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Exploration of effect of Odanacatib on inhibiting orthodontic recurrence in rats and on CatK and IGF-1 mRNA

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Abstract. – OBJECTIVE: This study aimed to investigate the inhibitory effect of Odanacatib on orthodontic recurrence in rats.

MATERIALS AND METHODS: Forty rats were selected to establish a planting anchorage molar movement model, and 50 g of force was used for the mesial movement of the right maxillary first molar. Forty rats were randomly divided into the observation group (n=20) and control group (n=20). Odanacatib (60 μl, 1.25 μM) was locally injected into the mucoperiosteum around the right maxillary first molar of rats in the experimental group, and an equal amount of normal saline was injected into rats in the control group. A Vernier caliper was used for measuring the recurrence movement distance and recurrence rate of rats, Micro-CT for scanning the bone mineral density (BMD) and bone volume fraction (BVF) of the alveolar bone, TRAP special staining for observing changes in osteoclasts and quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) for detecting the mRNA expressions of cathepsin K (CatK) and insulin-like growth factor 1 (IGF-1) in periodontal tissues.

RESULTS: After 3 weeks of modeling, the movement distance of the first molar of rats in the two groups was 1.16±0.19 mm. The molar movement distance and recurrence rate of rats were significantly higher in the control group than those in the observation group ($p<0.05$). The BMD and BVF of the alveolar bone of rats were markedly lower in the control group than those in the observation group ($p<0.05$). There was no statistically significant difference in the number of osteoclasts between the observation group (26.15±3.92) and the control group (27.01±2.74) (t=0.882, $p=0.383$). The CatK mRNA expression of rats was remarkably lower in the observation group than that in the control group ($p<0.05$). The IGF-1 mRNA expression of rats was significantly higher in the observation group than that in the control group ($p<0.05$).

CONCLUSIONS: By promoting the IGF-1 mRNA expression and increasing the BMD and BVF of the alveolar bone, Odanacatib inhibits orthodontic recurrence and has no effect on osteoclast activity.

Key Words
Odanacatib, Orthodontics, Rat model, CatK, IGF-1.

Introduction

Orthodontics is the most commonly used surgery in dental correction by clinicians. The correction of deformed teeth with long-term external traction provides an aesthetic and balanced effect, and keeps teeth in a stable arrangement. This enhances the aesthetics of patients' maxillofacial region, and ensures their orthodontic stability[1,2]. It has been reported[3] that no matter which kind of orthodontic treatment is impossible to achieve absolute stability. Therefore, one of the problems that clinicians need to solve urgently is how to avoid orthodontic recurrence.

At present, clinical orthodontic treatment is divided into an active correction stage and orthodontic recurrence prevention stage, of which the most difficult to be treated is the orthodontic recurrence prevention[4]. There is a study[5] showing that more than 50% of patients have a recurrence within 10 years after orthodontic treatment, whose periodontal tissues are greatly damaged after recurrence. Orthodontic recurrence is more common in adults, because adult orthodontic patients have a long correction period and unstable orthodontic, who are prone to recurrence[6]. Odanacatib is a non-lysosomal reversible inhibitor developed by Merck & Co Company for treating osteoporosis and bone metastasis cathepsin K (CatK)[7]. Law et al[8] has shown that it is a reversible and time-dependent human CatK inhibitor. CatK, a cysteine protease, is mainly present in the lysosome, and specifically expressed in osteoclasts[9]. Drake et al[10] have shown that after the CatK gene in mice is knocked out, bone absorption in mice is signifi-

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cantly reduced, but the rate of bone formation is increased, leading to osteosclerosis. Panwar et al[11] have previously shown that Odanacatib inhibits osteoclast-mediated bone absorption *in vitro* experiments. Based on this, Odanacatib is speculated to have an inhibitory effect on the orthodontic tooth movement stage.

Therefore, in this study, the inhibitory effect of Odanacatib on orthodontic recurrence in rats was investigated, to provide a reference for clinical treatment.

Materials and Methods

Rat Basic Data

In this work, 40 SPF rats were purchased from the Guangdong Medical Laboratory Animal Center, weighed 210±10 g and aged 8 weeks, who had normal periodontal and dental body. They were fed in the animal laboratory, with a laboratory temperature of 20-25°C, a balanced humidity of 45-60% and a noise <50 decibels, free to drink and eat, 12 h of light/dark circle.

