ACTA ODONTOLOGICA SCANDINAVICA, 2017
VOL. 75, NO. 8, 595–602
https://doi.org/10.1080/00016357.2017.1365375

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ORIGINAL ARTICLE

Effects of calcitonin on orthodontic tooth movement and associated root resorption in rats

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ABSTRACT

Objectives: Our main aim was to evaluate the effects of calcitonin (CT) on orthodontic tooth movement (OTM) and orthodontic root resorption in a rat model.

Material and methods: Eighty male Wistar rats were randomly divided into five groups. Rats in the negative control group were not given any appliances or injections. All the remaining rats were used to establish a model of OTM. The positive control group were then injected with normal saline, while rats in the three experimental groups were injected with 0.2 IU, 1 IU or 5 IU/kg/day CT. Nickel-titanium closed-coil springs were used to deliver an initial 50 g mesial force to the left maxillary first molar for 14 days in rats in the positive control group and the experimental groups. Each group was randomly subdivided into two groups, one for analysis of tooth movement, tissue changes and tartrate-resistant acid phosphatase (TRAP)-positive cells in alveolar bone, the other to examine root resorption by scanning electron microscopy.

Results: The OTM distance, the number of force-induced osteoclasts and root resorption areas were significantly decreased in CT-injected rats in a dose-dependent manner.

Conclusions: Administration of CT reduces the root resorption area and may therefore be effective as a novel adjunctive orthodontic approach to diminish undesired tooth movement via enhancing anchorage or preventing relapse after OTM.

ARTICLE HISTORY
Received 12 February 2017
Revised 30 July 2017
Accepted 4 August 2017

KEYWORDS
Calcitonin; orthodontic tooth movement; root resorption

Introduction

Orthodontic tooth movement (OTM) is a harmonious process of periodontal and alveolar reconstruction. Osteoblasts form new bone on the tension side and osteoclasts absorb alveolar bone on the pressure side [1]. The extent of tooth movement depends on the number of osteoclasts on the pressure side [2]. Recent studies support the utility of biological regulators that inhibit osteoclasts as novel adjunctive therapeutic agents for orthodontic treatment, such as retarding undesired tooth movement [3–6] or preventing retention after OTM [7–9].

Calcitonin (CT), a 32-amino-acid peptide hormone, is a therapeutic agent used to treat osteoporosis that inhibits osteoclastic bone resorption and stimulates bone formation [10]. CT inhibits bone resorption by acting directly on osteoclasts to suppress cell motility [11,12] and reduce their number and activity [13]. Previous studies have demonstrated that CT affects dental eruption [14] and modulates root resorption activity by protein kinase A [15]. Other studies indicate that CT regulates appetite, lactation, and pain perception in areas of the central nervous system [16], suggestive of analgesic effects [17–19]. In view of these findings, we speculate that CT may reduce orthodontic tooth pain to some extent. However, limited studies to date have focused on the effects of CT on OTM and force-induced root resorption. In the present experimental setup, CT was administered to rats via local injection and the effects of the biological modulator on OTM and root resorption were assessed, along with the corresponding pathological changes.

Material and methods

Laboratory animal model of OTM

Eighty healthy 7-week-old male Wister rats weighing 200–250 g each were purchased from Chang Sheng Biotechnology Co. Ltd. (Liaoning, China) and maintained according to the guidelines of the institutional ethics committee of Harbin Medical University. Rats were kept in 10 cages (eight per cage) in a room maintained at 20–24 °C with a 12-h/12-h light/dark cycle and were randomly divided into five groups. Rats were fed a diet of commercially manufactured standard laboratory granules and water, to prevent them exerting an excessive chewing force, and acclimatized for at least 7 days before the experiment. An experimental model of OTM was established in all rats except the negative control group according to the method described in previous reports [20,21]. Briefly, under anesthesia, Ni-Ti closed-coil

