ISSN 2347-6893

ZJXG Decoction Promoted the Fracture Healing by Reducing the Decomposition of BMP-7, IGF-1, bFGF and TGFβ1 in Fracture Rats

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ABSTRACT

The aim is to investigate the effect of Zhuang Jin Xu Gu (ZJXG) decoction on serum levels of bone morphogenetic protein-7 (BMP-7), insulin-like growth factor-1 (IGF-1), basic fibroblast growth factor (bFGF) and transforming growth factor β1(TGFβ1) and to explore its promoting femoral fracture healing in rats. Femur fractures were generated in 72 male adult Wistar rats by cutting femur transversely at middle point. ZJXG decoction was administered orally after surgery for 28d. The fracture healing process was analyzed by X-ray, gross anatomy and hematoxtlin-eosin (HE) staining. The serum levels of BMP-7, IGF-1, bFGF and TGFβ1 were detected by enzyme linked immunobsorbent assay (ELISA). X-ray imaging and gross anatomy indicated that the fibrous callus tissue at the femoral fracture-end increased and the fracture line became fuzzy at 7-14 d following treatment with ZJXG Decoction. HE staining showed that the fibrous-granular tissue at the fracture-end changed gradually to fibrous, cartilaginous and zosseous callus tissues from 7d to 28d. ELISA results showed that the serum levels of BMP-7, IGF-1, bFGF and TGFβ1 increased significantly 28 days following treatment with ZJXG decoction, compared to model group. It is concluded that ZJXG decoction could enhance the fracture healing by reducing the decomposition of BMP-7 and IGF-1, bFGF and TGFβ1 and enhancing their activities.

Indexing terms/Keywords

ZJXG decoction; fracture; X-ray; BMP-7; IGF-1; bFGF; TGFβ1; rats

Academic Discipline And Sub-Disciplines

Osteology,clinical medicine and Integrative Medicine

SUBJECT CLASSIFICATION

Biology and Medicine

TYPE (METHOD/APPROACH)

Quasi-experimental

ISSN 2347-6893

INTRODUCTION

The biological factors of body cells occurred stress change at the molecular level after fractures[1]. All necrotic bone cells, osteoblasts and absorbed bone matrix in fracture-end released endogenous growth factors[2] to adjust the process of fracture healing with the environment change[3]. Of the numerous cell factors, bone morphogenetic protein-7 (BMP-7)[4], insulin-like growth factor-1 (IGF-1)[5], basic fibroblast growth factor (bFGF) [6] and transforming growth factor (TGF β 1) [7-8] played particularly prominent roles. Fracture was treated with the main principle of restoration, fixation and functional exercise in modern medicine, while in traditional Chinese medicine, fracture treatment was insisted on four principles consisting of combination of dynamics and statics (combination of fixation and functional movement), laying equal emphasis on the consideration of muscular and bony elements for the treatment (fracture healing and functional recovery at the same time), treatment from inside and outside (taking regional and whole treatments into consideration), medical care and patient cooperation (combination of medical treatment assay with patient's activity) [9]. Zhuang Jin Xu Gu (ZJXG) decoction, a traditional Chinese medicine originated from "Shang Ke Da Cheng" written by Doctor Zhao Lian in Qing Dynasty, has been clinically used for promoting fracture healing for many years with unique advantages. Animal experiments showed that ZJXG decoction could effectively promote the femoral fracture healing of rats [10], but it failed to fully reflect the fracture healing process because the observation time only 2 weeks [11]. This experiment aimed to elucidate if the effect of ZJXG decoction in fracture repair was related to the serum levels of BMP-7, IGF-1, bFGF and TGFβ1, and to investigate its mechanism of enhancing fracture healing.

MATERIALS AND METHODS

1.1 Animal models

Total of 72 male adult Wistar rats purchased from Experiment Animal Center of Qingdao Drug Inspection Institute, SCXK (LU) 20120010), weighted 230-250g. All rats were given time to adapt to the laboratory environment for 7 days, then divided randomly into control group (n=24), model group (n=24) and treated group (n=24). The rats were anesthetized with intraperitoneal injection of 100g/L chloral hydrate (300 mg/kg) and then restrained in a prone position for operation. The femoral fracture model was established by cutting the femur transversely with low speed dental drill（JBX-NE22，NSK Co. Ltd. Japan）at the middle section (about 1.0cm below the great trochanter) from medial parapatellar incision [12]. After the manual reduction, the fractured femur was fixed with intramedullary Kirschner wires (diameter 1.0mm, Shanghai Medical Apparatus Co. Ltd.). The sham group was subjected to the same procedure except without cutting femur. Animals were allowed to drink and eat freely after surgery. The survival rate is 100%.

