Effect of parathyroid hormone on experimental tooth movement in rats

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Introduction: The aim of this study was to investigate the effect of parathyroid hormone injection on experimental tooth movement in rats. Methods: Sixty male Wistar rats were randomly divided into 2 groups. Their maxillary right first molars were moved mesially with nickel-titanium closed-coil springs. The experimental group received daily subcutaneous injections of parathyroid hormone at a dose of 4 μg per 100 g of body weight for 12 days, and the control group received vehicle injections. The results were evaluated by intraoral measurements and by hematoxylin and eosin, tartrate-resistant acid phosphatase, and immunohistochemistry staining. Results: The tooth movement and osteoclast numbers were significantly increased in the parathyroid hormone group compared with the control group. The expressions of receptor activator of nuclear factor kappa B ligand and insulin-like growth factor-I were significantly stimulated in the parathyroid hormone group. Conclusions: The data suggest that short-term parathyroid hormone injection might be a potential method for accelerating orthodontic tooth movement by increasing the alveolar bone turnover rate. (Am J Orthod Dentofacial Orthop 2013;144:523-32)

During orthodontic treatment, appliances usually cause inconveniences in patients’ daily lives and hinder proper oral hygiene, increasing the risk of dental caries and periodontal disease. Also, orthodontists are usually confronted with difficult situations of ineffective tooth movement, such as uprighting of tipping molars, or space closure in patients with high alveolar bone density. Therefore, it is a primary concern for orthodontists to develop a method of accelerating tooth movement to make orthodontic treatment more effective. Since orthodontic tooth movement is achieved through alveolar bone remodeling, medicines stimulating bone metabolism might promote tooth movement. As an important regulator of bone metabolism, parathyroid hormone could be a potentially valuable agent for facilitating tooth movement.

Parathyroid hormone, as a major regulator of calcium and phosphate homeostasis, has gained particular attention for its paradoxical effects on bone metabolism. Relative studies have confirmed that parathyroid hormone could stimulate both osteoclast-mediated bone resorption and osteoblast-mediated bone formation, therefore accelerating the bone turnover rate. The ultimate effect of parathyroid hormone on bone remodeling is determined by the administration protocol. Continuous infusion of parathyroid hormone results in a catabolic effect, whereas intermittent injection leads to an anabolic effect. Actually, intermittent low-dose parathyroid hormone analogs have been widely administered in the clinical treatment of osteoporosis.

Previous studies have investigated the effect of different administration patterns of parathyroid hormone on orthodontic tooth movement rate and periodontal repair. The results suggested that systemic continuous infusion or local chronic application of parathyroid hormone could accelerate tooth movement in rats through enhancement of alveolar bone resorption, whereas long-term intermittent injection of parathyroid hormone facilitated periodontal repair of
bone or root resorption after orthodontic tooth movement through activation of osteoblastic cells. However, the effect of intermittent injection of parathyroid hormone on the tooth movement rate has not been clarified yet. Salazar et al evaluated the tooth movement in ovariectomized rats treated with intermittent parathyroid hormone injections and found that they did not hinder tooth movement; on the contrary, those rats showed greater tooth movements than did the control group. The authors mentioned that unlike other osteoporosis treatment drugs such as bisphosphonates, calcitonin, and calcium with vitamin D, which diminish bone resorption, parathyroid hormones stimulated osteoblast function with no interference in osteoclast activity and facilitated bone remodeling. This could explain the acceleration effect of intermittent parathyroid hormone.

The precise mechanism underlying the effect of intermittent parathyroid hormone on bone metabolism has not been clarified yet. However, it is verified that under intermittent parathyroid hormone administration, both osteoblast and osteoclast activities are stimulated. Insulin-like growth factor-1 (IGF-I) and receptor activator of nuclear factor kappa B ligand (RANKL)/osteoprotegerin system are essential to the biologic processes, respectively. The receptors of parathyroid hormone are only expressed on the cell membrane of osteoblasts. After the binding of parathyroid hormone molecules to their receptors, the osteoblasts are stimulated to produce more IGF-I via a cyclic adenosine monophosphate (cAMP)-dependent mechanism, which functions as an autocrine/paracrine factor and activates its adaptor molecule insulin-receptor substrate-1 in osteoblast precursors in bone marrow, and causes osteoblast proliferation, differentiation, and function.

