Differences in accelerated tooth movement promoted by recombinant human parathyroid hormone after mandibular ramus osteotomy

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Introduction: This study investigated the effects of different doses of parathyroid hormone (PTH) on orthodontic tooth movement after mandibular ramus osteotomy and the associated dose-response relationship. **Methods:** One-hundred twenty rabbits were divided into 2 experimental groups (A and B) and 2 control groups (control group and negative control group). An experimental model of mandibular ramus osteotomy with installation of an orthodontic tooth movement device was established in groups A and B and the control group. After surgery, groups A and B received intermittent subcutaneous injections of PTH, 20 and 40 μg/kg, respectively, and the control group received injections of normal saline solution. The negative control group underwent installation of the orthodontic tooth movement device without mandibular ramus osteotomy and received normal saline solution after surgery. Changes in expression of RANKL and RUNX2 in the periodontal tissues of the first molars were evaluated by means of immunohistochemical analysis and quantitative fluorescence polymerase chain reaction. **Results:** Movement of the first molars was more rapid in group B than in group A in the 21 days after surgery. Significantly higher *RANKL* mRNA levels and lower *RUNX2* mRNA levels were detected on the compression side of the periodontal tissues in groups A and B than in the control groups. There was a significant difference in RANKL and RUNX2 expression levels between group B and the control groups at all time points. **Conclusions:** Mandibular ramus osteotomy combined with high-dose PTH can increase catabolism on the compressed periodontal tissues, thereby accelerating remodeling of periodontal bone and promoting orthodontic tooth movement after surgery. (Am J Orthod Dentofacial Orthop 2019;155:670-80)

The concept of a surgery-first approach (SFA) in orthognathic surgery has attracted attention from clinicians and researchers because it can shorten the course of orthodontic treatment in patients with dentomaxillofacial deformity.[1] In most reports, the patient's lateral profile and occlusion can be improved significantly early in the treatment course, and the treatment time is markedly shortened. SFA is thought to activate factors related to bone metabolism that adjust the alveolar bone conversion rate and accelerate orthodontic tooth movement postoperatively.[2-4] As such, SFA differs from the conventional periodontal procedures performed to facilitate rapid tooth movement, in which bone surgery is performed around the target teeth and the alveolar bone is weakened by cutting or penetration. However, there is controversy concerning the outcomes of SFA. Occlusion is unstable in the immediate postoperative period in most cases, so accelerated tooth movement during this time is a major concern and postoperative orthodontic movement must be planned very carefully. Moreover, a systematic review and meta-analysis concluded that although SFA is an effective alternative to conventional surgery, with a shorter total treatment duration, the postoperative orthodontic treatment time is longer.[5] The velocity of orthodontic tooth movement is related to the rate of periodontal bone remodeling and is regulated by hormones, in particular parathyroid hormone (PTH). PTH is secreted by the parathyroid glands, regulates the balance of calcium and phosphorus metabolism

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in the body, and is involved in the synthesis and catabolism of bone. The effect of exogenous PTH on bone remodeling depends on the dose administered and the route of administration. Continuous administration of PTH mainly produces a catabolic effect, whereas the effects of intermittent administration are mainly anabolic.[6,7] Rapid bone formation on the tension side and resorption on the compression side of periodontal bone tissues inevitably lead to accelerated orthodontic tooth movement. However, to date, there has been no report on the use of PTH for promoting orthodontic tooth movement after orthognathic surgery.

Although experimental research has demonstrated that PTH enhances orthodontic tooth movement,[8-11] the optimal dose and route of administration remain unclear. Soma et al[8,9] reported that continuous administration of 10 mg PTH accelerated orthodontic tooth movement in rats, whereas intermittent administration of PTH did not. Furthermore, Li et al[10] reported that daily subcutaneous injection of 4 μg/100 g PTH accelerated orthodontic movement of the maxillary first molars in rats. Salazar et al[11] reported that subcutaneous administration of 30 μg/kg/d PTH was associated with a greater increase in the velocity of postsurgical orthodontic tooth movement by day 7 in ovariectomized osteoporotic rats than in nonovariectomized osteoporotic rats.

Several clinical studies and basic research experiments have found that intermittent administration of PTH can accelerate the repair of bone defects and promote healing of fractures and formation of new bone during distraction osteogenesis.[12-17] In view of the reports demonstrating that administration of PTH can accelerate tooth movement, we hypothesized that administration of PTH after orthognathic surgery would be beneficial not only for postoperative bone healing at the osteotomy site, but also for regulation of periodontal bone remodeling of orthodontically treated teeth, thereby accelerating orthodontic tooth movement and shortening the course of orthodontic treatment. No study to date has identified the optimal dose of PTH after orthognathic surgery or whether PTH can further accelerate orthodontic tooth movement. We embarked on the present research to provide theoretical support and experimental evidence of acceleration of orthodontic tooth movement with the administration of PTH. First, we established a rabbit model of orthodontic movement of the mandibular first molars after mandibular ramus osteotomy that incorporated intermittent subcutaneous injections of different doses of recombinant human PTH (rhPTH). To assess the effects of different doses of rhPTH on orthodontic tooth movement and to identify the mechanisms underlying this phenomenon, we then evaluated the velocity of mandibular first molar movement by means of histomorphometric analysis and analyzed the expression of receptor activator of nuclear factor kappa-B ligand (RANKL) and runt-related transcription factor 2 (RUNX2) on the compression side of the periodontal tissues after intermittent postoperative administration of PTH at 2 dose strengths.

MATERIAL AND METHODS

One hundred and twenty 6-month-old specific pathogen–free New Zealand white rabbits (60 male, 60 female; mean weight 2.5 ± 0.1 kg) were selected for this study. The rabbits were housed individually in a specific pathogen–free animal facility at a temperature of 22 ± 2°C, a humidity of 50 ± 10%, ventilation ≥14 times/h, and on a 12-h dark-light cycle. Each rabbit was provided with 350 g standard pellets daily, along with free access to tap water. The experimental protocol was reviewed and approved by the Animal Experiment Ethics Committee of Guizhou Medical University.