Main Reagents and Materials

Odanacatib (Hangzhou RaystarBio, MK-0822, China), sodium pentobarbital (Sigma-Aldrich, St. Louis, MO, USA, USA), TRAP kit (Beijing Baiao Laibo Technology Co., Ltd., HR0561, China), PreReal-Time cDNA Synthesis Master Mix kit (Biomiga, RT0214-02, San Diego, CA, USA), 2×SYBR Mixture kit (RT0415-02; Biomiga, San Diego, CA, USA), TRIzol extraction reagent (Invitrogen, Carlsbad, CA, 15596018, USA). Primers for CatK and insulin-like growth factor 1 (IGF-1) were designed and synthesized by Shanghai Shenggong Bioengineering Co., (Shanghai, China; Table I). ABI 7900 PCR instrument (Applied Biosystems, Foster City, CA, USA), Orthodontic materials (Jiangsu Hangzhou Xinya Company, China), Micro-CT (Siemens AG, Germany), the Vernier caliper (Vogel, Germany), orthodontic spring dynamometer (Jinan Yongke Testing Instrument Co., Ltd., China).

Animal Modeling

All rats were modeled after being fed for a week. They were fixed in the supine position and anesthetized with 2% sodium pentobarbital. A high-speed diamond bur was used for grinding out 0.4 mm groove along the cervical margin of the maxillary incisor. The maxillary first molar of rats was used as the experimental tooth, and implanted with the micro-planting nail (1.6 mm in diameter and 6 mm in length). Incisors were used as anchorage teeth, and the ligation wire was used to pass through the gap between the first and second left molars. The orthodontic spring dynamometer was used to adjust the orthodontic nickel-titanium spiral tension spring to 50 g, one end of which was connected to the micro-planting nail, and the other end to the incisors. The modeling time was 3 weeks, during which the rat modeling was observed day and night, and the device was reset in time if falling off. Three weeks later, the device was dissected. 40 rats were randomly divided into the observation group and the control group. Odanacatib (0.06 mL, 1.25 μmol/L) was injected into rats in the observation group[12], and an equal amount of normal saline was injected into rats in the control group. The injection sites of rats in the two groups were placed in the mucoperiosteum around the right maxillary first molar, once of injection every 3 days, for 2 weeks. Rats were anesthetized with 4% sodium pentobarbital for 2 weeks and then sacrificed by cervical dislocation to collect their periodontal tissues.

Recurrence Distance Measurement

After the device in rats was dissected, the vernier caliper was used for measuring the molar movement distance from the day to the 2nd week after dissection. All measurements were repeated 3 times to obtain an average value. The recurrence rate was calculated (recurrence rate = recurrence distance/tooth movement distance *100%).

Micro-CT Detection

After rats were sacrificed, three molars and alveolar bone tissues of their maxillary were collected and fixed with 10% formalin. Micro-CT was

Table I. Primer sequence.

Gene	Upstream primer	Downstream primer
CatK	5'-CAGTGAAGAGGTGGTTCAGA-3'	5'-TTCCATCTCGGGGTCTGAGA-3'
IGF-1	5'-TTACCCAACAGCAGTCCACT-3'	5'-AGCAGGCTGACGTTCGCACT-3'
GAPDH	5'-GGGTGATGCTGGTGCTGAGTATGT-3'	5'-AAGAATGGGTGTTGCTGTTGAAGTC-3'

used to scan specimens to detect the bone mineral density (BMD) and bone volume fraction (BVF) of the alveolar bone of rats in the two groups. Micro-CT specific parameters: scanning conditions were 80 kv and 500 μA, and pixel was 15 μm. The image was reconstructed after scanning.