On the other hand, osteoblasts stimulated by parathyroid hormone molecules also express more RANKL on the cell membrane, which binds to the receptor activator of nuclear factor kappa B (RANK) on the cell membrane of osteoclastic precursors through cell-to-cell contact and stimulates osteoclast proliferation, differentiation, and activation. Osteoprotegerin, the decoy receptor of RANKL, is secreted by osteoblasts and inhibits the binding of RANKL and RANK. The RANKL/osteoprotegerin ratio is an indirect reflection of the actual functional RANKL molecules and the activity of osteoclastogenesis. The osteoprotegerin expression level is not significantly changed by parathyroid hormone administration; therefore, the resultant RANKL/osteoprotegerin ratio is remarkably increased.

The anabolic effect of intermittent parathyroid hormone in the clinical treatment of osteoporosis involves not only osteoblastic bone formation, but also osteoclastic bone resorption. The ultimate increase of bone density is achieved through the "anabolic window," which means that bone formation is greater than bone resorption during the first 6 to 18 months. Actually, some researchers suppose that active osteoclastic resorption is necessary for the effect of the parathyroid hormone on bone formation in a remodeling system. After intermittent parathyroid hormone administration, RANKL expression is greatly enhanced, resulting in an increase in osteoclastic resorptive activity. In turn, the resorptive activity increases the release of osteogenic growth factors from bone matrix and osteoclasts, and it stimulates bone remodeling.

Based on the previous literature, intermittent parathyroid hormone administration stimulates both osteoblast and osteoclast activity, and it promotes bone formation via enhanced bone remodeling. We hypothesize that intermittent parathyroid hormone could also facilitate orthodontic tooth movement through stimulation of alveolar bone remodeling. The purpose of this study was to investigate whether intermittent parathyroid hormone administration can accelerate orthodontic tooth movement and its impact on the expression of the RANKL/osteoprotegerin system and IGF-I in the periodontal area.

**MATERIAL AND METHODS**

All experimental procedures were approved by the animal experiment ethics committee of the State Key Laboratory of Oral Diseases of Sichuan University in China. Sixty 8-week-old male Wistar rats weighing 200 ± 10 g were obtained from the university’s experimental animal center. The animals were randomly divided into 2 groups of 30 animals each: the parathyroid hormone group and the control group. They were kept in plastic cages with a standard 12-hour light-and-dark cycle and fed a soft diet with water ad libitum. During the experiment, their weights were recorded every day.

An orthodontic elastic closed-coil spring (Grikin Advanced Materials, Beijing, China) was fixed between the maxillary right first molar and the incisors with ultraviolet curable resin (MB 4403; 3M Unitek, Monrovia, Calif) under anesthesia with intraperitoneal injection of 2% ketamine hydrochloride at 2 mL per kilogram of body weight. The maxillary first molars were moved mesially with a force of 40 g (Fig 1). The appliances were activated immediately upon insertion, and the fit was checked daily. No reactivation was performed during the experimental period.

Starting 1 day before the installation of the orthodontic appliance, the animals in the experimental group...
received a daily injection of recombinant human para-
thyroid hormone (1–34) (PTHP-002; Chinese Peptide, Hangzhou, China) at a dosage of 4 μg per 100 g of body weight dissolved in phosphate-buffered saline (PBS) at a concentration of 1 μg per milliliter. The animals in the control group received the same volume of vehicle (PBS). The protocol of parathyroid hormone (1–34) injection was decided according to previous studies.14,21

Six animals from each group were killed on day 0 (the day before orthodontic force application) and on days 3, 6, 9, and 12. After that, the maxilla of each animal was dissected and divided into halves and prepared for light microscopic observation. The distance of tooth movement was determined by measuring the separation between the first and second maxillary molars using a vernier caliper with an accuracy of 0.02 mm.

