Original Article

Effects of BMP-2 compound with fibrin on osteoporotic vertebral fracture healing in rats

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Abstract

Objectives: To investigate the effects of bone morphogenetic protein-2 (BMP-2) compound with fibrin on osteoporotic vertebral fracture healing in rats. **Methods**: For the present study 160 Specific-Pathogen Free 32-week-old female Sprague-Dawley rats were used. 120 rats were randomly divided in three groups (experimental, model and sham operation group- n=40 per group) and were ovariectomized to establish the osteoporosis model. 40 rats served as a control group without treatment. The expression of BMP-2 in the fracture zone at the 4th, 6th, 8th, and 12th weeks was detected by qRT-PCR. The expression of BALP and CTX-I in serum at the 12th week was detected by Elisa. **Results**: At week 8, the morphology of the sham operation group was the same and the fracture healing occurred more slowly than in the other groups. At week 12, the expression of BMP-2 in the model group was significantly higher than that in the other three groups (p<0.05). At week 12, the maximum load, maximum strain, and elastic modulus of model group were significantly lower than those of the other three groups. **Conclusions**: BMP-2 compound with fibrin can enhance the timing and quality of bone fracture healing in rats.

Keywords: Bone Morphogenetic Protein-2, Fibrin, Fracture Healing, Osteoporosis, Vertebral Fracture

Introduction

Osteoporosis is a systemic skeletal disorder characterized by low bone mass, compromised bone strength and increased fracture risk. Osteoporosis is becoming an increasing burden on health care globally in view of the demographic changes[1]. Fracture incidence increases due to the ageing of population. A report from the United States shows that[2,3] osteoporotic patients over 50 years old account for about 3% of the total population in the United States, and another 10% of the population have low bone mass[4,5]. Osteoporosis, as a multifactorial disorder, has a complex pathogenesis and is the consequence of genetic, hormonal, dietary, lifestyle and physical factors[6]. Bone morphogenetic protein (BMP) is the largest subgroup of Transforming growth factor-β superfamily[7]. BMPs play a key role in bone remodeling and are related to the occurrence and development of osteoporosis. Signaling by BMPs plays an important role in a variety of bone cell-types. The reduction of BMP will cause changes in the number and function of bone cells[8]. BMP-2 belongs to one of BMP family members and is a key factor in bone healing[9]. U.S. FDA approved in 2004 that rhBMP-2 (recombinant human Bone Morphogenetic protein-2) and collagen sponge composite can be used in clinical treatment of open fractures[10]. However, bone morphogenetic protein alone will be diluted by tissue fluid and decomposed by protease after implantation, and cannot maintain a sustained drug concentration. Therefore, we chose to combine fibrin gel to achieve the effect of improving drug durability[11]. However, there are few related researches on whether BMP-2 compound can also improve the condition of osteoporotic vertebral fracture. This study aims to explore the effects of BMP-2 compound fibrin on promoting osteoporotic vertebral fracture healing in rats.

Materials and methods

Animals

160 Specific-Pathogen Free (SPF) grade 32-week-old female Sprague-Dawley (SD) rats (Beijing Weitonglihua Experimental Animal Technology Co., Ltd., SCXK (Beijing) 2016-0006,101) with a mass of 240~260 g were reared in the animal research center of Affiliated Xuzhou Hospital of Jiangsu University after purchase. The rats were exposed to 12 hours of circulating light at a temperature of 21-25°C, a relative humidity of 50-60%, with free to food and water.

Main reagents and materials

RhBMP-2 (China Beijing Zhongke Wuyuan Biotechnology Company, H5001-50), transScript Green Two-step qRT-PCR SuperMix, EasyPure RNA Kit (China Beijing TransGen Biotech company, AQ201-01, ER101-01), rat bone alkaline phosphatase (BALP), rat type I collagen cross-linked carboxy-terminal peptide (CTX-I), Elisa Kit (Shanghai Enzyme-linked Biological Research Institute, China, ml003415, ml003405), chloral hydrate (Sigma company, USA, 47335-U), fibrinogen (self-configured by our hospital), BMP-2 primer sequence designed and synthesized by Shanghai Bio-engineering Biotechnology Co., Ltd., PCR instrument (Applied Biosystems company, USA, 7500).

