Original Research

Parathyroid hormone promotes cartilage healing after free reduction of mandibular condylar fractures by upregulating Sox9

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Impact statement

Condylar cartilage is the development center of mandibles. The mandibular condylar cartilage healing states after injury has a significant influence on the appearance and function of maxillofacial. In this study, we found that intermittent subcutaneous injection of parathyroid hormone (PTH) accelerates cartilage healing after the free reduction and internal fixation of high fractures of the mandibular condyle. This phenomenon may be due to that PTH administration promotes Sox9 expression to activate Col2a1 and suppresses matrix metalloproteinase-13 expression. These findings provide preliminary theoretical support and further clinical use for high fractures of the mandibular condyle.

Abstract

After high fractures of the mandibular condyle, the insufficient blood supply to the condyle often leads to poor bone and cartilage repair ability and poor clinical outcome. Parathyroid hormone (PTH) can promote the bone formation and mineralization of mandibular fracture, but its effects on cartilage healing after the free reduction and internal fixation of high fractures of the mandibular condyle are unknown. In this study, a rabbit model of free reduction and internal fixation of high fractures of the mandibular condyle was established, and the effects and mechanisms of PTH on condylar cartilage healing were explored. Forty-eight specific-pathogen-free (SPF) grade rabbits were randomly divided into two groups. In the experimental group, PTH was injected subcutaneously at 20 μg/kg (PTH (1–34)) every other day, and in the control group, PTH was replaced with 1 ml saline. The healing cartilages were assessed at postoperative days 7, 14, 21, and 28. Observation of gross specimens, hematoxylin eosin staining and Safranin O/fast green staining found that every-other-day subcutaneous injection of PTH at 20 μg/kg promoted healing of condylar cartilage and subchondral osteogenesis in the fracture site. Immunohistochemistry and polymerase chain reaction showed that PTH significantly upregulated the chondrogenic genes Sox9 and Col2a1 in the cartilage fracture site within 7–21 postoperative days in the experimental group than those in the control group, while it downregulated the cartilage inflammation gene matrix metalloproteinase-13 and chondrocyte terminal differentiation gene ColX. In summary, exogenous PTH can stimulate the formation of cartilage matrix by triggering Sox9 expression at the early stage of cartilage healing, and it provides a potential therapeutic protocol for high fractures of the mandibular condyle.

Keywords: Mandibular condyle fracture, cartilage healing, parathyroid hormone, Sox9, Col2a1

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Introduction

The mandibular condyle is the site predisposed to mandibular fracture.[1–3] For the condylar head fractures with severe displacement, the fractured bone fragments may become free due to disruption of the blood supply to the bone marrow and the tearing of the lateral pterygoid muscle. Therefore, the reduction and fixation of fractures are difficult, and the delayed healing of unhealed condylar factures, condylar bone absorption, and reduced ramus height of the mandible often occur after surgery.[4–6] High fractures of the mandibular condyle often involve condylar cartilage. Condylar cartilage is characterized histologically by few cells and few blood vessels. When the blood supply is affected, cartilage repair ability is greatly reduced. How to accelerate the repair of cartilage tissue after high fractures of the mandibular condyle, alleviate the degradation

of cartilage matrix, and thus promote the healing of condylar fractures has been a problem that needs to be solved in clinical practice.

PTH is a peptide hormone of 84 amino acids secreted by parathyroid chief cells. PTH regulates calcium/phosphorus metabolism and bone remodeling. Basic research and clinical studies have confirmed that intermittent and low-dose PTH can promote fracture healing and increase bone density.[7,8] PTH can also promote the healing of unhealed factures or fractures with delayed healing.[9,10] Our previous studies showed that every-other-day subcutaneous injection of PTH at 20 μg/kg can promote mandibular defect repair and accelerate new bone formation during mandibular distraction osteogenesis and orthodontic tooth movement.[11–13] Intermittent injection of PTH can promote the osteogenesis and mineralization of the mandibular condyle, stimulate condylar cartilage proliferation, and protect condylar cartilage.[14,15] However, the impact of PTH on cartilage healing after the free reduction and internal fixation of high fractures of the mandibular condyle remains unclear.

Cartilage healing after condylar fractures depends on chondrocyte proliferation and cartilage matrix formation. Sex-determining region on Y chromosome-box transcription factor 9 (Sox9) is a transcription factor essential for early chondrocyte development and differentiation.[16,17] Sox9 can promote the transcription of collagen type II α1 (Col2a1), the main component in the articular cartilage matrix, and inhibit the degradation of collagen type II.[18–20] Nakazawa *et al.*[21] found that exogenous PTH can increase the expression levels of Sox9 and collagen type II in the early femoral fracture healing process. Matrix metalloproteinase-13 (MMP-13) is a specific collagenase that causes the degradation of collagen type II, while Sox9 has a negative regulatory effect on MMP-13 expression.[21–23] Collagen X (ColX) is the hallmark of hypertrophic cartilage and is specifically expressed as cartilage converts to bone during development and endochondral fracture healing.[24] A number of studies indicated that PTH has potential for cartilage repair through promoting the proliferation of hypertrophic chondrocytes[25] and inhibiting chondrocyte hypertrophy.[26–28] The changes in the expression levels of Sox9, Col2a1, MMP-13, and ColX during the cartilage healing process after mandibular condylar fractures and whether the application of PTH can regulate Sox9 expression and promote condylar cartilage healing need to be studied in more depth.