1.2 Treatment methods

The ZJXG Decoction was decocted according to the Standard of Decocting Herbal Medicine promulgated by Chinese Administration Department of Traditional Chinese Medicine. The mixture of all herbal plants were cooked to the boil, kept on simmer for 10-15 min to concentrate the extracts, protecting and maintaining all essential ingredients. The same procedure was repeated for 2 times. The two extractions yielded an amount of 224ml liquid medicinal decoction containing 112g of dry weight (concentration of 0.5g/ml). Rats in treatment group were administered orally with ZJXG decoction of 1.25g/kg according to the previous research results [12], one time a day for 28 days, while the saline solution was given at the same volume to sham and control group rats.

1.3 Evaluation index

Six rats in each group were selected to observe after treated 7, 14, 21, 28 days respectively.

1.3.1 X-ray examination

The rats were anesthetized by injecting intraperitoneally 10% chloral hydrate (300 mg/kg) for X-ray evaluation (GE Revolution RE/d, USA).

1.3.2 Gross observation

After x-ray evaluation, the rats were sacrificed and taken out the femurs, removed the excess of soft tissue, washed with normal saline for general observation. About 4 ml blood was aseptically collected from abdominal aorta of each rat for analysis.

1.3.3 Histological staining

The femur were incubated in 4% formaldehyde solution for 24 h and decalcificated for 15 days in 20% ethylenediamine tetraacetic acid (EDTA). The interception of the fracture (including hematoma, callus) was dehydrated using graded ethanol, immersed in dimethylbenzene, embedded by paraffin. The 5μm thickness slices were made by mirotome (Leica RM2015, Shanghai Leica Instruments, China), the sections were stained with Hematoxylin-Eosin (HE).

1.3.4 Enzyme linked immunosorbent assay (ELISA)

About 4 ml blood were centrifugalized for 10 minutes at 4000 r/min at 4°C to separate the serum (2ml). The serum level of BMP-7, IGF-1, bFGF and TGFβ1 measured using commercially available ELISA kits (Blue Gene Co. Ltd). The procedure was performed following manufacturer's instruction. The OD was calculated with Bio-Rad 550 microplate reader (USA) set to 450 nm to reflect the level of BMP-7, IGF-1, bFGF and TGFβ1(ng/L).

1.4 Statistical analysis

ISSN 2347-6893

The data was expressed by mean ± standard deviation (X ± s) and analyzed with SPSS 16.5 statistical software. P<0.05 was considered significantly.

RESULTS

2.1 X-ray films

X-ray films revealed the femoral cortical bone was integrity and continuous in control group rats. The fracture-end was filled with fibrous tissue after fracture 7 days and became fibrous callus at fracture 14 days, and bony callus formatted at fracture 28 days. There were no statistical difference of callus structure existed between treated group and model group during fracture 7-21 days, but the bone callus structure in treatment group was better than that in model group after treatment 28 days.

2.2 Gross anatomy

Cortical bone was completely in control group rats while the fracture fragments separated in fracture group rats. The fracture-end was surrounded by fibrous granulation tissue (pinprick through easily) at fracture 7 days, formed fibrous callus and turned hard (pinprick through difficultly) at fracture 14 days. At fracture 21 days, fibrous callus and cartilage callus increased and more hard (the resistance force of pinprick) and fibrous callus was gradually replaced by cartilage and bone callus (pinprick through hardly) at fracture 28 days. There was no statistical difference between treatment groups and model group during fracture 7-21 days, but callus in treatment group became more hard (pinprick not through) than that in model group.

2.3 Histopathology

The bone structure of rats was normal in control group. The breaking-ends of rats in model group rats were filled with hematoma organization. On fracture 7 days, inflammatory cells infiltrated and granulation tissues occurred between fracture gaps. On day 14, fibroblast and osteoblasts proliferated of under periosteum in fracture fragments. Osteoclasts and osteoblasts were activated and trabecular bone formed at fracture 21 days, and trabecular bone was clearly visible until fracture 28 days. In treatment group, callus structure had no significant difference compared with model group at the same time during fracture 7-21 days, but by 28 days it was significantly better than that in model group.