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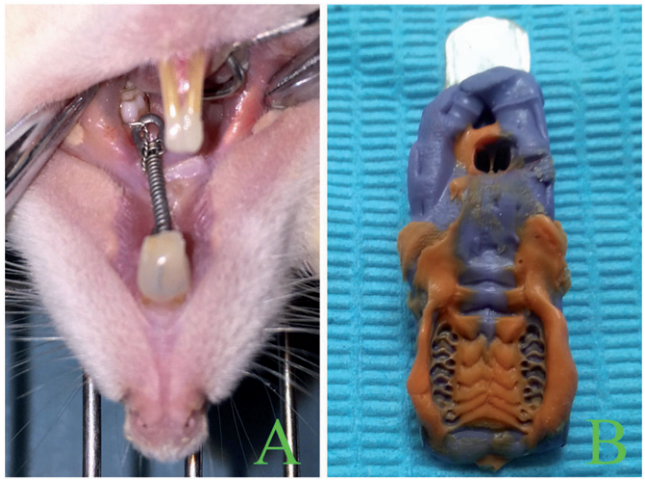

Figure 1. The OTM model in rat (A) and the maxillary impression made by individual tray loaded with silicone rubber impression material (B).

springs (Shinye Odontology Materials Corporation Company, Hangzhou, China) were fixed between the maxillary incisors and the left first molars, producing a mesial force of 50 g to move the first molars for 14 days. To prevent detachment of the appliance, a 0.5-mm groove was prepared on the cervical line of the incisors where the ligation wire (Grikin Advanced Materials, Beijing, China) was tied and bonded with composite resin (Figure 1(A)). The lower incisors were ground using a low-speed dental handpiece every 3 days to prevent damage to the coiled spring during mastication. In order to prevent the dentin allergy of the lower incisors, we covered them with composite resin after grinding the lower incisors. Every day each rat was checked to ensure the spring remained in place, and if the spring fell off, the rats were immediately sacrificed and the animal model of OTM was reconstructed using other rats.

Effects of CT on OTM after mechanical loading

For evaluation of the dose-dependent effects of CT on OTM and periodontal tissue changes, five groups of eight rats each were examined. Eight rats comprising the negative control group were not injected with CT. The rats ($n = 32$) in the other four groups were injected with normal saline or with 0.2, 1, or5 IU/kg/day CT for 14 days. Each dose of CT was added to normal saline to prepare the individualized solutions for injection in a volume of 40 μL. CT (Miacalcic, Novartis, Switzerland) was injected into the buccal subperiosteum adjacent to the furcation of the maxillary right first molar with a 0.1-mL micro syringe. Rats were deeply anaesthetized and sacrificed via cardiac perfusion with 4% paraformaldehyde at 14 days after the start of OTM. After dissection of the maxilla, silicone rubber impression material (O Type, Huge Co., Shanghai, China) was used to prepare maxillae and teeth impressions with individual trays (Figure 1(B)). Tooth movement on the left side was measured from the plaster model under a stereoscopic microscope (SAX7; Olympus, Tokyo, Japan). The closest distance between the first and second molars represented the amount of OTM, which was measured by the same investigator at three different times, and the average value was used.

Preparations for histological and immunohistochemical analyses

After all the impressions were perfused with hard plaster, maxillae were fixed in 4% paraformaldehyde for 24 h at 4 °C and decalcified in 10% EDTA, pH 7.4, for 2 months. Following decalcification, specimens were dehydrated and embedded in paraffin. Each block was cut into 5 μm thick mesiodistal serial sections using a microtome, with each section including distobuccal and mesial roots of the maxillary first molar. Next, sections were employed for hematoxylin–eosin (HE) and tartrate-resistant acid phosphatase (TRAP) immunohistochemical staining. Changes in periodontal tissues were examined in sections stained with HE and the number of osteoclasts counted from TRAP-positive cells in alveolar bone around the pressure side of the root under an optical microscope.

For TRAP immunohistochemical staining, sections were immersed in xylene for 10 min twice to eliminate paraffin, dehydrated in absolute alcohol, and rehydrated through a graded ethanol series to double-distilled water. To inhibit endogenous peroxidase activity, sections were incubated with 3% hydrogen peroxide for 10 min, followed by three washes in distilled water. Complex digestive enzymes (Boster Biotechnology, Wuhan, China) were used for antigen retrieval of the sections. Briefly, sections were incubated with complex digestive enzymes at 37 °C for 10 min, followed by polyclonal anti-TRAP antibody (ab185716; Abcam, Cambridge, UK) at 1:400 dilution for 12 h. Subsequently, sections were incubated with secondary HRP-linked anti-rabbit antibody (SV0002; Boster Biotechnology) for 20 min. Positive expression was visualized by reaction with 3.3-diaminobenzidine (Boster Biotechnology) for 1 min. Counterstaining was performed with hematoxylin for 10 s.