In this study, a rabbit model of free reduction and internal fixation of high fractures of the mandibular condyle was established, and the effects and mechanisms of PTH on cartilage healing after high condylar fractures were explored. Our findings provide a theoretical and experimental basis for clinical application of PTH to promote the healing of high fractures of the mandibular condyle.

Materials and methods

Experimental animals

The experimental animals were 6-month-old specific-pathogen-free New Zealand big-eared rabbits, with a body weight of 2.5 ± 0.2 kg and a male-to-female ratio of 1. The animal certificate No. is SCXK (Yu) 2007–0005. Animals were kept in in individual cages in the Animal Experimental Center of Guizhou Medical University, with a temperature of $(22 \pm 2)^{\circ}$C, a humidity of (50 ± 10), ventilation ≥ 14 times/h, and artificial lighting for 12 h/day. Animals were given the standard rabbit feed at 350 g per rabbit per day, with free access to water. The experimental protocol was reviewed and approved by the Animal Experimental Ethics Committee of Guizhou Medical University.

Experimental grouping

A total of 48 rabbits were used to establish an animal model of free reduction and internal fixation of high fractures of the mandibular condyle. The experimental animals were randomly divided into two groups of 24. In the experimental group, PTH was injected subcutaneously at 20 μg/kg (PTH (1–34) (Tocris, Bristol, UK)) every other day, and in the control group, PTH was replaced with 1 ml saline.

Establishment of an experimental animal model of free reduction and internal fixation of high fractures of the mandibular condyle

Sodium pentobarbital anesthesia (3%) was performed via the auricular vein, and skin in the surgical area was prepared and disinfected. The mandibular condyle on one side was selected as the surgical side, and a transverse incision approximately 2.5 cm long was made at the zygomatic arch. The skin and subcutaneous tissue were incised and separated to the bone surface. The ascending ramus of the mandible and the lateral surface of the condyle were exposed, and the periosteal soft tissue attachment on the anteromedial surface of the condyle was removed (Figure 1(a)). An L-shaped osteotomy line was created on the lateral surface of the condyle. The osteotomy line passed through the condylar articular surface to allow the anteromedial parts of the condyle to become free bone fragments (Figure 1(b)). After reduction, the fractured bone ends were fixed with titanium micromesh (Figure 1(c)), and the incision was sutured in layers.

Specimen collection and morphological observation of condylar tissue

Six experimental animals were sacrificed in each group at postoperative days 7, 14, 21, and 28 under anesthesia. Gross morphological observation was performed on the condylar fracture specimens at the surgical side. Three specimens were fixed with 4% paraformaldehyde for 24 h, decalcified with 10% ethylenediaminetetraacetic acid, and then embedded in conventional paraffin. Paraffin-embedded tissues were cut into 5-μm histological sections, dewaxed in xylene, and gradually hydrated in ethanol, followed by hematoxylin eosin (HE) staining and Safranin O/fast green staining. Under an optical microscope (Axio Scope A1, Carl Zeiss, Oberkochen, Germany), the morphological changes in the healing process of the condylar fractures were observed. The other three tissue specimens at each

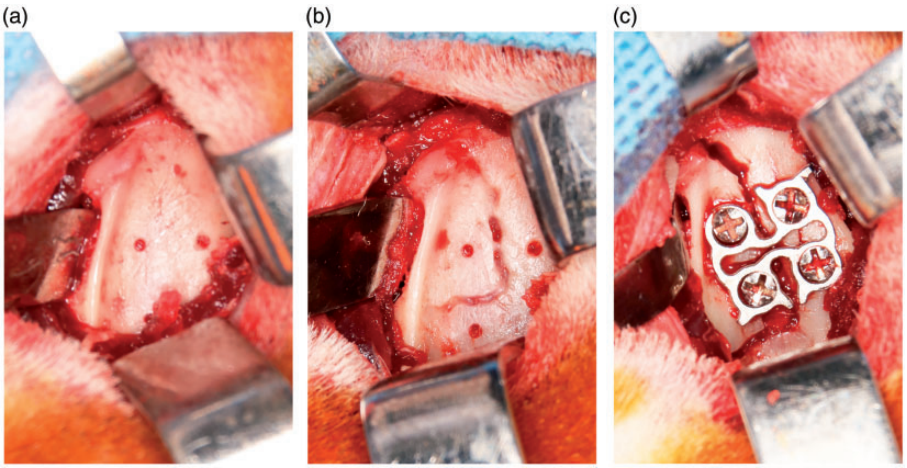

Figure 1. The rabbit model of free reduction and internal fixation of the mandibular condylar fractures. (a) Dissection of the periosteal soft tissue attachment on the anteromedial surface of the condyle. (b) The fracture line. (c) Fixation with titanium micromesh.

time point were stored in liquid nitrogen in a –80°C freezer for the polymerase chain reaction (PCR) detection of chondrogenic factors.

