Effects of extracts from Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) on excisional wound healing in a rat’s model

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Abstract

OBJECTIVE: To investigate the effects of extracts from Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) on the excisional wound healing in a rat’s model.

METHODS: Rats were performed a surgical lesion with a 2.0 cm resected tissue in the dorsal fascia. Following, animals were randomly divided into model group, YNB group and APE group those were respectively treated with saline, 1 mg/mL of Yunnan Baiyao and 1 mg/mL of Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) extracts. Wound contractions in days 0, 3, 7, 14 and 21 were calculated by an image analyzer. Histological analysis was analyzed using hematoxilin and eosin. Levels of tumor necrosis factor α (TNF-α), interleukin-2 (IL-2), IL-4, IL-10, transforming growth factor-β 1 (TGF-β1) and basic fibroblast growth factor (bFGF) were determined by real-time quantitative PCR.

RESULTS: Compared with that of YNB group and APE group, the skin of rats showed poor re-modeling and re-epithelization characterized by a significant decrease of neovascularization, epithelialization and fibroblast in the model group. In the APE group, levels of TNF-α and IL-2 were significantly down-regulated and IL-4 and IL-10 significantly up-regulated in contrasted with that of model group. In addition, levels of TGF-β1 and bFGF in the APE group were significantly induced compared with that of model group.

CONCLUSION: The results suggest that the extracts from Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) promote wound healing in the rats, which is associated with enhancing anti-inflammatory ability and inducing fibroblast formation.
tion, and remodeling. In the clinical, few strategies are involved into wound care and treatment in order to accelerate wound healing, such as preventing infection, attenuating swelling and reducing inflammation. Anti-microbial agents are widely used to prevent and treat infections. Anti-inflammatory drugs are recommended to reduce swelling and inflammation of the skin. Traditional Chinese Medicine (TCM) and natural compounds derived from a variety of herbs, as alternative and complementary therapies drugs, are gained great concern. In addition, numerous studies have demonstrated the effectiveness of extracts from plants such as Luhui (Aloe), Jizhuanhua (Herba seu Radix Calendulae e Officialiae), Jigucao (Herba Abri Cantoniensis), and Bai-lian (Radix Ampelopsis japonicae) in treating skin lesions. Roots of Chuanwu (Aconitum Carmichaelii) has been extensively used to treat colds, polyarthralgia, pain, heart failure, beriberi, and edema for thousands of years. Rootstock of Banxia (Pinellia ternata), has a therapeutic effect on treatment of cough, infection and inflammation. In the Shennong’s Herbal Classic of Materia Medica, it is suggested that some herbs can’t be used together to treat a well-defined disease in vivo because application of combination can result in negative effect in the body. Meanwhile, some incompatibilities of medicine are formed according long-term practices, such as Shiba Fan and Shijiu Wei. Therefore, incompatibility of Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) is example of Shiba Fan. Notably, application of Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) remained unclear in vitro. Considering here, the current study was performed to investigate the effects of the extracts from Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) extracts on wound healing and remodeling. Four animals per group were deeply anesthetized with chloralhydrate at a concentration of 350 mg/kg. Wound assessment starting from day 0, digital photograph was taken at days 0, 3, 7, 14 and 21 (Figure 1). Wound size was measured by an image analyzer (image measurement standard v 4.0.1, Bersoft, Puerto Plata, Dominican Republic) to assess the size changes during wound healing process. The full-thickness biopsy specimens of dorsal skin including wounded margin were dissected either for histological analysis, and immediately frozen in liquid nitrogen and then stored at −80 °C for real-time PCR analysis. Wound contraction % = (original wound area – specific day wound area) / original wound area × 100.

**METHODS**

### Preparation of aqueous extract

Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) were kindly provided by Hospital of Traditional Chinese Medicine of Nanyang Medical University. 350 g Chuanwu (Aconitum Carmichaelii) and 50 g Banxia (Rhizoma Pinelliae) were weighted, chopped and dried in shade, and powered mechanically. Power was immersed in water room temperature for 4 h with constant stirring, boiled distilled water for 2 h, filtered through a filter paper, concentrated by rotary evaporator R52002K (Changyu Biochemical Instrument Factory, Shanghai, China). Residual was collected and dissolved in sterilized water to form Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) extracts with a concentrate at 1 mg/mL.