Establishment of rat animal model

A total of 160 rats were randomly divided into 4 groups: control group, sham operation group, model group and experimental group. All rats were anesthetized with 10% chloral hydrate (300 mg/kg) intraperitoneally. Ovaries were removed in the experimental group and the model group. Only part of soft tissues around the ovaries were removed in the sham operation group, and then the incision was sutured. The control group was not interfered and the rats were kept in cages. Three months later, the rats in the control group did not undergo surgery. The sham operation group, model group and experimental group were anesthetized with 10% chloral hydrate (0.3 mL/100 g) intraperitoneally. The lateral position was taken and disinfected. The external oblique muscle and internal oblique muscle were surgically incised. The fat layer and sacrospinalis muscle gap were bluntly separated until the sacrospinalis muscle root and paravertebral muscle border. A fenestration was performed on the side of L5 vertebral body, with a size of about 4.5 mm^2 (1.5*3.0) and a depth of vertebral cancellous bone diameter. The cancellous bone near the vertebral body in the visual field was scraped to establish a fracture model. Gelatin sponge was used for hemostasis. The experimental group was treated with rhBMP-2 20 µg+ fibrin 20 µg gel mixture. The model group and the sham operation group were treated with equal volume of physiological saline, the window bone flap was sealed, and the incision was sutured layer by layer. After the operation was completed, the rats were kept in cages. All the operations were performed under aseptic conditions. After the operation, neomycin (2 mg/mL) was added to the drinking water for 7 days to prevent bacterial infection.

Collection of samples

The rats were sacrificed at the 4th, 6th, 8th and 12th weeks of the rat modeling respectively. 5 rats were sacrificed at a time, 10% chloral hydrate (300 mg/kg) was used to anesthetize them; the rats were decapitated, the L5 vertebral body of the rats was taken for subsequent detection, and the peripheral blood of the rats at the 12th week was collected to detect the expression of BALP and CTX-I.

Observation indicators

qRT-PCR detection

The collected tissues were extracted with EasyPure RNA Kit reagent to extract total RNA. The purity, concentration and integrity of the extracted total RNA were detected by UV spectrophotometer and agarose gel electrophoresis. TransScript Green Two-Step qRT-PCR SuperMix was used to reverse transcription of total RNA, and the specific steps were carried out according to the kit instructions. 5X TransScript® II All-in-One SuperMix for qPCR and gDNA Remover kits to carry out reverse transcription operation procedures in strict accordance with manufacturers' kits. Then PCR amplification experiment was carried out. PCR reaction system was as follows: cDNA 1µL, upstream and downstream primers 0.4µL each, 2XTransScript® Tip Green qPCR SuperMix 10µl, Passive Reference Dye (50X). Finally, Nuclease-free Water was added to make up to 20µL. PCR reaction conditions were as follows: 94°C pre-denaturation for 30s, 94°C denaturation for 5s, 60°C annealing extension for 30s, for a total of 40 cycles. Each sample was provided with 3 repeated wells, and the experiment was carried out 3 times. GAPDH was used as an internal reference and $2^{-\Delta CT}$ was used to analyze the data. GAPDH upstream primer 5'-AAGAAGGTGGTGAGCAGG-3', downstream primer 5'-TCCACCACCCTGTGTGTGTA-3', BMP-2 upstream primer 5'-CGTGAGATTAGCAGTTT-3', downstream primer 5'-GGCGTTTCCGCTTTG-3'.

