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A Preliminary Mechanism Study on the Treatment of Rabbit Bone Defect Bone Nonunion with Xuduan Jiegu Decoction

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Abstract

Objective: To investigate the curative effect and mechanism of Xuduan Jiegu Decoction in the treatment of rabbit bone defect bone nonunion. **Methods:** The experiment was randomly divided into three groups; 1) The control group that was given neither the modeling nor the treatment; 2) The model group that was not given the treatment after the operation to model a bone defect; 3) The treatment group that was given intermittent bone decoction after modeling (*i.e.*, the operation), the dose was 105 g/piece, twice a day, for 4 consecutive weeks. For the three groups, Anterolateral X-rays of the left forearm were taken 14 days after the surgery to observe the bone nonunion healing with pretherapy and post-treatment. The expression levels of TGF-β, BMP-2 and VEGF in the blood of each group were measured by ELISA at 4 weeks after treatment. The peri-fracture histopathological changes between each group of pretherapy and post-treatment were evaluated by using tissue sections. **Results:** Compared with the control group, there was no obvious healing in the model group on the 14th day after the operation. Compared with the model group, the treatment group was treated with Xuduan Jiegu Decoction, and there was a trend of healing and callus formation on the 14th day. HE staining showed that the cells in the control group were closely arranged without any pathological changes. In the model group, the tissues around the fracture end were arranged loosely, and the cells were vacuolated and infiltrated by inflammatory cells. Compared with the model group, the peripheral cell arrangement was better and the peripheral lesions were reduced in the treatment group. The content of TGF-β, BMP-2 and VEGF in blood detected by ELISA was significantly higher in the treatment group than in the control group and

the model group, with statistical significance. **Conclusion:** Xuduan Jiegu Decoction can promote callus formation and accelerate fracture healing in rabbit radius bone defect, and the mechanism of promoting fracture healing is related to the increases of TGF-β, BMP-2 and VEGF levels.

Keywords

Xuduan Jiegu Decoction, Bone Nonunion, TGF-β, BMP-2, VEGF

1. Introduction

In our attempt to find a less traumatic and effective treatment for patients with bone nonunion, an animal model was used to verify the curative effect of the decoction on rabbit bone defect nonunion, and to investigate the peri-fracture histopathological and imaging changes with pretherapy and post-treatment of rabbit bone defect nonunion. The expression levels of TGF-β, BMP-2 and VEGF in the blood of each group were measured to gain insights into the mechanism of the decoction promoting bone nonunion healing.

2. Materials and Methods

2.1. Design

Randomized controlled animal experiment.

2.1.1. Experimental Materials

Male New Zealand White rabbit, VEGF kit (MM-0210O2, Jiangsu Enzyme Free Industry Co., Ltd.), TGF-β kit (MM-2677O2, Jiangsu Enzyme Free Industry Co., Ltd.), BMP-2 kit (MM-36851O2, Jiangsu Enzyme Free Industry Co., Ltd.).

2.1.2. Experimental Methods

After 7 days of adaptive feeding, the group was randomly divided into control group, model group and treatment group, with 3 animals in each group. Steps to establish the left radius bone defect model: For the model group and the treatment group, after anesthetizing the animals, a straight incision of about 3 cm was made on the inside of the left radius to separate and protect important blood vessels and nerves layer by layer, exposing the middle part of the radius. A miniature handheld cranial drill was used to cut the 15 mm radial shaft from the central part of the radius with the largest curvature as the midpoint. The length was measured with vernier caliper, and the broken end was repaired with a bone masseur to make it as flat as possible. At the same time, all the amputation sites were located in the middle of the radius as far as possible. Bone wax was used to seal the pulp cavity of the broken end of the bone, and the bone debris was rinsed and sutured layer by layer to complete the bone nonunion model of the rabbit radius bone defect. The control group and model group were not treated with the Xuduan Jiegu Decoction; after modeling, the treatment group was given Xuduan Jiegu Decoction

twice a day for 4 consecutive weeks by intragastric administration. The dose was calculated according to the equivalent dose ratio of the body surface area between humans and animals, and the dose was 105 g/animal. 14 days after surgery, anterior-to-lateral X-rays of the left forearm of each group were taken to observe the healing of bone nonunion with pretherapy and post-treatment for the three groups, and verify the efficacy of the decoction for rabbit bone defect nonunion. 3 rabbits in each group were killed after 4 weeks of treatment, and the expression levels of TGF-β, BMP-2 and VEGF in the blood of each group were measured by ELISA.