2.4 Enzyme linked immunosorbent assay (ELISA)

2.4.1 The serum level of BMP-7: Compared from the aspect of treatment times, there was no significant difference of serum levels of BMP-7 in control group during fracture 7-28 days (t=0.54-1.03, P>0.05). And also no significant difference of serum level of BMP-7 both in model group and treatment group during fracture 7-21days (t=0.29-0.87, P>0.05), while it significantly decreased at fracture 28 days (t=16.47-16.55, P<0.05). In paired comparisons of groups, the serum levels of BMP-7 in the treatment group and model group were significantly higher than those in control group during 7-28 days (t=20.46-21.07, P<0.05). There was no significant difference of serum levels of BMP-7 in model group and treatment group in the corresponding time during fracture 7-21 days (t=0.64-0.93, P>0.05), but still significantly higher than those in the model group (t=12.92, P<0.05) and control group (t=8.63, P<0.05) at treatment 28 days.(Table 1).

Table 1 Serum levels of BMP-7 ($\bar{x}$ ±SD, ng/L)

Groups	n	7d	14d	21d	28d
Control group	6	428.24±11.15	430.94±11.26	433.14±10.65	432.83±10.53
Sham group	6	538.85±12.17 [a]	540.42±12.22 [a]	543.14±13.28 [a]	450.69±11.65 [a c]
Treated group	6	530.69±14.36 [a]	534.70±13.55 [a]	536.42±13.32 [a]	493.27±10.59 [a b c]

[a] Compared with control group, *t*=20.46-21.07, *P*<0.05; [b] Compared with model group, *t*=12.92, *P*<0.05; [c] Compared with the 21 days, *t*=16.47-16.55, *P*<0.05.

2.4.2 The serum level of IGF-1: In control group, no significant difference of serum level of IGF-1 existed between 7 days to 28 days (t=0.26-1.18, P>0.05). Also there was no significant difference of serum level of IGF-1 both in model group and treatment group during fracture 7-21 days (t=0.28-1.59, P>0.05), while it decreased significantly at fracture 28 days (t=2.52-6.11, P<0.05). Compared in paired groups, the serum level of IGF-1 in treatment group and model group was significantly higher that in control group during fracture 7-28 days (t=7.59-9.18, P<0.05). There was no significant difference of serum level of IGF-1 between 7-21 days in model group and treatment group (t=0.09-1.92, P>0.05), but at fracture 28 day, they were still higher than those in the model group (t=5.94, P<0.05) and control group (t=7.51, P<0.05)(Table 2).

Table 2 Serum levels of IGF-1 ($\bar{x}$ ±SD, ng/L)

Groups	n	7d	14d	21d	28d
Control group	6	17.35±0.71	17.43±0.65	17.62±0.70	17.71±0.65
Sham group	6	21.08±1.07 [a]	21.22±1.13 [a]	20.47±0.96 [a]	18.02±0.83 [a c]
Treated group	6	20.35±0.95 [a]	20.39±0.97 [a]	21.25±0.92 [a]	20.25±0.85 [a b c]

[a] Compared with control group, *t*=7.59-9.18, *P*<0.05; [b] Compared with sham control group, *t*=5.94, *P*<0.05; [c] Compared with the 21 day, *t*=2.53-6.11, *P*<0.05.

2.4.3 The serum level of bFGF: There was no significant difference of serum level of BFGF in control group among fracture 7-28 days (t=0.36-1.47, P>0.05). And also no significant difference of serum level of BFGF both in model group and treatment group during fracture 7-21 days (t=0.29-0.87, P>0.05), while decreased significantly at fracture 28 days (t=5.78-16.47, P<0.05). In paired comparisons of groups, the serum level of BFGF in treatment group and model group was significantly higher than that in control group among 7-28 days (t=11.37-13.21, P<0.05). There was no significant difference of serum level of BFGF both in model group and treatment group among 7-21 days (t=0.38-1.60, P>0.05), but in treatment group it was still significantly higher than that in model group (t=10.34, P<0.05) and control group (t=11.42, P<0.05) at fracture 28 day.(Table 3).

Table 3 Serum levels of bFGF ($\bar{x}$ ±SD, ng/L)

Groups	n	7d	14d	21d	28d
Control group	6	18.36±0.74	18.23±0.89	17.89±0.87	18.07±0.73
Sham group	6	22.54±0.82 [a]	22.61±0.86 [a]	23.24±0.94 [a]	18.16±0.56 [a c]
Treated group	6	22.63±0.77 [a]	22.76±0.75 [a]	23.22±0.88 [a]	21.23±0.64 [a b c]

[a] Compared with control group, *t*=11.37-13.21, *P*<0.05; [b] Compared with sham control group, *t*=10.34, *P*<0.05; [c] Compared with the 21 day, *t*=5.78-14.68, *P*<0.05.