ELISA detection

5 mL of collected peripheral blood was allowed to stand for 30 min and then was centrifuged at 3000 rpm for 10 min. The collected supernatant was used to detect the expression of BALP and CTX-I in rats by ELSA kit. The ELISA detection method was as follows: Blank wells, standard wells and sample wells to be detected were respectively arranged. Accurately 50 µl of standard substance was added to the enzyme-labeled coating plate, 40 µl of sample diluent was added to the sample well to be tested, and then 10 µl of sample to be tested was added. The sample was added to the bottom of the well of the enzyme-labeled plate. Try not to touch the wall of the well, and it was gently shaken and mixed. After sealing the plate with sealing plate membrane, it was incubated at 37°C for 30 minutes. The sealing film was removed, the liquid was discarded, then it was spin-dried. Each well was filled with washing liquid, standing for 30 seconds, and then it was discarded, repeating this for 5 times, and then it was patted dry. 50 µl of enzyme-

labeled reagent was added to each well, except for blank wells. After sealing the plate with sealing plate membrane, it was incubated at 37°C for 30 minutes. The sealing film was removed, the liquid was discarded, and then it was spin-dried. Each well was filled with washing liquid, standing for 30 seconds, and then it was discarded, repeating this for 5 times, and then it was patted dry. First, A50 μl of developer was added. Then, B50 μl of developer was added to each well. They were mixed with gentle shaking, and developed color at 37°C in dark for 15 minutes. 50 μl of stop solution was added to each well to stop the reaction. The absorbance (OD value) of each well was measured in sequence at zero and 450 nm wavelength of blank air conditioner. The determination shall be carried out within 15 minutes after the termination liquid was added.

HE staining

The L5 vertebral bodies of rats were collected at the 4th and 8th weeks after the operation and decalcified, dehydrated and embedded in paraffin. According to the size of the fracture zone of the rats, the size of the fracture zone was determined by comparing with the size of the fracture zone of the rats. It was divided into two points, 3 pieces of 5 μm slices were made, the slices were placed on glass slides, protein glycerin was added dropwise, and they were baked for 15 min, and HE was used to observe the fracture healing of rats.

Detection of maximum load, maximum strain and elastic modulus

The L5 vertebral bodies of rats were collected at 6, 8 and 12 weeks old, the articular processes, transverse processes, spinous processes and cartilage were removed, and the 3*3*6 mm vertebral bodies (length, width and height) were prepared by rough sandpaper polishing. The maximum load, maximum strain and elastic modulus of the vertebral bodies were detected by electronic universal mechanical testing machine (Instron, 5900, USA).

Detection of volume percentage, surface percentage, average width and mineralization deposition rate of rat trabecular bone

Qwin image analysis system (Leica, Germany) was used to detect morphological parameters (trabecular volume percentage, surface percentage, average width, mineralization deposition rate detection) of L5 vertebral body in rats at 12th week.

Statistical analysis

In this study, SPSS20.0 (Shanghai Cabe Information Technology Co., Ltd., China) software package was used for statistical analysis on the collected data. GraphPad Prism 7 (Shenzhen Qiruitian Software Technology Co., Ltd., China) was used to draw the data picture. Chi-square test was used in the usage (%) of the counting data, and the counting data were expressed by X^2. K-S test was used to analyze the data distribution. Measurement data were expressed by mean±standard deviation (Meas±SD). The comparison of normal distribution data between the two groups was conducted by independent sample T test, expressed by T. The comparison between multiple groups was conducted by one-way analysis of variance. The comparison between the following two groups was conducted by Bonferroni. $P<0.05$ was considered as statistically significant difference.
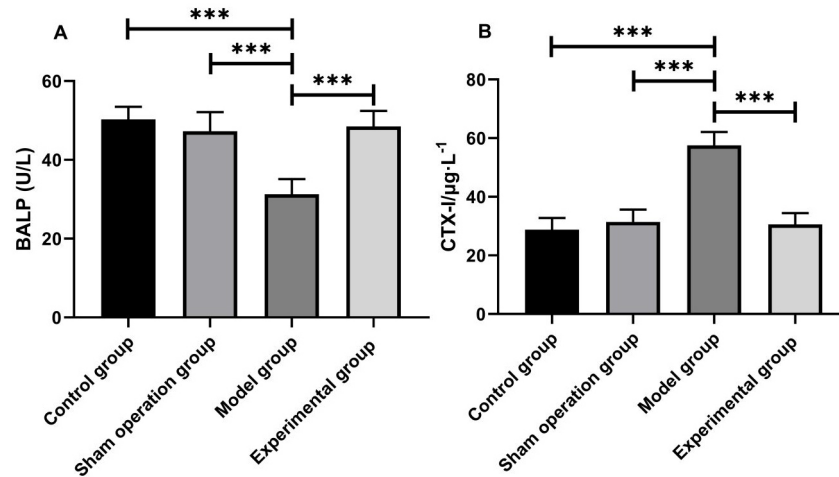
Results