Immunohistochemistry detection of the expression levels of Sox9 and MMP-13

Paraffin-embedded tissue of the condylar cartilage healing region at each time point was sliced, deparaffinized, and hydrated according to the aforementioned steps. After antigen retrieval in pH 6.0 citrate buffer for 3 min, the sections were rinsed with sterile water. After soaking in 3% hydrogen peroxide for 10 min, the sections were rinsed with phosphate-buffered saline (PBS). The primary antibody (Sox9: working concentration of 1:50; MMP-13: working concentration of 1:100; Bioss Antibodies, Inc., Wuhan, China) was added dropwise, and the sections were cultured overnight at 4°C in a refrigerator. After washing with PBS, horseradish peroxidase-polymer goat anti-rabbit secondary antibody was added, and sections were incubated with diaminobenzidine for 3 min. After washing with pure water, the sections were restained with HE for 60–90 s. The sections were dehydrated by a gradient ethanol series. They were transparent after applying xylene as the clearing agent. They were mounted with neutral balsam, and the expression levels of Sox9 and MMP-13 were observed under an optical microscope. The average optical density (AOD) was measured using Image-Pro Plus 6.0 software, and the mean value was used to determine the expression intensity of each group at each time point.

Detection of mRNA expression levels of Sox9, MMP-13, Col2a1, and ColX using quantitative PCR

At the condylar cartilage fracture site, approximately $2 \times 2 \times 3$ mm^3 tissue was harvested and ground in liquid nitrogen. TRIzol (Thermo Fermentas, USA) was used to extract the total RNA in samples, and the total RNA concentration was measured using a nucleic acid analyzer. RevertAid First Strand cDNA Synthesis Kit, a reverse transcription kit (Thermo Fisher, USA), was used to obtain complementary DNA (cDNA) from mRNA. The cDNA of samples was collected. The NeuroScript RT Master Mix (Perfect Real Time) kit (TaKaRa, JAN) was used to detect the expression levels of Sox9, MMP-13, Col2a1, and ColX. Glyceraldehyde-3-phosphate dehydrogenase served as an internal control. The reaction conditions were as follows: denaturation at 95°C for 30 s and 40 cycles of 95°C for 3 s and 60°C for 30 s (Table 1).

Statistical analysis

Measurement data of each group are expressed as the mean ± standard deviation. SPSS 25.0 software was used for statistical analysis. The t test was performed on the data of each group. $P < 0.05$ indicated that the difference was statistically significant.

Results

Gross observation of the condylar fracture site

All animals underwent successful surgery and received a daily intramuscular injection of 800,000 U penicillin for three consecutive days after surgery. No surgical site infections were noted. Gross morphological observation was performed on the condyle. (1) On postoperative day 7, the fracture line in both groups was clearly visible, with the obvious movement of free bones. Callus filling was seen in the fracture line. The condylar cartilage surface was not smooth (Figure 2(a) and (b)). (2) On postoperative days 14 and 21, a small amount of cartilaginous matrix was filled in the fracture line, which was more evident in the experimental group than in the control group. The fracture line was observed in the control group (Figure 2(c) and (e)), but no obvious fracture line marks were observed in the experimental group (Figure 2(d) and (f)). (3) On postoperative day 28, cartilage tissue had filled in the condylar cartilage fracture site in both groups, but the condylar surface was not smooth in control group, and there were depression defects in some condyles (Figure 2(g)). In the experimental group, the surface of the condylar cartilage was

Table 1. Primer design in qPCR.

Gene	Primer sequence	bp
Sox9	Forward primer 5′-CAGTACCCGCACCTGCACAAC-3′ Reverse primer 5′-CCGCTCCGCCTCCTCCAC-3′	105
MMP-13	Forward primer 5′-TCTACACCTACACCGGCAAGAGTC-3′ Reverse primer 5′-CGGAGACTGGTAATGGCATCAAGG-3′	163
Col2al	Forward primer 5′-CCACCGTGCCCAAGAAGAACTG-3′ Reverse primer 5′-GAAGCCGCCATTGATGGTCTCC-3′	86
ColX	Forward primer 5′-GCCCTTCTGCTGCTAGTGTCTTTC-3′ Reverse primer 5′-ACTGTGTCTTGGTGTTGGGTTGTG-3′	109
GAPDH	Forward primer 5′-CAAGGCTGTGGGCAAGGTCATC-3′ Reverse primer 5′-TTCTCCAGGCGGCAGGTCAG-3′	111

MMP-13: matrix metalloproteinase-13; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

