provides physiological barrier against infections [1,2]. Acute skin wounds usually occur in field battles, traffic accidents and in daily life [3]. Normally, small wounds heal without much medical intervention, but large wounds require timely medical attention [4]. Most of the available drugs currently used for wound healing are synthetic chemicals [5]. These drugs are expensive and are associated with some side-effects [6,7]. Thus, there is needed to evolve natural, newer and safer methods for treating acute wounds.
In recent years, herbal therapy have been shown to be beneficial in curing various diseases [8,9]. In addition, these alternative herbal remedies are reliable, inexpensive and of low toxicities [9,10].

Sanqixiantao dressing is a preparation in Teaching Hospital of Chengdu University of Traditional Chinese Medicine (Chengdu, PR China) and it is used in the hospital for treating acute wounds. The dressing is derived from six herbs (Panax notoginseng, Veronica peregrine, Rhus chinensis, Bletilla striata, Rheum palmatum, Glycyrrhiza uralensis); polyvinyl alcohol, carboxymethylcellulose sodium (CMC-Na), gelatin, and glycerol. Although Sanqixiantao dressing is an effective alternative clinical remedy for treating acute skin wounds, there are so far, no experimental data regarding its pharmacological activities.

In this study, the hemostatic, anti-inflammatory and antibacterial effects of Sanqixiantao dressing were investigated.

**EXPERIMENTAL**

**Plant materials**

The herbs that make up Sanqixiantao extract (Panax notoginseng, Veronica peregrine, Rhus chinensis, Bletilla striata, Rheum palmatum, Glycyrrhiza uralensis) were obtained from the dispensary of Traditional Chinese Medicine of Teaching Hospital of Chengdu University of Traditional Chinese Medicine (Chengdu, China).

**Chemicals**

Tumor necrosis factor (TNF) α and interleukin (IL) 6 ELISA kits were purchased from the Biosource International Co. (Camarillo, CA, USA). Yunnanbaiyao powder was purchased from the Yunnanbaiyao Co. (Kunming, China). Polyvinyl alcohol, CMC-Na, gelatin and glycerol were purchased from the Sigma China Co. (Shanghai, China). All other used regents were analytical grade.

**Bacterial strains and animals**

Bacterial strains used in this research were obtained from ATCC (MD, USA). SD rats, KM mice and rabbits were obtained from the Shanghai Laboratory Animal Research Center (Shanghai, China). The animal protocols were approved by the Animal Care and Use Committee of Teaching Hospital of Chengdu University of Traditional Chinese Medicine (approval no. 201607-JA-034). The animals were handled according to the standard protocols for the use of laboratory animals [11].

**Preparation of Sanqixiantao extracts and Sanqixiantao dressings**

The six herbal were ground and decocted 3 times by deionized water. Thereafter, the extracts were pooled and filtrated, and the filtrate was dried by vacuum drying apparatus under 50°C to yield the Sanqixiantao extract.

The Sanqixiantao dressing was prepared by mixing the extract (at 8 % incorporation) with forming membrane matrix (5:4:9:2 volume ratio of Polyvinyl alcohol: CMC-Na: gelatin: glycerol).

**Determination of therapeutic effects of Sanqixiantao dressing on localized surface wound in rat**

A total 30 SD rats were divided into 3 groups namely: control groups, positive group and Sanqixiantao dressing group (n = 10). For the positive group, Yunnanbaiyao powder, a well-known traumatic drug in China [12], was used as the positive drugs.

Rats with local wound rats were prepared as follows: The rats were anesthetized by intraperitoneal injection (i.p.) of 1 % pentobarbital sodium at doses of 40 mg/kg. Thereafter, a 1.5 × 1.5 cm bleeding wound was made on the backside of the rat. Subsequently, drugs were administered topically on the wounds, and hemostatic time, wound healing time and infection rate were evaluated in over a period of 10 days [13]. During the study, blood sample from each rat was collected 5 days after surgery by orbital blood sampling, and the serum levels of TNF-α and IL-6 were determined by commercial ELISA kits.