The rabbits were randomly divided into 2 experimental groups (groups A and B), a control group, and a negative control group (30 rabbits per group). Mandibular ramus osteotomy was performed on the ipsilateral side of the orthodontic tooth in groups A and B and the control group but not in the negative control group. After surgery, the animals received subcutaneous injections of 20 μg/kg rhPTH (group A), 40 μg/kg rhPTH (group B), or normal saline solution (control and negative control groups) once daily in the neck area. The rhPTH (1-34 N-terminal residues of human PTH) was obtained from Tocris Bioscience (Bristol, U.K.).

Sodium pentobarbital 3% was administered through the auricular vein for anesthesia. A 2-cm unilateral skin incision was made along the lower edge of the mandible. The skin, subcutaneous, and muscle tissues were excised until the bone surface was exposed. The periosteum was excised and the lateral mandibular periosteum stripped. In the anterior region of the mandibular angle, an arcuate osteotomy line was made along the posterior edge of the ramus and lower edge of the mandibular body. A solid internal fixation device with a titanium plate (Ningbo Cibei Medical Devices Co, Zhejiang, China) was used to fix the posterior edge of the ramus and the lower edge of the mandibular body (Fig 1, *A-C*). A fixation groove was prepared in the lower third of the neck of each mandibular central incisor and each mandibular first molar on the surgical side. A 0.2-mm orthodontic ligation wire was used to fix a nickel-titanium tension spring between the mandibular central incisors and the mandibular first molars, thus establishing an orthodontic model of mesial movement of the mandibular first

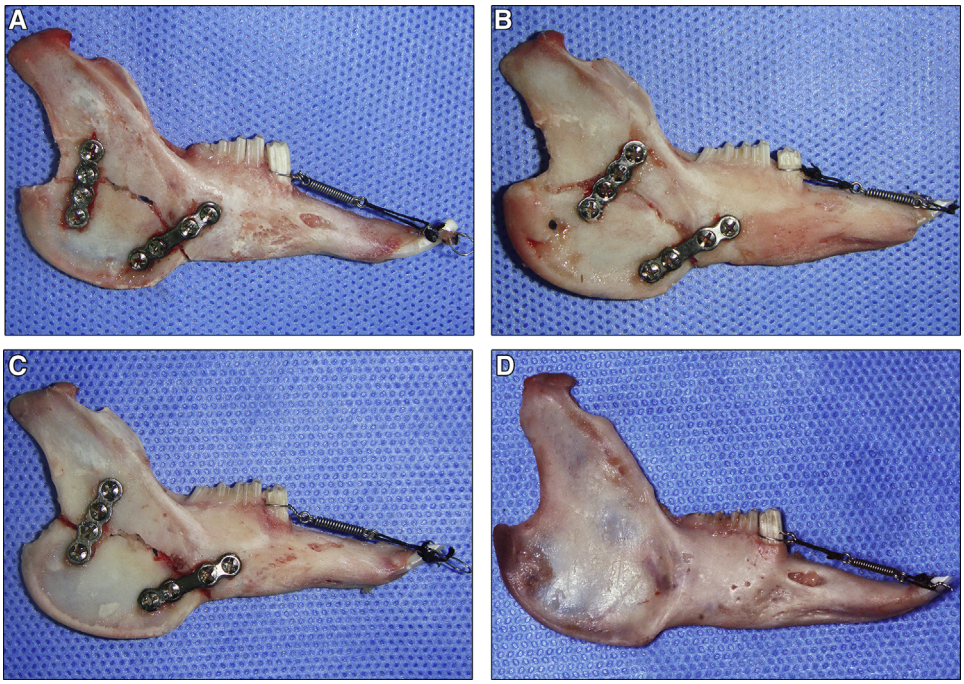

Fig 1. Specimens taken from the mandible on postoperative day 7 in **A,** group A, **B,** group B, **C,** the control group, and **D,** the negative control group. Accumulation of new bone tissue around the osteotomy line was observed at this time. More new bone tissue is present in group B than in group A. The amount of movement of the first molar in group B was the greatest among the groups (**B**). Group A: mandibular ramus osteotomy, installation of an orthodontic tooth movement device, and administration of 20 μg/kg rhPTH after surgery. Group B: mandibular ramus osteotomy, installation of an orthodontic tooth movement device, and administration of 40 μg/kg rhPTH after surgery. Control group: mandibular ramus osteotomy, installation of an orthodontic tooth movement device, and administration of normal saline solution after surgery. Negative control group: installation of the orthodontic tooth movement device and administration of normal saline solution after surgery.

molars (Fig 1, *A-D*). On postoperative day 1, the orthodontic traction force of the tension spring was adjusted to ~80 g to induce mesial movement of the mandibular first molars.

A Vernier caliper was used to measure the distance between the midpoints of the first and second molar crowns at the different time points. The average distance was calculated from 3 measurements recorded by the same investigator. The velocity (mm/d) of mesial movement of the mandibular first molars was then calculated.

Six animals in each group were killed on postoperative days 0, 5, 7, 14, and 21. Bone fragments containing the mandibular first molars and their proximal and distal alveolar bones were harvested. Three samples in each group were fixed with 4% paraformaldehyde for 24 hours and 20% ethylenediamine tetraacetic acid for 4 weeks and then embedded in paraffin per the conventional procedure. Histologic sections (5 μm thick) in the mesiodistal direction parallel to the direction of the orthodontic force application were prepared and stained with hematoxylin and eosin. Osteoclasts were stained with tartrate-resistant acid phosphatase (TRAP). Morphological changes in the periodontal tissues were observed under a microscope at low magnification (×100). Expression levels of RANKL and RUNX2 were determined. The remaining 3 periodontal samples from each group were preserved in RNA preservation solution for quantification of *RANKL* and *RUNX2* mRNA.