TRAP Staining

The maxillary molar and maxillary bone were separated and fixed with paraformaldehyde (4%). Forty hours later, samples were decalcified at room temperature. Sixty days later, the samples completely decalcified were rinsed with water and dehydrated with gradient ethanol. Then, xylene was used for transparency and embedding. Three slices were randomly selected for dewaxing and TRAP staining was performed according to the TRAP kit instructions.

qRT-PCR Detection

The collected periodontal tissues were used for extracting total RNA with TRIzol extraction reagent, ultraviolet spectrophotometer and agarose gel electrophoresis for determining the purity, concentration and integrity of the extracted total RNA. The total RNA was reversely transcribed by using Pre-Real Time cDNA Synthesis Master Mix kit in strict accordance with the kit instructions. Polymerase Chain Reaction (PCR) reaction system was as follows: 25 μL of 2×SYBR Mixture, each of 1 μL of upstream and downstream primers, 2 μL of Template DNA, and finally RNase-Free Water to complete up to 50 μL. PCR reaction conditions were as follows: pre-denaturation at 95°C for 10 min, at 95°C for 15 s, and 60°C for 60 s, for a total of 40 cycles. Three replicate wells were set up in each sample, and the experiment was repeated 3 times. In this study, GAPDH was used as an internal reference, and $2^{-\Delta\Delta ct}$ for analyzing the data.

Outcome Measures

The recurrence movement distance and recurrence rate of rats in the two groups were observed from the day to the 2nd week after injection. The BMD and BVF of the alveolar bone, changes in osteoclasts and mRNA expressions of CatK and IGF-1 in periodontal tissues of rats in the two groups were observed.

Statistical Analysis

In this study, SPSS 20.0 software package (SPSS IBM, Armonk, NY, USA) was used for statistically analyzing the data, GraphPad Prism 7 (La Jolla, CA, USA) for plotting the figures. Count data were expressed as a rate (%), tested by and expressed in chi-square. Measurement data were expressed as mean ± standard deviation (mean ± SD), analyzed by the *t*-test and expressed in *t*. When $p<0.05$, there is a statistically significant difference.

Results

Basic Data and Post-Modeling Conditions of Rats in Two Groups

Rats in the two groups were randomly divided. Altogether 20 rats were in the control group including 12 males and 8 females, and 20 rats were in the observation group including 8 males and 12 females. There were no statistically significant differences in the gender, weeks of age and body weight of rats between the two groups ($p>0.05$; Table II). There was no bad condition during rat modeling. Rats were healthy, and no rat died after modeling. There was a significant gap between the first and second molars of rats. This indicates successful modeling.

Rat Molar Movement Distance and Recurrence

The results of the Vernier caliper detection showed that after 3 weeks of modeling, the movement distance of the first molar of rats in the two groups was 1.16±0.19 mm. The first molar of rats in the two groups showed movement after admin-

Table II. General data of rats.

Data		Control group (*n*=20)	Observation group (*n*=20)	c^2/t	*p*-value
Gender				1.600	0.206
	Male	12	8		
	Female	8	12		
Age (week)		8.05±0.49	7.84±0.59	1.225	0.228
Weight (g)		210.26±8.98	208.64±10.61	0.521	0.605

Table III. Comparison of recurrence movement distance and recurrence rate between two groups of rats.

Group	Recurrence movement distance (mm)	Recurrence rate (%)
Control group	0.89±0.17	81.86±0.20
Observation group	0.62±0.13	52.18±0.11
t-value	5.630	5.783
p-value	0.000	0.000

istration. The molar movement distance and recurrence rate of rats were markedly higher in the control group than those in the observation group, with statistically significant differences ($p<0.05$; Table III, Figure 1).

BMD and BVF of Rat Alveolar Bone

Micro-CT was used to detect the BMD and BVF of the alveolar bone of rats in the two groups. The results showed the BMD and BVF of the alveolar bone of rats in the control group were remarkably lower than those in the observation group, with statistically significant differences ($p<0.05$; Table IV, Figure 2).

TRAP Staining in Two Groups of Rats

TRAP staining and count analysis were performed on rat osteoclasts in the two groups. The results showed that there was no statistically significant difference in the number of osteoclasts between the observation group (26.15±3.92) and the control group (27.01±2.74) ($t=0.882$, $p=0.383$; Table V).