### Animals and treatment

Adult male Sprague-Dawley rats (150-200 g) were obtained at Henan Animals Center for Medical Science and Research, housed under standard conditions of temperature (22 ± 2) °C, relative humidity (55% ± 5%) and light (12 h light/dark cycles). The animals care and use in the experiment were approved by Nanyang Medical University Animal Care and Use Committee. Wound healing activity was evaluated using excisional wound model. On the day 0, animals were anesthetized with 80 mg/kg ketamine and 10 mg/kg xylazine intramuscularly, and placed on operation table in its natural position. An impression was performed on area of 5 cm long × 4 cm wide, located in the thoracolumbar region. In the center trichotomized area was treated antiseptic with alcoholic clorexedine and fulfilled a surgical lesion with metallic punch with 2.0 cm in diameter resected all tissue in the dorsal fascia. The rats were randomly divided into 3 groups 20 animals per group, including model group, YNB group treated with 1 mg/mL of Yunnan Baiyao, and APE group administrated 1 mg/mL of Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) extracts. All drugs were sprayed on wound by injector for twice daily in a volume of 100 μL.

### Histological analysis

The specimen sample were fixed in fixative (60% of absolute alcohol, 30% of formaldehyde, 10% of glacial-acetic acid) and embedded with paraffin. The specimens were cut in 4 um thick sections, and then stained by routine hematoxilin and eosin (HE) for histological analysis. Briefly, the slides were deparaffinized in xylene, and rehydrated in descending order of ethanol. Following, the slides were dipped in hematoxylin, washed in tap water and dehydrated in ascending order of ethanol. Finally, the slides were stained with eosin and washed with absolute ethanol and xylene. The sections were analyzed under a light microscope (Olympus BX50, Barcelona, Spain) at 200 × magnifications.

### Quantitative real-time PCR

Total RNA was isolated from the wound tissue using TRIzol (Invitrogen Life Technologies, Shanghai, Chi-
Model group administrated with nature healing state; YNB group treated with 1 mg/mL of Yunnan Baiyao; APE group administrated 1 mg/mL of Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) extracts. YNB group: Yunnan Baiyao group; APE group: Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) extracts group. TNF-α: tumor necrosis factor α. Data are presented as mean ± standard error of mean (n = 4). Significant differences of 3, 7, 14 and 21 d compared with 0 d are designated as *P < 0.05 or **P < 0.01. Significant differences of YNB group and APE group with model group are marked as *P < 0.05 or **P < 0.01.

Table 1 Primes used in this study

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5'-3')</th>
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<tbody>
<tr>
<td>TNF-α-F</td>
<td>GAAGAGTAGTAGATGAAAGTGCACAG</td>
</tr>
<tr>
<td>TNF-α-R</td>
<td>TTACTTCCTGCCTCGTCCCTC</td>
</tr>
<tr>
<td>IL-2-F</td>
<td>GGTACGTTGCTACGTCAC</td>
</tr>
<tr>
<td>IL-2-R</td>
<td>TTACTTCCTGCCTCGTCCCTC</td>
</tr>
<tr>
<td>IL-4-R</td>
<td>GCTCCGGGTCCTCTTCCTTGTC</td>
</tr>
<tr>
<td>IL-10-F</td>
<td>CATGGGCGATACACGGGTTCCTT</td>
</tr>
<tr>
<td>IL-10-R</td>
<td>AGATCCGGTTTACCTGTTCCTTCCA</td>
</tr>
<tr>
<td>TGF-β-F</td>
<td>CCACGAGAAGAAGCGAAATGC</td>
</tr>
<tr>
<td>TGF-β-R</td>
<td>AAGTCAACCTCCTCTCCTCGG</td>
</tr>
<tr>
<td>bFGF-F</td>
<td>CCACGAGAAGAAGCAGAAATGC</td>
</tr>
<tr>
<td>bFGF-R</td>
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<tr>
<td>β-actin-F</td>
<td>GAAGAGTAGGATGAAAGTCACAG</td>
</tr>
<tr>
<td>β-actin-R</td>
<td>GAGACACCGTACAC</td>
</tr>
</tbody>
</table>

Notes: TNF-α: tumor necrosis factor α; IL: interleukin; TGF-β: transforming growth factor-β; bFGF: basic fibroblast growth factor.