Morphological changes in the periodontal tissues were observed in hematoxylin and eosin–stained histologic sections at low magnification. In TRAP-stained sections, osteoclasts on the alveolar bone surface on the compression side of the orthodontically treated teeth were counted in 5 evenly and well stained areas selected in a ×100 field of view. The mean number of osteoclasts was then calculated.

For analysis of RUNX2 and RANKL expression in the periodontal tissues on the compression side of the orthodontically treated teeth, immunohistochemical staining of the histological sections of periodontal tissue was

performed in accordance with the manufacturer's instructions (Wuhan Boster Bio-Engineering Co, Wuhan, Hubei, China). Briefly, after deparaffinization, dehydration, and antigen retrieval, the sections were reacted with H_2O_2 3% and incubated at 37°C for 10 minutes, and then incubated with the required primary antibodies (RUNX2 at a concentration of 1:30 and RANKL at a concentration of 1:100) at 4°C overnight. Next, they were washed with phosphate-buffered saline solution (PBS) and incubated at 37°C for 2 hours after drop-adding the secondary antibody and washed with PBS. After treatment with DAB solution, the sections were flushed completely, counterstained with hematoxylin and washed with water, treated with dehydration to render them transparent, and then mounted on slides and observed under a microscope. Under a ×400 field of view, 5 areas of identical size (200 × 200 μm) with cells that showed intense staining were selected to determine the integrated optical density with the use of Image-Pro Plus 6.0 image analysis software (Media Cybernetics, Rockville, Md). The average integrated optical density of each section was then calculated.

For quantification of *RANKL* and *RUNX2* mRNA by real-time polymerase chain reaction (PCR), alveolar bone tissue sections (3 × 3 mm^3) were excised from the compression side of the mandibular first molars and ground in liquid nitrogen. A magnetic bead–based RNA isolation kit (Life Technologies, Carlsbad, Calif) was used to extract total RNA from 1 mm^3 of ground tissue. Complementary DNA was synthesized with the use of the Primescript RT Reagent Kit (Takara, Beijing, China). The reaction mixture (10 μL; Table I) was prepared in reaction tubes on ice, and the tubes were placed in a quantitative fluorescence PCR instrument (Bio-Rad Laboratories, Hercules, Calif). The reaction conditions were set as follows: 95°C for 5 minutes, followed by 40 cycles of 95°C for 10 seconds, 58°C for 20 seconds, and 72°C for 30 seconds. At 60°C and 95°C, melting curve analysis was performed for each target gene and an internal reference gene. Relative expression levels of *RANKL* and *RUNX2* mRNA were determined by quantitative analysis of the PCR data with the $2^{-\Delta\Delta Ct}$ method. The qPCR primers for both genes (Table II) were designed and synthesized by Shanghai Sangon Bioengineering Technology and Services Co (Shanghai, China).

Statistical analysis

The results are presented as mean ± SD. One-way analysis of variance was performed with the use of IBM SPSS Statistics version 23.0 software (IBM Corp, Armonk, NY). The Student-Newman-Keuls multiple range test and least significant difference were used to examine the differences between groups. *P* values <0.05 were considered to be statistically significant.

Table I. Fluorescence quantitative PCR mix

Reagent	*Reaction system*
SYBR Green I Master	5.0 μL
Forward primer (10 μmol/L)	0.5 μL
Reverse primer (10 μmol/L)	0.5 μL
cDNA	0.25 μL
ddH_2O	3.75 μL
Total	10.0 μL

PCR, polymerase chain reaction; *cDNA*, complementary DNA; *ddH_2O*, double-distilled water.

Table II. Primer sequences

Gene	*Primer sequence*
RANKL	Forward 5′-CGCAGATGGATCCTAACAGA-3′
	Reverse 5′-TGCTTCTGTGTCTTCGCTCT-3′
RUNX2	Forward 5′-ATGACACTGCCACCTCTGAC-3′
	Reverse 5′-CCTGCCTGGCTCTTCTTACT-3′
GAPDH	Forward 5′-TCTGGCAAAGTGGATGTTGT-3′
	Reverse 5′-GTGGGTGGAATCATACTGGA-3′

RESULTS

All animals (except for those in the negative control group) 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. One rabbit in group B died from unknown causes on postoperative day 12, and the orthodontic device was irretrievably lost in one rabbit in the control group on postoperative day 6, leaving data for 118 rabbits available for inclusion in the analysis. Food consumption decreased to below normal in all rabbits that underwent surgery in the 2 days after surgery but returned to normal on postoperative day 3. By day 7, more new bone tissue was detected in the osteotomy gap in group B than in group A and the control group (Figs 1, *A-C*). By day 21 after surgery, bone healing at the site of the mandibular ramus osteotomy was good and the osteotomy line had disappeared. The gingival tissue between the incisors and first molars was slightly inflamed because of mechanical stimulation by the orthodontic device during mesial movement of the mandibular first molars.

After 5, 7, 14, and 21 days of application of orthodontic force, the amount of movement of the mandibular first molar in groups A and B was significantly greater than in the control groups. In addition, the distance of tooth movement in group B was the greatest

Table III. Intergroup comparison of distance of tooth movement at different time points, mm, mean ± SD

	Distance of orthodontic tooth movement			
Group	*Day 5*	*Day 7*	*Day 14*	*Day 21*
Negative control group	0.59 ± 0.02	0.91 ± 0.05	1.46 ± 0.04	1.87 ± 0.01
Control group	0.72 ± 0.04	1.17 ± 0.05*	1.63 ± 0.12*	2.27 ± 0.08*
Experimental group A	0.80 ± 0.06†‡	1.30 ± 0.08†‡	1.92 ± 0.10†‡	2.42 ± 0.07†‡
Experimental group B	0.95 ± 0.05†‡	1.52 ± 0.08†‡§	2.35 ± 0.14†‡§	2.78 ± 0.04†‡§

*$P < 0.05$, comparison between the control group and the negative control group at each time point; †$P < 0.05$, comparison between the experimental groups and the control group at each time point; ‡$P < 0.05$, comparison between the experimental groups and the negative control group at each time point; §$P < 0.05$, comparison between experimental group A and experimental group B at each time point.

among the groups ($P < 0.05$; Table III). In all of the groups, the most rapid orthodontic tooth movement was observed from days 5 to 7 after surgery, after which the movement decreased. In the 21 days after surgery, the movement of the first molars in groups A and B was significantly more rapid than in the control group ($P < 0.05$) and was more rapid in the control group than in the negative control group ($P < 0.05$). The velocity of orthodontic tooth movement was also more rapid in group B than in group A ($P < 0.05$; Fig 2).