2.4.4 The serum level of TGFβ1: There was no significant difference of serum level of TGFβ1 between day 7 and day 28 in control group (t=0.09-0.84, P>0.05). Also no significant difference of serum level of TGFβ1 between day 7 and day 21 in model group and treatment group (t=0.29-0.38, P>0.05), while decreased at fracture 28 days (t=5.78-7.54, P<0.05). In paired comparisons of groups, the serum level of TGFβ1 both in treatment group and model group was significantly higher that in control group between day 7 and day 28 (t=9.02-9.93, P<0.05). There was no significant difference of serum level of TGFβ1 between day 7 and day 21 in model group and treatment group (t=0.45-0.93, P>0.05), but on day 28, the levels was still significantly higher than that in model group (t=6.31, P<0.05) and control group (t=7.25, P<0.05) (Table 4).

Table 4 Serum levels of TGFβ1 ($\bar{x}$ ±SD, ng/L)

Groups	n	7d	14d	21d	28d
Control group	6	122.01±8.14	124.96±7.54	123.61±8.42	124.66±7.01
Sham group	6	162.55±10.19 [a]	161.08±12.26 [a]	163.09±11.21 [a]	127.28±7.43 [c]
Treated group	6	154.52±13.93 [a]	157.69±10.86 [a]	160.04±12.44 [a]	149.29±8.15 [a b c]

[a] Compared with control group, *t*=9.02~9.93, *P*<0.05; [b]Compared with sham control group, *t*=6.31, *P*<0.05; [c] Compared with the 21 day, *t*=2.29.54, *P*<0.05.

DISCUSSION

Fracture healing is an extremely complex process which is reportedly influenced by multiple cytokines and growth factors. Urist [1] discovered firstly BMP and then puts forward "bone induction" theory. BMP-7 is a positive modulator of fracture healing, induced osteogenic differentiation of mesenchymal stem cells by regulating the transcription factors Runx2 and Osterix, and cooperated with other regulatory factors involved in bone tissue formation [4]. IGF-1 is an important factor in regulating bone cell function and metabolism, it can reduce the collagen degradation, increase bone deposition, promote osteoblast differentiation, maturation and supplement [13], and in a time-and dose-dependent manner affected the proliferation and metabolism [14]. IGF-1 bund to receptors in bone tissue, occurred receptor tyrosine autophosphorylation to activate protease, induce phosphorylation of insulin receptor substrate, which regulated cartilage cells, bone growth, proliferation and metabolism [15-17].

The biological activity of endogenous bone growth factor has a high and strong physiological action to accelerate fracture healing [18], but exogenous cytokines can not effectively promote fracture healing because its short half-life and high clearance rate. In addition to relieving spasm and pain, promoting blood circulation and removing blood stasis, promoting granulation and other conventional role, traditional Chinese medicine also directly or indirectly affected the bone growth factor secretion, degradation and regulation of activity. Previous experiment [9] showed that the traditional Chinese medicine in the treatment of fractures could improve the volume and density of the external callus, connecting callus, bridging callus and mineralized callus, so that increased significantly the callus bone strength, tensile strength, flexural strength, load capacity and other mechanical properties. The experimental results of Wang et al. [19] indicated that ZJXG decoction promoted the proliferation of osteoblasts, Cells in S phase increased obviously. It suggested that ZJXG decoction could promote the synthesis of DNA in osteoblasts by promoting the proliferation of osteoblasts to accelerate the healing of fracture. Wang et al [12] reported that ZJXG decoction could significantly evaluated the serum levels of BMP-7 and NPY in rats at the initial stage of fracture and promote the fracture healing in rats.

By the way of regulation of cell proliferation, differentiation and the cell product synthesis, bFGF not only promoted bone cell growth, but also promoted the hyperplasia of the capillary vessels, improved blood supply, enhanced bone cells

adhering to scaffold materials [20]. Research of Tatsuyama et al. [3] showed that TGFβ1 and bFGF had synergistic effect in fracture healing, and TGFβ1 is the regulating factor of bFGF [21]. Applying TGFβ1 and bFGF at same time could stimulate synergistically the proliferation of osteoblasts and accelerated endochondral ossification process[5]. bFGF expressed constantly in various stages of fracture healing, it usually expressed strongly at the early stage of bone injury and decreased gradually after reaching a peak. Kawaguchi et al [6] confirmed that endogenous bFGF persists in the fracture site after fracture 1-3 weeks. Wang et al [22] reported that after fracture 4 days to 3 weeks, bFGF expressed strongly and decreased gradually with prolong of time. The early expression of bFGF could make various cells division and proliferation in fracture-end, hematoma organize into granulation tissue, while in late stage bFGF mainly maintained active proliferation for interstitial cells or fibroblasts.