**RESULTS**

**Wound healing**

Compared with that of model group, the percentage of wound contraction increased 8.9%, 56.14% (*P < 0.05), and 16.91% (*P < 0.05) at days 3, 7, and 14 in the APE treated group, respectively (Table 2). In the APE treated group, the percentage of wound contraction increased 6.95 times at day 7 compared with that of day 3 (Table 2). In addition, compared with that of day 7, the percentage of wound contraction increased 47.71% at day 14 (Table 2). On the day 21, the wounds had nearly recovered in the APE group and YNB group (Table 2).

**Histological observation of healing wound area**

Compared with that of YNB group, poor re-modeling and re-epithelialization were detected in the model group. Faster keratinization characterized with minor intraepithelial cornification was occurred in the YNB group and the APE treated group. Histopathological sections derived from APE group showed significant increase of neovascularization, epithelialization and fibroblast including of matured epidermis with keratiniza-
tion and mature hair follicles, fibroblasts in dermis.

**mRNA levels of TNF-α and IL-2**

Compared with that of day 0, expressions of TNF-α and IL-2 were significantly increased from days 3 to 21 in the model group, YNB group and APE group (Figures 1, 2). During experiment observed, expressions of TNF-α and IL-2 of model group were significantly increased in contrasted with that of YNB group (Figures 1, 2). Compared with that of model group, expressions of TNF-α and IL-2 were significantly decreased in the APE group (Figures 1, 2). mRNA level of TNF-α in the APE group decreased 46.06% ($P < 0.05$), 47.25% ($P < 0.05$) and 68.94% ($P < 0.01$) at days 7, 14 and 21 compared with that of model group, respectively (Figure 1). Expression of IL-2 in the APE group decreased 47.69% ($P < 0.05$) and 40.67% ($P < 0.05$) at days 7 and 14 with respected with that of model group, respectively (Figure 2).

**mRNA levels of IL-4 and IL-10**

Compared with that of day 0, expressions of IL-4 and IL-10 were significantly increased from days 3 to 21 in the model group, YNB group and APE group (Figure 3, 4). Compared with that of YNB group, expressions of IL-4 were significantly down-regulated in the model group at days 3 and 7 (Figure 3). In addition, expressions of IL-10 were significantly inhibited at days 3, 7 and 14. Notably, expression of IL-4 in the model group was significantly up-regulated at days 14 and 21 compared with that of YNB group. IL-10 at day 21 (Figure 3, 4). Compared with that of model group, expressions of IL-4 in the APE group increased 1.67 times ($P < 0.01$) and 77.07% ($P < 0.05$) at days 3 and 7, respectively (Figure 3). In addition, levels of IL-10 respectively increased 1.91 times ($P < 0.01$), 28.35% ($P < 0.05$) and 77.71% ($P < 0.05$) at days 3, 7 and 14 (Figure 4).

**mRNA levels of TGF-β1 and bFGF**

Compared with that of YNB group, expression of TGF-β1 was significantly down-regulated in the model group. Compared with that of model group, expressions of TGF-β1 increased more than 69.29% ($P < 0.05$) in the APE group from days 3 to 14 (Figure 5). Compared with that of YNB group, expression of bFGF was significantly inhibited in the model group. Compared with that of model group, expressions of bFGF increased more than 58.11% ($P < 0.05$) in the APE group from days 3 to 21 (Figure 6).

**DISCUSSION**

Tissue repair in the wound healing is a complex cascade involved into numerous cytokines and growth fac-

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**Table 2 Effects of Chuanwu (Aconitum carmichaelii) and Banxia (Rhizoma Pinelliae) extracts on wound contraction of rats (% ± s)**

<table>
<thead>
<tr>
<th>Group</th>
<th>0 d</th>
<th>3 d</th>
<th>7 d</th>
<th>14 d</th>
<th>21 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>0</td>
<td>6.94±1.52</td>
<td>38.51±3.71</td>
<td>75.97±2.66</td>
<td>99.34±0.65</td>
</tr>
<tr>
<td>YNB</td>
<td>0</td>
<td>7.81±1.93</td>
<td>56.64±4.18</td>
<td>90.46±1.47</td>
<td>99.96±0.04</td>
</tr>
<tr>
<td>APE</td>
<td>0</td>
<td>7.56±1.43</td>
<td>60.13±3.38</td>
<td>88.82±1.59</td>
<td>99.92±0.08</td>
</tr>
</tbody>
</table>