Bone resorption and formation were evident in the orthodontically treated periodontal tissues in all the study groups. After 5 days of application of orthodontic traction, all 4 groups showed a narrowed periodontal ligament space, disordered collagen fibers, and multiple osteoclasts in the bone resorption lacunae on the alveolar bone surface on the compression side. After 7 days of orthodontic traction, active bone remodeling without hyalinization was found to have caused a further increase in bone resorption lacunae, and more osteoclasts were observed on the compression side (Fig 3). The number of osteoclasts was higher in groups A and B than in the control group, and higher in the control group than that in the negative control group. Furthermore, a greater number of osteoclasts were detected in group B than in group A (Fig 4). After 14 and 21 days of orthodontic traction, the numbers of osteoclasts present in bone resorption lacunae on the compression side had decreased in all 4 groups. On continued application of orthodontic traction, the periodontal ligament gradually widened on the tension side of the orthodontically treated teeth and new bone formed. osteoblasts were detected around the newly formed bone.

The number of osteoclasts in all 4 groups peaked after 7 days of application of orthodontic traction. The number of osteoclasts was significantly higher in groups A and B than in the control group ($P < 0.05$), and significantly higher in the control group than in the negative control group ($P < 0.05$). In addition, a greater number of osteoclasts was found in group B than in group A (Table IV).

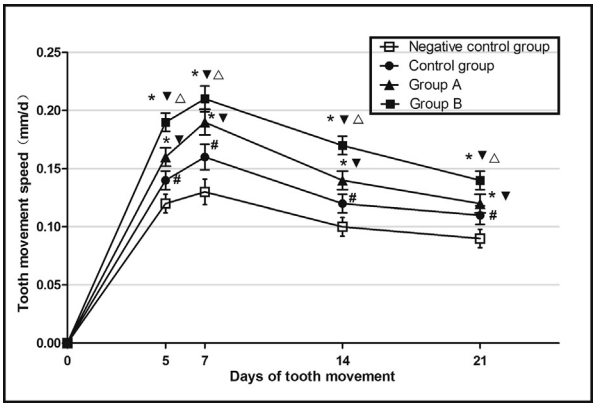

Fig 2. Intergroup comparison of the velocity of orthodontic tooth movement at different time points in the study. *$P < 0.05$, comparison between the experimental groups and the control group at each time point. ▼$P < 0.05$, comparison between the experimental groups and the negative control group at each time point. △$P < 0.05$, comparison between experimental group A and experimental group B at each time point. #$P < 0.05$, comparison between the control group and negative control group at each time point.

Immunohistochemistry revealed that RANKL expression was localized in the cytoplasm of the osteoblasts, osteoclasts, and fibroblasts in the periodontal tissues. After 5 and 7 days of orthodontic traction, the RANKL expression level (average optical density) in each group increased on the compression side of the alveolar bone, reaching a peak on day 7 and then gradually decreasing thereafter (Fig 5). At all time points, the intensity of staining was significantly higher in groups A and B than in the control group ($P < 0.05$), and higher in the control group than in the negative control group ($P < 0.05$). The staining intensity was also significantly higher in group B than in group A ($P < 0.05$; Table V).

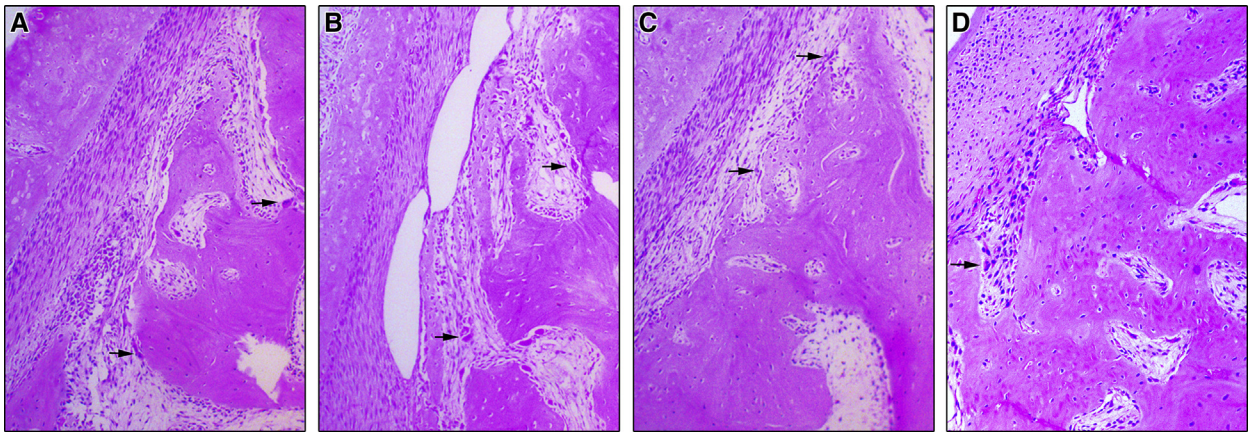

Fig 3. Morphologic changes in periodontal tissues (hematoxylin-eosin staining, magnification ×100) on the compression side on postoperative day 7 in **A,** group A, **B,** group B, **C,** the control group, and **D,** the negative control group. Bone resorption was evident on the compression side of periodontal tissues in each group. Osteoclasts (*arrows*) accumulated in bone resorption lacunae, and irregular periodontal ligament fibers were observed on postoperative day 7. In group B (**B**), osteoclasts formed relatively large bone resorption lacunae and degraded the surrounding extracellular matrix.

