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Effect of cyclosporine-A on orthodontic tooth movement in rats

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Structured Abstract

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Objective – The objective of this study is to examine the effect of cyclosporine-A (CsA) on the rate of orthodontic tooth movement in rats.

Setting and Sample Population – This is a randomized controlled trial with a split-mouth design in Sprague–Dawley rats.

Material and Methods – Eighteen rats, divided at random in two groups, were fed with 8 mg/kg CsA (experiment) or mineral oil (control) daily after initial healing of bilateral maxillary second molar removal. All rats received orthodontic coil springs (10 cN) secured to the maxillary incisors and first molars at the rights side, while no springs were placed at the left. Distances between first and third molars were measured on days 0, 3, 6, and 12. After sacrificing on day 12, the alveolar ridges of the maxillae were sectioned and blood samples were collected for serum tartrate-resistant acid phosphatase (TRAP)-5b level detection and for histology, respectively.

Results – Significantly larger changes in intermolar distances were found after orthodontic force application in the CsA group at days 3 and 12 when compared with the control group. The inter-radicular dental alveolus of CSA-fed rats was osteopenic. Significantly increased TRAP-5b serum level was noted in the CsA group when compared with the control group.

Conclusions – We suggest that CsA enhanced the rate of orthodontic tooth movement. The osteopenia and the increased osteoclastic activity could be the underlying factors.

Key words: cyclosporine-A; orthodontics; osteoclasts; tooth movement

Introduction

Cyclosporine A (CsA) has been widely used as an immunosuppressant to prevent organ transplant rejection and is used to treat a variety of disorders (1). However, after the administration of CsA, varied physiological and pathological reactions to the drug other than immunosuppression may be observed (2, 3). Enhanced osseous resorption during CsA therapy

in long bones has been reported in human clinical observations (4, 5), as well as in animal studies (6, 7). In the alveolar process, a pattern of osteopenia was observed in animals that received CsA (8, 9). Studies also suggest that the use of CsA may influence both alveolar bone healing in tooth-extraction sockets (10), as well as around titanium implants (11). In our previous study, we found that an increased interincisor diastema appeared after the overgrowth of the papilla in animals taking CsA. We consequently proposed that the enhanced alveolar remodeling induced by CsA might be the undetermined factor for the tooth movement (12). However, direct evidence of the effect of CsA on orthodontic tooth movement is still lacking. To test the effect of CsA on tooth movement by an orthodontic force, the changes of intermolar distances after orthodontic springing, the serum levels of tartrate-resistant acid phosphatase (TRAP-5b, a marker of osteoclastic activity), and the histological alterations of dental alveolar bone were examined in young rats after a short-time administration of CsA.

Materials and methods

Extraction of maxillary second molars and animal grouping

Eighteen male 4-week-old Sprague–Dawley rats, weighing 180–210 g, were selected. In order to easily insert the orthodontic appliances, as well as measure the intermolar distances, bilateral maxillary second molars were extracted from all the animals under general anesthesia induced by an intra-peritoneal injection of Zoletil (Virbac Laboratories, Carros, France) as described previously (13). The extractions also minimized the chance of development of CsA-induced papillary overgrowth which could cause a diastema and become a confounder (12). Following the extractions, animals underwent a 7-day period of initial wound healing before the commencement of feeding of CsA or oil. Animals were then randomly divided into two groups. Animals in CsA group received a daily dose (8 mg/kg body weight) of CsA by gastric feeding, while rats in the oil group received the vehicle (mineral oil) of CsA. Body weights of all rats were measured daily. Ethical approval for this study was obtained according to the guidelines for animal experiments of the National Defense Medical Center, Taiwan.

Fig. 1. Orthodontic appliance, with spring, attached to the incisors of a rat. The distance between the parallel arrows indicates the interdental distance.

Orthodontic appliance

Ten days after the first administration of CsA, the orthodontic appliance was placed under general anesthesia. A transverse hole was drilled through both the maxillary incisors. The stainless steel ligature wires (Dentaurum, Pforzheim, Germany) were inserted through the holes and secured with orthodontic appliances.