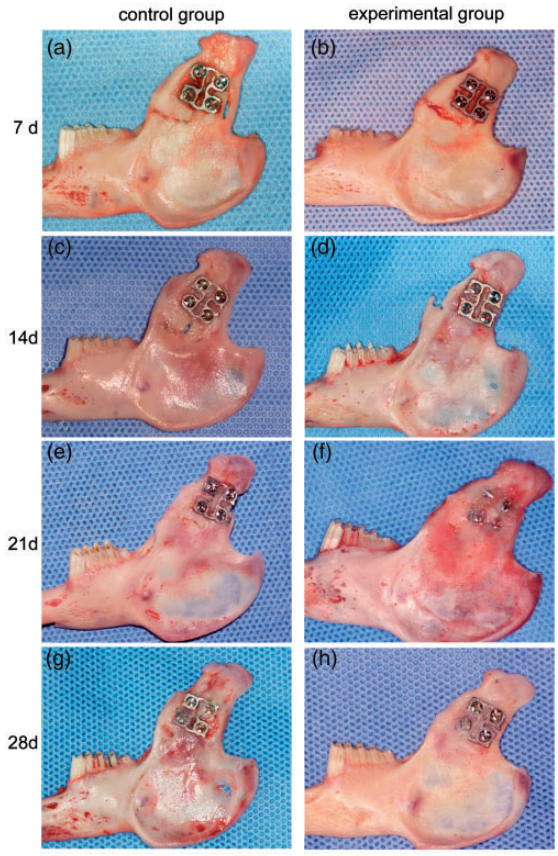

Figure 2. Observation of cartilage healing of condylar specimens at different time points. The fracture line gradually healed over time. In the experimental group, the healing of the fracture line was earlier than that in the control group, and the morphology of the condyle in the experimental group was mostly restored. In the control group, the cartilage surface was rough, and condylar bone absorption was found. (a, b). Postoperative day 7: (a) control group and (b) experimental group. (c, d). Postoperative day 14: (c) control group and (d) experimental group. (e, f). Postoperative day 21: (e) control group and (f) experimental group. (g, h). Postoperative day 28: (g) control group and (h) experimental group. Control group: every-other-day subcutaneous injection of saline (1 ml). Experimental group: every-other-day subcutaneous injection of PTH at 20 μg/kg.

smooth, the condylar morphology was mostly restored, and the fracture line disappeared (Figure 2(h)).

Histomorphological observation results

Safranin O/fast green staining can distinguish cartilage tissue from bone tissue, since the basophilic cartilage is red when stained with the alkaline dye Safflower O, and the eosinophilic bone is green or blue when stained with the acid dye solid green. On postoperative day 7, HE staining showed that bone trabeculae were small in size, disordered in arrangement, and poor in continuity in the control group (Figure 3(a)). In the experimental group (Figure 3(b)), there was focal deposition of new bone matrix, and some osteoblasts were arranged in a band shape. Safranin O/fast green staining showed that the structure of the chondrocytes in the cartilage fracture site was disordered, some of the chondrocyte nuclei had disappeared, the light green tissue was filled inside, and proteoglycans were sparse (Figure 3(c), control group; and Figure 3(d), experimental group). On postoperative day 14, HE staining showed that a large amount of reactive hyperplasia of bone was observed in the experimental group, and Safranin O/fast green staining showed green fibrous tissue filling in the cartilage fracture site, with a small amount of red cartilage matrix in between. Both were more abundant in the experimental group than the control group.

On postoperative days 21 and 28, HE staining showed a gradual increase in fused trabeculae. In the control group (Figure 3(e), 21d and Figure 3(i), 28d), there were lamellar structures, the uniformity and intensity of bone calcification were lower than those in the experimental group (Figure 3(f), 21d and Figure 3(j), 28d), and angiogenesis was observed. Safranin O/fast green staining showed that in the control group (Figure 3(g), 21d and Figure 3 (k), 28d), a large amount of fibrous tissue had filled the cartilage area, with less cartilage matrix. In the experimental group (Figure 3(h), 21d and Figure 3(l), 28d), the amount of new cartilage matrix was significantly increased, the number of cells in the proliferating layer was significantly increased, many chondrocytes and much cartilage matrix were found in the fracture site, many hypertrophic chondrocytes were found to fill the fracture site along the fracture line, and many bone trabeculae were found in the subchondral bone.

Expression levels of osteogenic factors of condylar cartilage by immunohistochemistry

Sox9 expression in the cartilage fracture site. The immunohistochemistry (IHC) results showed that on postoperative day 7, a small amount of Sox9 was expressed in the chondrocytes in the proliferating and the mature layers of