Observation of the root resorption area under a scanning electron microscope

After OTM, maxillae were dissected and immersed in 10% sodium hypochlorite for several hours until the first molar could be easily removed from alveolar bone. The first molar was carefully extracted and periodontal ligament around it removed. For better exposure of the distal buccal root, we carefully ground the middle buccal root between the mesial and distal buccal roots with a dental handpiece. The mesial side of distal buccal roots was observed under a scanning electron microscope (s-3400N; HITACHI, Tokyo, Japan) at the same distance and orientation. The root resorption area was measured with software Image J (EASYBIO, Beijing, China). The resorption area ratio was calculated by dividing the resorbed area by the entire mesial surface area [22].

Statistical analysis

All data were analyzed using the statistical package SPSS21.0 (IBM, New York, NY) and expressed as mean values ± standard deviations (MEAN ± SD). When all data were tested for normality and homogeneity of variance, one-way analysis of variance (ANOVA) was performed. Based on the result of

Table 1. The results of Tukey's multiple comparisons about the distance of OTM (μm), the number of TRAP(+) cells along the alveolar bone and root resorption area ratio (%).

		p value		
Group	Comparative group	Distance of OTM	TRAP(+) cells	Resorption area ratio
Negative control	Positive control	7.111E-13	7.111E-13	7.111E-13
	CT (0.2 IU)	7.111E-13	7.111E-13	7.111E-13
	CT (1 IU)	7.111E-13	9.758E-13	5.963E-10
	CT (5 IU)	8.956E-13	5.627E-5	.04003
Positive control	CT (0.2 IU)	.001418	.03769	7.112E-13
	CT (1 IU)	7.172E-13	2.041E-12	7.111E-13
	CT (5 IU)	7.111E-13	7.111E-13	7.111E-13
CT (0.2 IU)	CT (1 IU)	1.866E-10	3.179E-9	1.115E-11
	CT (5 IU)	7.112E-13	7.112E-13	7.112E-13
CT (1 IU)	CT (5 IU)	6.161E-6	3.829E-7	3.060E-6

ANOVA, we performed Tukey's multiple comparisons test. *p* values less than .05 were considered significant.

Results

Conditions of rats

During our 2-week experiment, the orthodontic appliances were fall off in two of eighty rats, which were sacrificed and replaced by other two rats to ensure eighty rats available for final analysis. No appreciable inflammatory response was observed at the local injection site in any of the eighty animals examined. Application of orthodontic force did not affect body weight in rats.

Inhibitory effect of CT on OTM

On Day14 after attachment of the orthodontic appliance, the distance of OTM was measured. The result of analysis of variance (ANOVA) showed statistically significant differences ($p = 2.416E\text{-}27$) and the Tukey's multiple comparisons test revealed statistically significant differences between each group (Table 1). The OTM distance was 0.891 ± 0.067 mm in the positive control group. The distance was significantly reduced to 0.342 ± 0.039 mm in the experimental group injected with 5 IU/kg CT, and to a lesser extent to 0.501 ± 0.055 mm in the group injected with 1 IU/kg CT and to 0.775 ± 0.075 mm in the group injected with 0.2 IU/kg CT. The distance of OTM showed a dose-dependent relationship with CT concentration (Figure 2).

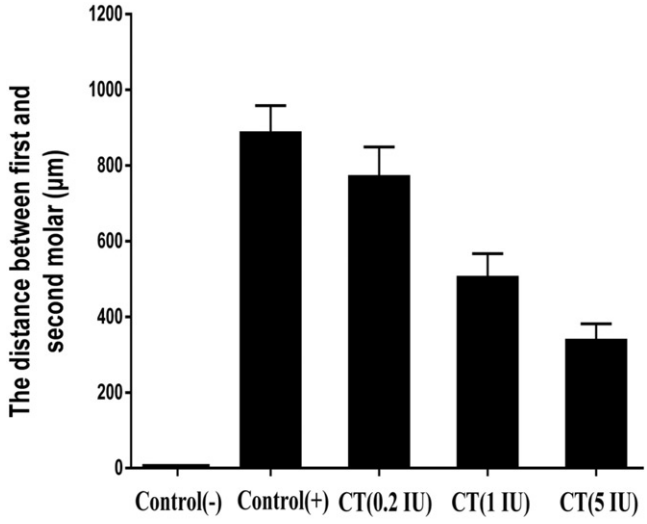

Figure 2. The distance of tooth movement (mean) in five groups: negative control group (no OTM and no injection), positive group (OTM and saline injection) and experimental groups injected with 0.2 IU, 1 IU and 5 IU/kg/day of CT, respectively after 14 days of loading. $n = 8$ for each group.

