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Original Article

Changes in BMP-2 expression and mechanical properties during treatment of rats with osteoporotic hindlimb fracture with strontium ranelate

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Abstract

Objectives: This study aims to investigate the changes in bone morphogenetic protein-2 (BMP-2) expression and mechanical properties in the healing process of rats with osteoporotic hindlimb fracture. **Methods:** 120 rat models of osteoporotic hindlimb fracture were established and randomly divided into experimental group and control group. Quantitative real-time polymerase chain reaction (PCR) used to detect the BMP-2 expression in the rat's callus tissue on the fractured side. The mechanical properties of rat's hindlimb skeleton were examined using a universal material mechanics testing machine. **Results:** The BMP-2 expression in the experimental group was higher than that in the control group (p<0.05). The linear correlation analysis showed that the BMP-2 was positively correlated with healing time (r=0.87, p<0.05). The mechanical properties were markedly improved at T2, T3 and T4, which peaked at T4 (p<0.05). However, the mechanical properties in the rats in the experimental group were notably superior to those in the control group at T2, T3, and T4 (p<0.05). **Conclusions:** The treatment with strontium ranelate can effectively improve the BMP-2 and bone mechanical properties of the rats with osteoporotic hindlimb fracture in the healing stage and accelerate the healing progress, which could be proved to be an efficacious means in treating osteoporotic fracture.

Keywords: Bmp-2, Fracture, Mechanical Properties, Osteoporosis, Strontium Ranelate

Introduction

The degeneration of the metabolic functions and bone structures in the body of older people can easily cause a series of coexisting illnesses[1]. With the acceleration of population aging around the globe in recent years, osteoporosis has become the leading disease threatening the physical health of the elderly[2]. The study of Weaver et al[3] showed that more than 80% of people aged over 60 years were osteoporotic in 2015, and the trend keeps rising over the past few years. Some data have indicated that[4,5] osteoporosis is not only limited to elderly, but also a percentage of middle-aged patients also suffer from the disease due to the social development and fast-paced city life. The degeneration of skeletal tissue in osteoporosis individuals makes them extremely vulnerable to fracture. Moreover, the recovery period of the patients is prolonged, and the difficulty of cure is increased because of the sparse original bone microarchitecture[6,7]. The statistical results of Saag et al[8] showed that over 50% new osteoporotic patients around the world were complicated with fracture in 2015. Facing the increasingly serious onset of osteoporosis, consistent efforts have been made in exploring means that can efficiently improve the patients' prognosis in clinical practices.

Currently, numerous studies in China and other countries[9,10] have demonstrated that the prognosis of patients with osteoporotic fracture can be significantly ameliorated through medicine intervention, but what type of treatment has the best efficacy remains controversial. As a type of drug capable of promoting bone formation and inhibiting bone resorption, strontium ranelate has a very high application value for the treatment of fracture[11]. Also, bone morphogenetic protein-2 (BMP-2) is the most important regulatory factor for bone formation, which participates in the skeletal development and repair of bone

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Table 1. Primer sequences.

	BMP-2	GAPDH
F	5'-CTGTCCCTACTGATGAGTTTCT-3'	5'-TCCCTCAA-GATTGTCAGCAA-3 '
R	5'-CATGCCTTAGGGATTTTGGA-3'	5'-GCCATGACGGTAACCACGG-3'

defect since the embryonic development in human body and plays crucial roles in the development of fat, kidney and nervous system[12]. There are few studies on the application of strontium ranelate in osteoporotic fracture at present, so rat models of osteoporotic hindlimb fracture were established and then treated with strontium ranelate in this research, so as to analyze the efficacy of strontium ranelate in treating osteoporotic fracture and its effect on BMP-2, thus providing reference and guidance for clinical treatment of such patients in the future.

Materials and methods

Animal models

A total of 120 clean female Sprague-Dawley (SD) rats aged 8 months old and weighing 220-260 g were purchased from the Laboratory Animal Center of Central South University and raised normally at room temperature.