The right half of the maxilla of each animal was fixed in 4% paraformaldehyde in 0.1 mol/L of PBS for 24 hours and decalcified in neutral 10% ethylenediamine tetra-acetic acid at room temperature for at least 2 months. This solution was changed every other day. A methyl red-ammonia-ammonium oxalate solution test was carried out to determine whether decalcification was completed. After dehydration and paraffin embedding, 5-μm serial sections in a mesiodistal direction parallel to the long axis of the distal root of the first molar were cut on a microtome (HM 355S; Microm International, Walldorf, Germany) and mounted on glass slides. Selected sections were stained with hematoxylin and eosin and tartrate-resistant acid phosphatase (TRAP).

Tissue sections were placed in a tris–hydroxy methyl aminomethane buffered saline solution (TBS) at pH 7.4 for 10 minutes. Thereafter, endogenous peroxidase activity was blocked in methanol/hydrogen peroxide for 10 minutes in the dark. Subsequently, sections were rinsed and then preincubated with TBS containing 4% bovine serum albumin (TBS/BSA) for 20 minutes to prevent unspecific background staining. Then, sections were incubated with a polyclonal primary antibody of rabbit origin raised against peptide mapping at the carboxy terminus of the protein (ZSGB-BIO, Beijing, China) in a 1:200 working solution (osteoprotegerin) or 1:50 working solution (RANKL) of TBS/BSA at 4°C overnight in a humidified chamber. The slides were rinsed again and incubated for 30 minutes with a rabbit anti-goat immunoglobulin diluted 1:100 in TBS/BSA (ZSGB-BIO) as secondary antibody. Incubation was stopped in TBS before the peroxidase-antiperoxidase complex was administered for 30 minutes. After another rinse, the tissue sections were stained in a 3,3′-diaminobenzidine solution for about 5 minutes, rinsed, and then counterstained with Mayer’s hematoxylin, dehydrated, and cover-slipped for light microscopic analysis. To prove the specificity of the immunoreactions, negative controls were carried out by omitting the primary antibody or using nonimmune immunoglobulin G instead, omitting both the primary and secondary antibody, and using TBS/BSA instead.
The light microscopic images were captured per specimen on the former compression side of the distal root of the maxillary first molar at the mesiocoronal area at magnification of 100 times, and near the bifurcation area at the mesiocoronal region at magnification of 400 times (Fig 2). Among the serial sections in the mesiodistal direction from each specimen, several central sections were selected for histomorphometric and immunohistochemical measurements: 3 sections for each measurement. The number of TRAP positive cells in the periodontal area was counted and expressed as cell numbers per millimeter of root length. The immunoreactivity of molecules was evaluated using an automatic image analysis system (HPIAS-1000, version 6.0; Media Cybernetics, Silver Spring, Md), and the immunoreactive intensity was converted to gray-scale values. All measurements were performed by 2 operators (H.H., R.L.), and any disagreement was resolved through discussion or assessment by a third investigator (J.C.). Both operators were blinded to the treatment allocation.

### Statistical analysis

The data were processed with SPSS software (version 11.5; SPSS, Chicago, Ill). The results were expressed as means and standard deviations. For histologic and immunohistochemical data, the results for each group were the means and standard deviations of the 6 measurements, each as an average of the 3 sections from a specimen. The data of tooth movement distance, osteoclast numbers, osteoprotegerin, and RANKL immunoreactivity between groups were evaluated by 1-way analysis of variance. The significance level was set at $P < 0.05$.

### RESULTS

During the early phase of the experiment, there was no significant difference of total tooth movement between the parathyroid hormone group and the control group, with approximate averages of 0.20 ± 0.04 and 0.22 ± 0.03 mm (for the parathyroid hormone and control groups, respectively) on day 3, and 0.30 ± 0.04 and 0.28 ± 0.02 mm (for the parathyroid hormone and control groups, respectively) on day 6. However, during the late phase, the differences between the 2 groups became significant. On day 9, the total tooth movement amounts were 0.48 ± 0.04 and 0.40 ± 0.03 mm on average for the parathyroid hormone and control groups, respectively ($P < 0.05$). On day 12, the differences between the 2 groups became greater, and the total tooth movement amounts were 0.72 ± 0.03 and 0.52 ± 0.04 mm on average for the parathyroid hormone and control groups, respectively ($P < 0.05$) (Fig 3).