Notes: model group administrated with nature healing state; YNB group treated with 1 mg/mL of Yunnan Baiyao; APE group administrated 1 mg/mL of Chuanwu (Aconitum Carminichaelii) and Banxia (Rhizoma Pinelliae) extracts. YNB group: Yunnan Baiyao group; APE group: Chuanwu (Aconitum Carminichaelii) and Banxia (Rhizoma Pinelliae) extracts group. Data are presented as mean ± standard error of mean (n = 4). Significant differences of YNB group and APE group with model group are marked as $\beta P < 0.05$ or $\alpha P < 0.01$.

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**Figure 2 Effects of Chuanwu (Aconitum carmichaelii) and Banxia (Rhizoma Pinelliae) extracts on the IL-2 mRNA level in wound healing rats**

Model group administrated with nature healing state; YNB group treated with 1 mg/mL of Yunnan Baiyao; APE group administrated 1 mg/mL of Chuanwu (Aconitum Carminichaelii) and Banxia (Rhizoma Pinelliae) extracts. YNB group: Yunnan Baiyao group; APE group: Chuanwu (Aconitum Carminichaelii) and Banxia (Rhizoma Pinelliae) extracts group. IL-2: interleukin-2. Data are presented as mean ± standard error of mean (n = 4). Significant differences of 3, 7, 14 and 21 d compared with 0 d are designated as $\beta P < 0.01$ or $\alpha P < 0.05$. Significant differences of YNB group and APE group with model group are marked as $\beta P < 0.05$. 
In the TCM, many herbs play a positive role in the wound healing through acting on cytokines and growth factors. Our data revealed that treatment with Chuanwu (*Aconi-
Our results showed that the administration of Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) and Banxia (Rhizoma Pinelliae) extracts group than in model group, Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) extracts group was significantly higher than that of the model group from days 3 to 14. Since stage of between days 7 falls in the inflammation and proliferation phases, these results suggest that the application of Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) extracts in wound rats has an anti-inflammatory role on wound healing. Inflammation cascade is mainly occurred in the original stage of wound healing and initiated by the innate immune system that pro-inflammatory cytokines, such as TNF-α, IL-2, IL-6 and IL-8. Inflammatory response is taken as a direct pathogenic-signal in the traumatised tissues. It has demonstrated that over-expression of these pro-inflammatory mediators can cause wound condition deterioration. Meanwhile, some cytokines function as anti-inflammatory player in wound healing and play a protecting role from negative impact, such as IL-4 and IL-10. Therefore, inflammatory response of wound tissue is coordinated by the interplay of anti-inflammatory cytokines and pro-inflammation cytokines, where a low level of pro-inflammation cytokines should be helpful to tissue repair. In the early stage of wound healing, the antibacterial, anti-inflammatory, and antioxidant properties are close related to the improvement of wound healing. Similar result is also observed in other report. Promoting role of wound healing derived from Baizhi (Angelicae Formosanae) and Dahuang (Radix et Rhizoma Rhei Palmati) extracts treatment is associated with the antibacterial, anti-inflammatory, and antioxidant activities. So, one key factor of wound healing acceleration from Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) extracts treatment is attribute to down-regulation of TNF-α and IL-2 expression as well as up-regulation of IL-2 and IL-10 expression. These results suggest that the application of Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) extracts could result in a down-regulation of TNF-α and IL-2 expression as well as up-regulation of IL-2 and IL-10 expression. These results suggest that the application of Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) extracts could result in an accelerating role for the healing of excisional wounds in the early stage of the wound rats. The wound healing process consists of four continuous and overlapping phases. The hemostasis phase starts immediately after injury, followed by the inflammation phase, which continues for approximately 10 d. Meanwhile, the proliferative phase starts on the third day after wounding and continues for approximately 2 weeks. Remodeling and scar maturation starts during the proliferative phase and continues for several months. In our data, the wound contraction rate of the Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) extracts group was significantly higher than that of the model group from days 3 to 14. Since stage of between days 3 and 14 falls in the inflammation and proliferation phases, these results therefore suggest that the wound healing effect of Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) extracts occurred within these phases.