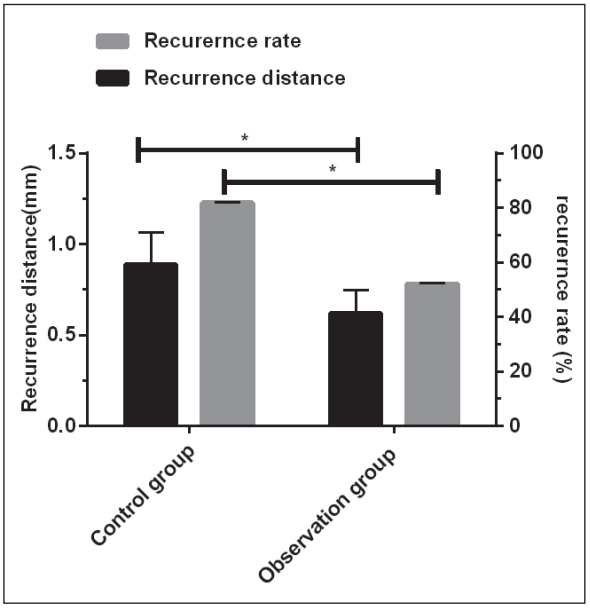

Figure 1. Comparison of recurrence movement distance and recurrence rate of rats be-tween two groups. The recurrence movement distance and recurrence rate of rats were compared between the two groups. The results showed that the recurrence movement distance and recurrence rate of rats were significantly lower in the observation group than those in the con-trol group, with differences. *indicates that there is a difference between the two groups, $p<0.05$.

mRNA Expressions of CatK and IGF-1 in Periodontal Tissues of Rats in Two Groups

qRT-PCR was used to detect the mRNA expressions of CatK and IGF-1 in periodontal tissues of rats in the two groups. The results showed that the CatK mRNA expression of rats was markedly lower in the observation group than that in the control group, with a statistically significant difference ($p<0.05$). The IGF-1 mRNA expression of rats was remarkably higher in the observation group than that in the control group, with a statistically significant difference ($p<0.05$; Table VI, Figure 3).

Discussion

The dentognathic deformity is mainly treated by teeth-maxilla orthodontic in clinical practice, whose therapeutic effect has been clinically proven for a long time[13]. Orthodontic treatment mainly adjusts the dental arch, jaw bone and malposed teeth of patients through external force, and guides their teeth to move, to inhibit the abnormal growth of the jaw bone and remodel their periodontal tissues and jaw bone, finally establishing a balanced occlusion. As a result, the normal physiological function of patients' mouth is improved, and patients' self-confidence is also enhanced[14]. Orthodontics is mainly divided into the active correction stage and the orthodontic recurrence prevention stage. The former is to correct patients' deformed teeth, and the latter is to maintain the position of their corrected teeth through the holding device, thereby preventing tooth movement[15]. Steinnes et al[16] showed that the recurrence rate is the highest within 1 year after orthodontics. In the study by Liou et al[17], patients after orthodontics wear a retainer for at least 2 years to maintain stability, mainly because of the

Table VI. Bone mineral density and bone volume fraction value of alveolar bone in two groups of rats.

Group	Bone mineral density of alveolar bone (g/cm³)	Bone volume fraction value (%)
Control group (n=20)	1.004±0.036	1.036±0.065
Observation group (n=20)	0.741±0.157	1.418±0.169
t-value	7.304	9.425
p-value	0.000	0.000

agitation balance that is not fully established, unbalanced periodontal fiber membrane tension and patients' bad habits.

Odanacatib, a new reversible non-peptide biaryl CatK inhibitor in recent years, is linked into benzene ring through the P2~P3 position and has an extremely high selective specificity[18]. Bone et al[19] have shown that Odanacatib induces osteoclasts and causes their superficies to have concave surfaces, thereby reducing absorption area and bone absorption. Jensen et al[20] have shown that Odanacatib does not affect osteoclast activity while inhibiting bone absorption. CatK is a protease that enters the lysosome to degrade proteins. Kim et al[21] believe that CatK is the key of osteoclast-mediated bone absorption and specifically expressed in bone cells. IGF-1 is a multi-functional proliferation regulator. Cai et al[22] have shown that IGF-1 plays an important regulatory role in periodontal tissue remodeling. O'Neill et al[23] have shown that IGF-1 also promotes the growth, development and proliferation of individual cells. Besides, IGF-1 increases the osteogenic differentiation of periodontal fibroblasts and accelerates protein mitosis[24]. However, there is no study clearly demonstrating whether Odanacatib inhibits orthodontic recurrence. Therefore, in this work, the inhibitory effect of Odanacatib on orthodontic recurrence in rats was explored, to provide a reference for its clinical treatment.

