Original Article
Therapeutic effect of artificial bone powder complex combined with recombinant human bone morphogenetic protein-2 on tibial bone defect in rats

Liping Kuang[1*], Huai'an Zhu[2*], Miao Yin[3]

[1]Wuhan Dazhong Dental Medical Co., Ltd., Wuhan, Hubei Province, China; [2]Department of Stomatology, Wuhan Children's Hospital (Wuhan Maternal and Child Healthcare Hospital), Tongji Medical College, Huazhong University of Science & Technology, Wuhan, Hubei Province, China; [3]Hubei Tumor Biological Behavior Key Laboratory, Center of Stomatology, Zhongnan Hospital of Wuhan University, Wuhan, Hubei Province, China. *Equal contributors and co-first authors.

Abstract: Objective: To study the therapeutic effect of artificial bone powder complex combined with recombinant human bone morphogenetic protein-2 (rhBMP-2) on tibial bone defect in rats. Methods: Altogether 80 SD rats were selected to establish a tibial bone defect model, which was divided into the model group, the artificial bone powder group, the rhBMP-2 group and the combined group, with 20 rats in each group. The model group was not filled with any material. The artificial bone powder group was given 100 μg/mL artificial bone powder complex, the rhBMP-2 group was given 100 μg/mL rhBMP-2, and the combined group was given 100 μg/mL rhBMP-2 combined artificial bone powder. Histology, X-ray film of bone defect position, bone density value of osteogenic region, alkaline phosphatase and calcium content were observed at 6, 12 and 18 weeks after operation. Core binding factor α1, zinc finger structure transcription factor, osteocalcin and bone sialoprotein were detected at 18 weeks after operation. Results: Compared with the model group, TbAr, dense bone area, bone mineral density in osteogenic region, ALP activity and callus Ca content were increased in the combined group, the artificial bone meal group and the rhBMP-2 group 6, 12 and 18 weeks after operation. The TbAr, compact bone area, bone density in osteogenic region, ALP activity and Callus Ca content in the combined group were higher than those in artificial bone meal group and rhBMP-2 group at 6, 12 and 18 weeks after operation ($P < 0.05$). The TbAr and ALP activities in the combined group, rhBMP-2 group, artificial bone powder group and model group at 18 weeks after operation were all lower than those in the four groups at 6 and 12 weeks after operation, and the compact bone area, bone density in osteogenic region and Callus Ca content were higher than those in the four groups at 6 and 12 weeks after operation. The TbAr of the combined group, rhBMP-2 group, artificial bone meal group and model group at 12 weeks after operation was lower than that of the four groups at 6 weeks after operation, and the compact bone area, bone density value in osteogenic region, ALP activity and Callus Ca content of the four groups at 12 weeks after operation were higher than that of the four groups at 6 weeks after operation ($P < 0.05$). The Runx2, Ostenix, OCN and BSP mRNA in the combined group were higher than those in the artificial bone meal group, rhBMP-2 group and model group 18 weeks after operation ($P < 0.05$). Conclusion: Artificial bone powder combined with rhBMP-2 has positive therapeutic effects on tibial bone defects in rats. It can promote osteogenesis and participate in the repair process of bone defects by increasing Runx2, Ostenix, OCN and BSP mRNA.

Keywords: Artificial bone powder complex, recombinant human bone morphogenetic protein-2, tibial bone defect, repair

Introduction

Tibia is the central part of weight-bearing bone in lower limbs. Traffic accidents, mechanical injuries, and falls can lead to tibial fractures. Tibia fractures have shown an upward trend in recent years. As there is only one layer of skin on the anterior medial side and anterior crest of tibia, the subcutaneous tissue coverage is relatively thin. Once tibial fractures occur, it will affect blood circulation, easily lead to infection and affect fracture healing of patients [1]. The

