Effect of Gu Tong Xian capsule on expression level of type I, II collagen and BMP-2 mRNA in rabbits with fracture during long-distance running

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Abstract

The study aims to analyze and investigate the effects of Gu Tong Xian Capsule on the expression level of type I, II collagen and BMP-2 mRNA in rabbits with fracture during long-distance running. 60 adult healthy rabbits were selected as research objects, and then randomly divided into three groups including model group, positive control group and treatment group, each containing 20 rabbits. The three groups were treated with saline gastric lavage, powder for fracture and trauma, and Gu Tong Xian capsule, respectively. The rabbits of the three groups were respectively sacrificed at 1st week, 2nd weeks and 4th week after operation for sample collection. After that, the expression levels of bone collagen type I, II and BMP-2 of three groups were measured and compared with each other. At all stages, the transcriptional level of type I collagen mRNA in the treatment group were significantly higher than that in the positive control group and model group (p < 0.05); Transcriptional level of type II collagen mRNA in the treatment group increased significantly in the first week, then gradually declined in the 2nd and 4th week after operation for sample collection. At all stages, the transcriptional level of type II collagen mRNA in the treatment group were significantly lower than that in the positive control group and model group (p < 0.05); In addition, the transcriptional level of bone morphogenetic protein BMP-2 mRNA at fracture site of the treatment group was higher than that of model group and positive control group (p < 0.05). Gu Tong Xian Capsule can significantly promote fracture healing of experiment rabbits and reduce fracture healing time. Moreover, it can well regulate the expression levels of type I, II collagen and transcriptional level of BMP-2 mRNA in experiment rabbits with fracture.

1. Introduction

Fracture is a common traumatic disease, which will not only bring varying degrees of pain, but also some psychological pressure, affecting work and life quality of patients. Fracture, also called discontinuity of bone continuity, can be categorized into cortical bone fracture and bone trabecular interruption (Jamal et al., 2017; Sindhu et al., 2017). Fracture healing basically refers to repairing the tissue between the fracture ends. To restore the bone to normal function and state is the ultimate outcome of fracture healing.

Currently, treatment for fracture has attracted increasing attention in clinics, as research on fracture healing has been continuously deepened. A large amount of clinical practice experience shows that Gu Tong Xian capsule can nourish liver and kidney, strengthen the bones and muscles, while promoting the circulation of qi and blood to arrest pain, which can effectively treat osteoporosis, aseptic necrosis of femoral head, and delayed union of fracture (Ali et al., 2017; Abbas et al., 2017; Dong et al., 2006). In this study, the effects of Gu Tong Xian Capsule on expression level of type I, II collagen and BMP-2 mRNA in rabbits with fracture during long-distance running were analyzed and discussed. Figs. 1-2.

2. Materials and methods

2.1. General information

In this study, 60 healthy New Zealand white rabbits were selected as research objects. Aged 6–8 months, the rabbits were (6.7 ± 0.8) months old in average; weighting between 2 kg and 3 kg, the rabbits weighted (2.6 ± 0.4) kg in average. The rabbits...
were randomly divided into three groups: model group, positive control group and treatment group, each containing 20 experiment rabbits.

2.2. Methods

2.2.1. Experimental materials

Applied experimental reagents were in situ hybridization kits, i.e. in situ hybridization detection kits for Collagen Type I, Collagen Type II and BMP-2. Auxiliary reagents included ethylenediaminetetraacetic acid disodium, poly-lysine, etc.; Experimental drugs included Gu Tong Xian capsule, powder for fracture and trauma; Experimental apparatus and equipment included surgical instruments, research optical microscope, rotary tissue slicer and electric thermostat incubator.