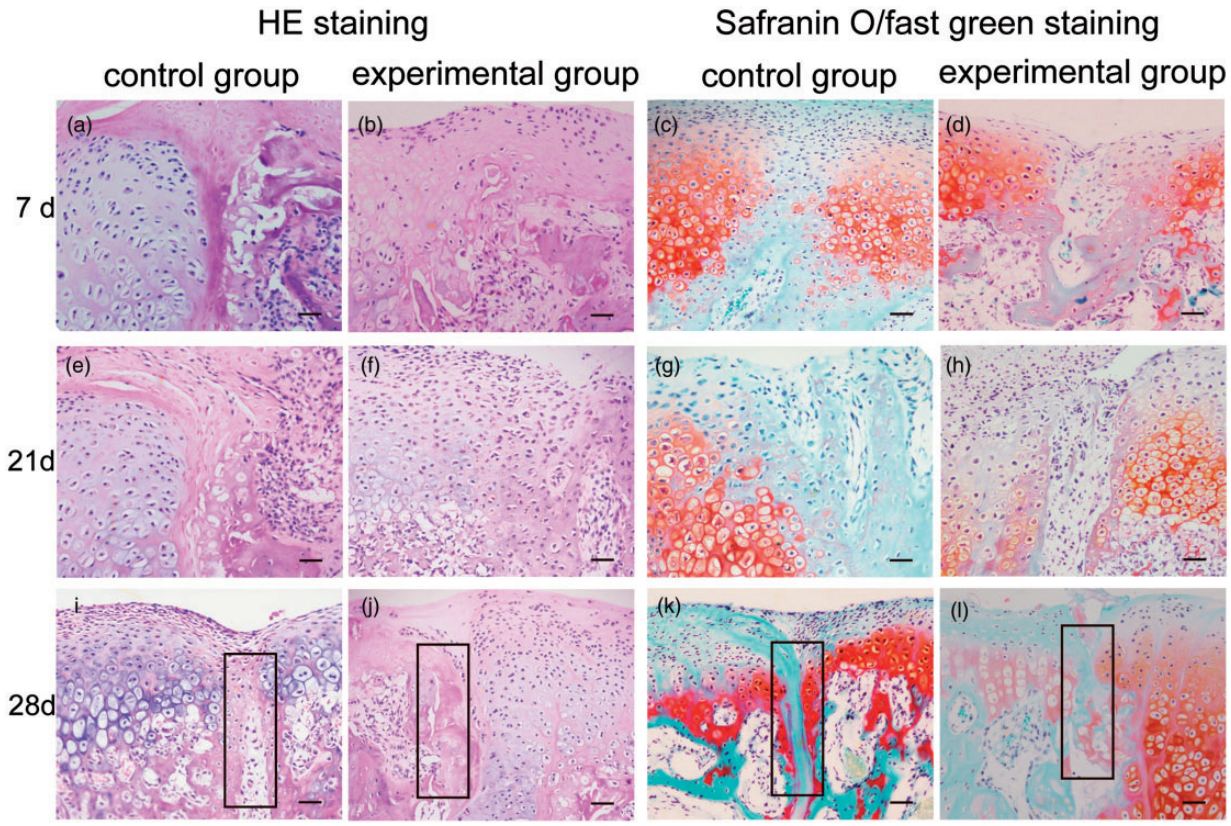

Figure 3. HE staining and Safranin O/fast green staining results of the condylar fracture site. With increasing the fixation time, the new cartilage matrix in the experimental group was more abundant than that in the control group, the cell proliferation in the proliferating layer was more obvious, and a large amount of trabecular bone formation was observed in the subchondral bone. (In the Safranin O/fast green staining, the red part is cartilage, and the green or blue part is the bone tissue.) (a, b). HE staining on postoperative day 7: (a) control group and (b) experimental group. (c, d). Safranin O/fast green staining on postoperative day 7: (c) control group and (d) experimental group. (e, f). HE staining on postoperative day 21: (e) control group and (f) experimental group. (g, h). Safranin O/fast green staining on postoperative day 21: (g) control group and (h) experimental group. (i, j). HE staining on postoperative day 28: (i) control group and (j) experimental group. (k, l). Safranin O/fast green staining on postoperative day 28: (k) control group and (l) experimental group. The black box indicates the fracture line area. Scale bars = 100 μm.
HE: hematoxylin eosin.

the cartilage fracture site, and the Sox9 expression was stronger in the proliferating layer cells (Figure 4(a) and (b)). During postoperative days 14–21, Sox9 expression continuously increased and peaked in the experimental group (Figure 4(c) to (f)). On postoperative day 28, Sox9 expression was slightly decreased in the experimental group (Figure 4(h)), while it was increased in the control group (Figure 4(g)). The staining intensity AOD results showed that on postoperative days 7, 14, and 21, the Sox9-positive staining intensity in the experimental group was stronger than that in the control group ($P < 0.05$). On postoperative day 28, there was no significant difference in the positive staining intensity between the experimental and control groups (Table 2).

Mmp-13 expression in the cartilage fracture site. IHC showed that MMP-13 was expressed in the cytoplasm of chondrocytes and mast cells in the mature layer. On postoperative day 7, MMP-13 expression in the control group was greater than that in the experimental group (Figure 5(a) and (b)). On postoperative day 14, MMP-13 expression was slightly decreased in both groups (Figure 5(c) and (d)). On postoperative days 21 and 28, MMP-13 expression showed an upward trend, and the staining in the control group was darker (Figure 5(e) to (h)). The staining intensity AOD results showed that on postoperative days 7, 14, and 21, the positive staining intensity in the experimental group was lower than that in the control group ($P < 0.05$). On postoperative day 28, there was no significant difference in the positive staining intensity between the experimental and control groups (Table 2).

The mRNA expression levels of Sox9, MMP-13, Col2a1, and ColX in the cartilage fracture site

The relative mRNA expression of Sox9 in both groups showed an increasing trend at the early postoperative time (Figure 6(a)). The expression peaked on postoperative day 21 and slightly decreased on day 28 in the experimental group. The relative mRNA expression of Sox9 in the experimental group on postoperative days 7, 14, and 21 was significantly different from that in the control group. There was no significant difference between the two groups on day 28.

The relative mRNA expression of MMP-13 in the experimental group was low on postoperative days 7 and 14 and increased on postoperative days 21 and 28. In the control group, the relative mRNA expression of MMP-13 was high on postoperative day 7, then decreased, and then increased