research on bone defect repair is gradually deepening. The advantages of ideal bone repair materials are mainly embodied in biocompatibility, bone guidance, absorbed degradability, good bone induction, being able to be replaced by host bone, wide selection of materials, easy processing, low price, easy disinfection, and convenient clinical operation [2]. In the bone defect model, recombinant human bone morphogenetic protein-2 (rhBMP-2) intervention alone has a fast diffusion and decomposition rate, and cannot act on more target cells within an effective time, making it difficult to give full play to its induction activity [3]. Studies have shown that the source of autogenous bone is less and the absorption is fast. Allogeneic and xenogeneic bones have immune rejection reaction and have potential risk of infectious diseases. All kinds of artificial bone substitute materials only play the role of bone scaffold and have certain limitations in osteoinductive activity [4]. rhBMP-2 belongs to bone morphogenetic proteins (BMP), which is the strongest osteogenic factor, but it is easy to lose. Guo pointed out that rhBMP-2 compound has a significant effect on repairing bone defects of dental implants. By inducing the occurrence of rhBMP-2, it can make up for the deficiency of rapid absorption and difficult formation of rhBMP-2 [5]. At present, there is little research on the mechanism of rhBMP-2 combined with artificial bone powder in repairing tibial bone defect. This paper aimed to study the therapeutic effect of rhBMP-2 combined with artificial bone powder on tibial bone defect in rats.

Materials and methods

Research animals

In this study, 80 SD rats, aged 6-10 weeks, with an average age of (8.0±1.2) weeks and a body weight of (220.5±10.2) g, were selected and provided by the Animal Experimental Center of Zhongnan Hospital of Wuhan University (Animal License No.: SYXK (Wuhan) 2015-0025). The animal studies have been approved by the Animal Ethics Committee of Wuhan Children's Hospital (Wuhan Maternal and Child Healthcare Hospital), Tongji Medical College, Huazhong University of Science & Technology.

Main reagents and instruments: rhBMP-2 with purity > 95% (Beijing Bioss Biotechnology Co., Ltd., China); artificial bone powder complex (Bsy (Dalian) International Trade Co., Ltd., China); Semi-automatic image digitizer (Beijing Sartorius Instrument System Co., Ltd., China); EPEX DR machine (Beijing Perlong Technology Co., Ltd., China); LX-20 Automatic Biochemical Analyzer (Beckman, USA); Runx2, Ostenix, OCN, BSP primary antibody (Wuhan Boster Biological Technology Co., Ltd., China); pentobarbital sodium (Beijing Huaye Huanyu Chemical Co., Ltd., China); microwave digestion instrument (Changsha Yongle Kang Instrument Co., Ltd., China); RT-PCR kit (Shanghai Kule Biotechnology Co., Ltd., China).

Methods

Model establishment and intervention: Referring to Li et al., 80 rats were used to establish tibial bone defect model: rats were anesthetized with 3% pentobarbital sodium (30 mg/kg), hair was cut short in the middle and upper segment of each rat's left leg, the anterolateral straight incision was taken, skin, fascia and muscle were cut in turn, the muscle was separated, the middle and upper segment of tibia was exposed and was cut off horizontally, a 4 mm gap was taken, the model was established, the wound was washed with 0.9% physiological saline, no internal and external fixation was performed, and the musculocutaneous membrane, fascia and skin were sutured layer by layer [6]. The rats were randomly divided into the model group, the artificial bone powder group, the rhBMP-2 group and the combined group, with 20 rats in each group. The left tibial bone defect in the model group was not filled with any material. Artificial bone powder group was treated with 100 μg/mL artificial bone powder compound into the left tibial bone defect of rats. In rhBMP-2 group, rhBMP-2 lyophilized powder was dissolved in sterile water for injection (100 μg/mL), centrifuged, mixed evenly, and placed for 72 h to fill rhBMP-2 at a concentration of 100 μg/mL into the left tibial bone defect of rats. The combined group was given rhBMP-2 (100 μg/mL) combined with artificial bone powder for intervention.

HE dyeing: Rats were euthanized with 200 mg/kg pentobarbital sodium injection 18 weeks after operation [7]. After dissection, the osseous delusion tissue was completely sepa-

Table 1. Primer sequences used in this study

Term	Sequence
Runx2 F	GAATGATGAGAACATACTCTGCCG
Runx2 R	GGATTTGTGAAGACCGTTATGG
Ostenix F	CGCATCTGAAAGCCCACTTG
Ostenix R	CAGCTCGTCAGAGCGAGTGAA
OCN F	CAACAATGGACTTGGAGCCC
OCN R	ATAGATGCGCTTGTAGGCGT
BSP F	CTGACCAGTTATGGCACCAC
BSP R	TAATCCTGACCCTCGTAGCC
GAPDH F	CCTCGTCTCATAGACAAGATGGT
GAPDH R	GGGTAGAGTCATACTGGAACATG

Note: Runx2: runt-related transcription factor 2; OCN: osteocalcin; BSP: bone sialoprotein; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

rated, the surface soft tissue was peeled off, fixed in 4% paraformaldehyde for 24 h, decalcified and washed, dehydrated step by step in ethanol, sliced, and stained with routine HE.