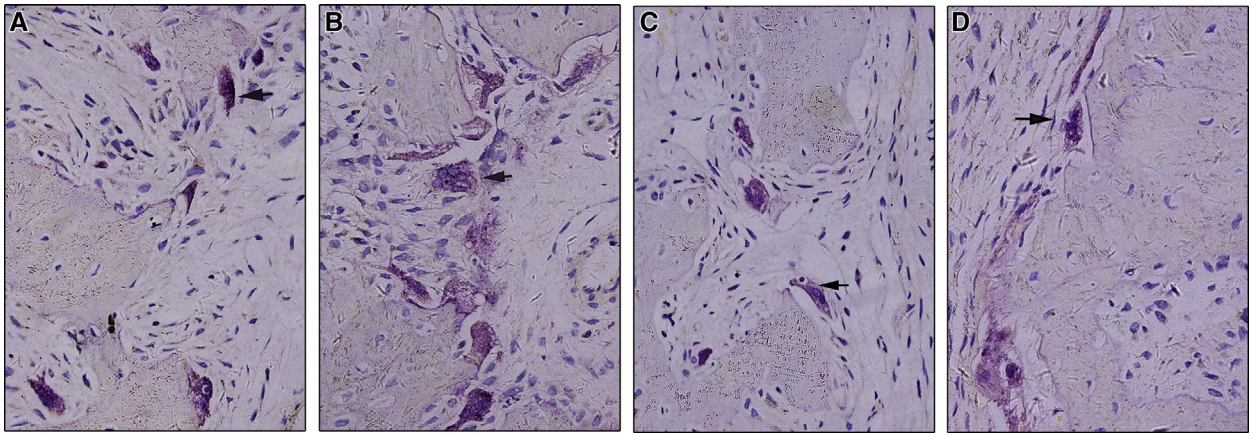

Fig 4. Osteoclasts observed on the compression side of the periodontal tissues (TRAP staining, magnification ×200) on postoperative day 7 in **A,** group A, **B,** group B, **C,** the control group, and **D,** the negative control group. Osteoclasts (*arrows*) are seen as large cells with an irregular shape, rich cytoplasm, and multiple dark-stained nuclei. Group B contained a greater number of osteoclasts than group A.

RUNX2 was expressed in the nuclei of osteoblasts and fibroblasts present around the newly formed bone trabeculae (Fig 6). The expression levels peaked after 7 days of orthodontic traction and decreased gradually thereafter. After 5, 7, and 14 days orthodontic traction, the staining intensity values for RUNX2 in groups A and B were significantly lower than those in the control groups ($P < 0.05$), and were lower in the control group than in the negative control group ($P < 0.05$). After 21 days of orthodontic traction, there was no significant difference in staining intensity between group A and the control groups ($P > 0.05$). However, the staining intensity was significantly higher in group A than in group B on days 7 and 14 ($P < 0.05$; Table VI).

The findings of real-time quantitative fluorescence PCR showed that *RANKL* and *RUNX2* mRNA levels on the compression side of the alveolar bone increased with time, peaking after 7 days of orthodontic traction and decreasing gradually thereafter. The *RANKL* mRNA level in alveolar bone was significantly higher in

Table IV. Intergroup comparison of number of osteoclasts on the compression side at different time points, mean ± SD

	Number of osteoclasts, tooth movement day				
Group	*Day 0*	*Day 5*	*Day 7*	*Day 14*	*Day 21*
Negative control group	1.53 ± 0.17	5.66 ± 0.35	7.89 ± 0.51	4.04 ± 0.32	3.63 ± 0.13
Control group	1.50 ± 0.19	6.67 ± 0.12	9.78 ± 1.39*	5.67 ± 0.67*	5.11 ± 0.51*
Experimental group A	1.48 ± 0.32	13.55 ± 0.39†‡	18.66 ± 0.58†‡	14.11 ± 0.84†‡	8.89 ± 1.84†‡
Experimental group B	1.55 ± 0.53	16.22 ± 0.51†‡	20.89 ± 1.17†‡§	15.00 ± 1.00†‡	11.22 ± 1.50†‡§

*$P < 0.05$, comparison between the control group and negative control group at each time point; †$P < 0.05$, comparison between the experimental groups and the control group at each time point; ‡$P < 0.05$, comparison between the experimental groups and the negative control group at each time point; §$P < 0.05$, comparison between experimental group A and experimental group B at each time point.