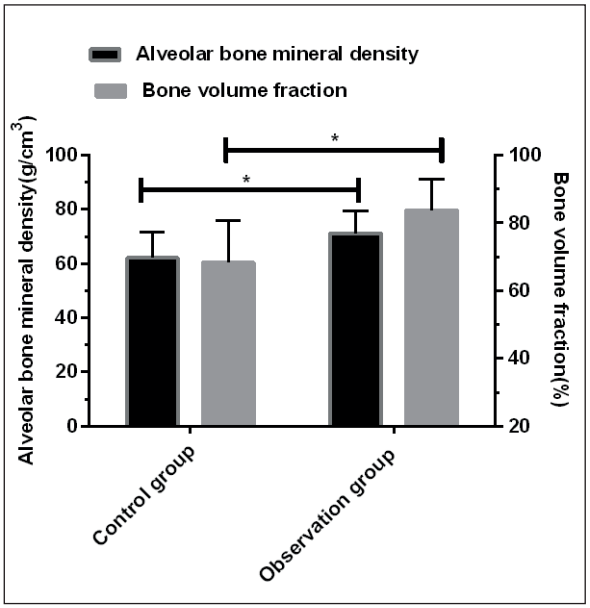

Figure 2. BMD and BVF of alveolar bone of rats in two groups. The BMD and BVF of the alveolar bone of rats were compared between the two groups. The results showed that the BMD and BVF of the alveolar bone of rats were markedly higher in the observation group than those in the control group, with differences. *indicates that there is a difference between the two groups, $p<0.05$.

Primates are the most suitable animals for the orthodontic model, because their structure is basically consistent with that of human, with a high degree of similarity. However, they are expensive and not conducive to modeling. Rodents, the most commonly used animal models in clinical experiments, are cheap and easy to feed. In this work, the orthodontic model of SD rats was established. After 3 weeks of modeling, there was a significant gap between the first and second molars of rats. All rats were well tolerated and no rat died. Then, rats in the two groups were injected with normal saline and Odanacatib every 3 days, respectively, to measure their molar movement. The results showed that the molar movement distance and recurrence rate of rats were markedly higher in the control group than those in the observation group. It is indicated that the injection of Odanacatib has a significant inhibitory effect on orthodontic recurrence in rats. The BMD and BVF of the alveolar bone are indicators that directly reflect the state of bone metabolism[25]. Therefore, we detected the BMD and BVF of the alveolar bone, and the results showed that the BMD and BVF of rats were significantly higher in the observation group than those in the control group. It is indicated that the injection of Odanacatib can increase the BMD and BVF of the alveolar bone and inhibit orthodontic recurrence. Moreover, the mRNA expressions of CatK and IGF-1 in periodontal tissues of rats were detected. It was found that the

Table V. Comparison of the number of osteoclasts between the two groups.

Group	Number of osteoclasts	*t* value	*p*-value
Control group (*n*=20)	27.01±2.74	0.882	0.383
Observation group (*n*=20)	26.15±3.92		

Table VI. Expressions of CatK and IGF-1 in periodontal tissues of two groups of rats.

Group	CatK mRNA	IGF-1mRNA
Control group (n=20)	1.004±0.036	1.036±0.065
Observation group (n=20)	0.741±0.157	1.418±0.169
t-value	7.304	9.425
p-value	0.000	0.000

CatK mRNA expression in periodontal tissues of rats was remarkably higher in the control group than that in the observation group, but the IGF-1 mRNA expression was markedly lower in the control group than that in the observation group. The increase in the mRNA expression of IGF-1 may be due to the fact that Odanacatib inhibits its degradation. Sims et al[26] show that the release of IGF-1 can be stimulated by CatK inhibitor, which is consistent with the results of this study. TRAP staining was used for observing the number of osteoclasts in periodontal tissues of rats. The results showed that there was no significant difference in the number of osteoclasts in periodontal tissues of rats between the two groups. This may be due to the fact that CatK degrades TRAP, which is inhibited by Odanacatib. Cornish et al[27] have shown that the increase in IGF-1 accelerates the proliferation of osteoblasts while promoting the synthesis of osteocalcin and collagen. In this work, the injection of Odanacatib reduced the CatK mRNA expression but increased the IGF-1 mRNA expression in periodontal tissues of rats, which had no effect on the osteoclast activity in periodontal tissues and effectively inhibited orthodontic recurrence.