The orthodontic appliance was first inserted on the maxillary right first molar. A 10 cN mesially directed force was then applied (Fig. 1). The force level was verified using a Dynamometer (Correx; Dentarum, Newtown, PA, USA) measuring gauge. The orthodontic appliance which consisted of a stretched 10 cN closed coil spring (GAC, New York, NY, USA) was then ligated to the stainless steel ligature wires between the maxillary right first molar and two maxillary central incisors (14). Molars on the left side were used as non-appliance controls.

In summary, animals were randomly divided into two groups with different feedings: CsA and oil groups. Within the two groups, the maxillae were further divided as a split-mouth design for orthodontic treatment on the right and non-orthodontic treatment on the left. Hence, in total, there are four experimental categories of the maxillae: 1) oil; 2) oil + spring; 3) CsA; 4) CsA + spring.

Intra-oral measurements

Impressions (Vinyl Polysiloxane Imprint-II, VPS) of both right and left sides of the molars were taken at days 0, 3, 6, and 12. The intermolar distances between

the most distal points of the first molars and the most mesial points of the third molars were measured from the converted plaster casts under a microscope (Fig. 1).

Serum level of osteoclast-derived tartrate-resistant acid phosphatase (TRAP-5b)

On day 12, 1 ml blood from the tail vein was drawn from each animal prior to it being sacrificed. To evaluate the activity of osteoclasts on bone surfaces, the serum was separated by 1100 *g* centrifugation for 15 min at 4°C and then stored at 80°C for titrating the TRAP. Serum level of TRAP-5b, a marker of osteoclastic activity, was determined using a solid-phase immunofixed-enzyme activity assay (RatTrap Assay; SBA Sciences, Oulu, Finland; IDS, Cat. SB-TR102). The assay uses a specific monoclonal antibody prepared with baculovirus-generated recombinant rat TRAP as the antigen.

Histological preparation and histometric analysis

On day 12, all animals were sacrificed with carbon dioxide inhalation and the maxillae were dissected and fixed in 4% formalin for 4–8 h. Four categories of jaw specimens were obtained: the negative control (oil), the CsA, the orthodontic, and the CsA-ortho-combined jaw specimens. All tissue specimens were decalcified in 0.25 M ethylenediaminetetraacetic acid (EDTA; 10%, pH 7.2; USB Corporation, Cleveland, OH, USA) for 21 days and embedded in paraffin. Six micrometer serial sagittal sections were made parallel to the long axis of the molars. Ten consecutive sections closest to the midline of the crowns containing the mesial and distal root, and the inter-radicular alveolar bone were selected for histological examination and histometric analysis (Fig. 2). The dental alveolar bone volume (V-bone; mm^3/mm^3), bone marrow volume (V-marrow; mm^3/mm^3), alveolar bone-specific surface area (Sv; mm^2/mm^3), and alveolar surface-to-volume ratio (S/V; mm^2/mm^3) were measured in the areas at the coronal and apical inter-radicular bones (Fig. 2). This measurement was made by superimposing the image with a calibrated micrometer disc under ×200 magnification, as in previous studies (10).

Statistical analysis

The repeated measure analysis of variance was selected to evaluate the body weight changes for the factors of CsA treatment (between-subjects factor) and experimental duration (dependent variable). The student t-test was used to compare the difference in the serum level of TRAP 5b (U/l) between the control and CsA groups. To evaluate whether the changes in intermolar distance (dependent variable) were related to the CsA feeding (between-subjects factor) and the orthodontic application (within-subject factor), the repeated measures analysis of variance was used. The independent *t*-test was further used to examine the difference of the changes in intermolar distance in CsA with and without appliances, in non-CsA with and without appliances, with orthodontic appliances under oil and CsA treatments, and without orthodontic appliances under oil and CsA treatments. The repeated measures analysis of variance was also used to evaluate the differences of the histometric measurements (dependent variables) in the inter-radicular dental alveoli at coronal and apical zones (the within-subject factor) and among four categories of jaw specimens according to CsA and