Bone morphology of rats

The histological morphology of the fracture zone of the four rats groups at the 4th and 8th weeks was observed. The histological morphology of the fracture zone of the sham operation group and the experimental group was basically the same at the 4th week, with chondrocytes, cartilage islands and bone fibers appearing, and reticular bone-like trabecular structure formation (fibrocallus) appeared between the fibers and chondrocytes, while a large number of chondrocytes were formed in the model group after 4 weeks of fracture, but only a small part of fibrocallus and structural disorder existed. At the 8th week, the bony callus of the sham operation group and the experimental group was completely mature, the fibrous callus disappeared, and the fracture had basically healed. Compared with the bone tissue of the control group, the morphology of the sham operation group was basically the same. At the 8th week, the model group had partial bone defects, a large number of fibrous callus, and no mature callus. Compared with the sham operation group and the experimental group, the fracture healing was slower. The bone tissue of rats in the control group was normal.

Expression of BMP-2 in bone tissue of rats at different time points

The expression of BMP-2 in rat tissues at various time points were detected, and it was found that there were statistical differences between groups at various time points ($p<0.001$). At the 4th, 6th and 8th weeks, the expression of BMP-2 in sham operation group, model group and experimental group was significantly higher than that in control group, and there were differences. The expression of BMP-2 in sham operation group and experimental group was significantly lower than that in model group ($p<0.05$). There were no statistical differences among the other groups ($p>0.05$). At the 12th week, the expression of BMP-2 in the model group was significantly higher than that in the other three groups ($p<0.05$), and there were no significant differences among the other groups ($p>0.05$). The comparison in each group showed that there were no significant differences in BMP-2 expression in the control group ($P>0.05$). BMP-2 peak value in the sham operation group and the experimental group reached and then gradually decreased at the 6th week, while BMP-2 peak value in the model group reached and then gradually decreased at the 8th week.

Figure 1. Expression analysis of BALP and CTX-I in rat serum. A. ELISA test results showed that the expression of BALP in model group was significantly lower than that in other three groups (p<0.001), ***indicated p<0.001. B. ELISA results showed that the expression of CTX-I in model group was significantly higher than that in other three groups (p<0.001), ***indicated p<0.001.

Table 1. Expression of BMP-2 in the four rats groups at different time points.

Group	Week 4	Week 6	Week 8	Week 12	F value	P value
Control group	1.038±0.061	1.053±0.085	1.013±0.120	1.067±0.112	1.775	0.229
Sham operation group	1.430±0.059*	1.636±0.085*	1.503±0.054*	1.145±0.125	84.946	<0.001
Model group	1.247±0.075*,#	1.455±0.056*,#	1.695±0.085*,#	1.403±0.066*,#	38.941	<0.001
Experimental group	1.515±0.086*,Δ	1.729±0.082*,Δ	1.522±0.051*,Δ	1.182±0.077Δ	94.865	<0.001
F value	44.495	73.917	63.211	11.764		
P value	<0.001	<0.001	<0.001	0.001		
*indicates that there is a difference compared with the control group (p<0.05), #indicates that there is a difference compared with the sham operation group (p<0.05), Δ indicates that there is a difference compared with the model group (p<0.05).						

Expression of BALP and CTX-I in rat serum

The results of detecting the expression of BALP and CTX-I in serum of rats in each group at the 12th week showed that the expression of BALP and CTX-I in serum of rats in the sham operation group and the experimental group had no significant differences with those in the control group (p>0.05), while the expression of BALP and CTX-I in serum of rats in the model group were significantly lower than those in other groups, and the expression of CTX-I was significantly higher than that in other groups (p<0.05), as shown in Figure 1.