Detection of bone histomorphometry related indicators: The bone histomorphometry indicators (including trabecular area and dense bone area) were measured by Bioquant OSTEO bone histomorphometry image analysis system at 6 weeks (6 euthanasia rats), 12 weeks (6 euthanasia rats) and 18 weeks (8 euthanasia rats) after operation in each group using 50 times ordinary light microscope field of view.

Determination of bone mineral density, ALP and Ca content in osteogenic region: Before the rats were executed, the lower limb positive X-ray films of rats were taken by EPEX DR machine, and the bone mineral density in the osteogenic region of rats was measured by semi-automatic image digitizer. Extraction of rat osteoblast bone, centrifugation (centrifugation radius 15 cm, 3,000 r/min, 15 min), ALP determination were performed: 100 μL of fresh prepared substrate was taken; the cell lysis products were added, incubated in 37°C incubator for 30 min; sodium hydroxide was added to terminate the reaction; ELISA was used to detect ALP level at 410 nm. Determination of calcium content in bone of rat osteogenic region: Formic acid and concentrated nitric acid were dropped into the collected blood sample, shaken and mixed, cooled after reaction. Concentrated nitric acid was added, and then hydrogen peroxide was added after cooling. The reactant was poured into a polytetrafluoroethylene digestion cup, placed into an

outer tank and covered tightly. Microwave digestion was performed in two steps in a microwave digestion instrument to obtain a yellowish clear transparent sample solution, and the calcium content was measured.

Runx2, Ostenix, OCN, BSP mRNA detection: Fluorescent quantitative RT-PCR was used to separate mononuclear cells from bone samples in osteogenic region with Ficoll solution. TRIzol was applied to extract RNA, which was detected by electrophoresis and quantified, and cDNA was synthesize by reverse transcription with RT-PCR kit. After that, real-time fluorescence quantitative PCR experiment was carried out. The probe or SYBR was reacted with 25 uL, and the reaction conditions were as follows: 94°C for 5 min, 94°C for 45 s, 60°C for 1 min, for a total of 40 cycles. Standard group and blank control were set. A total of 3-15 cycle fluorescence signals before PCR reaction experiment were used as fluorescence bottom signals, the baseline was adjusted, and Runx2, Ostenix, OCN, BSP mRNA expression was analyzed by $2^{-\Delta\Delta Ct}$. GAPDH was applied as the internal reference. See **Table 1**.

Statistical method

SPSS 20.0 was used for statistical analysis. The measurement data were represented by mean ± standard deviation ($\bar{x}$ ± sd). The comparison among groups was conducted by one-way ANOVA, and post hoc Bonferroni test was used for pair-wise comparison. The P value less than 0.05 was regarded as statistical significance.

Results

Effects of artificial bone powder complex and rhBMP-2 on bone metrology related indexes in model rats

As shown in **Table 2**, compared with the model group, TbAr and dense bone area increased in the combined group, artificial bone powder group and rhBMP-2 group at 6, 12 and 18 weeks after operation. The TbAr and compact bone area in the combined group were higher than those in the artificial bone meal group and rhBMP-2 group at 6, 12 and 18 weeks after operation ($P < 0.05$). There was no statistical difference in TbAr and compact bone area between rhBMP-2 group and artificial bone meal group at 6, 12 and 18 weeks after

Table 2. Comparison of the related indexes of rat bone metrology in various groups ($\bar{x} \pm sd$)

Group	Model group (n = 20)	Artificial bone powder group (n = 20)	rhBMP-2 group (n = 20)	Combined group (n = 20)	F	P
TbAr (%)						
6 weeks after operation (n = 6)	10.17±1.05	20.38±2.65*,#	22.06±2.89*,#	70.22±3.85*	55.289	0.001
12 weeks after operation (n = 6)	8.82±0.89	13.39±2.52*,#	14.14±2.66*,#	19.16±1.05*	27.601	0.001
18 weeks after operation (n = 8)	8.05±0.28	8.26±1.85*,#	9.06±2.02*,#	17.74±0.78*	49.658	0.001
F	8.285	15.173	14.915	57.037		
P	0.001	0.001	0.001	0.001		
Dense bone area (%)						
6 weeks after operation (n = 6)	1.85±0.56	4.95±0.92*,#	5.11±1.05*,#	13.05±0.75*	43.965	0.001
12 weeks after operation (n = 6)	10.75±1.77	38.20±2.25*,#	39.45±2.47*,#	62.12±1.98*	71.096	0.001
18 weeks after operation (n = 8)	14.48±2.21	40.45±5.58*,#	41.01±5.60*,#	68.86±3.96*	50.875	0.001
F	20.322	22.914	23.026	50.607		
P	0.001	0.001	0.001	0.001		