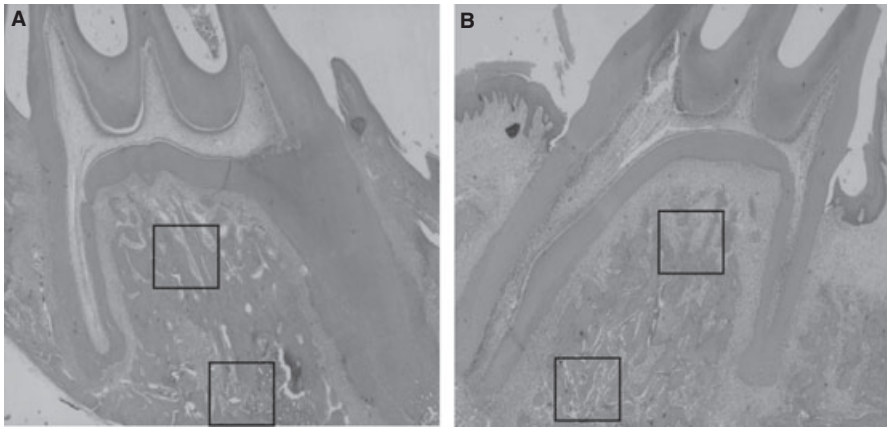


Fig. 2. Histological pictures representing the maxillary molars and their surrounding dental alveolus in jaws that received no treatment (A) and combined (cyclosporine A plus orthodontic) treatments (B). Histomorphometric analysis was performed at the coronal and apical zones of the inter-radicular bone (the rectangular areas indicate the zones for histometric measurements; H & E staining, original magnification × 5).

orthodontic treatments: negative control, orthodontic treatment, CsA treatment, and combined treatment (between-subjects factor). $p < 0.05$ was considered statistically significant.

Results

Of the 18 animals at the start, 16 (89%) survived with their appliances intact for the 12-day measurement (9 for control and 7 for CsA group). Two animals were lost because of anesthetic complications. The body weight of the rats increased continuously during the experiment of 22 days, and there was no significant difference between the control and CsA rats (Fig. 3). However, a significantly higher TRAP-5b serum level was found in CsA group when compared with the control group (0.92 ± 0.04 and 1.99 ± 0.06 U/l, means and standard errors, for control and CsA groups, respectively; Fig. 4).

The change of intermolar distance at day 3 was significantly increased by the orthodontic force application and CsA treatment ($p = 0.001$ for orthodontic force application and $p = 0.002$ for CsA treatment, Fig. 5). On the side without the appliance, a similar change of distance was observed in the two animal groups (−0.028 ± 0.025 and 0.029 ± 0.029 mm for vehicle and CsA groups, respectively). On the side with the appliance, however, the change of distance was significantly larger in animals that received CsA when compared with the group that received the vehicle (0.321 ± 0.062 and 0.067 ± 0.049 mm for CsA and control groups, respectively, $p = 0.007$). In both animal groups, the change in distance significantly increased on the side that received orthodontic appliances when compared with those on the side without orthodontic treatment. Similar results were found at days 6 and 12, but no significant difference was observed between the side with orthodontic force application from the CsA and control/oil groups at day 6 ($p = 0.096$).

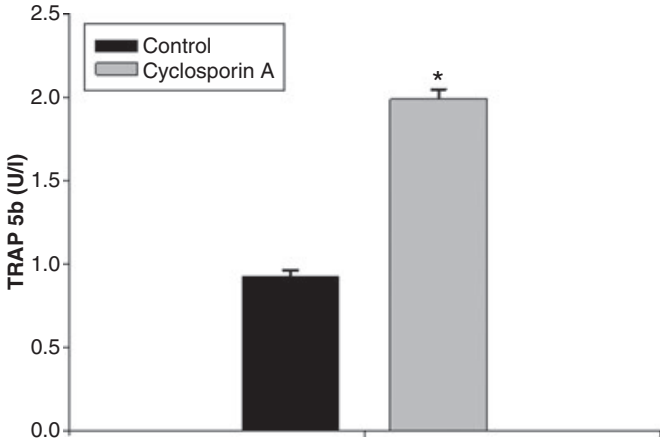


Fig. 4. Serum levels of tartrate-resistant acid phosphatase 5b (U/l) in the control and cyclosporine A groups (means and standard errors, *significantly different from the control at $p < 0.01$).