Multinucleated elastic cells appeared on day 3 and increased on days 6 and 9. These cells formed the resorption lacunae around themselves on the surface of the alveolar bone. The resorption was more obvious in the mesiocoronal and distoapical regions of the distal root,

![Fig 3. Time course of orthodontic tooth movement in the rats treated with vehicle injection and intermittent parathyroid hormone (1-34) injection respectively. The data shown represent the means and standard deviations. $^a P < 0.05$, intermittent parathyroid hormone (1-34) group vs vehicle injection group.](image-url)
indicating tipping movement of the first molar. In the parathyroid hormone group, there were more functional multinucleated clastic cells on the surface of alveolar bone on days 6 and 9, and the resorption lacunae were larger (Fig 4).

The multinucleated osteoclasts and a few mononucleated osteoclast precursors in the periodontal ligament area were all stained positive for TRAP. Most of the TRAP positive cells were located in the resorption lacunae on the alveolar bone surface. Only a few mononuclear TRAP positive cells were located near vessels in the periodontal ligament, resembling hematopoietic osteoclast precursors. On day 3, a few osteoclasts appeared on the surface of the alveolar bone, but the resorption was not active, and few resorption lacunae were formed. On day 6, the number of osteoclasts increased remarkably, and the average cell numbers per millimeter of root length were 8.55 ± 1.83 and 6.72 ± 1.58 for the parathyroid hormone and control groups, respectively (P > 0.05). On day 9, the osteoclast numbers increased to average cell numbers per millimeter of root length of 13.72 ± 1.62 and 9.85 ± 1.34 for the parathyroid hormone and control groups, respectively, with a significant difference between the groups (P <0.05). On day 12, the average osteoclast numbers per millimeter were 11.96 ± 1.02 and 7.56 ± 1.42 for the parathyroid hormone and control groups, respectively, and the difference between the groups remained significant (P <0.05). It seems that the difference between the parathyroid hormone and the control groups became larger over time (Figs 5 and 6).

The immunoreactivity of RANKL was mainly observed in the osteoblasts and osteoclast-like cells on the surface of alveolar bone with strongly positive staining in the cytoplasm and weakly positive staining in the extracellular matrix of the periodontal ligament; the immunoreactivity of osteoprotegerin was mainly observed in some osteoblasts with strongly positive staining in the cytoplasm and weakly positive staining in the extracellular matrix of the periodontal ligament.
The expression of osteoprotegerin did not change significantly by parathyroid hormone administration, and the gray-scale value of osteoprotegerin immunoreactivity fluctuated from 115.93 to 124.08. The expression of RANKL was greatly enhanced in the parathyroid hormone group, and the difference between the 2 groups

Fig 5. Number of TRAP positive osteoclasts per millimeter of root length. Data are expressed as means and standard deviations. *P <0.05, intermittent parathyroid hormone (1-34) group vs vehicle injection group.

Fig 6. Histologic sections captured on the compression side of the distal root of the maxillary first molar (TRAP staining): A, day 6, the number of active osteoclasts (arrows) on the alveolar surface was approximately equal between the 2 groups (magnification, 100 times); B, day 9, active resorption by osteoclasts (arrows), more osteoclasts appeared in the parathyroid hormone group (magnification, 100 times); C, day 12, large resorption lacunae were formed after active resorption, and osteoclasts were still active (magnification, 100 times); D and E, day 9, TRAP positive osteoclasts (arrows) on the bone surface (magnification, 400 times).
was significant on days 9 and 12. The gray-scale values of RANKL were 157.62 ± 3.72 and 134.38 ± 1.37 for the parathyroid hormone and control groups, respectively, on day 9 ($P < 0.05$), and 142.77 ± 2.36 and 129.34 ± 3.62 for the parathyroid hormone and control groups, respectively, on day 12 ($P < 0.05$) (Tables I and II, Fig 7).