Note: Compare with model group, *$P < 0.05$; compared with the combined group, #$P < 0.05$. rhBMP-2: recombinant human bone morphogenetic protein-2.
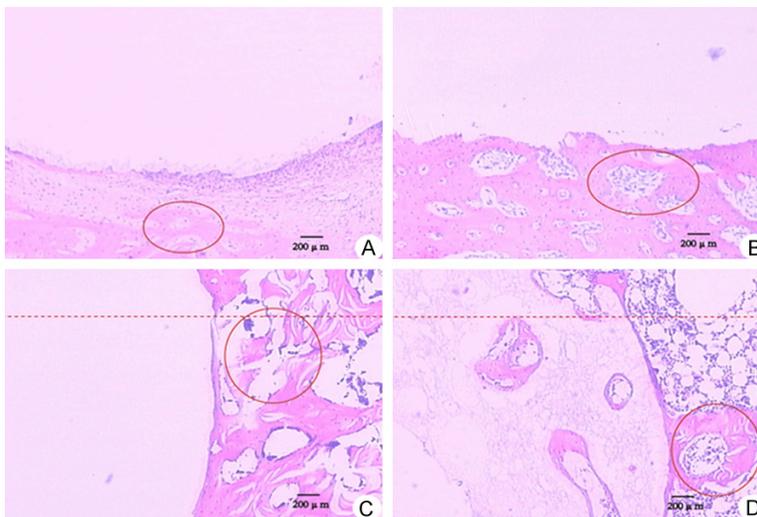

Figure 1. After 18 weeks of intervention, the pathological changes of rats in each group were observed (HE staining, × 50) A: Model group; B: Artificial bone powder group; C: rhBMP-2 group; D: Combined group. rhBMP-2: recombinant human bone morphogenetic protein-2; HE: hematoxyiin-eosin.

bone was higher than that of the four groups at 6 weeks after operation ($P < 0.05$).

Effects of artificial bone meal complex and rhBMP-2 on pathological changes in model rats

As shown in **Figure 1A**, after 18 weeks of intervention, no osteocytes were produced in the model group. **Figure 1B** and **1C** showed the results of the artificial bone meal group and rhBMP-2 group respectively. The new osteocytes in the two groups were relatively active and abundant, with fibrous tissue hyperplasia at the interface position and relatively complete bone cortex. As shown in **Figure 1D**, the number of new osteocytes in the combined group increased significantly, a large number of new osteocytes were generated at the interface position, and bone integration was better.

Effects of artificial bone powder complex and rhBMP-2 on bone mineral density in osteogenic region of model rats

As shown in **Table 3**, compared with the model group, the bone mineral density in the bone formation area of the combined group, rhBMP-2

operation ($P > 0.05$). The TbAr of the combined group, rhBMP-2 group, artificial bone meal group and model group at 18 weeks after operation was lower than that of the four groups at 6 and 12 weeks after operation, and the area of compact bone was higher than that of the four groups at 6 and 12 weeks after operation. The TbAr of the combined group, rhBMP-2 group, artificial bone meal group and model group at 12 weeks after operation was lower than that of the four groups at 6 weeks after operation, and the area of dense

Table 3. Comparison of bone mineral density in osteogenic region of rats in each group ($\bar{x} \pm sd$)

Group	Bone mineral density in osteogenic region (mg/cm^2)			F	P
	6 weeks after operation (n = 6)	12 weeks after operation (n = 6)	18 weeks after operation (n = 6)		
Model group (n = 20)	127.75±4.05	152.90±3.29	163.75±2.58	30.543	0.001
Artificial bone powder group (n = 20)	141.28±2.15*,#	169.56±3.45*,#	177.39±1.82*,#	51.060	0.001
rhBMP-2 group (n = 20)	141.56±2.28*,#	169.79±2.55*,#	177.79±1.97*,#	47.810	0.001
Combined group (n = 20)	146.42±2.45*	186.78±3.46*	202.66±4.05*	44.963	0.001
F	14.492	26.073	34.378		
P	0.001	0.001	0.001		

Note: Compare with model group, *$P < 0.05$; compared with the combined group, #$P < 0.05$. rhBMP-2: recombinant human bone morphogenetic protein-2.