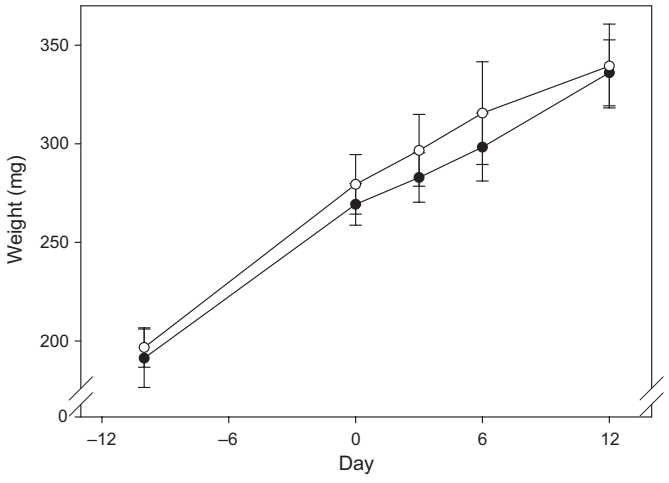
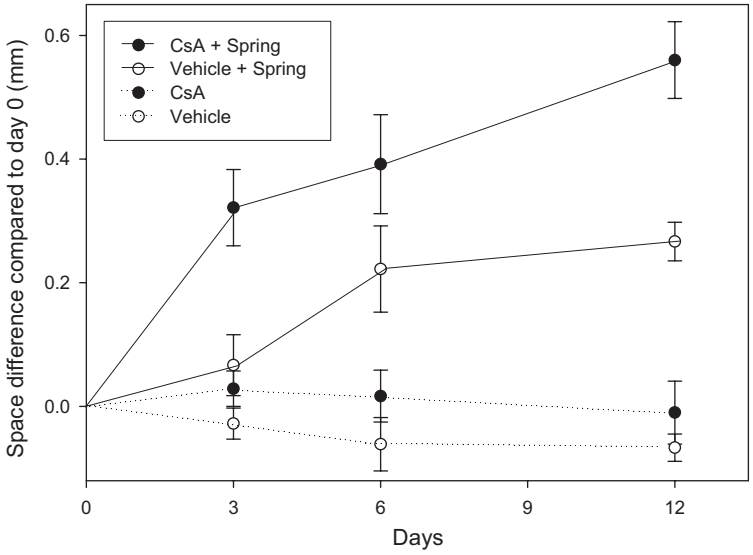


Fig. 3. Body weight of the rats during the 22-day experiment (day 0 = the day of applying the orthodontic force; open circles = weights of control rats; solid circles = weights of rats feeding with cyclosporine A; means ± standard deviations).

In general, histologically osteopenia in both coronal and apical zones of the inter-radicular bone was observed for the jaws that received CsA (Fig. 6C and c) when compared with the group that had neither CsA nor orthodontic treatment (negative control, Fig. 6A and a). An increased remodeling (increase of reverse lines, for example) was seen in jaws that had orthodontic treatment (Fig. 6B, b, D, and d). As shown by histomorphometry, the dental alveolar V-bone was significantly smaller in the group that received only CsA when compared with the group that had no treatment (negative control) and the group that received orthodontic force application. However, the bone volume rebounded with statistical significance in the jaws that had combined therapies, but was still significantly less than the negative control and the group that had orthodontics without CsA (Fig. 6). Similar but opposite results were observed for the means of marrow volume and V-marrow. The bone of the specific area (Sv) showed no difference among the four jaw groups, but the mean alveolar bone S/V was significantly greater in the jaws that received CsA only when compared with