3. Results

3.1. X-Ray Detection of Bone Nonunion Healing

After the establishment of the left radius bone defect model, anteroposterior and lateral X-rays of the left forearm were taken 14 days after surgery to observe the bone nonunion healing with pretherapy and post-treatment for the three groups. The results were shown in Figure 1. Compared with the control group, there was no significant healing in the model group on the 14th day (As shown by red arrow). Compared with the model group, the treatment group was treated with Xuduan Jiegu Decoction, and there was a healing trend on the 14th day (As shown by white arrow).

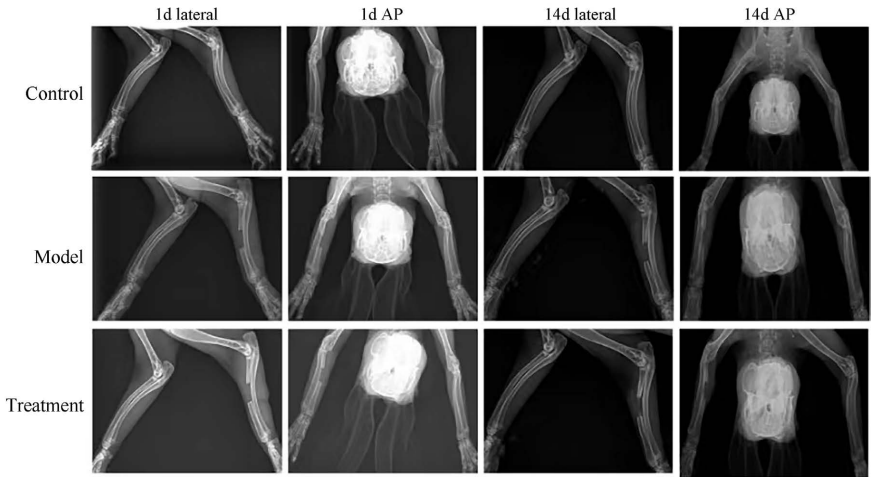

Figure 1. X-ray.

3.2. HE Staining Was Used to Observe the Histopathology around the Peri-Fracture

The left radius bone defect model was constructed and treated with Xuduan Jiegu Decoction for 4 weeks. HE staining was performed to observe the histopathology around peri-fracture. The results were shown in Figure 2. In the control group, the cells were closely arranged and no lesions were found (As shown by black arrow). In the model group, the tissues around the peri-fracture were arranged loosely, and the cells were vacuolated and infiltrated by inflammatory cells (As

shown by blue arrow). Compared with the model group, the peri-fracture cell arrangement was better and the peri-fracture lesions were reduced in the treatment group (As shown by red arrow).

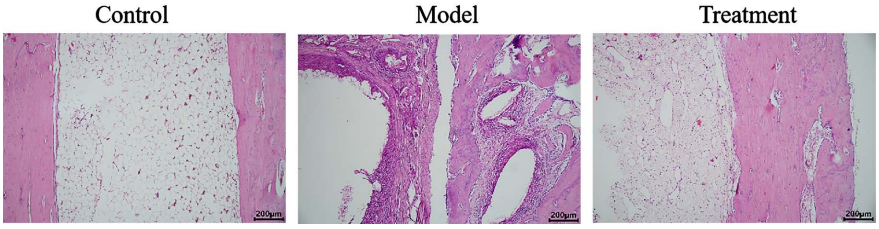

Figure 2. HE staining of peri-fracture.

