Journal of Orthodontics, Vol. 30, 2003, 31–37

SCIENTIFIC SECTION

Inactivated periods of constant orthodontic forces related to desirable tooth movement in rats

T. Kameyama, Y. Matsumoto, H. Warita and K. Soma
Tokyo Medical and Dental University, Japan

Abstract

Aim: To examine the effects of inactive periods of force on the amount of tooth displacement and root resorption during experimental tooth movement in rats.

Sample: Sixty 11-week-old male Sprague-Dawley rats.

Method: The maxillary first molar (M1) was moved mesially using a removable titanium-nickel alloy closed coil spring for 14 days. The rats were divided into four groups with, 0, 1, 4, and 9 hours of inactivation per day.

Results: Tooth displacement in the 0- and 1-hour groups was significantly greater than that in the 9-hour group. The area of root resorption in the 4- and 9-hour groups was significantly less than that in the 0- and 1-hour groups. There was no significant difference in root resorption between 0- and 1-hour groups, and also between 4- and 9-hour groups.

Conclusion: The distance of tooth displacement gradually decreased as the inactive period increased, whereas root resorption suddenly decreased between 1 and 4 hours of inactive orthodontic force.

Index words: Experimental tooth movement, intermittent force, titanium-nickel (Ni-Ti) alloy closed coil spring, root resorption, titanium implant

Received 14 February 2002; accepted 11 July 2002

Introduction

It is generally accepted that tooth pain, widening of periodontal ligament (PDL) space, and root resorption are produced less frequently by light forces.[1,2] However, it is still not known whether the optimal orthodontic force should be, continuous, intermittent, or interrupted. Some studies have demonstrated that light continuous forces produce effective tooth movement with minimum tissue damage.[3–7] Reitan[8,9] suggested that hyalinized tissues are resorbed more rapidly if a given force is applied intermittently, rather than in a continuously.

In addition, some studies have observed less root resorption in tooth movement produced by interrupted forces. For example, Levander *et al.*[10] demonstrated that the amount of root resorption is significantly less in patients treated with a pause of 2–3 months compared to those treated without interruption. Moreover, Rygh[11] reported that when the orthodontic force is discontinued, any root resorption is repaired.

In recent studies of intermittent forces, Igarashi *et al.*[12] reported that the application of 20gf of force with a resting period of 12 hours per day in rats produces more effective movement than full time forces. Konoo *et al.*[13] found that the application of 40gf of force for 1 hour per day in rats is ineffective at moving teeth. These studies, however, did not examine the effect of force duration on tooth movement. In the experiments reported here, we improved the tooth movement model developed in our previous study. The appliance comprised a nickel-titanium (Ni-Ti) alloy closed coil spring and a titanium screw implant.[14]

The aim of this study was to examine the effects of force duration, that is, inactive periods of force application on the amount of (i) tooth displacement and (ii) root resorption during experimental tooth movement.

Address for correspondence: Dr T. Kameyama, Orthodontic Science, Department of Orofacial Development and Function, Division of Oral Health Sciences, Graduate School, Tokyo Medical and Dental University, 1–5–45 Yushima, Bunkyo-ku, Tokyo, 113-8549, Japan. E-mail: kame.orts@tmd.ac.jp

Materials and methods

Animals

Sixty 11-week-old male Sprague–Dawley rats (body weight 360 ± 18 g) were used. All rats were fed a solid diet and given water *ad libitum* during the period of the experiment. To assess whole-body effects, the animals were weighed before each treatment. All procedures followed the guidelines of the Tokyo Medical and Dental University for Animal Research. The experimental protocols had the approval by the local Ethics Committee.

Experimental tooth movement

We improved the method of tooth movement from our previous report.[14] Eleven-week-old rats were intraperitoneally anaesthetized with ketamine hydrochloride (Ketalar 50, Sankyo Co. Ltd, Tokyo, Japan) containing 20% xylazine hydrochloride (Celactal 2% injection, Bayer-Japan Co. Ltd., Tokyo, Japan) as a muscle relaxant, after inhalant anaesthesia with diethyl ether. A titanium screw implant, 1.0 mm in diameter and 4.0 mm in length (Shioda Co. Ltd, Tochigi, Japan), with a titanium plate was placed just behind the right maxillary incisor for anchorage (Figure 1). Tooth movement was started after the implant had been allowed to stabilize for 1 week. The titanium plate and molar clamp allowed the easy removal of the a nickel-titanium (Ni-Ti) alloy closed coil spring[14] (diameter: 0.015 mm; lumen: 0.9 mm; length: 2.0 mm; load: 10 gf; Figure 2), and the maxillary left first molar (M1) was moved in four patterns of continuation and intermittence for 14 days.