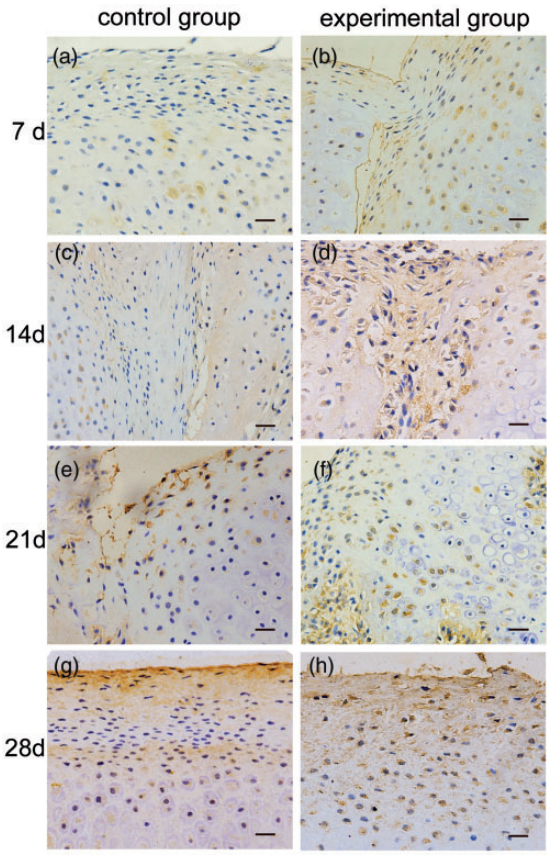

Figure 4. Sox9 expression in new cartilage in the condylar fracture site by IHC. (a, b). Postoperative day 7: (a) control group and (b) experimental group. (c, d). Postoperative day 14: (c) control group and (d) experimental group. (e, f). Postoperative day 21: (e) control group and (f) experimental group. (g, h). Postoperative day 28: (g) control group and (h) experimental group. On postoperative days 7, 14, and 21, the IHC staining of Sox9 in the experimental group was darker than that in the control group. Scale bars = 50 μm.

again on postoperative days 21 and 28. On days 7, 14, and 21, the mRNA expression of MMP-13 in the experimental group was lower than that in the control group, and the differences were statistically significant. There was no significant difference in the mRNA expression of MMP-13 between the two groups on postoperative day 28 (Figure 6(b)).

The relative mRNA expression of Col2a1 showed an increasing trend after surgery. In the experimental group, the expression continued to increase on postoperative days 7, 14, and 21, peaked on day 21, and decreased on day 28. The relative mRNA expression of Col2a1 in the control group showed an increasing trend from day 7 through day 28. On days 14, 21, and 28, the mRNA expression of Col2a1 in the experimental group was significantly different from that in the control group (Figure 6(c)).

In the control group, the relative mRNA expression of ColX showed an overall upward trend after surgery and peaked on postoperative day 21, then decreased on day 28. In the experimental group, the mRNA expression of ColX was not high during the first 21 days after surgery but increased on postoperative day 28. At days 14, 21, and 28, the differences in mRNA expression of ColX in condylar cartilage between the two groups were statistically significant (Figure 6(d)).

Discussion

When high fractures of the mandibular condyle occur, it is easy for the lateral pterygoid muscle attached to the condyle to become detached so that the bone fragments become free, without a blood supply. Toure[29] showed that when condylar fractures are displaced, the blood supply from the inferior alveolar artery and the superficial temporal artery is damaged or broken, while the lateral pterygoid muscle can provide a blood supply for condylar bone. This study dissected the periosteal soft tissue attachment on the anteromedial surface of the condyle to simulate the impaired blood supply after high fractures of the mandibular condyle and established a rabbit model of free reduction and fixation of high fractures of the mandibular condyle.

PTH is one of the key hormones regulating the growth and regeneration of bone. PTH also plays a dual regulatory role in cartilage regeneration. Low doses of PTH can promote the differentiation of mesenchymal stem cells (MSCs) into cartilage, promote the proliferation of cartilage, and reduce the conversion of cartilage into osteoblasts. High-dose, continuous PTH can inhibit the proliferation of cartilage and cause endochondral ossification.[30,31] Based on the finding that PTH can promote mandibular regeneration,[11,12] this study explored the effect of intermittent injection of 20 μg/kg PTH on high fractures of the mandibular condyle. Histomorphological observations suggested that intermittent subcutaneous injection of PTH can not only promote condylar fracture healing but also alleviate the degradation of cartilage matrix. Safranin O/fast green staining and HE staining suggested that intermittent injection of low-dose PTH could increase the number of chondrocytes in the cartilage fracture site, promote cartilage matrix formation, and accelerate cartilage repair.

As condylar cartilage is the developmental center of the mandible, damage to it could not only cause osteoarthritis but also cause mandible growth and development stagnation, or even condylar bone absorption. Sox9 regulates the development and regeneration of cartilage through different signal transduction pathways. Sox9 is a major regulator of the chondrogenic differentiation of MSCs. Sox9 can bind to chondrocyte-specific enhancers, thereby promoting the secretion of collagen type II.[18] PTH promotes condylar cartilage hyperplasia and increases condylar bone density through PTH receptor 1.[15,25,32] Dutra *et al.*[15] confirmed that intermittent injection of PTH can raise Sox9 expression and thereby promote the formation of condylar cartilage. Zhang *et al.*[30] showed that low-dose PTH could increase the expression levels of Sox9, Col2a1, and type 1 PTH receptor (PTH1R) during the chondrogenic differentiation of MSCs. In this study, Sox9 expression was significantly increased in the experimental group at postoperative days 14 and 21 over the control group. Therefore, it was speculated that PTH upregulated Sox9 expression. In addition, Col2a1 expression in the experimental group showed an overall upward trend after surgery, and the differences between

Table 2. Staining intensities of Sox9 and MMP-13 in condylar cartilage tissue at different time.