Histological changes in periodontal tissue examined via HE staining during OTM

Periodontal destruction and resorption of the distobuccal root of the right maxillary first molar decreased with increasing doses of CT. In the negative control group, the periodontal ligament was arranged in an orderly manner with no destruction (Figure 3(A)), and the root surface was smooth (Figure 3(F)). In the positive control group, periodontal destruction was the most significant, the periodontal ligament fibers were disoriented to the greatest extent (Figure 3(B)), and root resorption (Figure 3(G)) was the largest and deepest up to the dentin. The least extent of destruction of periodontal tissue with no obvious root resorption was observed in the 5 IU/kg group (Figure 3(E,J)), which was better than that of the 1 IU/kg group (Figure 3(D,I)). In the 0.2 IU/kg treatment group, periodontal damage (Figure 3(C)) and root resorption (Figure 3(H)) were greater than in the 1 IU/kg group and slightly less than the positive control group.

TRAP expression in alveolar bone during OTM

We examined osteoclast formation in alveolar bone surrounding the mesial side of the distobuccal root by TRAP immunohistochemical staining. TRAP was expressed in the cytoplasm of osteoclasts in alveolar bone where bone resorption occurred on the mesial side (Figure 4). The result of ANOVA revealed statistically significant differences ($p = 8.853E\text{-}24$) and the Tukey's multiple comparisons test revealed statistically significant differences between each group (Table 1). No osteoclasts were found in the negative control group (Figure 4(A,F)). The number of TRAP-positive cells was highest in the positive control group (18.0 ± 2.0) (Figure 4(B)). Within the three experimental groups, osteoclast number decreased (15.8 ± 1.7, 9.3 ± 1.7, 4.0 ± 1.3) with increasing concentrations of CT (Figure 4(C–E)). Osteoclasts can be clearly seen in the higher magnification images (Figure 4(G–J)). The number of