Modeling methods

By reference to the rat modeling scheme for osteoporotic fracture of Li et al[13], the ovaries of the 120 rats were removed with dorsal bilateral ovariectomy after anesthesia with an intraperitoneal injection of ketamine (0.1 g/kg). 3 months later, the rats surviving in operation were randomly divided into experimental group (n=60) and control group (n=60) to establish models of osteoporotic fracture. After anesthesia and disinfection with ketamine injected intraperitoneally, the rats were fixed in the lateral position, and a 0.5 cm longitudinal incision was made on the right lateral femur to expose the middle femur that was cut by a pair of scissors to cause transverse fracture. Then a small incision was made from the medial patella to expose the femoral condyle. After that, a 14-gauge needle was retrogradely implanted and fixed at the broken end of fracture, serving as an intramedullary fixation nail. Next, the incisions were sutured after the broken end of fracture was well fixed. After the operation was accomplished, the rats were put into a rearing cage with free movement and food. The aseptic procedures were strictly followed during the operation.

Experimental methods

The rats in the experimental group were treated with strontium ranelate (1 g/kg) once a day through gavage method from the 3rd day after modeling, while those in control group recovered spontaneously without any treatment. 10 rats in each group were killed by decapitation before treatment (T1) and at the 3rd (T2), 6th (T3), and 9th (T4) weeks of treatment, respectively. Then the rats' callus tissues on the fractured side were taken and stored in liquid nitrogen. Furthermore, the skeleton of the hind limb was fetched for mechanical property test. The callus tissues were added and mixed with TRIzol at a proportion of 1 mL: 100 mg to extract total ribonucleic acid (RNA), which was synthesized into first-strand complementary deoxyribonucleic acid (cDNA) through reverse transcription and detected using fluorescence quantitative polymerase chain reaction (PCR) according to the instructions. Reaction conditions: at 50°C for 2 min, 95°C for 10 min, 95°C for 15 s and 60°C for 1 min, 45 cycles in total. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the internal reference gene and amplified together with the genes to be detected to calculate the Ct value (the number of amplification cycles in which the fluorescence signal of amplification product reached the set threshold). The primer sequences are shown in Table 1. On the premise of maintaining intact cortical shell of the lumbar vertebra taken out, the spinous process, soft tissue, articular process, and transverse process were completely removed, and then the lumbar vertebra was made into a cuboid (6 mm ×3 mm ×3 mm) and subjected to vertical compression test using a universal material mechanics testing machine (manufactured by Shimadzu Corporation, Japan), with a loading speed of 2 mm/min.

Observation indexes

Relative expressions of the BMP-2 in bone tissues of the two groups of rats at T1, T2, T3 and T4, the maximum load, elastic modulus and maximum strain of the vertebrae detected by the universal material mechanics testing machine at T1, T2, T3 and T4, and the correlation between BMP-2 and healing time.

Statistical methods

Statistical Product and Service Solutions (SPSS) 22.0 software [Yijun (Shanghai) Information Technology Co., Ltd.] were used for data analysis and processing. All the results were expressed as mean ± standard deviation. Repeated measures analysis of variance was adopted for comparison among multiple groups, t-test was performed for comparison between two groups, and Pearson analysis was conducted for correlation analysis. $p<0.05$ suggested that the difference was statistically significant.

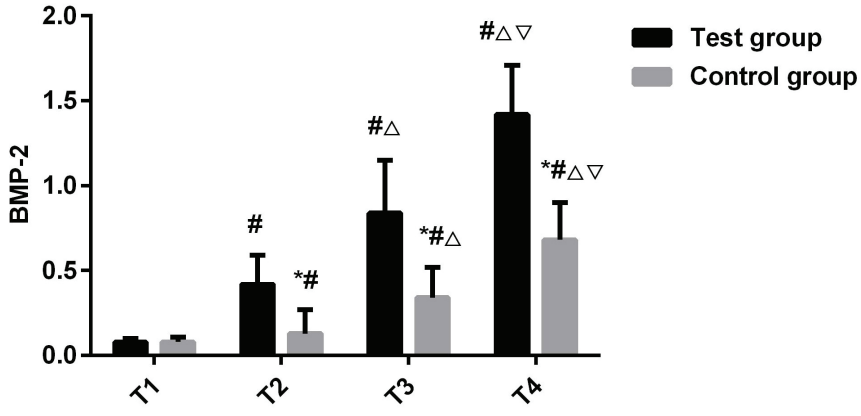

Figure 1. BMP-2 expression in callus tissues on the fractured side of rats in experimental group and control group. The results of qRT-PCR show that there is no significant difference at T1 between the two groups (p>0.05). The BMP-2 expression increases notably at T2, keeps rising at T3 and peaks at T4 (p<0.05). The rats in experimental group have remarkably higher BMP-2 expression at T2, T3 and T4 than control group (p<0.05). *p<0.05 vs. BMP-2 expression in experimental group, #p<0.05 vs. BMP-2 expression in the same group at T1, $^{\triangle}p$<0.05 vs. BMP-2 expression in the same group at T2, and p<0.05 vs. BMP-2 expression in the same group at T3.