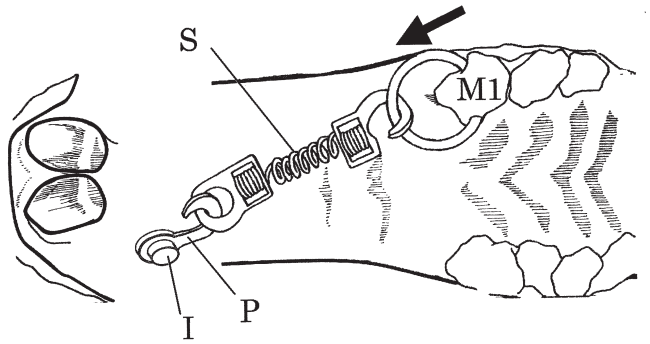

Fig. 1 A schematic drawing of a occlusal view of the appliance for tooth movement. The arrow shows the direction of load by Ti-Ni alloy closed coil spring (S) in the maxillary left first molar (M1) with an anchorage unit made up of a titanium screw implant (I) and a titanium plate (P).

The rats were randomly divided into one control and four experimental groups (12 animals in each group). They were designated according to inactive periods of 0, 1, and 4 (Figure 3). Zero-hour means continuous force. In 1-, 4-, and 9-hour groups, Ni-Ti alloy closed coil springs were set and removed everyday under ether anaesthesia. A previous study showed that under physiological conditions, both bone formation and resorption are more intense in the day-time than in the night time,[15–17], and these are the resting and active periods for rats, respectively. Therefore, we set the inactive period at night to evaluate the most effective

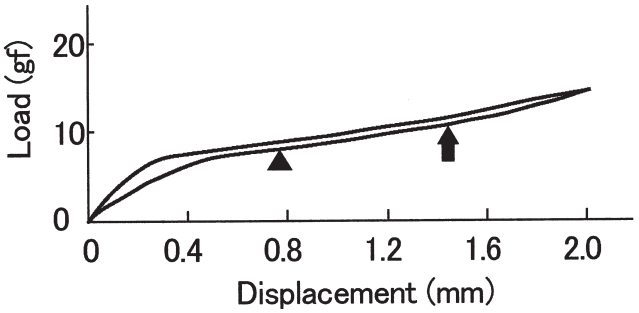

Fig. 2 A load-displacement curve of the super-elastic Ti-Ni alloy closed coil spring. The curve between an arrow and an arrowhead indicate the magnitude of orthodontic force at the beginning (arrow) and at the end (arrowhead) of tooth movement used in this study.

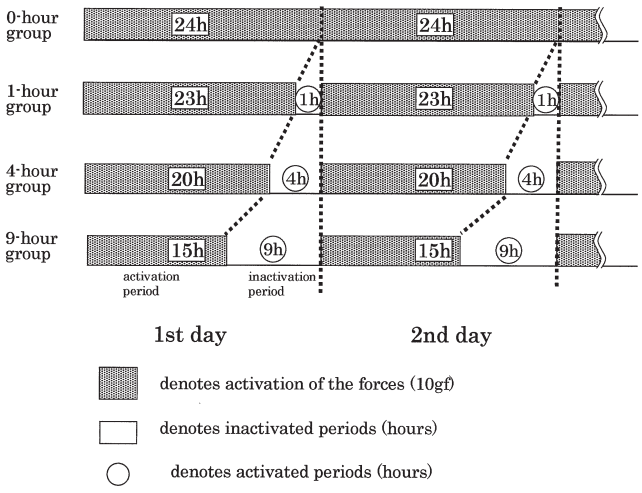

Fig. 3 Time schedule of four experimental groups according to inactive periods of 0, 1, 4, and 9 hours per day, and they were named 0-, 1-, 4-, and 9-hour group. During inactive periods, the Ti-Ni alloy closed coil spring was removed and no orthodontic force was applied. The inactive periods were 22:00–23:00 in the 1-hour group, 20:00–24:00 in the 4-hour group, and 22:00–7:00 in the 9-hour group. Continuous force was applied to animals of the 0-hour group throughout the experimental period of 14 days.

bone remodelling within the diurnal rhythm. The inactive periods were 22:00–23:00 in the 1-hour group, 20:00–24:00 in the 4-hour group, and 22:00–7:00 in the 9-hour group. During inactive periods, the Ni-Ti alloy closed coil spring was removed and no orthodontic force was applied. Non-treated rats were used as the control group.