The immunoreactivity of IGF-1 was mainly observed in the mononucleated cuboidal osteoblasts on the surface of alveolar bone with strongly positive staining in the cytoplasm and weakly positive staining in the extracellular matrix of the periodontal ligament area. The cuboidal profile of the osteoblasts indicated their active function. The expression of IGF-1 was remarkably increased by the parathyroid hormone on days 9 and 12. The gray-scale values of IGF-1 were 137.01 ± 4.23 and 116.72 ± 3.41 for the parathyroid hormone and control groups, respectively, on day 9 ($P < 0.05$), and 148.26 ± 2.76 and 119.17 ± 0.78 for the parathyroid hormone and control groups, respectively, on day 12 ($P < 0.05$) (Table III, Fig 8).

**DISCUSSION**

In this study, the acceleration effect of intermittent parathyroid hormone on tooth movement has been shown in a rat model. The results demonstrated that the change of tooth movement rate was not significant until day 9, probably because the accumulation of parathyroid hormone took several days before it reached the pharmacologically effective concentration in the periodontal ligament. The difference between the 2 groups increased during the late phase, possibly because of the constant action of the parathyroid hormone. On days 9 and 12, the actual differences of the average tooth movement amounts between the groups were 0.08 and 0.2 mm, respectively. These differences might be too small for a clinical orthodontic patient, and orthodontists might consider it valueless. But on a rat model, the mesiodistal size of the first molar crown was 2 to 3 mm, and the difference of 0.2 mm equaled 10% of the molar crown size. For a clinical patient, whose first molar is more than 10 mm, the difference of 0.2 mm in rats might equal a 1-mm difference of tooth movement. A difference of 1 mm of tooth movement in orthodontic treatment might be significant. Many other studies investigating the medication effect on the tooth movement rates on rat models obtained similar results; the differences between the experimental and control groups ranged between 0.05 and 0.30 mm, and the researchers believed that the statistical significance suggested the acceleration effect.\textsuperscript{14,22,23}

The molecules of osteoprotegerin, RANKL, and IGF-1 were synthesized by differentiated osteoblasts in membranous and soluble forms.\textsuperscript{4,24} Therefore, the molecules exist not only on the cell membranes and in the cytoplasm, but also in the matrix as secreted molecules. As shown in Figures 7 and 8, the intracellular and extracellular cytoplasm of immunoreactive cells was stained strongly positive for the higher concentration of detected molecules, and the extracellular matrix was stained weakly positive for the lower concentration. The number or proportion of positive cells was not enough to reflect the expression level. Therefore, the immunoreactivity of these molecules in the periodontal ligament area was analyzed by a computer-based image analyzing system and converted into gray-scale values. This measurement method detected the molecules both intracellularly and extracellularly.

RANKL/osteoprotegerin and IGF-1 are essential molecules for the effect of parathyroid hormone on bone metabolism, but on different aspects. RANKL/osteoprotegerin mediates osteoclastogenesis, whereas IGF-1 mediates osteoblastogenesis. In this study, we measured the expression levels of the 3 molecules to reflect the effects of parathyroid hormone on osteoclasts and osteoblasts. The results showed that the expression levels of both RANKL and IGF-1 increased, indicating that intermittent parathyroid hormones stimulated both osteoclastogenesis and osteoblastogenesis. This biphasic effect of parathyroid hormone is not contradictory; instead, we hypothesize that the increases of RANKL and IGF-1 might interact with each other. Previous researchers demonstrated that intermittent parathyroid hormone administration caused an increase of RANKL and resulted in osteoclastic resorptive activity. With bone matrix degradation, the osteoblastogenic growth factors (including IGF-1) were released; this in turn activated osteoblast

**Table I. Comparison of RANKL gray-scale values in the periodontal ligament**

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 6</th>
<th>Day 9</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTH</td>
<td>122.47 ± 8.34</td>
<td>157.62 ± 3.27*</td>
<td>142.77 ± 2.36*</td>
</tr>
<tr>
<td>Control</td>
<td>117.76 ± 4.27</td>
<td>134.38 ± 1.37</td>
<td>129.44 ± 3.62</td>
</tr>
</tbody>
</table>

$P < 0.05$ compared with the control group.