Results

Modeling results

Among the 120 rats, a total of 110 rat models were established successfully, with a success rate of 91.67%, (110/115), including 55 rats in the experimental group and 55 rats in control group.

BMP-2 expression level

There was no significant difference in BMP-2 expression at T1 between the experimental group and the control group (p>0.05). The BMP-2 expression levels were (0.42±0.17), (0.84±0.31) and (1.42±0.29) in experimental group at T2, T3 and T4, respectively, which were remarkably higher than those in control group [(0.13±0.14), (0.34±0.18) and (0.68±0.22)] (p<0.05). In experimental group, the BMP-2 level at T2, T3, and T4 were higher than that at T1, which was the highest at T4, followed by at T3 and T2 (p<0.05) (Figure 1).

Correlation of BMP-2 with healing time

The linear correlation analysis showed that BMP-2 was positively correlated with the healing time (r=0.87, p<0.05) (Figure 2).

Mechanical properties of vertebrae

The maximum strains were (3.07±0.84) N/mm^2, (8.64±1.86) N/mm^2, (16.34±1.94) N/mm^2 and (24.83±2.87) N/mm^2 at T1, T2, T3 and T4, respectively, in experimental group, which were (3.14±0.78) N/mm^2, (5.33±1.46) N/mm^2, (8.68±2.07) N/mm^2 and (13.26±1.84) N/mm^2, respectively, in control group. The maximum loads were (12.33±1.86) N/mm^2, (32.24±1.63) N/mm^2, (69.83±6.72) N/mm^2 and (102.34±8.64) N/mm^2 at T1, T2, T3 and T4, respectively, in experimental group, which were (13.01±1.94) N/mm^2, (28.14±3.12) N/mm^2, (45.16±3.69) N/mm^2 and (59.64±4.59) N/mm^2, respectively, in control group. The elastic moduli were (26.09±2.43) N/mm^2, (41.62±1.86) N/mm^2, (55.17±3.08) N/mm^2 and (64.27±4.11) N/mm^2 at T1, T2, T3 and T4, respectively, in experimental group, which were (26.12±2.63) N/mm^2, (31.42±1.59) N/mm^2, (39.57±3.28) N/mm^2 and (45.72±2.88) N/mm^2, respectively, in control group. There were no significant differences in the above-mentioned indexes at T1 between the two groups (p>0.05). The levels of those indexes started to rise markedly at T2 and peaked at T4 in both groups (p<0.05). Moreover, the experimental group had notably higher indexes than control group at T2, T3, and T4, displaying statistically significant differences (p<0.05) (Figures 3, 4 & 5).

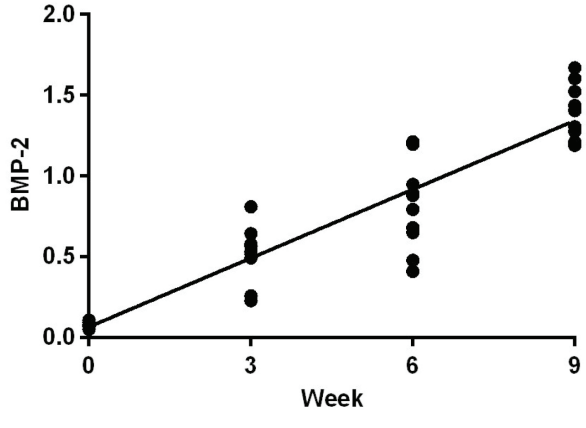

Figure 2. Correlation of BMP-2 with healing time. According to the linear correlation analysis, the BMP-2 is positively correlated with healing time (r=0.87, p<0.05).