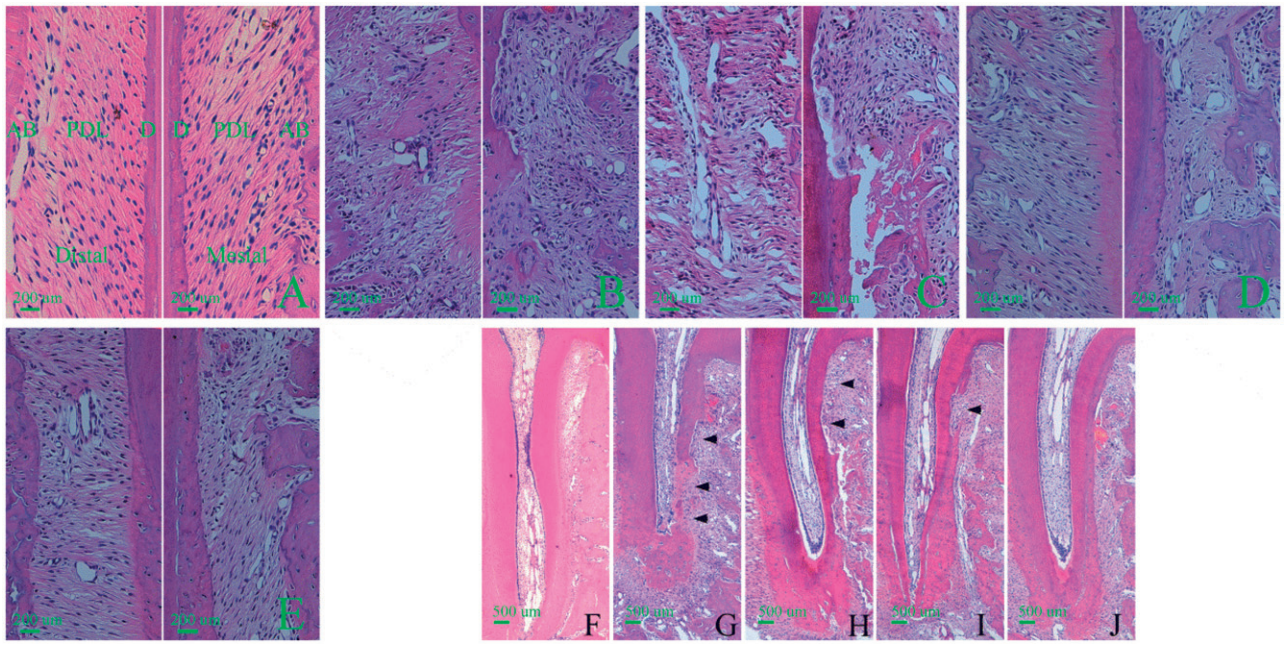

Figure 3. Representative images of periodontal tissue (A–E) and root resorption (F–J) stained by HE at Days 14 after the application of force. Periodontal tissue of the teeth with no OTM and no injection (A), the loaded teeth injected with saline (B) and O.2 IU,1 IU, 5 IU/kg/day of CT, respectively (C–E). Root resorption of loaded teeth in the pressure side with no OTM and no injection (F), the loaded teeth injected with saline (G) and the loaded teeth injected with O.2 IU, 1 IU and 5 IU/kg/day of CT respectively (H–J). Mesial: the pressure side; distal: the tension side; AB: alveolar bone; D: dentin; PDL: periodontal ligament. Black arrows: root resorption.

osteoclasts showed a dose-dependent relationship with CT dose (Figure 5).

Effects of CT on orthodontic force-induced root resorption

On Day 14 after fixation of the orthodontic appliance, we measured orthodontic force-induced root resorption on the mesial side of the distobuccal root under a scanning electron microscope (Figure 6(A,B)). The root resorption rate was calculated. The result of ANOVA revealed statistically significant differences ($p = 2.691E-29$) and the Tukey's multiple comparisons test showed statistically significant differences between each group (Table 1). The root resorption rate showed a dose-dependent relationship with CT (Figure 6(C)), consistent with the results of HE analysis. The root surface in the negative control group was smoother than the other groups (Figure 6(D)). In the positive control group, the root resorption rate was 31.44 ± 2.90%, with the greatest degree of resorption (Figure 6(E)). The root resorption rate was reduced to 17.44 ± 1.96% and 8.05 ± 1.49% in animals injected with 0.2 IU/kg CT and 1 IU/kg CT (Figure 6(F,G)), and was more significantly reduced in the 5 IU/kg CT treatment group (2.58 ± 0.80%) (Figure 6(H)).

Discussion

Rats have many advantages as OTM models and are generally considered to be the best choice for studying this phenomenon. First, the periodontal tissue and anatomical structure in rats are similar to those in humans. Second, rats are relatively inexpensive and easy to breed, making them suitable for a large sample study. Finally, orthodontic histological changes in the periodontal tissue and the mechanism are the same as in humans, but change faster.

CT, a therapeutic agent for osteoporosis, acts by inhibiting osteoclastic bone resorption. CT is reported to affect dental eruption [14] and to regulate root resorption activity [15,16]. Osteoclasts are involved in dental eruption and root resorption, which are similar to the processes of OTM. However, the specific effects of CT on OTM are unknown at present. In this study, we have demonstrated for the first time that application of CT significantly inhibits OTM in a rat model, partly due to its inhibitory effect on osteoclastic bone resorption. In this study, the doses of CT used were chosen in reference to other studies and were in the range used by other authors [23–25]. Moreover, the maximum dosage applied was less than that in some studies [26–28]. All dosages applied in this study correspond to physiological concentrations in rats. Our results support the application of CT as a novel adjunctive orthodontic approach to diminish undesired tooth movement, such as enhancing anchorage or preventing retention after OTM.

TRAP, an isoenzyme of acid phosphatase found mainly in bone tissue and blood cells, also plays an important role in resorptive processes through osteoclastic activity [29]. In the current study, the number of osteoclasts on the pressure side was examined using TRAP immunohistochemistry. Orthodontic mechanical forces activate osteoclasts on the pressure side, resulting in OTM. Local application of CT in our experiments induced a decrease in the number of osteoclasts around the root and alveolar bone on the pressure side, and consequently, suppression of tooth movement. Additionally, different doses of CT exerted significantly variable inhibitory effects on OTM. Moreover, CT led to marked suppression of root resorption in a dose-dependent manner.