	Effect of Orthodontic Appliance			Effect of CsA Treatment		
	Without	With	*P* value	Vehicle	CsA	*P* value
Day 3	.000±.019	.194±.039	.001*	.019±.027	.175±.030	.002*
Day 6	−.022±.032	.307±.054	<.001*	.081±.044	.204±.053	.096
Day 12	−.038±.024	.413±.031	<.001*	.100±.024	.275±.032	.001*

Fig. 5. Effects of cyclosporine A (CsA) treatment and orthodontic force application on the changes of intermolar distances, compared with the distance at day 0 of applying the orthodontic force, in rats who received CsA (8 mg/kg/day) or mineral oil during the observation period of 12 days (means ± standard errors). Effects of factor of CsA or orthodontic force on the changes of intermolar distances (mm) at each observation interval of day 3, 6, and 12 were summarized in the bottom.

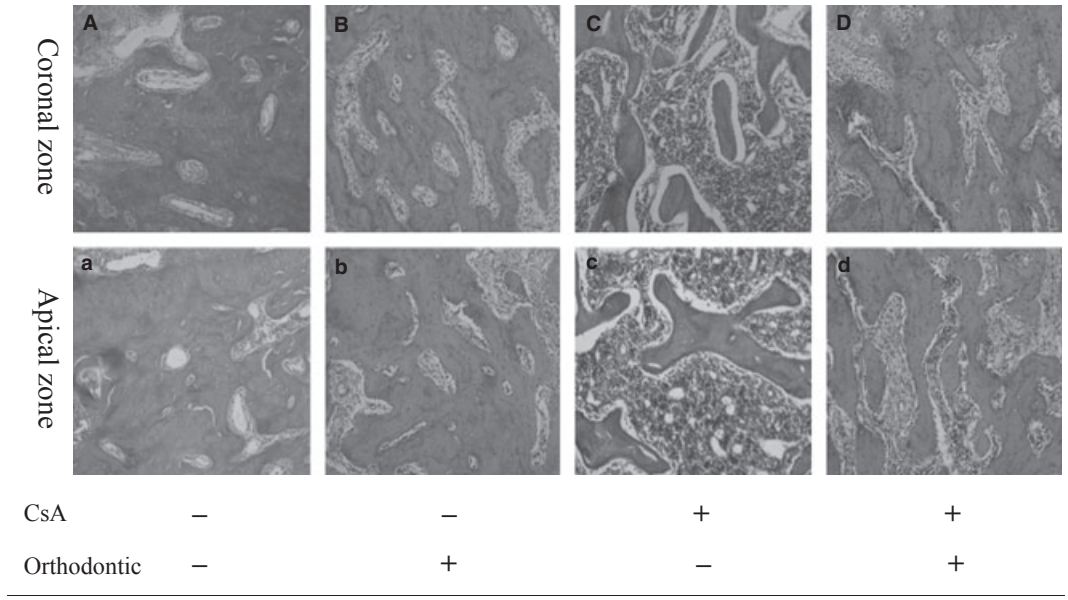
the rest of the three groups. The mean S/V in jaws that received combined therapies was lower than the group that had CsA, but higher than that in the jaws without CsA treatments.

Discussion

In this study, we examined the effect of CsA treatment on orthodontic tooth movement using a rat model. After a 12-day orthodontic treatment, the changes in intermolar distances increased in sites with orthodontic force application, regardless of whether CsA treatment was given or not (Fig. 5). However, the change in distance was larger in animals that received CsA than in animals that had the oil/solvent. On the side where no orthodontic force was applied, similar changes in distance were observed in the two groups with and without CsA. From a histological perspective, osteopenia was observed in the coronal and apical zones of the inter-radicular bone of the jaws that received CsA (Fig. 6C and c vs. Fig. 6A and a). In addition, a higher TRAP-5b serum level was recorded in CsA group when compared with the control group (Fig. 4). Therefore, we suggest that the rate of orthodontic tooth movement was enhanced during CsA therapy, and the altered osteoclast activity might play a role in this enhanced movement.