On days 0, 7, and 14 of tooth displacement, silicone impressions (Dent Silicone-V, Shofu Co., Ltd, Kyoto, Japan) of the maxillary left molars were taken and cast models were made with dental stone (New Plastone, GC Co., Ltd, Tokyo, Japan).[6,7] The interproximal spaces between M1 and second molar (M2) of the models were measured five times by each of the four investigators at intervals of 1 hour using a non-contact digital microscopic gage (MS-214; Fusoh Co., Ltd, Tokyo, Japan) and the mean value within the groups was taken as the tooth displacement.[7]

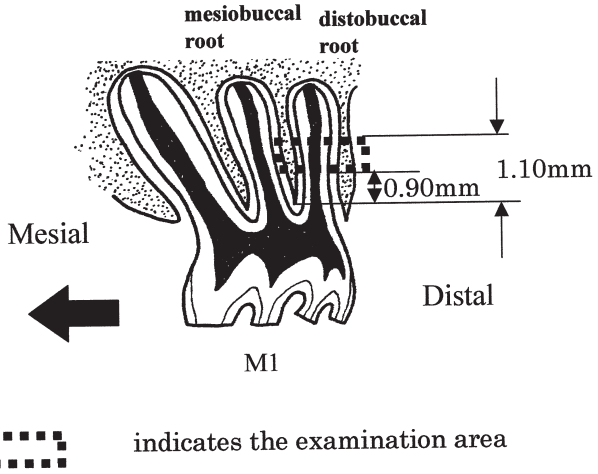

Fig. 4 A schematic drawing of the observation area. The mesial pressure side, 0.90–1.10 mm from the furcation of distobuccal root of the maxillary left first molar (M1) were observed. (Arrow: direction of force.)

Histological and histomorphometric examination

On day 14 of tooth displacement, the rats were sacrificed by decapitation and the maxillae were immediately removed, fixed in a solution of 10% neutral buffered formalin (Wako Pure Chemical Co. Ltd, Osaka, Japan) at 4°C overnight; decalcified in 10% EDTA solution at 4°C for 6 weeks; and embedded in paraffin with conventional methods. Seven-micrometre thick horizontal serial sections of the M1 were made with a microtome (RM2155, LEICA Co. Ltd., Nussloch, Germany). The mesial sides of distobuccal roots of M1 were selected for observation. They were stained with H&E for histomorphometric study. To identify osteoclasts and odontoclasts for active bone and root resorption, TRAP activity according to a previous method.[18] The sections were counterstained with haematoxylin. The observation area was the mesial pressure side, 0.90–1.10 mm from the furcation of distobuccal and mesiobuccal roots of M1 (Figure 4). Consequently, we defined a TRAP-positive multinucleated cell on the bone surface as an osteoclast and on the root surface of resorption lacuna as an odontoclast. The area of the root resorption lacuna, in which odontoclasts were recognized in a serial section, was measured five times by each investigator at intervals of one hour at the pressure side of the root using a computer image analysis software (Image-Pro Plus, Media Cybernetics, Maryland, USA); (Figure 5).[19] The mean root resorption area for each animal was the average of the values obtained from three sections selected at 70-μm intervals.

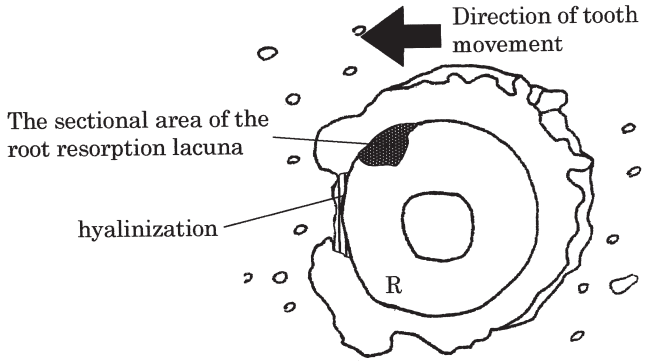

Fig. 5 A schematic drawing of a horizontal section. The sectional area of a root resorption lacuna on the pressure side for the disto-buccal root (R) in the experimental group was measured.