3.3. The Contents of TGF-β, BMP-2 and VEGF in Blood Were Measured by ELISA

The left radius bone defect model was constructed and treated with Xuduan Jiegu Decoction for 4 weeks, and the contents of TGF-β, BMP-2 and VEGF in the blood of each group were measured by ELISA. The results are shown in **Figure 3**. Compared with the control group, the contents of BMP-2 and TGF-β in the model group were significantly reduced, while the content of VEGF was decreased but c VEGF were significantly increased with statistical significance.

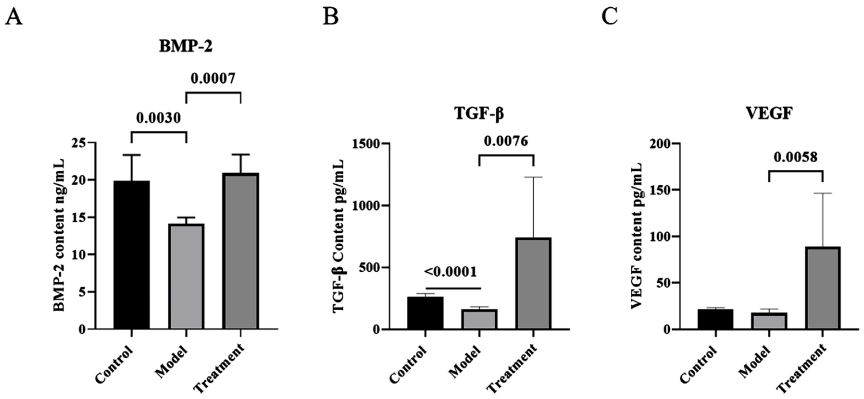

Figure 3. The contents of TGF-β, BMP-2 and VEGF.

4. Statistical Analysis

GraphPad Prism 9.0 software was applied to graph drawing and statistical analysis. All experiments were repeated 3 times, and the quantitative results were expressed as mean ± standard deviation ($\overline{x} \pm s$). One-way analysis of variance was used for quantitative comparison among multiple groups, and S-N-K method was used for two comparisons. $P < 0.05$ indicated significant difference.

5. Discussion

At present, there is no unified standard for the diagnosis of bone nonunion. It is generally believed if the fracture has not healed for more than 6 months, it is

considered to be bone nonunion [1]. According to the American Academy of Orthopaedic Surgeons (AAOS), the diagnosis of bone nonunion can be made only after at least 9 months after fracture and no obvious signs of fracture healing which have been observed dynamically for 3 months [2].

Traditional Chinese medicine generally believes that bone disconnection belongs to the category of "bone impotence", when the fracture occurs, the bone is broken and the tendon is injured, the meridians are damaged, and the blood spillage is outside the pulse, resulting in local Qi blockage, resulting in local Qi and blood ying wei imbalance, resulting in slow healing of the fracture, or even nonhealing, resulting in bone disconnection. From the dialectical point of view of traditional Chinese medicine, 6 months after surgery is the late stage of fracture, because of the tonifying liver and kidney, strong tendons and bones. Deficiency of Qi and blood is caused after postfracture, muscles and bones must not be cured, and blood stasis. Just as Chen Shiduo said in Qing Dynasty: "When the lid hits the servant and falls, there will be evil blood left inside; if the blood is not alive, the blood stasis will not be removed; if the blood stasis is not removed, the bones will not be connected." It is essential to promote blood circulation and remove blood stasis, tonify liver and kidney, and strengthen in the treatment of bone disconnection [3].