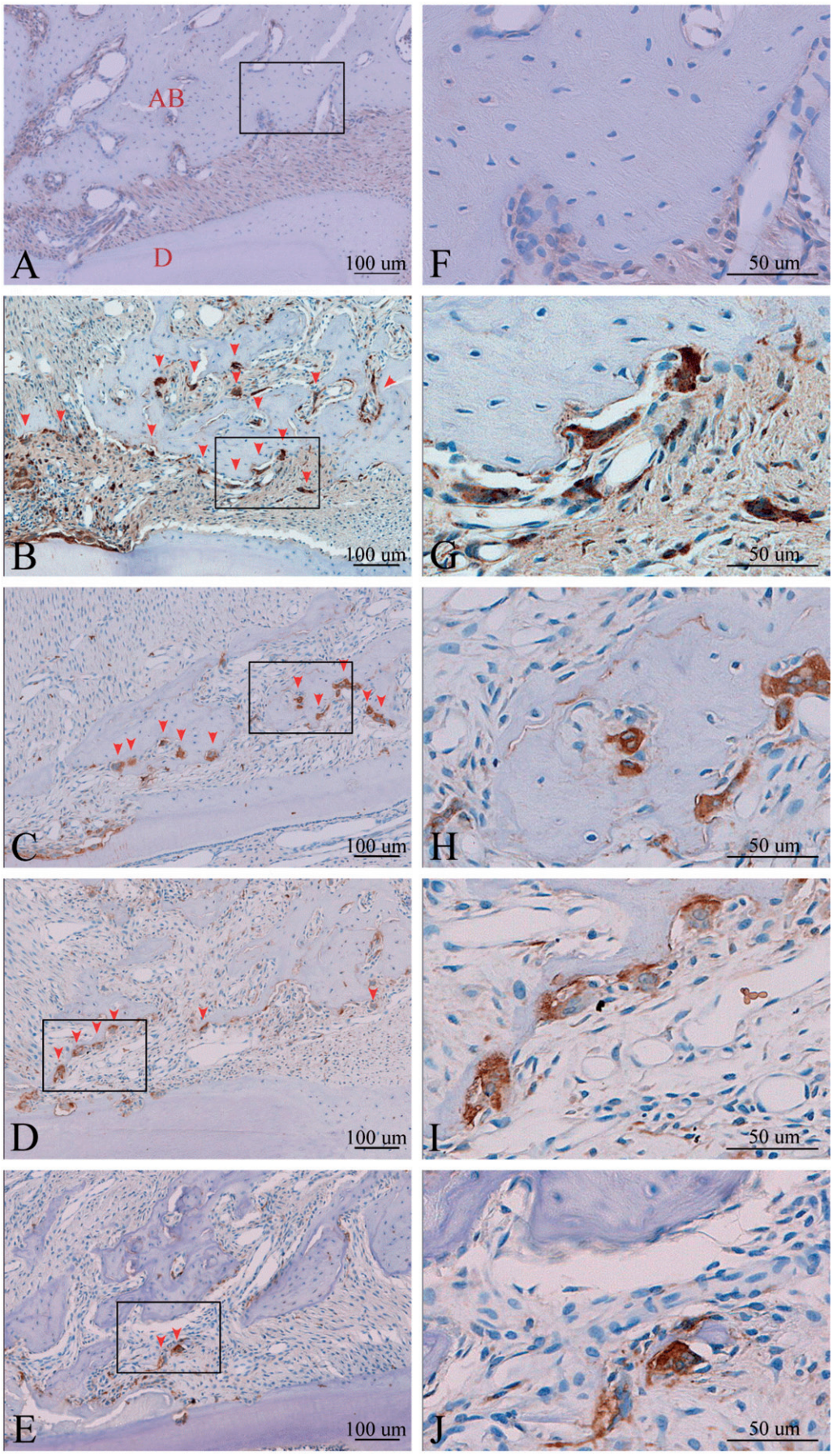

Figure 4. Representative images of TRAP immunohistochemistry staining of the teeth with no OTM and no injection (A), the loaded teeth injected with saline (B) and the loaded teeth injected with 0.2 IU,1 IU and 5 IU/kg/day of CT, respectively (C–E) at Days 14 after the application of force. F–J are the higher magnification images of the squares in A–E separately. AB, alveolar bone; D, dentin. Red arrows: osteoclasts.