The process of fracture healing process was divided into three stages of early, middle and later respectively according to the theories of traditional Chinese medicine "Liver governing sinew, kidney governing bone and engendering marrow, spleen governing muscle, injuring Qi to induce pain, injuring configuration to lead swelling", and three corresponding treatment principles of "activating blood circulation to remove blood stasis, joining bone and uniting sinew, nourishing liver and kidney" were applied to promote fracture healing. Western medicine considered that fracture was a process of bone integrity and continuity interrupted, and the fracture healing was divided into three stages of hematoma machining, original callus formatting, and callus remodeling according the changes of histology and cytology [2]. Huang [9] treated tibia plateau fractures by DJXG decoction with locking plate fixation and achieved an efficiency of 90%. Zhang et al. [23] treated humeral fracture nonunion by applying unilateral multifunctional external fixator with ZJXG decoction, and the fracture healing rate was as high as 97.4%. Liang [24] used dynamic hip screw fixation combining ZJXG decoction to treat senile intertrochanteric fractures, the result was satisfactory. Another research [25] showed that in the period of hematoma organization, the drugs of activating blood circulation and removing blood stasis (such as Radix salvia miltiorrhizae, Rhizoma Chuanxiong, Safflower, etc.) could improve regional blood circulation of the fracture-ends and provide conditions for callus formation. In the period of callus formation, Teasel root and Drynaria rhizome which contained rich collagen, calcium salts and trace elements involved in protein synthetase metabolism and beneficial to bone repair [26]. This experiment indicated that in the first 3 weeks after fracture, ZJXG decoction did not increase the serum levels of BMP-7, IGF-1, bFGF and TGFβ1, but could only maintained those bone growth factors in high level continuously after fracture 21 days. This showed that ZJXG decoction could not promote the secretion of endogenous bone growth cytokines, perhaps, only reduced down the decomposition rates of endogenous BMP7, IGF-1, bFGF and TGFβ1 prolonged their half life to enhance their activities, and thus promoted fracture healing.

ACKNOWLEDGMENTS

This work was granted for the National Natural Science Foundation of Shandong Provence (ZR2011HM050).

REFERENCES

1. Urist MR, Delange RJ, Finerman GA. Bone cell differentiation and growth factors. Science, 1983, 220(4598):680-686.

2. Croteau S, Ranch F, Silvestri A, Hamdy RC. Bone morphogenetic proteins in orthopedics: from basic science to clinical practice. Orthopedics, 1999, 22(7):686-695.

3. Tatsuyama K, Maezawa Y, Baha H, Imamura Y, Fukuda M. Expression of various growth factors for cell proliferation and cylodifferentiation during fracture repair of bone. Eur J Histochem, 2000, 44(3):269-278.

4. Kwong FN, Hoyland JA, Fremont AJ, Evans CH. Altered relative expression of BMPs and BMP inhibitors in cartilaginous areas of human fractures progressing towards nonunion. Orthop Res, 2009, 27(6):752-757.

5. Raschke M, Wildeman B, Inden P, Bail H, Flyvbjerg A, Hoffmann J, Haas NP, Schmidmaier G. Insulin-like growth factor 1 and transforming growth factor-beat 1 accelerates osteotomy healing using polylactide-coated implants as a delivery system a biomechanical and histology study in mini-pigs. Bone, 2002, 30(1):144-151.

6. Kawaguchi H，Kurokawa T, Hanada K, Hiyama Y, Tamura M, Ogata E, Matsumoto T. Stimulation of fracture repair by recombinant human basic fibroblast growth factor in normal and streptozotocin-diabetic rats. Endocrinology, 1994, 135(2):774-781.

7. Schmidmaier G, Wildemann B, Heejer J, Gäbelein T, Flyvbjerg A, Bail H J, Raschke M. Improvement of fracture healing by systemic administration of growth hormone and local application of insulin-like growth factor-1 and transforming growth factor-beat 1. Bone, 2002, 31(1):165-172.