**Determination of blood coagulation time in vitro**

Rabbit blood sample was collected form the ear vein using EDTA vacuum anticoagulant tubes. Then, the anti-coagulated plasma was prepared by centrifugation (3000 rpm, for 20 min). Subsequently, clotting time was determined in vitro using plate method for blood coagulation and test tube method according to the previous reports [13].

**Antibacterial assay**

The antibacterial activities of sanqixiantao extracts against 6 pathogenic bacteria that cause wound infection were determined. These bacteria
include *Escherichia coli* (ATCC 35218), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus epidermidis* (ATCC 12228), *Clostridium perfringens* (ATCC 13124), and *Clostridium tetani* (ATCC 19406). The final concentrations of the extract in bacterial culture medium were 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 and 12.8 mg/mL. The MIC and MBC values were determined as described earlier [14].

**Dimethylbenzene-induced ear edema test**

The effect of *Sanqixiantao* extract on dimethylbenzene-induced ear edema test in mice was according to the method outlined previously [15]. *Sanqixiantao* extracts and positive drugs (Indometacin) were administered orally. After 40 min of drug treatment, dimethylbenzene was topically applied to the right ear of mice, and the mice were sacrificed under anesthesia (1 % sodium pentobarbital, 40 mg/kg, ip). The degree of ear edema was determined.

**Acetic acid-induced abdominal writhing test**

The analgesic effect of *Sanqixiantao* extract was evaluated by acetic acid-induced abdominal writhing test according to previously reported method [16]. The *sanqixiantao* extract and positive drug (indometacin) were administered orally. After 40 min of the drug treatment, acetic acid (0.75 %, 10 mL/kg) was intraperitoneal administered to mice. The number of writhes was counted, starting 3 min after acetic acid treatment over a period of 12 min.

**Statistical analysis**

Data are expressed as mean ± SD. One way analysis of variance (ANOVA) was used to compare the means between two groups. Data analysis was carried out by SPSS 17.0 software. *P* < 0.05 was considered statistically significant.

**RESULTS**

**Effect of *Sanqixiantao* dressing on localized wound surface**

As shown in Table 1, in control rats, the hemostatic time was 95.83 ± 5.74 s; after treatment with *sanqixiantao* dressing, the hemostatic time significantly decreased (47.31 ± 4.85 s, *p* < 0.01) when compared with rats in the control group. Furthermore, would healing time was significantly decreased by treatment with *sanqixiantao* dressing (3.8 ± 0.80 day vs. 5.8 ± 1.32 day, *p* < 0.01). In addition, *sanqixiantao* dressing significantly decreased infection rate of the wounds (10 vs 100 %).

Results from ELISA (Table 2) show that serum levels of TNF-α (932.18 ± 374.49 pg/mL vs 217.81 ± 65.75 pg/mL, *p* < 0.01) and IL-6 (596.92 ± 147.36 pg/mL vs 132.86 ± 28.35 pg/mL, *p* < 0.01) were significantly decreased after treatment with *Sanqixiantao* dressing, when compared with control rats. This suggests that the dressing possesses significantly anti-inflammatory effect, which was better than that of the positive drug (*Yunnan Baiyao* powder).

**Sanqixiantao** extract shortened blood clotting time *in vitro*

The results of the plate method and tube method (Table 3) revealed that *sanqixiantao* extracts (0.2, 0.4, 0.8, 1.6, 3.2 and 6.4 mg/mL) had significantly higher blood coagulation effects (*p* < 0.01) when compared with control. This effect was also concentration-dependent.