2.2.2. Experimental methods

The model group, positive control group and treatment group were treated with saline gastric lavage, powder for fracture and trauma, and Gu Tong Xian capsule, respectively. Construction of animal model. Conduct anesthesia for all experiment rabbits by ear vein injection of 1% pentobarbital sodium (30 mg/kg), cut skin muscle, make a 3 mm-long bone defect in middle radius by rongeur, suture the incision, and dress the wound layer by layer (Zaidi et al., 2017; Razali and Said, 2017; Ding, 2016). Anti-infective therapy was given 3 days after the surgery with intramuscular injection of gentamicin of 40,000 units; rabbits in model group were given with 20 mL of saline for gavage, once a day. Mix 1.5 g of Gu Tong Xian capsule with distilled water, forming 20 mL of suspension which was given to rabbits in treatment group for gastric administration once a day. Mix 3.0 g of fracture and trauma powder with distilled water, forming 20 mL of suspension which was given to rabbits in control group for gastric administration once a day (Cahill et al., 2015a).

Before modeling, empty body weight of all the rabbits were measured weekly, and their spirit, activity, appetite, stool and hair gloss were closely observed. Sampling and preparation of specimen were done according to the standard processes, including preparation before sampling, sampling and fixture, glass slide preparation, decalcification after specimen fixture, dehydration by gradient alcohol and n-butanol, xylene transparent and paraffin embedding, section preparation (Dobson et al., 2015).

Fully bake the prepared paraffin sections for 6 h, observe HE staining under the light microscope, and perform photomicrography; Test was implemented in accordance with in situ hybridization operation specification and kit instructions. Conduct a parallel blank control test along with in situ hybridization by replacing the labeled probe with PBS buffer (Jia, 2007; Ghoneum et al., 2015).

2.3. Statistical methods

The quantitative data involved in this study were expressed with ($x \pm s$) and tested with t. The difference was of statistical significance when $p < 0.05$.

3. Results

3.1. Light microscopy results of HE staining

Within the 1st week, the treatment group showed significant proliferation of fibrous tissue and nascent capillaries as well as increased amount of osteoblasts at the adventitia. The control group had small amount of residual hematoma, with osteoblasts occasionally seen. In the model group, hematoma was typical, fibrous tissue was infrequent and blood cells and inflammatory cells infiltrated in the hematoma. Within the 2nd week, it observed in the control group that fibrous tissue was proliferated actively into bone cells and cartilage tissue, without hematoma any longer. The treatment group had a large amount of new trabecular bone, cartilage tissue, and osteoblasts and chondrocytes were active. There was a small amount of hematoma and significantly proliferated fibrous tissues in the model group. Within the 4th week, the control group featured osteoid tissue and new trabecular bone, and lamellar bone could be seen. In the treatment group, the lacelated trabecular bone was connected, medullary cavity was closed, and lamellar bone was formed. The model group still featured new trabecular bone and cartilage tissue.

3.2. The results of immunohistochemistry

The criteria were as follows: 10 high power fields were selected for each slice, and the positive staining intensity and positive staining area were used as the basis. Positive staining intensity included 0 point for negative, 1 point for light staining, 2 points for clear staining, 3 points for strong staining. The area of positive staining distribution included 0 point for less than or equal to 10%, 1 point for less than or equal to 25%, 2 points for less than or equal to 50%, and 3 points for more than 50%. The final determination score is the sum of the two indexes, as shown in the following Tables 1 and 2. The effect of Weitongxian capsule on expression of a protein by in situ hybridization was measured with an enzyme-linked immunosorbent assay (ELISA) kit.

Fig. 1. Cortical bone fracture.

Fig. 2. Bone trabecular interruption.
Table 1
Effect of Weitongxian capsule on expression level of type I collagen (x ± s).

| Group          | n  | 1st week | 2nd week | 4th week |
|----------------|----|----------|----------|----------|
| Treatment group | 10 | 2.7 ± 0.56 | 3.7 ± 0.54 | 4.9 ± 0.85 |
| Control group   | 10 | 1.2 ± 0.87 | 2.1 ± 0.43 | 3.5 ± 0.56 |
| Model group     | 10 | 0.3 ± 0.46 | 1.0 ± 0.72 | 2.4 ± 0.42 |

Table 2
Effect of Weitongxian capsule on expression level of type II collagen (x ± s).