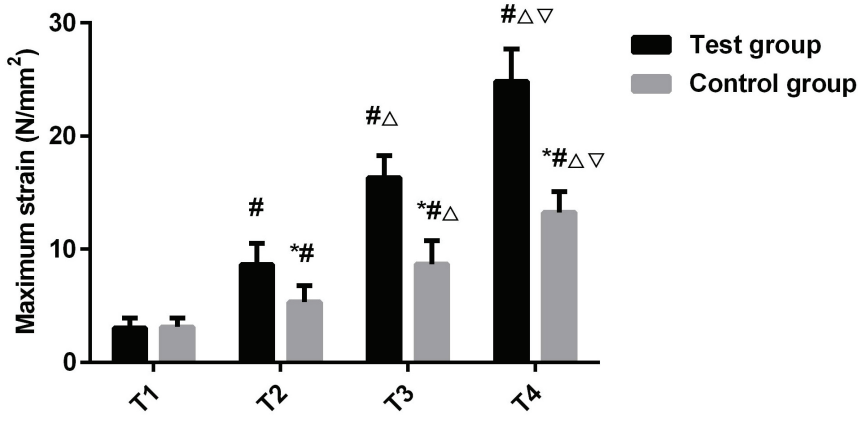

Figure 3. Detection results of maximum strain. There is no significant difference at T1 between the two groups (p>0.05). The maximum strain increases markedly at T2, keeps rising at T3 and reaches the peak at T4 (p<0.05). Compared with that in control group, the maximum strain in experimental group is significantly higher at T2, T3 and T4 (p<0.05). *p<0.05 vs. maximum strain in experimental group, #p<0.05 vs. maximum strain in the same group at T1, $^{\triangle}p$<0.05 vs. maximum strain in the same group at T2, and p<0.05 vs. maximum strain in the same group at T3.

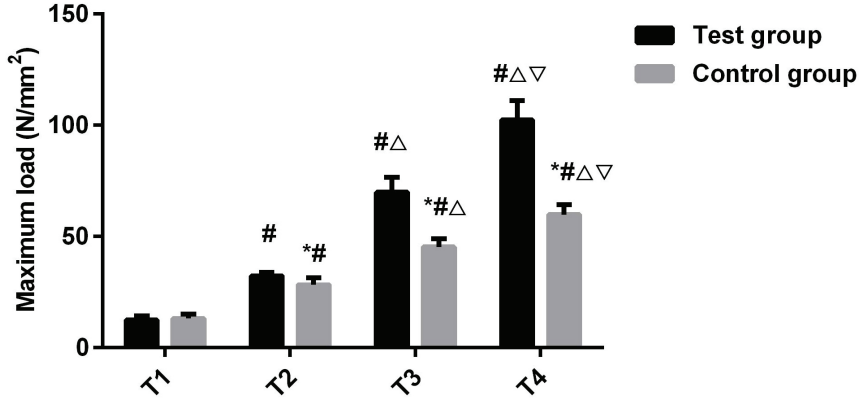

Figure 4. Detection results of maximum load. There is no significant difference at T1 between the two groups (p>0.05). The maximum load increases markedly at T2, keeps rising at T3 and peaks at T4 (p<0.05). The maximum load in experimental group is increased evidently at T2, T3 and T4 compared with that in control group (p<0.05). *p<0.05 vs. maximum load in experimental group, #p<0.05 vs. maximum load in the same group at T1, $^{\triangle}p$<0.05 vs. maximum load in the same group at T2, and p<0.05 vs. maximum load in the same group at T3.

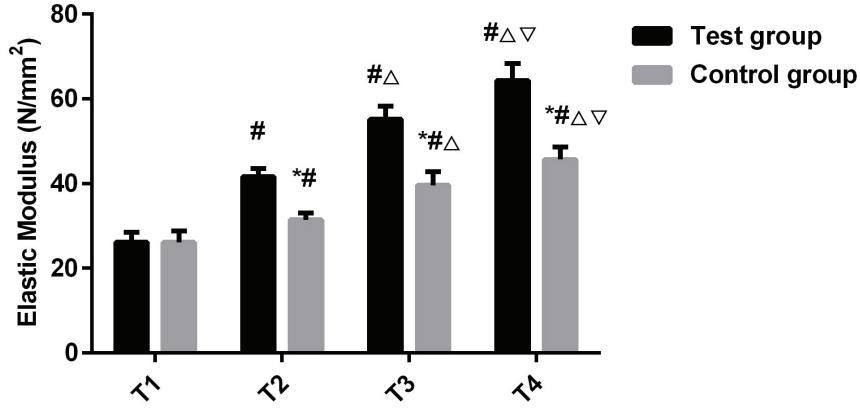

Figure 5. Detection results of elastic modulus. The difference is not significant at T1 between the two groups (p>0.05). The elastic modulus increases obviously at T2, keeps rising at T3 and reaches the peak at T4 (p<0.05). The rats in experimental group have remarkably higher elastic modulus than control group at T2, T3 and T4 (p<0.05). *p<0.05 vs. elastic modulus in experimental group, #p<0.05 vs. elastic modulus in the same group at T1, $^{\triangle}p$<0.05 vs. elastic modulus in the same group at T2, and p<0.05 vs. elastic modulus in the same group at T3.