<p>| Table 1: Effect of <em>sanqixiantao</em> dressing on hemostatic time, wound healing time and infection rate on local wound surface in rat |</p>
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hemostatic time (s)</th>
<th>Wound healing time (day)</th>
<th>Infection rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>95.83±5.74</td>
<td>5.8±1.32</td>
<td>100</td>
</tr>
<tr>
<td>Positive</td>
<td>80.96±4.92*</td>
<td>4.4±1.35*</td>
<td>30</td>
</tr>
<tr>
<td>Sanqixiantao dressing</td>
<td>47.31±4.85**</td>
<td>3.8±0.80**</td>
<td>10</td>
</tr>
</tbody>
</table>

*Yunnan Baiyao* was used as the positive drug; *p* < 0.05, **p** < 0.01, vs normal control

<p>| Table 2: Effect of <em>sanqixiantao</em> dressing on serum levels of TNF-α and IL-6 in local wounded rat |</p>
<table>
<thead>
<tr>
<th>Treatment</th>
<th>TNF-α (pg/mL)</th>
<th>IL-6 (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>932.18 ± 374.49</td>
<td>596.92 ± 147.36</td>
</tr>
<tr>
<td>Positive control</td>
<td>549.54 ± 144.38**</td>
<td>276.87 ± 59.34**</td>
</tr>
<tr>
<td>Sanqixiantao dressing</td>
<td>217.81± 65.75**</td>
<td>132.86 ± 28.35**</td>
</tr>
</tbody>
</table>

*Yunnan Baiyao* was used as positive control; *p* < 0.05, **p** < 0.01, vs control
Table 3: Effect of sanqixiantao extracts on clotting time of plasma in rabbit

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plate method (s)</th>
<th>Tube method (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>264.77 ± 13.64</td>
<td>294.81 ±16.34</td>
</tr>
<tr>
<td>Sanqixiantao extracts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2 mg/mL</td>
<td>206.21 ± 13.17**</td>
<td>221.02 ± 14.52**</td>
</tr>
<tr>
<td>0.4 mg/mL</td>
<td>164.28 ± 10.03**</td>
<td>175.39 ± 9.84**</td>
</tr>
<tr>
<td>0.8 mg/mL</td>
<td>142.07 ± 9.13**</td>
<td>150.63 ± 10.35**</td>
</tr>
<tr>
<td>1.6 mg/mL</td>
<td>129.38 ± 8.03**</td>
<td>132.04 ± 8.97**</td>
</tr>
<tr>
<td>3.2 mg/mL</td>
<td>100.46 ± 7.26**</td>
<td></td>
</tr>
<tr>
<td>6.4 mg/mL</td>
<td>79.18 ± 8.07**</td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.05, **P < 0.01, vs control

Table 4: Anti-bacterial effects of sanqixiantao extracts in vitro

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Concentration (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC</td>
</tr>
<tr>
<td>Escherichia coli ATCC 35218</td>
<td>0.4</td>
</tr>
<tr>
<td>Staphylococcus aureus ATCC 25923</td>
<td>0.8</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa ATCC 9027</td>
<td>0.8</td>
</tr>
<tr>
<td>Staphylococcus epidermidis ATCC 12228</td>
<td>1.6</td>
</tr>
<tr>
<td>Clostridium perfringens ATCC 13124</td>
<td>1.6</td>
</tr>
<tr>
<td>Clostridium tetani ATCC 19406</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Table 5: Anti-inflammatory and analgesic effects of sanqixiantao extracts in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Weight of ear edema (mg)</th>
<th>Number of writhings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13.71 ± 3.53</td>
<td>31.6 ± 6.22</td>
</tr>
<tr>
<td>Positive</td>
<td>10.02 ± 3.31*</td>
<td>25.8 ± 4.78*</td>
</tr>
<tr>
<td>Sanqixiantao extract</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>10.11 ± 3.66*</td>
<td>25.7 ± 5.48*</td>
</tr>
<tr>
<td>200 mg/kg</td>
<td>9.14 ± 2.76**</td>
<td>23.8 ± 5.35**</td>
</tr>
<tr>
<td>400 mg/kg</td>
<td>7.76 ± 2.54**</td>
<td>20.4 ± 2.59**</td>
</tr>
<tr>
<td>600 mg/kg</td>
<td>6.29 ± 2.71**</td>
<td>18.3 ± 3.09**</td>
</tr>
</tbody>
</table>