Detection of maximum load, maximum strain and elastic modulus

The maximum load, the maximum strain and the elastic modulus of the rats at the 6th, 8th and 12th weeks after the operation were detected and found that there were statistical differences between the groups at each time point (p<0.001). The maximum load, the maximum strain and the elastic modulus of the control group at the 6th and 8th weeks were significantly higher than those of the sham operation group, the model group and the experimental group (p<0.05). The maximum load, maximum strain and elastic modulus of the sham operation group and the experimental group were significantly higher than those of the model group (p<0.05), while there were no statistical differences among the other groups (p>0.05). At the 12th week, the maximum load, maximum strain and elastic modulus of the model group were significantly lower than those of the other three groups (p<0.05), and there were no significant differences among the other groups (p>0.05). The intra-group comparison found that the maximum load, maximum strain and elastic modulus of the sham operation group, model group and experimental group increased gradually with the increase of time, and there were statistical differences (p<0.001) in

Table 2. Comparison of maximum loads [(N/mm^2)].

Group	Week 6	Week 8	Week 12	F value	P value
Control group	120.95±6.50	121.76±7.17	120.11±5.72	0.294	0.626
Sham operation group	52.52±3.09*	82.09±4.89*	115.29±6.96	184.113	<0.001
Model group	31.08±3.63*,#	52.73±4.04*,#	63.10±4.04*,#	109.590	<0.001
Experimental group	51.32±3.09*,Δ	86.51±6.89*,Δ	116.99±5.63Δ	84.975	0.001
F value	280.751	91.523	161.225		
P value	<0.001	<0.001	<0.001		
*indicates that there is a difference compared with the control group (p<0.05), #indicates that there is a difference compared with the sham operation group (p<0.05), Δindicates that there is a difference compared with the model group (p<0.05).					

Table 3. Comparison of maximum strain [(N/mm^2)].

Group	Week 6	Week 8	Week 12	F value	P value
Control group	26.03±3.49	26.71±2.70	26.47±3.03	0.229	0.728
Sham operation group	13.46±2.24*	21.74±1.79*	25.88±2.73	146.565	<0.001
Model group	7.98±2.27*,#	10.46ω2.01*,#	14.02±2.61*,#	20.063	0.006
Experimental group	13.98±1.45*,Δ	21.28±1.74*,Δ	25.85ω2.49Δ	79.013	<0.001
F value	39.425	55.981	21.688		
P value	<0.001	<0.001	<0.001		
* indicates that there is a difference compared with the control group (p<0.05), #indicates that there is a difference compared with the sham operation group (p<0.05), Δindicates that there is a difference compared with the model group (p<0.05).					

Table 4. Comparison of elastic modulus [(N/mm^2)].

Group	Week 6	Week 8	Week 12	F value	P value
Control group	63.49±5.2	64.27±4.45	63.31±4.11	0.397	0.612
Sham operation group	51.28±3.71*	54.04±3.21*	63.12±2.41	15.544	0.007
Model group	24.97±2.15*,#	33.41±4.15*,#	44.12±5.87*,#	39.952	<0.001
Experimental group	50.37±4.1*,Δ	55.94±3.58*,Δ	64.78±3.46Δ	25.765	0.007
F value	100.642	35.703	10.268		
P value	<0.001	<0.001	0.001		
*indicates that there is a difference compared with the control group (p<0.05), #indicates that there is a difference compared with the sham operation group (p<0.05), Δindicates that there is a difference compared with the model group (p<0.05).					

the intra-group comparison, while there were no significant differences (p>0.05) in each time point of the control group (see Tables 2, 3 and 4).

Expression of volume percentage, surface percentage, average width and mineralization deposition rate of rat trabecular bone

The volume percentage, surface percentage, average width and mineralization deposition rate of trabeculae in each group of rats at the 12th week were detected. It was found that the volume percentage and average width of trabeculae in the control group, sham operation group and experimental

group were significantly higher than those in the model group (p<0.05). The surface percentage and mineralization deposition rate in the control group, sham operation group and experimental group were significantly lower than those in the model group (p<0.05). The indexes in the control group, sham operation group and experimental group had no difference (p>0.05) (Table 5).