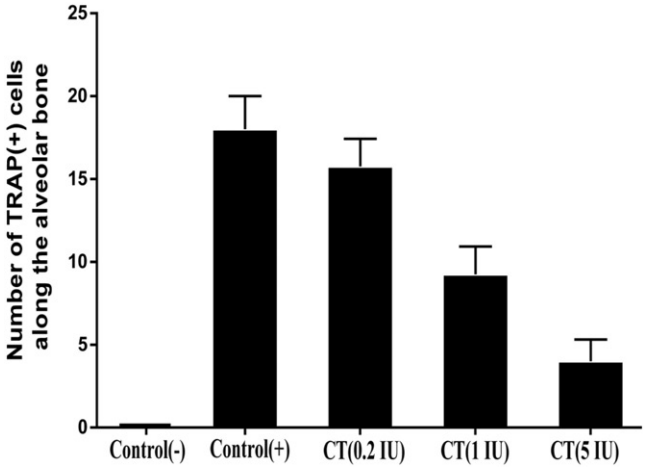

Figure 5. The number of osteoclasts (mean) along mesial alveolar bone in five groups: negative control group, positive group and experimental groups injected with 0.2 IU, 1 IU and 5 IU/kg/day of CT, respectively after 14 days of loading. $n = 8$ for each group.

Experimental OTM models have been established for more than 100 years [30], with application of different forces [4,21,31]. However, there must be some inevitable potential complicating factors in measurement of the distance of OTM, such as deformation of the appliance during mastication, mesial drift of the second molar and cranial growth during the experiment. All these factors could differ between individual animals and these effects take some time to occur, so they are ignored in this study. However, these effects should be taken into account in studies with longer treatment periods. Moreover, in order to avoid potential rater error, all the measurements were performed by one person. Nevertheless, the inevitable visual subjectivity also affects the accuracy of the results of measurement. In addition, this study used a level ruler to adjust the occlusal plane of models, which can minimize measurement errors due to different viewing angles. The closest distance was measured by the same investigator at three different times, and the mean value was used.