**Table II. Comparison of osteoprotegerin gray-scale values in the periodontal ligament**

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 6</th>
<th>Day 9</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTH</td>
<td>121.38 ± 4.55</td>
<td>120.74 ± 1.86</td>
<td>123.07 ± 7.26</td>
</tr>
<tr>
<td>Control</td>
<td>116.76 ± 3.22</td>
<td>121.36 ± 4.57</td>
<td>118.47 ± 6.33</td>
</tr>
</tbody>
</table>

**PTH**, Parathyroid hormone.
proliferation and differentiation.15,20 Because RANKL was synthesized by osteoblasts, the increased osteoblastic cells might induce the increase of RANKL production.

The biphasic effect of intermittent parathyroid hormone administration resulted in an increased bone turnover rate; this accelerated tooth movement in our study. However, the ultimate effect of long-term, low-dose intermittent parathyroid hormone injection is toward bone formation. This anabolic effect was not observed histologically in this study, although the immunohistochemistry results confirmed the enhanced expression of IGF-1. There were several possible reasons. First, the osteoblastic formation activity might have been enhanced as a result of the IGF-1 increase, but the periodontal tissues were under an orthodontic compression force; therefore, the resorptive activity still played a dominant role. Second, in clinical treatment, the significant anabolic effect of parathyroid hormone is achieved after at least 12 to 18 months, and the duration of our study was too short.9

Fig 7. Expression of RANKL and osteoprotegerin in the periodontal ligament area on the compression side of the distal root of the maxillary first molar (immunohistochemistry staining; magnification, 400 times). The immunoreactivity of RANKL was strong in osteoblasts (hollow arrows) and some osteoclast-like cells (arrows), and weak in the extracellular matrix of the periodontal ligament area; the immunoreactivity of osteoprotegerin was strong in some osteoblasts (hollow arrows) and weak in the extracellular matrix of the periodontal ligament. A and B, day 12, RANKL expression was higher in the parathyroid hormone group with more positive cells than in the control group; C and D, day 12, osteoprotegerin expression was not different in the 2 groups.

Table III. Comparison of IGF-1 gray-scale values in the periodontal ligament

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 6</th>
<th>Day 9</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTH</td>
<td>112.41 ± 6.41</td>
<td>137.01 ± 4.23*</td>
<td>148.26 ± 2.76*</td>
</tr>
<tr>
<td>Control</td>
<td>116.76 ± 3.22</td>
<td>116.72 ± 3.41</td>
<td>119.17 ± 0.78</td>
</tr>
</tbody>
</table>

PTH, Parathyroid hormone.
*P <0.05 compared with the control group.

Lossdörfer et al12 investigated the anabolic effect of intermittent parathyroid hormone on root resorption repair after orthodontic tooth movement in rats, and the increase of root formation was not observed until 21 days after the parathyroid hormone injections. Therefore, during a short term of intermittent parathyroid hormone injection, the anabolic effect was not achieved, and the general effect was an increase of the bone turnover rate. This indicates that short-term intermittent parathyroid hormone injection might be applicable for the acceleration of orthodontic tooth movement.
Our study demonstrated that short-term parathyroid hormone injection accelerated the orthodontic tooth movement in rats. However, if parathyroid hormone injection is applied in clinical treatment to accelerate tooth movement, it might have unwanted systemic effects on bone metabolism: e.g., increasing bone density. A limitation of this study was that the systemic effects of parathyroid hormone injection were not evaluated. Although unlike other osteoporosis-treating medicines (e.g., bisphosphonates), parathyroid hormone has a more balanced effect on bone metabolism, stimulating both osteoblastic and osteoclastic activities, systemic application of parathyroid hormone for a long time is still a risk for altered bone metabolism. Therefore, this study is just an explorative experiment about the acceleration effect of parathyroid hormone on orthodontic tooth movement; our future studies will explore the possibility of local injection of parathyroid hormone into the periodontal area and investigate whether local injection of parathyroid hormone has the same effect on orthodontic tooth movement.

CONCLUSIONS

One main concern of the orthodontist is to move teeth in the most effective manner and reduce the treatment duration. This study demonstrated that intermittent parathyroid hormone administration might be a solution to accelerate tooth movement through enhancement of alveolar bone remodeling.

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532  

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