	Sox9			MMP-13		
Group	**Control group**	**Experimental group**	***P***	**Control group**	**Experimental group**	***P***
7 days	0.005 ± 0.001	0.008 ± 0.002**	0.002	0.013 ± 0.004	0.003 ± 0.008***	0.000
14 days	0.002 ± 0.003	0.006 ± 0.011**	0.003	0.005 ± 0.002	0.002 ± 0.004*	0.034
21 days	0.008 ± 0.001	0.023 ± 0.005*	0.010	0.011 ± 0.001	0.005 ± 0.004*	0.028
28 days	0.019 ± 0.001	0.012 ± 0.006	0.231	0.011 ± 0.004	0.010 ± 0.004	0.077

MMP-13: matrix metalloproteinase-13.

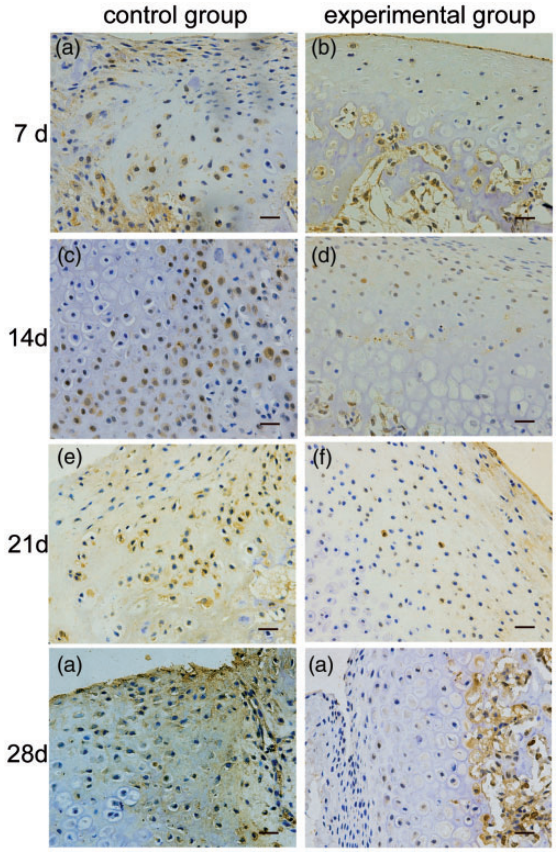

Figure 5. MMP-13 expression in new cartilage in the condylar fracture site by IHC. (a, b). Postoperative day 7: (a) control group and (b) experimental group. (c, d). Postoperative day 14: (c) control group and (d) experimental group. (e, f). Postoperative day 21: (e) control group and (f) experimental group. (g, h). Postoperative day 28: (g) control group and (h) experimental group. On postoperative days 7, 14, and 21, the IHC staining of MMP-13 in the control group was darker than that in the experimental group. Scale bars = 50 μm.

the two groups at postoperative days 14, 21, and 28 were statistically significant, indicating that the condylar cartilage matrix formed faster in the experimental group than the control group. The trend of the mRNA expression of Col2a1 in the experimental group was consistent with the Sox9 expression, suggesting that Col2a1 expression might be regulated by Sox9 after PTH administration.

Hypertrophic chondrocytes also play important roles in condylar cartilage regeneration. ColX is secreted by the chondrocytes in hypertrophic layer, is a marker of terminal differentiation during chondrogenesis, and is also the signal for the initiation of endochondral ossification.[25,33,34] Mature chondrocytes do not express ColX. During endochondral repair, prior to converting to the bone, chondrocytes undergo hypertrophic maturation. This process is characterized biologically by the presence of a provisional ColX extracellular matrix. Lydon *et al.*[35] found that osteochondral defects of the distal femur of the sheep heal through endochondral ossification as evidenced by chondrocyte hypertrophy and ColX expression. Hasegawa *et al.*[36] found that Sox9 expression was decreased in the endochondral ossification, leading to the transformation of cartilage into osteoblasts, which is conducive to the healing of fractures under the cartilage. Other studies suggested that in the late stage of chondrogenesis, Sox9 can directly inhibit the ColX expression in chondrocytes and delay the formation of subchondral bone.[37,38] Dutra *et al.*[15] found that after intermittent administration of PTH for 14 days, the ColX expression in condylar chondrocytes cultured in vitro significantly decreased. In this study, on postoperative days 7, 14, and 21, the mRNA expression of ColX was low in the experimental group. One reason may be that the high Sox9 expression early after intermittent administration of PTH can inhibit the mRNA expression of ColX. On day 28, Sox9 expression was decreased, while the mRNA expression of ColX was increased, which may be the result of the negative regulatory effect of Sox9 on ColX. Based on the histomorphological observations on postoperative day 28, we speculate that the mandibular condylar fractures entered the endochondral osteogenesis stage, which may have been due to the upregulation of the mRNA expression of ColX.