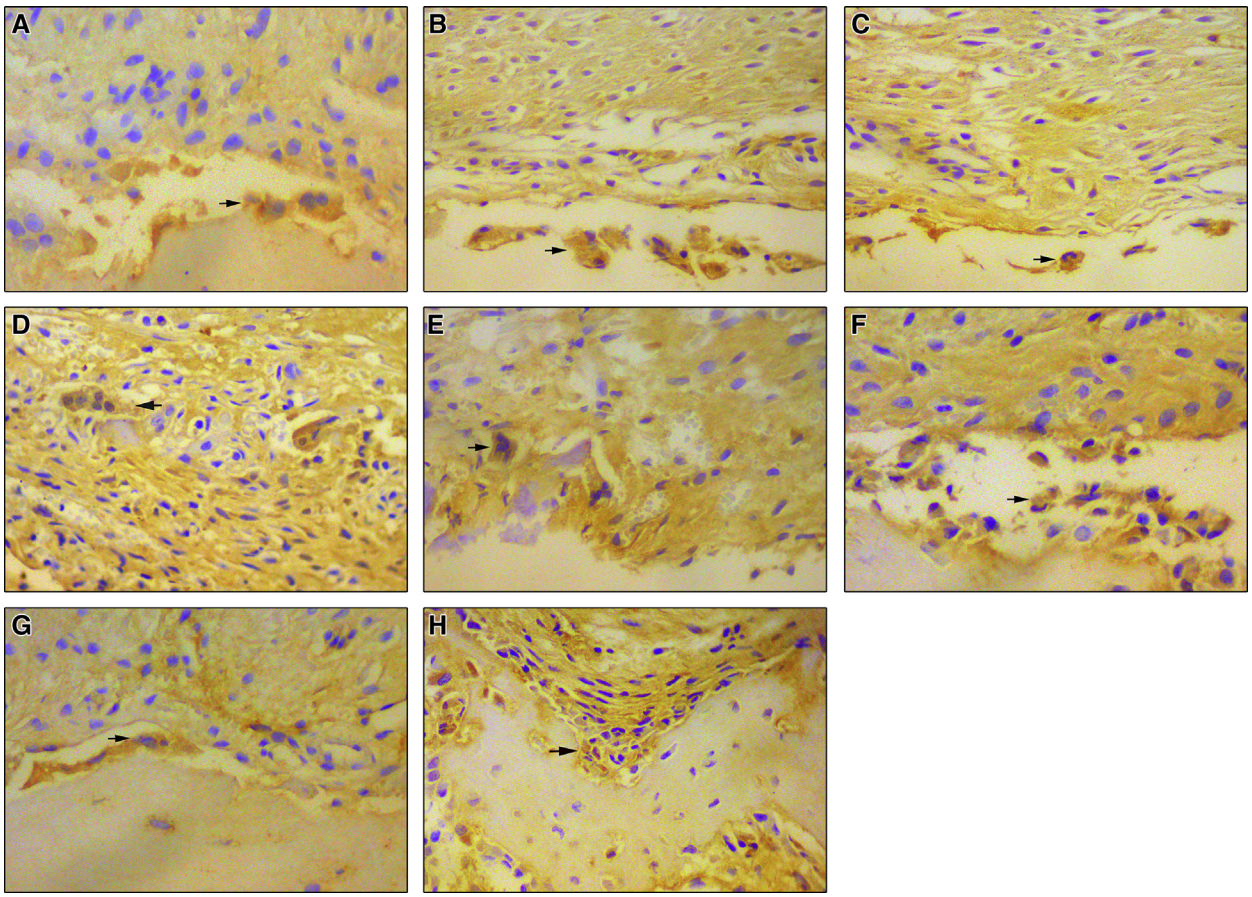

Fig 5. Comparison of RANKL expression on the compression side on postoperative days 7 and 14 (magnification ×400). **A-D,** Group A, group B, the control group, and the negative control group, respectively, on day 7. **E-H,** Group A, group B, the control group, and the negative control group, respectively, on day 14. Immunoreactivity against RANKL indicated expression in the cytoplasm (*arrows*) of osteoblasts and some osteoclast-like cells. Expression of RANKL in the extracellular matrix of the periodontal ligament region was relatively weak.

groups A and B than in the control group ($P < 0.05$), and significantly higher in the control group than in the negative control group ($P < 0.05$). The *RANKL* mRNA level was also higher in group B than in group A. In contrast, the *RUNX2* mRNA level on the compression side was significantly higher in the control group than

Table V. Intergroup comparison of RANKL expression on the compression side at different time points, mean ± SD

	Integrated optical density, tooth movement day				
Group	*Day 0*	*Day 5*	*Day 7*	*Day 14*	*Day 21*
Negative control group	0.043 ± 0.005	0.094 ± 0.004	0.157 ± 0.005	0.129 ± 0.003	0.109 ± 0.007
Control group	0.046 ± 0.004	0.119 ± 0.005*	0.173 ± 0.008*	0.146 ± 0.009*	0.133 ± 0.009*
Experimental group A	0.047 ± 0.003	0.144 ± 0.003†‡	0.202 ± 0.008†‡	0.168 ± 0.007†	0.147 ± 0.007†
Experimental group B	0.041 ± 0.003	0.166 ± 0.004†‡	0.245 ± 0.008†‡§	0.198 ± 0.007†‡§	0.166 ± 0.003†‡§

*$P < 0.05$, comparison between the control group and negative control group at each time point; †$P < 0.05$, comparison between the experimental groups and the control group at each time point; ‡$P < 0.05$, comparison between the experimental groups and the negative control group at each time point; §$P < 0.05$, comparison between experimental group A and experimental group B at each time point.