In our data the histological observation revealed more collagen fibers and more myoibroblasts were present in Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) extracts group than in model group, suggesting that Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) extracts treatment increase collagen fibers and myoibroblasts in the inflammation phase of wound healing process. Collagen fibers play key components in wound healing, which provide structural support of skin strength. Myoibroblasts are crucial cells involved in wound healing, which present more in the granulation tissue and disappeared after wound repair. The inflammatory cells, such as neutrophils and monocytes, are recruited to the wound site after injury. Our histological characterization revealed that infiltration of inflammatory cell was more in Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) extracts treated group than in model group. Our results showed that the administration of Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) extracts treatment that revealed

Figure 6 Effects of Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) extracts on the bFGF mRNA level in wound healing rats

Model group administrated with nature healing state; YNB group treated with 1 mg/mL of Yunnan Baiyao; APE group administrated 1 mg/mL of Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae). YNB group: Yunnan Baiyao group; APE group: Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) extracts group, bFGF: basic fibroblast growth factor. Data are presented as mean±standard error of mean (n = 4). Significant differences of 3, 7, 14 and 21 d compared with 0 d are designated as *P < 0.01 or **P < 0.05; significant differences of YNB group and APE group with model group are marked as #P < 0.05.
administration of Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) extracts can result in an acceleration of fibroblast formation in the wound healing through regulating certain genes expression. It has indicated that induction of TGF-β1 expression contributes to wound healing because TGF-β1 may initiate fibroblast, collagen synthesis and extracellular matrix formation. In addition, TGF-β1 also has an ability to stimulate the expressions of other cytokines, such as matrix metalloproteinase 9 vascular endothelial growth factor-A and monocyte/macrophage chemotactic protein-1. bFGF, an important fibroblast growth factor, can stimulate re-epithelialisation and mediates in mesenchymal-epithelial interactions to promote epithelial proliferation and migration within the wounded area, which facilitates differentiation of new epidermis once combination with its receptor through. Based on mentions, administration of Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) extracts causes the induction of TGF-β1 and bFGF that may lead to fibrosis in the cellular proliferation stage of wound rat.

Combination of Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) is considered as an instance of Shiba Fan in the classical TCM book. In this study, Chuanwu (Aconitum Carmichaelii) co-administration with Banxia (Rhizoma Pinelliae) plays a positive role in wound healing by application in vitro that is paradoxical with the traditional view. In the TCM, Chuanwu (Aconitum Carmichaelii) possesses an evil reputation because it is considered as an extremely toxic plant in which toxic aconite alkaloids are contained which serve as highly toxic to myocardium, nerves, stomach and intestine through hyperpolarization and activation effect on the voltage-dependent sodium channels of the. Banxia (Rhizoma Pinelliae) also has few side effects and can leads to tongue numbness, swelling, salivation, slurred speech, and hoarseness. For a long time, the exact reasons of what Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) can’t be used together in vivo remain large unknown. But, the medical affect of Chuanwu (Aconitum Carmichaelii) combination with other herbs in vitro are constantly explored in recent years. The combination of Chuanwu (Aconitum Carmichaelii) and Shaoyao (Paeonia lactiflora) was showed to produce better efficacy in preventing and curing secondary adjuvant arthritis in rats. Modern clinical studies have evidenced that combination of the lateral root of Chuanwu (Aconitum Carmichaelii) and other herbs have anti-arthritis property in the therapy of patients, indicating its promising therapeutic potential against arthritis. These studies reveal that the application of Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) should open new scenario in vitro. But, there is still a long and exciting road ahead to fully understand and explain the mechanism of Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) extracts in the wound healing.

In conclusion, it is demonstrated that treatment of Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) extracts can promote wound healing during the inflammation and proliferation phases in the rat’s model. Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) extracts may be useful for application in wound treatment in the early stage of wound healing. With respect of down-regulation of TNF-α and IL-2 expressions, and up-regulation IL-4, IL-10, TGF-β1 and bFGF expressions, it is proposed that Chuanwu (Aconitum Carmichaelii) and Banxia (Rhizoma Pinelliae) extracts may have multiple effects in the treatment of skin lesions, including anti-inflammatory activity, cell stimulation properties.

REFERENCES