Table 4. Comparison of ALP activity of rats in each group ($\bar{x} \pm sd$)

Group	ALP content (U/L)			F	P
	6 weeks after operation (n = 6)	12 weeks after operation (n = 6)	18 weeks after operation (n = 6)		
Model group (n = 20)	147.65±2.06	155.75±2.60	139.88±2.75	16.395	0.001
Artificial bone powder group (n = 20)	166.85±2.40*,#	174.65±4.50*,#	163.05±4.62*,#	7.049	0.001
rhBMP-2 group (n = 20)	167.17±2.54*,#	174.85±4.89*,#	163.88±4.97*,#	6.172	0.001
Combined group (n = 20)	206.65±7.79*	228.26±9.96*	185.07±4.80*	21.320	0.001
F	26.903	25.882	34.658		
P	0.001	0.001	0.001		

Note: Compare with model group, *$P < 0.05$; compared with the combined group, #$P < 0.05$. ALP, alkaline phosphatase; rhBMP-2: recombinant human bone morphogenetic protein-2.

group and artificial bone meal group increased at 6, 12 and 18 weeks after operation. Compared with the artificial bone meal group and rhBMP-2 group, the bone mineral density in the bone forming region of the combined group increased at 6, 12 and 18 weeks after operation ($P < 0.05$). There was no significant difference in bone mineral density between rhBMP-2 group and artificial bone meal group at 6, 12 and 18 weeks after operation ($P > 0.05$). Bone mineral density (BMD) in osteogenic region of combined group, rhBMP-2 group, artificial bone meal group and model group at 18 weeks after operation was higher than that of 4 groups at 6 and 12 weeks after operation. The bone mineral density (BMD) in the bone formation area of the combined group, rhBMP-2 group, artificial bone meal group and model group 12 weeks after operation was higher than that of the four groups 6 weeks after operation ($P < 0.05$).

Effects of artificial bone powder complex and rhBMP-2 on ALP activity in model rats

As shown in **Table 4**, compared with the model group, ALP activity in the combined group, rhBMP-2 group and artificial bone powder group increased at 6, 12 and 18 weeks after operation. Compared with artificial bone powder group and rhBMP-2 group, ALP activity in combined group increased at 6, 12 and 18 weeks after operation ($P < 0.05$). There was no significant difference in ALP content between rhBMP-2 group and artificial bone meal group at 6, 12 and 18 weeks after operation ($P > 0.05$). ALP activity in the combined group, rhBMP-2 group, artificial bone meal group and model group at 18 weeks after operation was lower than that in the four groups at 6 and 12 weeks after operation. The ALP activity in the combined group, rhBMP-2 group, artificial bone meal group and model group at 12 weeks after operation was higher than that in the four groups at 6 weeks after operation ($P < 0.05$).

Effect of artificial bone powder complex and rhBMP-2 on Ca content in callus of model rats

As shown in **Table 5**, compared with the model group, the Callus Ca content in the combined group, rhBMP-2 group and artificial bone powder group increased 6, 12 and 18 weeks after

Table 5. Comparison of the content of Ca in callus of rats in each group ($\bar{x} \pm sd$)

Group	Ca content in callus (mg/g)			F	P
	6 weeks after operation (n = 6)	12 weeks after operation (n = 6)	18 weeks after operation (n = 6)		
Model group (n = 20)	177.62±1.01	181.61±0.75	185.85±2.15	12.938	0.001
Artificial bone powder group (n = 20)	189.45±0.85*,#	193.16±0.75*,#	198.75±2.68*,#	12.189	0.001
rhBMP-2 group (n = 20)	189.99±0.89*,#	193.83±0.78*,#	198.98±2.84*,#	11.128	0.001
Combined group (n = 20)	202.98±1.08*	209.25±2.05*	220.87±2.25*	26.794	0.001
F	63.015	46.524	47.742		
P	0.001	0.001	0.001		