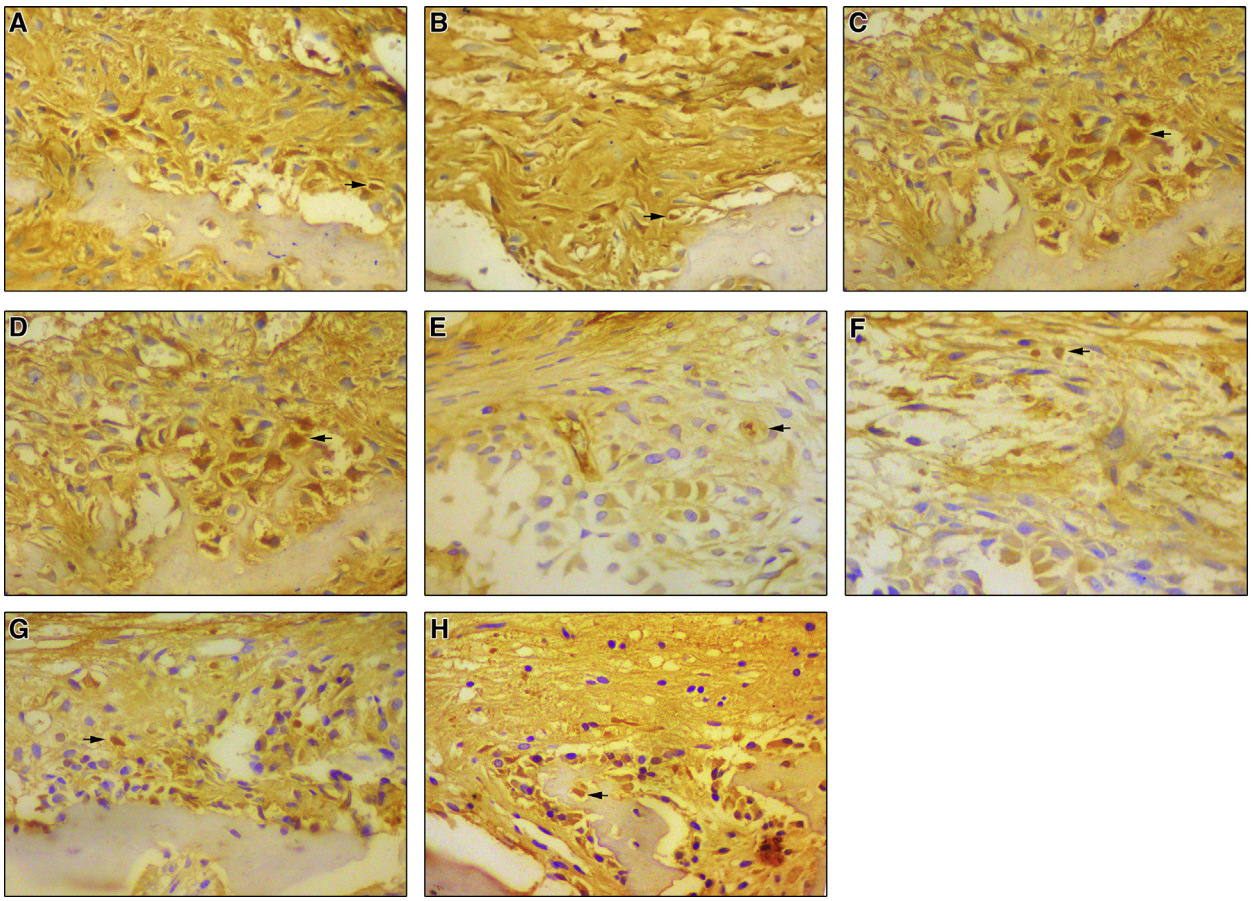

Fig 6. Comparison of RUNX2 expression on the compression side on postoperative days 7 and 14 (magnification ×400). **A-D,** Group A, group B, the control group, and the negative control group, respectively, on day 7. **E-H,** Group A, group B, the control group, and the negative control group, respectively, on day 14. Immunoreactivity against RUNX2 indicated strong expression of mononuclear cubic osteoblasts in the nuclei on the bone surface (*arrows*). Expression of RUNX2 was relatively weak in the extracellular matrix of the periodontal ligament region.

in groups A and B and was significantly lower in group B than in group A ($P < 0.05$). The *RUNX2* mRNA level was significantly higher in the negative control group than in the control group ($P < 0.05$; Figs 7 and 8).

DISCUSSION

Dentomaxillofacial deformities in adults can be corrected by a combination of orthognathic surgery and orthodontic treatment. However, remodeling of

Table VI. Intergroup comparison of RUNX2 expression on the compression side at different time points, mean ± SD

	Integrated optical density, tooth movement day				
Group	*Day 0*	*Day 5*	*Day 7*	*Day 14*	*Day 21*
Negative control group	0.035 ± 0.002	0.094 ± 0.002	0.123 ± 0.001	0.108 ± 0.002	0.087 ± 0.002
Control group	0.031 ± 0.002	0.083 ± 0.004	0.117 ± 0.001*	0.096 ± 0.002*	0.074 ± 0.001*
Experimental group A	0.036 ± 0.005	0.068 ± 0.005†	0.103 ± 0.006†‡	0.085 ± 0.003†‡	0.071 ± 0.006‡
Experimental group B	0.033 ± 0.001	0.054 ± 0.003†‡	0.084 ± 0.003†‡§	0.075 ± 0.001†‡§	0.068 ± 0.003†‡

*$P < 0.05$, comparison between the control group and negative control group at each time point; †$P < 0.05$, comparison between the experimental groups and the control group at each time point; ‡$P < 0.05$, comparison between the experimental groups and the negative control group at each time point; §$P < 0.05$, comparison between experimental group A and experimental group B at each time point.

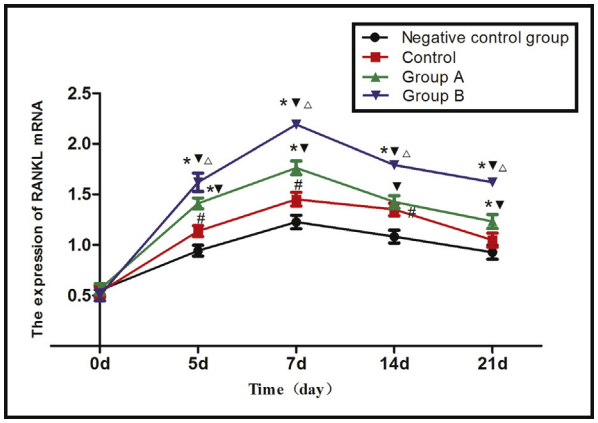

Fig 7. Comparison of relative expression of *RANKL* mRNA on the compression side between the study groups. #$P < 0.05$ for comparison between the control group and negative control group at each time point. *$P < 0.05$, comparison between the experimental groups and the control group at each time point. ▼$P < 0.05$, comparison between the experimental groups and the negative control group at each time point. △$P < 0.05$, comparison between experimental group A and experimental group B at each time point.