Discussion

Osteoporotic fracture refers to the bone damage caused by the breakage of bone microarchitecture, and the key to treatment lies in how to restore the damaged bone tissues[14]. The circulation of blood supply is the first problem to be solved in the healing process of fracture because it can provide massive oxygen and nutrients necessary for the healing of fractured sites and create a preferable microenvironment for rehabilitation[15,16]. Secondly, the treatment of fracture focuses on the reconstruction of bone marrow that is a structure composed of multiple organic matters and contains various cells (such as osteoprogenitor cell and endosteal cell) maintaining the integrity of bone microarchitecture[17]. Currently, many studies in China and other countries[18-20] have proven that strontium ranelate can not only promote the formation of vascular endothelial growth factor (VEGF) by its molecule cracking but also stimulate the differentiation of osteoblasts, effectively accelerating the reformation of skeleton. However, it is mostly applied to traumatic fractures in clinic, and there are still great doubts about its application value for osteoporotic fracture at present. Therefore, the changes in BMP-2 and bone mechanical properties in the healing process of rats with fractures were investigated by establishing rat models of osteoporotic hindlimb fracture and using strontium ranelate as the therapeutic drug in this research, to testify the application value of strontium ranelate in osteoporotic fracture.

According to the experimental results in this research, there were no differences in the examination results of BMP-2 and mechanical properties at T1 between the experimental group treated with strontium ranelate and control group treated without strontium ranelate. The levels of those indexes began to increase remarkably at T2, which were notably higher in experimental group compared with those in control group. The linear correlation analysis revealed that BMP-2 had a positive correlation with the fracture healing time of the rats. BMP-2 is recognized as a factor promoting bone formation at present, which regenerates the bone tissues by forming the cartilage tissues first. It has been proven that BMP-2 has the ability to induce ectopic bone formation at various sites of human body (muscle, subcutaneous tissue, fat, etc.)[21]. As for the rats in experimental group, the BMP-2 level was significantly elevated in the healing process, suggesting that strontium ranelate can provide large quantities of osteogenic factors for fracture. In addition, the mechanical properties in experimental group were superior to those in control group in the healing process, demonstrating that strontium ranelate has a higher application value for skeletal rehabilitation. Endochondral ossification plays the predominant role in the healing process of osteoporotic fracture[22], while the BMP-2 can activate the chemotaxis of mesenchymal cells, promote its decomposition into chondrocytes and accelerate the neo-bone formation through endochondral ossification and bone marrow[23]. It is presumed that strontium ranelate brings massive chondrocytes and osteoblasts to the site of fracture in the process of elevating the BMP-2 level, thus speeding up the progress of endochondral ossification and promoting the generation of new bone, but its mechanism of action still needs further investigations. Compared with those in control group, the excellent mechanical properties of the rats in experimental group indicated that the bone microarchitecture of the rat's vertebra was sounder and more compact in experimental group in the healing process. The increased resistance to load of the vertebra enhances the healing and treatment of fracture, greatly lowering the risk of refracture. It is also one of the key points to effectively ameliorate the prognosis of osteoporosis patients. These results are consistent with the findings of Müller et al[24], further evidencing the viewpoints in this experiment.

Deficiencies still exist in this experiment due to limited conditions. For example, the healing time of osteoporotic fracture is generally about 12 weeks, but only 9 weeks of data were recorded in this experiment. Moreover, possible differences between the human body and animal model could not be excluded, so human experiments should be carried out as soon as possible to perfect our analyses and achieve the optimum experimental results.

In conclusion, the treatment with strontium ranelate can effectively improve the BMP-2 level and bone mechanical properties of the rats with osteoporotic hindlimb fracture in the healing stage and accelerate the healing progress, which can be proved to be an efficacious means in treating the osteoporotic fracture.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

DW and XF performed PCR; CY and LZ were responsible for animal models construction. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by the ethics committee of the Affiliated Hospital of Taishan Medical University.

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