Note: Compare with model group, *$P < 0.05$; compared with the combined group, #$P < 0.05$. rhBMP-2: recombinant human bone morphogenetic protein-2.
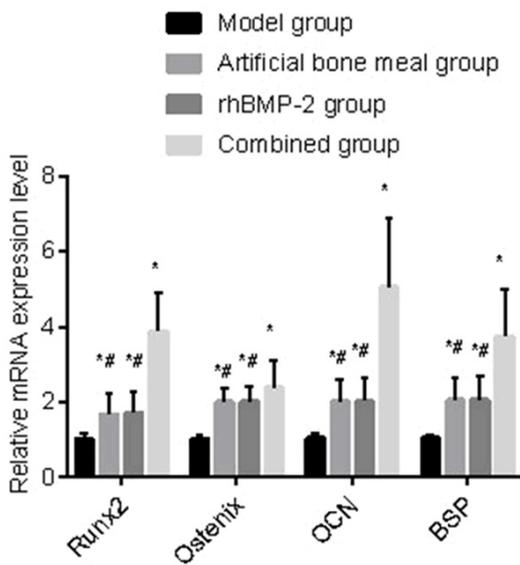

Figure 2. Expression of Runx2, Ostenix, OCN and BSP mRNA. Compare with model group, *$P < 0.05$; compared with the combined group, #$P < 0.05$. Runx2: runt-related transcription factor 2; OCN: osteocalcin; BSP: bone sialoprotein.

operation. Compared with artificial bone meal group and rhBMP-2 group, the Ca content of callus in the combined group increased at 6, 12 and 18 weeks after operation ($P < 0.05$). The Ca content of callus in rhBMP-2 group and artificial bone powder group at 6, 12 and 18 weeks after operation showed no statistical difference ($P > 0.05$). The Ca content of callus in the combined group, rhBMP-2 group, artificial bone meal group and model group at 18 weeks after operation was higher than that in the 4 groups at 6 and 12 weeks after operation. The Ca content of callus in the combined group, rhBMP-2 group, artificial bone meal group and model group 12 weeks after operation was higher than that in the four groups 6 weeks after operation ($P < 0.05$).

Effects of artificial bone powder complex and rhBMP-2 on Runx2, Ostenix, OCN, BSP mRNA in model rats

As shown in **Figure 2**, the artificial bone powder complex and rhBMP-2 were tested after 18 weeks of intervention. Compared with the model group, Runx2, Ostenix, OCN, BSP mRNA in the artificial bone powder group, rhBMP-2 group, and the combined group increased with statistical difference ($P < 0.05$). There was no statistical difference in Runx2, Ostenix, OCN, BSP mRNA between rhBMP-2 group and artificial bone meal group 18 weeks after operation ($P > 0.05$). Compared with artificial bone powder group and rhBMP-2 group, Runx2, Ostenix, OCN and BSP mRNA in the combined group were increased with statistical difference ($P < 0.05$).

Discussion

At present, bone defect repair materials are the main research content in bone defect repair. It will affect the repair quality and are affected by disease transmission and immune rejection [8]. rhBMP-2 has strong osteogenic induction and plays an important role in bone defect repair and fracture healing [9]. Some studies have shown that rhBMP-2 can promote bone defect, nonunion and fracture healing by inducing osteogenic activity, but its single use effect is not ideal because rhBMP-2 is easy to be absorbed and degraded, and also lacks suitable carriers, which has limitations in the process of new bone formation [10-12].

The results of this study showed that compared with the model group, the combined group, rhBMP-2 group and artificial bone powder group had increased TbAr and compact bone area at 6, 12 and 18 weeks after operation, indicating that rhBMP-2 combined with artificial bone powder and artificial bone powder alone could all improve the level of bone metrology related indexes in rats and promote new bone formation. The TbAr of the combined group, rhBMP-2 group and artificial bone powder group was the highest at 6 weeks after operation, indicating that the new bone grew and became active at the fastest speed in this period. The TbAr of the combined group, rhBMP-2 group and artificial bone meal group decreased and the compact bone area was relatively high at 18 weeks after operation, suggesting that the end of bone remodeling may have formed in this period. Therefore, rhBMP-2 combined with artificial bone powder can accelerate the process of bone defect repair. Through histopathological observation, it was found that the number of combined osteoblasts increased significantly, fibrous callus appeared earlier, and fracture healing speed was faster.