Indomethacin was used as the positive drug; *P < 0.05, **P < 0.01, vs control

In vitro antibacterial activity of sanqixiantao extract

The antibacterial activities of the sanqixiantao extract are presented in Table 4. The extract produced appreciable antibacterial activities against the six tested organisms viz E. coli (MIC: 0.4 mg/mL, MBC: 1.6 mg/mL); S. aureus (MIC: 0.8 mg/mL, MBC: 3.2 mg/mL); P. aeruginosa (MIC: 0.8 mg/mL, MBC: 3.2 mg/mL); S. epidermidis (MIC: 1.6 mg/mL, MBC: 3.2 mg/mL); C. perfringens (MIC: 1.6 mg/mL, MBC: 6.4 mg/mL) and C. tetani (MIC: 0.8 mg/mL, MBC: 3.2 mg/mL).

Anti-inflammatory and anti-nociceptive activities of sanqixiantao extract

The results of anti-inflammatory and anti-nociceptive properties of the extract which were evaluated by dimethyl-benzene-induced ear edema test and acetic acid-induced abdominal writhing test, are shown in Table 5. Sanqixiantao extract showed significant dose-dependent effects on ear edema at 100, 200, 400 and 600 mg/kg, when compared with mice in control group (p < 0.05, p < 0.01, p < 0.01, and p < 0.01, respectively). In addition, the extract (at 100, 200, 400 and 600 mg/kg) dose-dependently decreased abdominal writhes induced by acetic acid, relative to the control group (p < 0.05, p < 0.01, p < 0.01, and p < 0.01, respectively). These results suggest that sanqixiantao extract possesses potential anti-nociceptive anti-inflammatory properties.

DISCUSSION

From time immemorial, herbal derived medicines have been applied in the treatment or prevention of various diseases. Herbal medicines are known to produce reliable pharmacological effects [9,17,18]. In the present study, hemostatic, anti-inflammatory and antibacterial effects of sanqixiantao dressing were demonstrated in vivo and in vitro for the first time.

Studies have indicated that medical dressings have beneficial for wound healing, especially drug-loaded dressings [19,20]. These drug-loaded dressings not only inhibit the wound growth (p < 0.05, p < 0.01, p < 0.01, and p < 0.01, respectively). In addition, the extract (at 100, 200, 400 and 600 mg/kg) dose-dependently decreased abdominal writhes induced by acetic acid, relative to the control group (p < 0.05, p < 0.01, p < 0.01, and p < 0.01, respectively). These results suggest that sanqixiantao extract possesses potential anti-nociceptive anti-inflammatory properties. 

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infections, but also promote the healing of wounds [20]. In the treatment of acute skin wounds, rapid hemostasis is a very important property of the good wound dressings [21,22]. In the present study, Sanqixiantao dressing and its extract had significant blood coagulation activities in vivo and in vitro.

Infection and inflammatory reactions are the most common complications of skin wounds, and might result in high fever and even sepsis [22,23]. Therefore, controlling inflammatory reactions and infections are essential for wound healing. The present study revealed that Sanqixiantao extracts alleviated the inflammation and significantly decreased the serum levels of pro-inflammatory cytokines such as TNF-α and IL-6 [24]. Bacterial infections usually result in the exacerbation of wounds and severe inflammatory reactions [25]. The results also demonstrated that Sanqixiantao extract had significant antibacterial activities against six pathogenic bacteria that cause wound infection (E. coli, S. aureus, P. aeruginosa, S. epidermidis, C. perfringens and C. tetani). In addition, the present investigation indicated that Sanqixiantao extract possesses strong analgesic activity. Sanqixiantao dressing shortened wound healing time of local surface wound in rats. These results suggest that Sanqixiantao dressing is beneficial for treatment of skin wounds.

CONCLUSION

The findings of this study demonstrate that Sanqixiantao dressing has significant hemostatic, anti-inflammatory and antibacterial effects in vivo and in vitro, and thus lends some support for its therapeutic application in the treatment of skin wounds.

DECLARATIONS

Acknowledgement

None.

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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Trop J Pharm Res, September 2017; 16(9): 2243