Discussion

Osteoporosis is a primary disorder of the skeleton and is the most common cause of fractures. Due to the aging of the

Table 5. Comparison of volume percentage, surface percentage, average width and mineralization deposition rate of rat trabecular bone.

Group	Trabecular volume percentage (%)	Surface percentage (%)	Average width (μm)	Mineralization sedimentation rate (%)
Control group	19.68±2.44	3.30±1.16	200.40±7.86	0.72±0.08
Sham operation group	19.17±2.56	3.28±1.41	197.39±7.69	0.71±0.06
Model group	14.95±2.31*,#	8.54±1.43*,#	154.74±9.70*,#	1.15±0.14*,#
Experimental group	19.18±2.14△	3.20±1.07△	203.70±9.86△	0.74±0.10△
F value	5.374	18.612	27.991	27.636
P value	0.009	<0.001	<0.001	<0.001

*indicates that there is a difference compared with the control group (p<0.05), #indicates that there is a difference compared with the sham operation group (p<0.05), △indicates that there is a difference compared with the model group (p<0.05).

population, the prevalence of osteoporosis and low bone mass is expected to increase[12]. Gillespie et al. study[13] showed that the incidence rate gradually increased with the increase of patients' age through a 2-year survey of 1,638,454 women over 50 years old who had not experienced osteoporosis. At present, the clinical plan for osteoporotic vertebral fracture is limited by many factors, such as cement leakage. Although the pain of postoperative patients is relieved, kyphosis may still occur[14]. Therefore, it is hoped to find a new clinical plan for treatment.

Bone morphogenetic protein (BMP) is the earliest and most in-depth growth factor and has become the preferred protein factor for bone tissue engineering[15]. BMP plays a regulatory role in different stages of development of various tissues, especially in inducing bone formation[16]. *In vitro* studies show that[17,18] both embryonic cells and mature cells will increase their differentiation activity after being induced by BMP. BMPs are suggested to have therapeutic potential to enhance fracture healing in osteoporotic patients. BMP-2 is one of the most important extracellular signal molecules in BMP family to promote bone formation and induce osteoblast differentiation[19]. Some countries have approved the treatment of rhBMP-2 in open fractures[20], but it is not clear whether rhBMP-2 has the same efficacy in osteoporotic vertebral fractures. Therefore, this study aims to provide references for clinicians' treatment by exploring the improvement of BMP-2 compound fibrin on the condition of osteoporotic vertebral fracture in rats.

In the study of Cheng et al[21], BMP-2 delivered by sucrose acetate isobutyrate significantly improved bone repair in rats with open fractures. In this study, we established a rat model of osteoporotic vertebral fracture. After treatment with BMP-2 and fibrin, we observed the histological changes of the rats at the 4th and 8th weeks after the model was established. It was found that in the 4th week of the model group, there were a large number of chondrocytes, only a small amount of fibrous callus, and the structure was disordered. However, the experimental group and the sham operation group have the same tissue morphology in the fracture zone, with a small number of chondrocytes, cartilage islands and bone fibers,

and fibrous callus appeared on the fibers and chondrocytes. At the 8th week, some bone defects and a large amount of fibrous callus appeared in the fracture tissue area of rats in the model group, but no mature callus appeared. Compared with the model group, the sham operation group and the experimental group, the bony callus was completely mature, the fibrous callus disappeared, and the fracture basically healed. Compared with the bone tissue of the control group, the morphology was basically the same, which indicated that BMP-2 had the same effect in treating osteoporotic vertebral fracture. Furthermore, we further detected the expression of BMP-2 in fracture tissues of rats in each group at different time points, and found that the intra-group comparison found that the expression of BMP-2 in rats in each group increased continuously with the increase of time except for the control group, and the expression of BMP-2 in sham operation group and experimental group reached a peak in the 6th week and then gradually decreased, while BMP-2 in model group reached a peak in the 8th week. The inter-group comparison found that the rats in sham operation group and experimental group reached the 4th, 6th. Compared with the control group, it was obviously increased at the 8th week, but there were no differences at the 12th week. Compared with the control group, the model group had differences at all time points, which indicated that the metabolism of rats after ovariectomy was out of balance, and the BMP-2 growth factor released after fracture was obviously decreased, while the healing of osteoporotic rats' vertebral fracture was effectively promoted by exogenous increase of BMP-2 expression.