Many studies have shown that CsA induces high turnover osteoporosis and weight loss in rat tibiae (15–18). Both bone formation and resorption parameters are increased, but the resorption far exceeds formation (15). CsA, however, does not influence linear tibial bone growth or radiographic density. In the alveolar process specifically, a distinct pattern of osteoclasia and osteoid formation has been observed in CsA-exposed animals over a 6-week interval (19). CsA treatment is associated with alveolar bone resorption, which is represented by a decrease in bone volume, bone surface, and number of osteoblasts per bone surface. In addition, there is an increase in the number of osteoclasts per bone surface (20). If the same CsA treatment is given in the long term, the same phenomenon would be found as well (21).

It has been postulated that those effects of CsA on bone may be due to T cells rather than a direct effect on bone (22). On the other hand, it has been discussed whether CsA, which is a calcineurin inhibitor, has significant inhibitory effects on the differentiations of osteoblast and osteoclast through the regulation of NFAT-calcineurin signaling pathway (23–26). Interestingly, it has been shown that the sole presence of



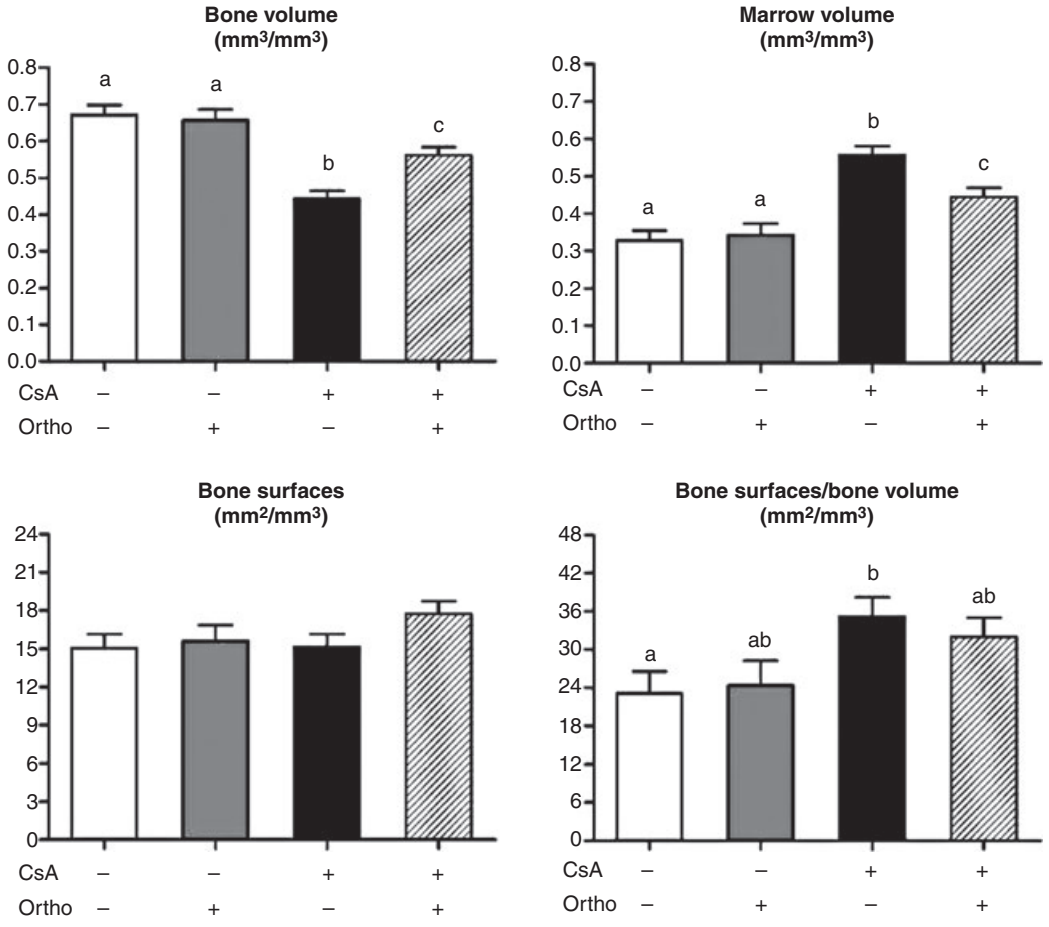


Fig. 6. Histological pictures presenting the bony morphology at the coronal (A–D) and apical (a–d) zones of the inter-radicular dental alveoli of maxillary first molars from four types of jaws according to cyclosporine A (CsA) and orthodontic treatments (no-treatment, orthodontic treatment, CsA treatment and combined treatments; H & E staining, original magnification × 25). Comparison of the histomorphometric measurements, including bone volume, marrow volume, bone-specific surface area and surface-to-volume ratio, among the four types of jaws was illustrated in the bottom (a, b and c: the subgroups by the *post-hoc* analysis, if $p < 0.05$).

T-cells is not enough to cause CsA-induced osteopenia *in vivo* (27). This supports the assumptions that other cells types or mechanisms may exist for CsA to affect osteoblastogenesis and osteoclastogenesis. These other possible mechanisms of CsA-induced high turnover osteopenia include decreased renal function, reduced $1,25(OH)_2D$, increased PTH secretion, and increased production of circulating proinflammatory cytokines other than direct effects on T cells (28). However, the exact mechanisms of CsA-induced osteoblastogenesis and osteoclastogenesis still remain uncertain, and further detailed research is therefore needed.

Certain drugs and systemic factors can also influence orthodontic tooth movement (29). Anti-inflammatory drugs, NSAIDs, and aspirin have been shown to reduce bone resorption and orthodontic tooth movement in animals (30–32). Similarly, the administration of bisphosphonates reduced the rate of orthodontic tooth movement and decreased the number of osteoclasts (33, 34). On the other hand, the experimental tooth movement rate was enhanced in ovariectomy animals (35). Thyroid hormones, parathyroid hormone, and vitamin D were also shown to enhance the rate of tooth movement in rats (36–38). Many pharmacological modulations modify not only the rate of bone turnover but also the local structure of bone, and may further influence the rate of orthodontic movement (39–43).