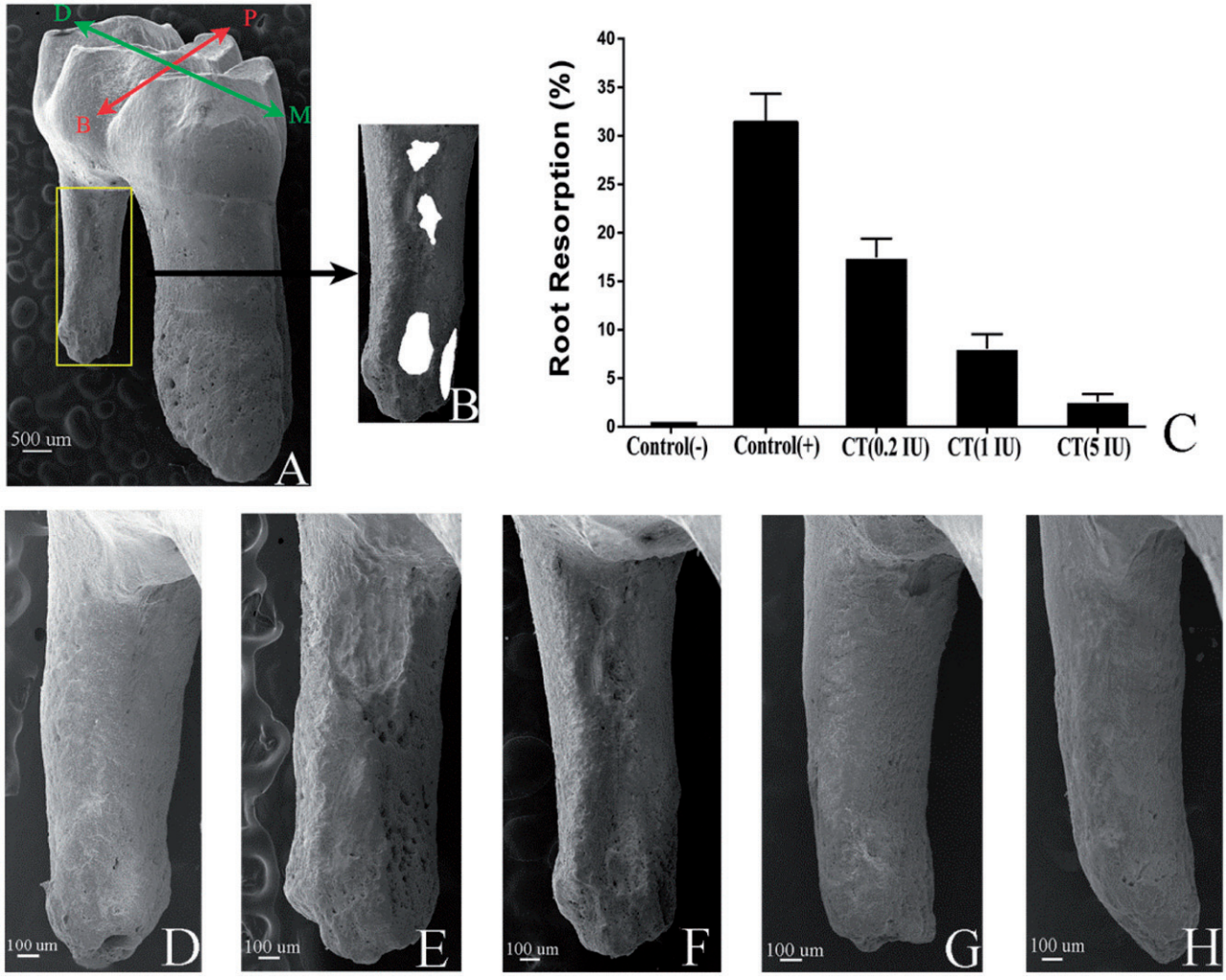

Figure 6. Effect of CT on root resorption after mechanical loading. Scanning electron micrographs of the maxillary first molars (A). Resorption area (white) and the total root surface area (grey) (B). The resorption area ratio was calculated by dividing the resorbed area by the total root surface area [resorption area ratio = white area/(white and grey area)]. Graph demonstrating the ratio of the root resorption area (mean) in all groups (C). $n = 8$ for each group. Representative images of scanning electron micrographs of the distobuccal roots of the maxillary first molars in the negative control group (D), positive control group loaded and injected with saline (E) and in experimental groups injected with 0.2 IU, 1 IU and 5 IU/kg/day of CT, respectively (F–H). M: mesial; D: distal; B: buccal; P: palatal.

The aim of this study was to investigate the effects of CT on OTM and root resorption, and to observe the dose-dependent relationship with CT. Twenty grams of orthodontic force could produce root resorption, but the area of root absorption was less than that induced by a 50 g force [32]. According to many studies that selected a large super-physiological force to ensure that a significant amount of root resorption occurred [33–36], this study selected 50 g of orthodontic force, making the different effects of different doses more obvious. Based on the degree of root resorption observed in SEM images (Figure 4(A)), we concluded that the orthodontic force used in the experimental model should be stronger than the appropriate force. In clinical practice, slightly stronger than appropriate orthodontic force is used at times, depending on the type of tooth to be moved and the manner of movement. Furthermore, to determine whether CT can reduce serious root resorption to the utmost extent, we selected high force to establish the root absorption model. Local application of CT retarded OTM and inhibited root resorption, even when higher orthodontic force was used.

Accumulating evidence has shown that CT exerts a direct stimulatory effect on bone formation and mineralization in osteoblastic cells [9]. Moreover, CT is reported to increase normal calcification of dentin matrix [37]. The promotion of distal alveolar bone remodeling and repair effect of CT on root resorption during orthodontic treatment require evaluation in future studies.

Several agents have been identified that inhibit bone resorption [3,38,39]. However, only CT has been shown to provide a significant analgesic benefit [17–19]. CT exerts an anti-nociceptive effect related to the serotonergic system [40]. The binding sites of CT in the hypothalamus, pituitary, pons, and medulla indicate a neuromodulatory function [41]. Previous findings indicate that CT and its receptors control appetite, lactation, and pain perception in the CNS [16]. Other studies have shown that the inhibitory effect of CT on pain is regulated via binding to its receptors in peripheral nerves [42]. During the process of orthodontic treatment, application of mechanical force can lead to tooth pain, so we propose that CT may reduce orthodontic tooth pain, which is better than other available drugs. Further research is required to establish whether CT can ease tooth pain caused by orthodontic stress in an orthodontic rat model. In the present study, OTM and root resorption are inhibited by CT in an orthodontic rat model, indicating that CT can effectively control the extent of OTM and undesirable root resorption. The effects of CT on orthodontic periodontal tissue remodeling during OTM still require extensive clarification.

Acknowledgements

We express our gratitude to Qi Huang and Xiang Ban for providing excellent technical assistance in this experiment.

Disclosure statement

We declare that we have no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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