Furthermore, we also tested the maximum load, maximum strain and elastic modulus of rats in each group at weeks 6, 8 and 12. We found that the maximum load, maximum strain and elastic modulus of rats in other groups at weeks 6, 8 and 12 except the control group increased gradually with time. The maximum load, maximum strain and elastic modulus of rats in the experimental group and the sham operation group showed no obvious difference at each time point, and showed no difference compared with the control group at week 12. Although the indexes of rats in the model group gradually improved, the degree of change was

obviously lower than that in the experimental group and the sham operation group. The maximum load, the maximum strain and the elastic modulus are important indexes for evaluating the biological function of bone[22]. Detection of these indexes also shows that the recovery of the biological function of bone in rats can be improved after treatment with BMP-2 composite fibrin. Studies by Huang et al[23] show that the maximum load, the maximum strain and the elastic modulus of the ovariectomized fracture mice treated with rhBMP-2 combined with psoralen are significantly improved compared with the model group. This is similar to the results of our study, but relative to his study, we have increased the detection at various time points, and observed the changes of maximum load, maximum strain and elastic modulus of rats through dynamic detection at multiple time points, further proving the role of BMP-2 composite fibrin in the treatment of osteoporotic vertebral fracture healing.

We also detected the expression of BALP and CTX-I in serum of rats in each group at the 12th week, as well as the volume percentage, surface percentage, average width and mineralization deposition rate of trabecular bone. BALP is secreted by osteoblasts, and its expression changes are markers reflecting the maturation and activity of osteoblasts[24]. CTX-I is a bone turnover marker and is an important predictor of bone loss rate and fracture risk[25]. The results showed that the expression of BALP and CTX-I in model group were significantly lower than that in other groups, and the expression of CTX-I was higher than that in each group. This indicated that the expression of BALP and CTX-I in rats were effectively improved after BMP-2 combined with fibrin therapy. This may be that BMP can indirectly stimulate osteoclast formation through the osteoblast nuclear factor receptor activator to stimulate κ-B (RANK)/RANKL ligand (RANKL) pathway, thus causing changes in the expression of BALP and CTX-I[26,27]. Volume percentage and average width of trabecular bone are important indicators to measure bone mass, while surface percentage and mineralization deposition rate are important parameters to reflect bone resorption[28]. However, we found that the volume percentage, surface percentage, average width and mineralized deposition rate of trabecular bone in the sham operation group and the experimental group were significantly higher than those in the model group at the 12th week, while the surface percentage and mineralized deposition rate in the sham operation group and the experimental group were significantly lower than those in the model group, and there were no significant differences in each index between the experimental group and the sham operation group and the control group, which indicated that BMP-2 composite fibrin could improve the relationship between bone resorption and bone formation, promote osteoporotic vertebral tissue to become compact and trabecular bone bulky, and thus improve the healing quality.

In this study, although we showed the effect of BMP-2 composite fibrin in promoting osteoporotic vertebral fracture healing in rats, however, there are several limitations. Firstly, as a basic experiment, whether the clinical efficacy of BMP-2 composite fibrin is consistent with the experimental result needs further research. Secondly, this study has not deeply explored the specific mechanism of BMP-2 in osteoporotic vertebral fracture healing. Therefore, we aim to add clinical experiments in future research and more related basic experiments to explore the specific mechanism of BMP-2 in osteoporotic vertebral fracture healing, so as to supplement and verify our experimental results. In conclusion, BMP-2 compound fibrin can promote the healing speed and improve the healing quality of osteoporotic vertebral fracture in rats.

Authors' contribution

XO, YD and LY conceived and designed the study. FX, XY and PS performed ELISA. ST, QC and YX contributed to observation indexes analysis. XO wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval

The study was approved by the Ethics Committee of Xuzhou Third Hospital, Affiliated Hospital of Jiangsu University.

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