In the present study, histological examination revealed that osteopenia was observed in the interradicular dental alveolus of CsA-treated rats, regardless of the orthodontic force application (Fig. 6). Orthodontic therapy in patients treated with CsA was firstly discussed in 1991. Findings, including the potential for orthodontic appliances to increase the severity of induced gingival enlargement, and the potential of having gingival hyperplasia to complicate orthodontic therapy, were discussed (44). In ligature-induced experimental periodontitis, however, significantly less alveolar bone loss and more alveolar bone formation were observed in rats treated with CsA than in those without (45). Recently, biphasic effect of CsA on osteoblast differentiation and bone formation was observed (46). The present study further showed that the CsA-induced osteopenia seems to be reduced when orthodontic force was applied. Although the exact cause is still unknown, an increased expression of VEGF in the gingiva of rats that received CsA was found (47). This may indicate that orthodontics is being performed in an altered metabolic state when CsA is administrated. It may partly explain why there are increased reverse lines with reduced osteopenia in the jaws that received orthodontics and combined treatments.

Gingival overgrowth is a local side effect in the oral cavity during CsA treatment. In animal studies, a dose-dependent effect in the development of gingival overgrowth has been widely noticed. A significant gingival overgrowth was easily observed when the dosages were >10 mg/kg (5, 48). In order to mimic the clinical use for post-transplantation and to avoid high-dose induced gingival overgrowth as in the previous study (30 mg/kg), a daily dose of 8 mg/kg body weight of CsA was chosen in this study (12, 49). In the current study, the maxillary second molars were also removed initially to avoid tooth movement induced by the enlarged papilla as a confounder, if overgrowth were to happen.

Conclusion

We suggest that CsA enhances the rate of orthodontic tooth movement. Osteopenia in the alveolar process and increased osteoclastic activity might be the underlying factors.

Clinical relevance

Cyclosporine A, an immunosuppressant, causes an increased systemic osteoclastic activity and a local osteopenia in the alveolar process. Accelerated tooth movement was also observed after applying an orthodontic force in an animal model. This might indicate that accelerated orthodontic movement should be taken into consideration for subjects taking this drug.

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