However, in this paper, there are still certain limitations. First, the measurement tool used in this experimental is the Vernier caliper, so there may be measurement errors. Second, whether the

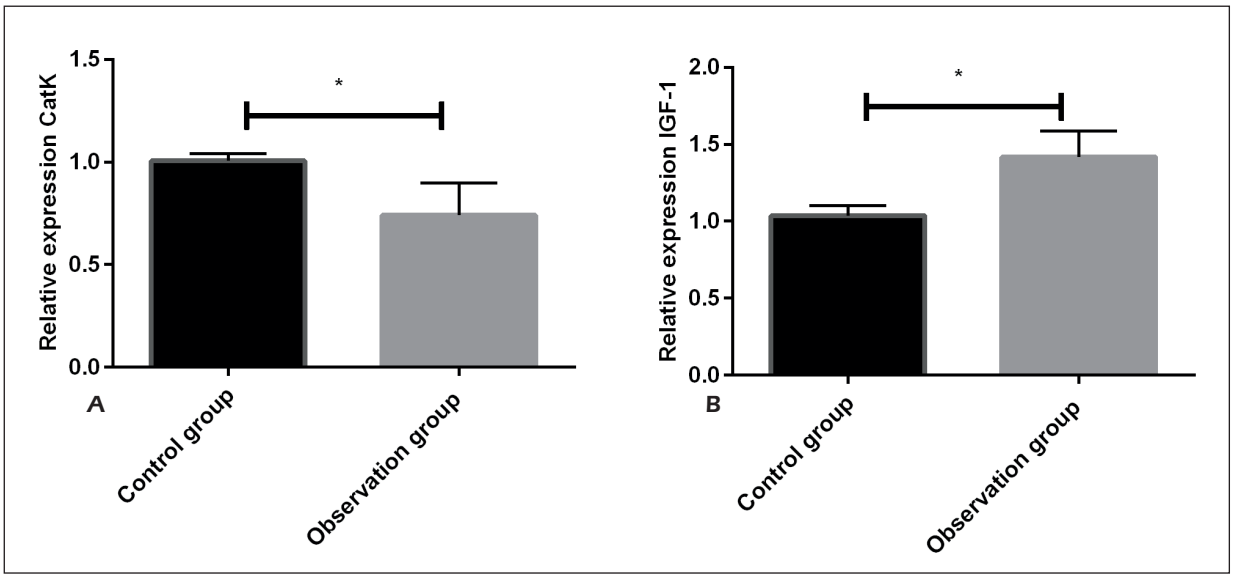

Figure 3. Expressions of CatK and IGF-1 in periodontal tissues of rats in two groups. **A**, The CatK expression in periodontal tissues of rats was remarkably lower in the observation group than that in the control group, with a difference. **B**, The IGF-1 expression in periodontal tissues of rats was significantly lower in the observation group than that in the control group, with a difference. *indicates that there is a difference between the two groups, $p<0.05$.

IGF-1 expression is increased by the injection of Odanacatib needs further verification. Finally, it remains unclear whether this study can improve patients' conditions in clinical practice. Therefore, it is hoped that in future studies more scientific methods will be used for measurement, how Odanacatib affects the IGF-1 expression will be further explored, and the corresponding clinical trials will be conducted to confirm the authenticity of the results of this work.

Conclusions

We showed that, by promoting the IGF-1 mRNA expression and increasing the BMD and BVF of the alveolar bone, Odanacatib inhibits orthodontic recurrence and has no effect on the osteoclast activity.

Conflict of Interests

The authors declare that they have no conflict of interest.

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