8. Blumenfeld I, Srouj S, Lanir Y, Laufer D, Livne E. Enhancement of bone defect healing in old rats by TGF-beat and IGF-1. Exp Gerontol, 2002, 37(4):553-565.

9. Huang PJ. ZJXG decoction combined with locking plate fixation in the treatment of tibial plateau fractures compression. Inner Mongol Journal of Traditional Chinese Medicine, 2012,31(14):20-21.

10. Guo YL, Wang XJ, Pan YX, Du ZX, Zhou Z. ZJXG decoction promotes the expression of bone morphogenetic protein-7 to enhance fracture healing in rats. J Trauma Treat, 2012, 1(1): 1-4.

11. Pan YX, Wang XJ, Du ZX, Zhou Z, Guo YL. Zhuang Jin Xu Gu decoction improves fracture healing in rats by augmenting the expression of NPY. J Dent Med Med Sci, 2012, 3(8): 517-521.

12. Wang XJ, Pan YX, Du ZX, Zhou Z,Liu M, Guo YL. Effects of Zhuangjin Xugu Decoction on the expressions of BMP-7 and NPY of fracture callus in rats. China Journal of Traditional Chinese Medicine and Pharmacy, 2013, 28(8):2420-2422.

13. Kurland ES, Rosen CJ, Cosman F, McMahon D, Chan F, Shane E, Lindsay R, Dempster D, Bilezikian JP. Insulin-like growth factor-I in men with idiopathic osteoporosis. J Clin Endocrinol Metab, 1997, 82(2):279-286.

14. Liu G, Hu YY, Ma P,Han YS. Effects of insulin-like growth factor -I on the proliferation and metabolism of rabbit articular chondrocytes. Chinese Journal of Reparative and Reconstructive Surgery, 2002, 16(3):228-230.

15. Schmidmaier G, Wildemann B, Ostapowicz D, Kandziora F, Stange R, Haas NP, Raschke M. Long-term effects of local growth factor (IGF-1 and TGF-beat 1) treatment on fracture healing, A safety study for using growth factors. J Orthop Res, 2004, 22(3):514-519.

16. Wildemann B, Bambad P, Homler CH, Haas NP, Raschke M, Schmidmaier G. Local delivery of growth factors from coat titanium plates increases osteotomy healing in rats. Bone, 2004, 34(5):862-868.

17. Wang M, Xin HX, Huang XY. Insulin like growth factor on the experimental study of the growth of osteoblasts. China Orthopedic Journal, 2004, 12(3):236-238.

18. Westerhuis IL, Van Bezooijen RL, Klcen P. Use of bone morphogenetic proteins in traumatology. Injury, 2005, 36(12):1405-1412.

19. Wang L, Tu H, Yang FY, Kong LS, Bao JW, Zheng S. The proliferation of rat osteoblasts cultured in serum containing ZJXG decoction. Journal of Clinical Rehabilitative Tissue Engineering Research, 2011, 15(37):6959-6962.

20. Liu Y and Shen HQ. Basic fibroblast growth factor role in tissue repair and reconstruction after fracture. Chinese Journal of Tissue Engineering, 2008,12(33):6522-6525.

21. Zeng J, Zhang HZ，Xu DL，Qiu JS，Zhang M，Liang HZ. Dynamic expression of osteoblast transplant promote osteoporotic fracture healing process-related factors. Chin J Clin Rehabilit, 2003, 7(3):448-473.

22. Wang LP, Dang GD. Basic fibroblast growth factor expression and synthesis in bone wound healing process. Chin J Orthop, 1999, 19(5):488-490.

23. Zhang XH, Guo XQ, Zeng SC, Xiao F, Chen SM，Zhang P，Kang L. Unilateral multifunctional external fixation combined with Chinese humeral nonunion 43 cases. Jiangxi Journal of Traditional Chinese Medicine, 2006, 37(8):37-38.

24. Liang SY, Liu ZJ. Integrative treatment of senile intertrochanteric fracture 42 cases. Forum on Traditional Chinese Medicine, 2005, 20(5): 45-46.

25. Liu ZH, Yang SR, Hui C. Promote healing of experimental Chinese medicine Research Survey. Journal of Changchun University of Traditional Chinese Medicine, 2004, 20(2):63-65.

26. Zhou QL, Wang LJ. Study on the therapeutic effect of pilose antler polypeptides on experimental fracture and its mechanism. Journal of Norman Bethune University of Medical Science, 1999, 25(5):586-588.