In this study, compared with the model group, the bone mineral density in the osteogenic region of the combined group, rhBMP-2 group and artificial bone powder group increased at 6, 12 and 18 weeks after operation, indicating that rhBMP-2 and artificial bone powder could promote the bone mineral density in the osteogenic region of rats to increase to different degrees. Among them, the combined group had the most obvious effect. And the longer intervention time was closely related to the higher bone mineral density in the osteogenic region, which is showing an increasing trend. This indicated that rhBMP-2 combined with artificial bone powder could promote osteogenic differentiation in rats. ALP is often used as a biomarker of osteoblasts to promote mineralization of extracellular matrix [13, 14]. Some studies have pointed out that ALP in osteoblasts is up-regulated and participates in cell differentiation and cell maturation [15]. The results of this study showed that compared with the model group, the combined group, rhBMP-2 group and artificial bone powder group had higher ALP activity and Callus Ca content at 6, 12 and 18 weeks after operation, and the combined group had significant effect. These results indicated that rhBMP-2 combined with artificial bone powder had significant osteogenic ability in inducing bone defect differentiation and had the function of promoting bone defect repair.

Runx2 participates in the differentiation process of osteoblasts. Studies believed that the knockout of Runx2 gene in mouse experiments would block the differentiation of osteoblasts and affect bone development [16]. Runx2 heterozygous mutation would lead to short stature, bone hypoplasia and redundant teeth [17]. Ostenix is mainly expressed in osteoblasts and participates in the differentiation process of osteoblasts. When Runx2 was over-expressed in mice, Ostenix was not found. In Ostenix mice, Runx2 is expressed, so there is a close connection between Runx2 and OSX [18, 19]. OCN belongs to vitamin k-dependent calcium binding protein, Runx2 can regulate the expression of OCN and ALP genes and promote osteoblast differentiation [20]. BSP plays an important regulatory role in the mineralization of bone matrix and the growth of bone tumors, participating in the pathogenesis and development [21, 22]. In this paper, after 18 weeks of intervention on tibial bone defect model, the three groups of Runx2, Ostenix, OCN and BSP mRNA increased to different degrees, which indicated that rhBMP-2 and artificial bone powder could promote Runx2, Ostenix, OCN and BSP mRNA to increase to different degrees, of which rhBMP-2 combined with artificial bone powder had the most prominent effect. These results suggested that rhBMP-2 promoted bone defect repair in rats by regulating Runx2, Ostenix, OCN and BSP genes.

However, the relationship between the optimal concentration of rhBMP-2 and bone defect repair has not been studied in this paper, so further research is needed to achieve the ideal bone defect repair effect.

To sum up, artificial bone powder compound combined with rhBMP-2 has certain therapeutic effects on tibial bone defects in rats. It can promote osteogenesis growth by increasing the expression of Runx2, Ostenix, OCN and BSP proteins, participate in the repair process of bone defects, and provide data reference for its clinical application. Furthermore, it

has great significance in promoting the healing of oral and maxillofacial trauma or tooth bone trauma or implant repair.

Disclosure of conflict of interest

None.

Address correspondence to: Huai'an Zhu, Department of Stomatology, Wuhan Children's Hospital (Wuhan Maternal and Child Healthcare Hospital), Tongji Medical College, Huazhong University of Science & Technology, No. 213 Qiuchang Road, Jiang'an District, Wuhan 430014, Hubei Province, China. Tel: +86-027-82433227; E-mail: zhuhuaianw3f2@163.com

References

[1] Liu JJ, Huang L, Han QB, Li XZ and Que XY. Effect of nerve growth factor on the healing of rat tibial fracture and its mechanism. Shandong Med J 2016; 56: 27-29.

[2] Matsumoto T and Goto D. Effect of low-intensity whole-body vibration on bone defect repair and associated vascularization in mice. Med Biol Eng 2017; 55: 2257-2266.

[3] Müller CW, Hildebrandt K, Gerich T, Krettek C, van Griensven M and Balmayor ER. BMP-2-transduced human bone marrow stem cells enhance neo-bone formation in a rat critical-sized femur defect. J Tissue Eng Regen Med 2017; 11: 1122-1131.

[4] Chen ZP, Zhang Y, Lin ZF, Ye XL, Zhang YQ, Xia YJ, Xia H and Yin QS. Radiological evaluation of dextran sulfate/recombinant human bone morphogenetic protein-2/chitosan composite microspheres combined with coral hydroxyapatite artificial bone in repairing large segmental bone defects. Chin J Reparative Reconstr Surg 2017; 31: 1384-1389.

[5] Guo L. Study on the effect of cpc combined with human recombinant bone morphogenetic protein-2(rhbmp-2) in repairing immediate implant bone defect. Mod Instru Med Treat 2015; 21: 104-105.