The early healing process after condylar fracture fixation is inevitably associated with inflammation in articular cartilage. Intermittent administration of PTH has a certain protective effect on cartilage with osteoarthritis and on degenerative lesions in subchondral bone.[39–41] MMP-13 is a major enzyme that degrades collagen type II and is one of the important factors promoting cartilage inflammation. Uchida *et al.*[41] showed that MMP-13 expression increased in the skull and femur of rats after PTH perfusion, suggesting that high-dose PTH can upregulated MMP-13 and stimulate bone resorption to some extent. Dutra *et al.*[15] showed that intermittent administration of PTH can reduce MMP-13 expression in condylar cartilage. By establishing an experimental model of osteoarthritis, Lugo *et al.*[42] showed that intermittent administration of PTH reduced MMP-13 expression and improved osteoarthritis. Yan *et al.*[39] found that after the application of PTH, the microstructural changes in subarticular trabecular bone in guinea pigs

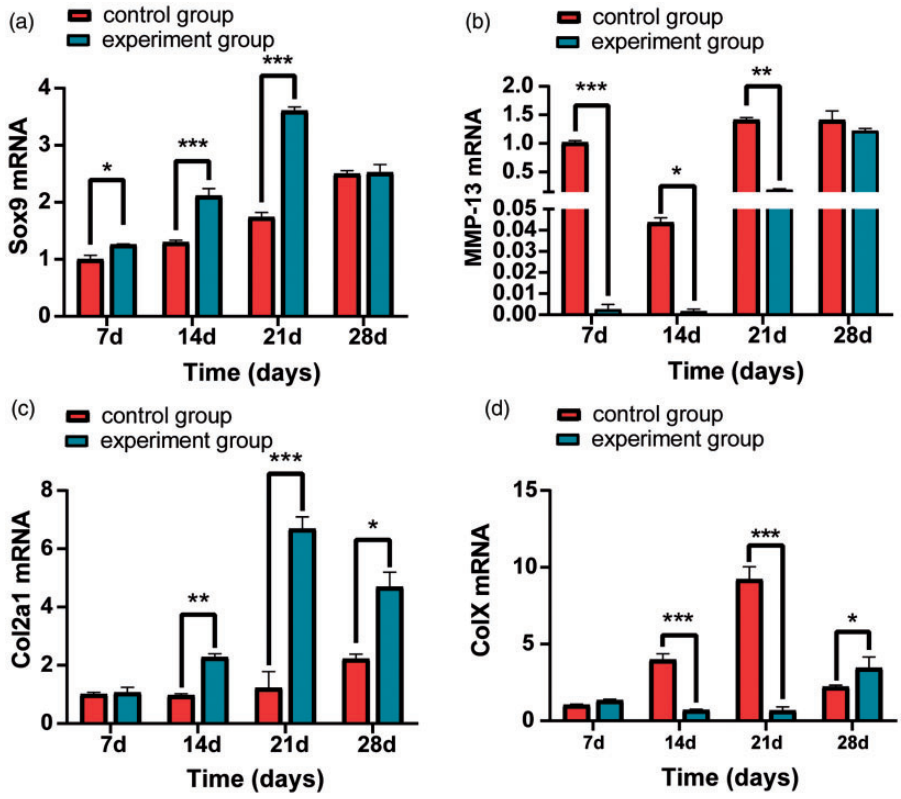

Figure 6. Expression levels of Sox9, MMP-13, Col2a1, and ColX in the healing region of the condylar cartilage by RT-qPCR. (a) mRNA expression of Sox9 in new cartilage in the condylar fracture site at different time points. (b) mRNA expression of MMP-13 in new cartilage in the condylar fracture site at different time points. (c) mRNA expression of Col2a1 in new cartilage in the condylar fracture site at different time points. (d) mRNA expression of ColX in new cartilage in the condylar fracture site at different time points. Statistical analysis was performed using the *t* test. * indicates a statistically significant difference compared with the control group (*$P<$ 0.05, **$P<0.01$, ***$P<0.001$).
MMP-13: matrix metalloproteinase-13.

were delayed, and the expression of collagen type II in the cartilage was increased, but the MMP-13 expression was decreased. In this study, MMP-13 expression in the control group was high in the early stage, suggesting that the degradation of the cartilage matrix in the condylar cartilage was active. The MMP-13 expression in the experimental group was lower than that in the control group at all postoperative time points, indicating that the every-other-day injection of PTH could reduce early MMP-13 expression, thereby reducing the degradation of cartilage matrix and the condylar bone absorption. On postoperative day 28, MMP-13 expression remained high in the two groups, suggesting that cartilage matrix degradation still existed at this time, cartilage matrix formation and degradation had not reached equilibrium, and cartilage healing was not complete.

In summary, exogenous PTH could promote cartilage healing after the fixation of high fractures of the mandibular condyle. The mechanism may be as follows: PTH upregulates Sox9 expression, activates Col2a1 expression, promotes the deposition of cartilage matrix, and repairs cartilage damage. The upregulated Sox9 in the early stage inhibits the expression of MMP-13 and ColX and inhibits the degradation of extracellular matrix and the terminal differentiation of chondrocytes to promote cartilage healing after condylar fractures.

Our observation period was relatively short, and there were no groups with different PTH doses, so the optimal dose of PTH to promote cartilage repair is not known. The exact regulatory mechanism by which exogenous PTH promotes cartilage healing after condylar fracture through Sox9 remains unclear, so further studies are needed.

AUTHORS' CONTRIBUTIONS

The authors' contributions were as follows: ZLT: conception and design the experiments; YYJ and LQX: implement the experiments and drafting the article; DXW, YLC, and QG: animal experimental study; YH: histopathological analysis; GXZ: data analysis; YYJ: interpretation the article; and ZLT: manuscript revision. All of the authors reviewed and approved the final manuscript.

DECLARATION OF CONFLICTING INTERESTS

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