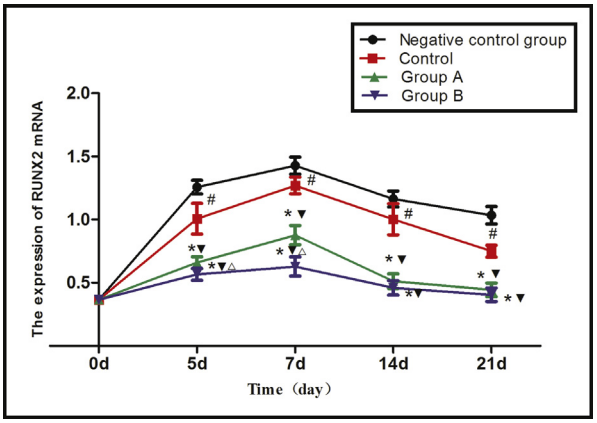

Fig 8. Comparison of relative expression of *RUNX2* mRNA on the compression side among the groups. #$P < 0.05$, comparison between the control group and negative control group at each time point. *$P < 0.05$, comparison between the experimental groups and the control group at each time point. ▼$P < 0.05$, comparison between the experimental groups and the negative control group at each time point. △$P < 0.05$, comparison between the experimental group A and experimental group B at each time point.

periodontal tissue in orthodontically treated teeth in adults is slow, so the course of orthodontic treatment is relatively long. Researchers have been exploring techniques for acceleration of orthodontic tooth movement. To date, the mainstream techniques and methods used have included administration of medication or hormones, device-assisted treatment, and surgery to accelerate orthodontic tooth movement.[18,19] The main surgical procedures used include interdental osteotomy, corticotomy, and orthognathic surgery. Interdental osteotomy or other periodontal surgeries[20,21] might accelerate orthodontic tooth movement by stimulating regional periodontal bone tissues to remodel the orthodontically treated teeth. The conventional orthognathic surgery procedures are Le Fort I osteotomy of the maxilla and sagittal split ramus osteotomy or intraoral vertical ramus osteotomy of the mandible. The Le Fort I osteotomy is a horizontal osteotomy of the maxilla above the roots of the teeth. Experimental studies have shown that maxillary Le Fort I osteotomy can accelerate orthodontic tooth movement[22]; however, the osteotomy site in mandibular ramus orthognathic surgery is a considerable distance from the mandibular orthodontic tooth, and the effect of mandibular orthognathic surgery on the velocity of orthodontic tooth movement remains unclear. We hypothesized that PTH can further accelerate orthodontic tooth movement after mandibular ramus osteotomy. The present study shows that administration of PTH can further accelerate movement

of the mandibular first molars after mandibular ramus osteotomy with good bone healing at the site of the osteotomy. The increased number of osteoclasts on the compression side of the periodontal tissue suggests that PTH accelerates orthodontic tooth movement further by remodeling of periodontal bone.

The dose of PTH affects the rates of orthodontic tooth movement, bone formation, and resorption of periodontal tissues; however, the appropriate dose of PTH for acceleration of orthodontic tooth movement has yet to be clearly established. The PTH doses and methods of administration used in various experimental animal models of orthodontic tooth movement have varied widely.[8-11] The present study was designed to investigate the effect of different doses of PTH on orthodontic tooth movement after mandibular ramus orthognathic osteotomy. Orthodontic tooth movement was accelerated in rabbits that received intermittent subcutaneous injections of PTH at doses of 20 μg/kg and 40 μg/kg, with more rapid movement in the group that received the higher dose. Furthermore, a greater number of osteoclasts were detected in the rabbits that received the higher dose of PTH than in the group that received the lower dose and the control group. These findings suggest that PTH can accelerate orthodontic tooth movement in a dose-dependent manner.

The velocity of orthodontic tooth movement depends on the rate of remodeling of periodontal bone tissues on the compression side. Periodontal tissue remodeling involves many cytokines produced by osteoblasts and osteoclasts, among which RUNX2 and RANKL are important regulators of osteoblastic and osteoclastic activity. Previous studies have reported increased expression of RANKL and RUNX2 during orthodontic tooth movement, suggesting that these factors play an important role in the remodeling of the periodontal tissues.[23-27] It has also been reported that RUNX2 expression is down-regulated and that RANKL expression is up-regulated on the compression side of periodontal bone tissues during orthodontic tooth movement.[28,29] The RANKL protein can activate osteoclasts to promote alveolar bone remodeling and orthodontic tooth movement.[11,24,25,30] However, there is no report on the expression of RUNX2 and RANKL in the periodontal tissue of orthodontically treated teeth after mandibular orthognathic surgery and concomitant intermittent administration of PTH. The present study analyzed mRNA and protein expression of *RANKL* and *RUNX2* on the compression side of orthodontically treated teeth at different time points after mandibular ramus osteotomy and intermittent injection of PTH after the surgery. We found increased expression of RANKL and RUNX2 on the compression side of the alveolar bone in both the experimental and the control groups. The increased expression of RANKL in the group that received PTH indicates enhanced osteoclast activity, which could cause increased catabolism on the compression side of the alveolar bone to promote rapid orthodontic tooth movement. The increased expression of RUNX2 in the control group suggests enhanced osteoblastic activity, which somewhat decreases the velocity of orthodontic tooth movement.

The present findings provide preliminary theoretical support and experimental evidence for administration of PTH to promote early tooth movement after mandibular ramus osteotomy. Intermittent administration of rhPTH 1-34 can accelerate orthodontic tooth movement in a dose-dependent manner, and higher doses of PTH can further accelerate orthodontic tooth movement at an early stage after mandibular ramus osteotomy. Mandibular ramus osteotomy combined with PTH administration can promote RANKL expression and suppress RUNX2 expression on the compression side of orthodontically treated teeth to accelerate periodontal bone tissue remodeling and orthodontic tooth movement.

The present study has some limitations. Only 2 daily doses of PTH were used, and the dosing regimen is one of the important factors when considering treatment with this agent. Therefore, large-scale studies are needed to investigate the optimal dose, route of administration, and duration of treatment needed to achieve optimal acceleration of tooth movement and bone healing after mandibular ramus osteotomy. Furthermore, the dose-response relationship and the mechanisms underlying the ability of PTH to accelerate tooth movement after mandibular ramus osteotomy were not fully investigated in this study.

CONCLUSIONS

In this study, we showed that administration of exogenous PTH can increase catabolic activity on the compression side of orthodontically treated periodontal tissues to promote tooth movement after mandibular osteotomy. Our findings indicate that different doses of PTH have different effects on the velocity of orthodontic tooth movement. However, orthodontic tooth movement was affected by both mandibular ramus osteotomy and administration of PTH, which suggests that the mechanism controlling orthodontic tooth movement is very complex.

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