[6] Li L, Qin YB, Ma G and Li B. Lithium chloride compound calcium phosphate bone cement can promote the repair of tibial bone defect in rats. J South Med Univ 2016; 36: 824-828.

[7] Zatroch KK, Knight CG, Reimer JN and Pang DS. Refinement of intraperitoneal injection of sodium pentobarbital for euthanasia in laboratory rats (rattus norvegicus). BMC Vet Res 2017; 13: 60.

[8] Wu JY, Chen LL, Wang RF and Song Z. Exosomes secreted by stem cells from human exfoliated deciduous teeth promote alveolar bone defect repair through the regulation of angiogenesis and osteogenesis. ACS Biomater Sci Eng 2019; 5.

[9] Xia YJ, Xia H, Chen L, Ying QS, Yu X, Li LH, Wang JH and Zhang Y. Efficient delivery of recombinant human bone morphogenetic protein (rh-BMP-2) with dextran sulfate-chitosan microspheres. Exp Ther Med 2018; 15: 3265-3272.

[10] Thoma DS, Cha JK, Sapata VM, Jung RE, Hüsler J and Jung UW. Localized bone regeneration around dental implants using recombinant bone morphogenetic protein-2 and platelet-derived growth factor-BB in the canine. Clin Oral Implants Res 2017; 28: 1334-1341.

[11] Herford AS. The use of recombinant human bone morphogenetic protein-2 (rhBMP-2) in maxillofacial trauma. Chin J Traumatol 2017; 20: 1-3.

[12] Cleemann R, Bechtold JE, Sorensen M, Soballe K and Baas J. Dose-dependent resorption of allograft by rhBMP-2 uncompensated by new bone formation-a canine study with implants and zoledronate. J Arthroplasty 2018; 33: 1215-1221.

[13] Ma XD and Sun HQ. Effect of the Jintiange capsule-assisted internal fixation on fracture healing and bone metabolism activity of patients with colles fracture. J Hainan Med Univ 2017; 23: 1926-1929.

[14] Aghyarian S, Bentley E, Hoang TN and Gindri IM. In vitro and in vivo characterization of pre-mixed PMMA-CaP composite bone cements. ACS Biomater Sci Eng 2017; 3: 2267-2277.

[15] Abdallah BM. Marrow adipocytes inhibit the differentiation of mesenchymal stem cells into osteoblasts via suppressing BMP-signaling. J Biomed Sci 2017; 24: 11.

[16] Ojima F, Saito Y, Tsuchiya Y, Ogoshi M, Fukamachi H, Inagaki K, Otsuka F, Takeuchi S and Takahashi S. Runx3 regulates folliculogenesis and steroidogenesis in granulosa cells of immature mice. Cell Tissue Res 2019; 375: 743-754.

[17] Lee JH, Koak JY, Lim YJ, Kwon HB, Kong H and Kim MJ. Effects of fluoride-modified titanium surfaces with the similar roughness on RUNX2 gene expression of osteoblast-like MG63 cells. J Biomed Mater Res A 2017; 105: 3102-3109.

[18] Baek JE, Choi JY and Kim JE. Skeletal analysis and differential gene expression in Runx2/Osterix double heterozygous embryos. Biochem Biophys Res Commun 2014; 451: 442-448.

[19] Li ZH, Wang W, Xu HJ, Ning Y, Fang WJ, Liao W, Zou J, Yang Y and Shao NS. Effects of altered CXCL12/CXCR4 axis on BMP2/Smad/Runx2/Osterix axis and osteogenic gene expressions during osteogenic differentiation of MSCs. Am J Transl Res 2017; 9: 1680-1693.

[20] Shen YS, Chen XJ, Wuri SN, Yang F, Pang FX, Xu LL, He W and Wei QS. Polydatin improves osteogenic differentiation of human bone mesenchymal stem cells by stimulating TAZ expression via BMP2-Wnt/β-catenin signaling pathway. Stem Cell Res Ther 2020; 11: 204.

[21] Hasegawa T. Ultrastructure and biological function of matrix vesicles in bone mineralization. Histochem Cell Biol 2018; 149: 289-304.

[22] Go YY, Kim SE, Cho GJ, Chae SW and Song JJ. Differential effects of amnion and chorion membrane extracts on osteoblast-like cells due to the different growth factor composition of the extracts. PLoS One 2017; 12: e0182716.