In Western medicine, the treatment of bone nonunion mainly adopts the method of stabilizing the peri-fracture and filling the bone defect, However, There are many methods to treat bone nonunion in traditional Chinese medicine, mainly taking traditional Chinese medicine, medicinal bath, fumigation, plasma introduction, acupuncture and so on. Using traditional Chinese medicine and acupuncture to promote local blood circulation and improve the activity of osteoblasts can achieve the above effects well [4]-[6]. Wang Shuxiang *et al.* [7] applied bushen Huoxue Decoction to treat 46 patients with bone nonunion after fracture operation, and found that the cure rate reached 76.09% after following-up. Liang Guisheng *et al.* [8] treated 44 patients with tibia-fibular nonunion of lower limbs by acupuncture and moxibustion, and found that only two patients had no callus formation after 30 weeks of treatment.

Xuduan Jiegu Decoction is a commonly used prescription which includes Xuduan 15 g, Danshen 30 g, Dragon's blood 10 g, Chuanxiong 15 g, Angelica 15 g, psoralen 15 g, *Drynaria fortunei* 15 g, Astragalus 15 g and natural copper 10 g [9] in clinical treatment of bone nonunion. Among them, Astragalus and Angelica supply Qi and blood, Chuanxiong, Danshen line Qi and blood activating, Chuanxiong, natural copper, Xuduan, Dragon's blood disper blood stasis, *Drynaria fortunei*, psoralen supply liver and kidney, with Qi and blood activating blood stasis, kidney replenishing tendon and bone replenishing effect. This is consistent with the experimental results. The results of this experimental study showed that: compared with the model group, after the treatment group was treated with Xuduan Jiegu Decoction, the X-ray showed a healing trend at the peri-fracture at 14 days. HE staining of the peri-fracture showed that the cell arrangement around the peri-fracture in the treatment group was better and the surrounding lesions were reduced, similar to that in the control group, indicating that the peri-fracture had a

healing trend. However, in the model group, the tissue arrangement around the peri-fracture was loose, there was vacuolation in the cells, and there was inflammatory cell infiltration, indicating that the peri-fracture had no obvious healing.

Modern pharmacological techniques have shown that *Drynaria fortunei* can significantly up-regulate the expression of vascular epithelial growth factor (VEGF) and fibroblast growth factor (FGF) [10], promote the proliferation and differentiation of osteoblasts, accelerate the formation of capillaries, and thus promote fracture healing. Xuduan [11] can promote the proliferation and differentiation of osteoblasts, improve the activity of osteoblasts, and accelerate callus remodeling. Dragon's blood can regulate TGF-β/Smad pathway [12], while TGF-β plays an important role in fracture healing. Bone Morphogenetic Protein (BMP), as the initiator of fracture healing, can induce mesenchymal cells to transform into bone tissue and cartilage. BMP has many family members, except BMP1, the rest belong to TGF-β superfamily members, and BMP2 is the only subtype that can induce bone formation alone. The extract from *Salvia miltiorrhiza* can effectively promote the proliferation of rat bone marrow mesenchymal stem cells and the differentiation of osteoblasts [13]. Astragalus and Angelica [14] promote angiogenesis by increasing the expression of VEGF. In this subject research, the content and expression of TGF-β, BMP-2 and VEGF in blood were measured by ELISA. Compared with the model group, TGF-β, BMP-2 and VEGF were significantly increased in the treatment group, with statistical significance; compared with the control group, TGF-β, BMP-2 and VEGF were also increased in the treatment group. Therefore, the results of this study showed that the Xuduan Jiegu Decoction promoted callus formation and accelerated fracture healing by increasing the content and expression of TGF-β, BMP-2 and VEGF.

In summary, Chinese medicine treatment of bone nonunion has its unique advantages, which is conducive to reducing local swelling and pain, improving blood circulation, promoting fracture healing, improving patients' confidence and initiative in rehabilitation training, and has little toxic and side effects, so as to help patients carry out rehabilitation training as soon as possible and promote the recovery of joint function [15]. The curative effect of the Xuduan Jiegu Decoction on bone defect nonunion is observed. Xuduan Jiegu Decoction plays a role in the treatment of bone nonunion by promoting the expression of local TGF-β, BMP-2 and VEGF, and the mechanism of promoting fracture healing has been elucidated from the molecular level. This study used a limited number of animals and therefore preliminary.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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