| Group          | n  | 1st week | 2nd week | 4th week |
|----------------|----|----------|----------|----------|
| Treatment group | 10 | 4.0 ± 0.56 | 1.7 ± 0.55 | 0.5 ± 0.53 |
| Control group   | 10 | 1.9 ± 0.85 | 3.3 ± 0.86 | 1.6 ± 0.88 |
| Model group     | 10 | 0.7 ± 0.46 | 4.9 ± 0.89 | 3.1 ± 0.72 |

Table 3
Effect of Gu Tong Xian capsule on transcriptional level of type I collagen mRNA (x ± s).

| Group          | n  | 1st week | 2nd week | 4th week |
|----------------|----|----------|----------|----------|
| Treatment group | 10 | 2.6 ± 0.87 | 4.1 ± 0.72 | 4.9 ± 0.85 |
| Control group   | 10 | 1.2 ± 0.85 | 2.5 ± 0.87 | 3.9 ± 0.42 |
| Model group     | 10 | 0.3 ± 0.46 | 1.3 ± 0.44 | 2.5 ± 0.56 |

Table 4
Effect of Gu Tong Xian capsule on transcriptional level of type II collagen mRNA (x ± s).

| Group          | n  | 1st week | 2nd week | 4th week |
|----------------|----|----------|----------|----------|
| Treatment group | 10 | 3.6 ± 0.87 | 1.7 ± 0.86 | 0.7 ± 0.56 |
| Control group   | 10 | 2.2 ± 0.73 | 3.7 ± 0.88 | 1.9 ± 0.87 |
| Model group     | 10 | 0.9 ± 0.80 | 5.1 ± 0.73 | 3.5 ± 0.78 |

4. Discussion

Regarding the effect of Gu Tong Xian capsule on transcriptional level of collagen I, II and BMP-2 mRNA, the results showed that at all stages, the transcriptional level of type I collagen mRNA in the treatment group was significantly higher than the other two groups; the transcriptional level of type II collagen mRNA in the treatment group increased significantly within the 1st week, and then gradually declined in the 2nd and 4th week, which was significantly different from the model group and the positive control group. The transcriptional level of bone morphogenetic protein BMP-2 mRNA at fracture site of the treatment group was higher than the other two groups (p < 0.05), which fully indicated that the treatment group enjoyed a quicker bone formation, and entered into ossification and plasticity phase earlier.

To promote fracture healing, passage of channels and collaterals (as shown in Fig. 3) is of great significance. Gu Tong Xian capsule contains ingredients such as Rhizoma Drynariae, deer-horn glue, Morinda officinalis, pseudo-ginseng, turtle shell, radix aconiti preparata, Astragalus membranaceus, etc. In the recipe, various drugs complement each other and give full play to the effect of nourishing liver and kidney, strengthening the bones and muscles, while promoting the circulation of qi and blood to remove stasis, activating meridians to stop pain, warming channel and expelling cold, which is consistent with traditional concept of “stasis removal, new bone growth, fracture” in fracture treatment (Guan, 2004; Yung et al., 2015).

Clinical practice has proved that Gu Tong Xian capsules can promote fracture healing. By observing the changes of serum alkaline phosphatase, phosphorus, zinc, calcium and body weight as well as the radiology and histomorphology at fracture site in the experimental animal, it showed that Gu Tong Xian capsule exerted multi-level and multi-faceted effect in fracture healing promotion (Cahill et al., 2015b). The compound ingredients of Gu Tong Xian capsule as well as the multi-level interaction between ingredients were deeply analyze using serum pharmacological method, in order to continuously innovate the study on bone formation mechanism of traditional Chinese medicine (Zhao, 2004).

5. Conclusion

To sum up, the traditional Chinese medicine Gu Tong Xian capsule can regulate the transcriptional level of type I, II collagen and
BMP-2 mRNA in rabbits with fracture during long-distance running, significantly shorten the fracture healing period, and promote the conversion from fibrous callus to bone callus.

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