Statistical analysis

The means and the standard errors were calculated for body weight, tooth displacement, and root resorption area on days 7 and 14. The statistical process was performed using Stat View version 5.0 (SAS Institute Inc. Cary, NC, USA). The body weight difference between experimental and control groups were analysed using an unpaired two group *t*-test, while tooth displacement and root resorption area were analysed by analysis of variance (ANOVA) followed by a Scheffe *post-hoc* test. A level of $P < 0.05$ was considered to indicate a significant difference.

Results

Animals

The weight in the experimental group did not increase during the week after the implantation, in addition, there was no significant difference in weight between the control and the experimental groups over the whole experimental period (Figure 6).

Change in distance of tooth displacement

Figure 7 shows the amount of tooth displacement in the animals of each group on days 7 and 14. The tooth movement distance of the 0- and 1-hour groups was significantly greater than that of the 9-hour group ($P < 0.05$). There was no significant difference between 0- and 1-hour groups, or between the 1- and 4-hour groups.

Histological evaluation

In the control group, osteoclasts and odontoclasts were rarely seen on the mesial root surface of the M1, where neither bone nor root resorption was found (Figure 8e). In the 0- and 1-hour groups hyalinization of PDL and undermining bone resorption were found (Figure 8a,b). The root surface facing opposite the area of undermining bone resorption was resorbed by odontoclasts. Some of the root resorption occurred not only in cementum, but also in dentine. Observation of 4- and 9-hour groups revealed that hyalinized tissue and undermining bone resorption were rarely found and direct bone resorption was recognized as the duration of forces become short (Figure 8c,d). Less root resorption was found in the 4- and 9-hour groups. Figure 9 shows the mean area of root resorption lacunae. There was a significant difference in the area of root resorption lacunae between 0- and 1-hour groups, and 4- and 9-hour groups. On the other hand, there was no significant difference between the 0- and 1-hour groups, and also between 4- and 9-hour groups.

The margin of error for the measurements of tooth displacement and root resorption area was calculated for intra- and inter-investigator reproducibility. They were compared by analysis of variance (ANOVA) followed by a Scheffe *post-hoc* test. No significant differences were found among the five measurements of the experiment or between the collective results for all investigators ($P > 0.05$).

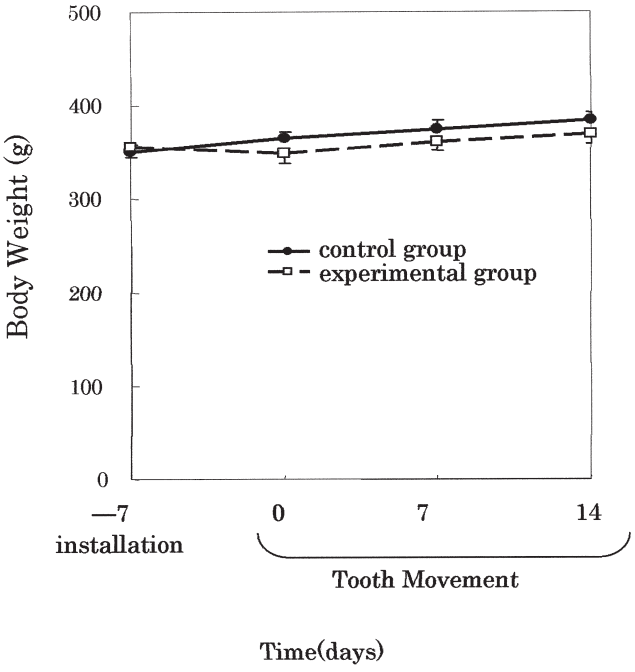

Fig. 6 The change of body weight. There was no significant difference in weight between groups (mean ± SE)—7days: installation of a screw implant. 0 day: beginning of experimental tooth movement. No significant difference between the control and experimental groups.

Discussion

We focused on differences for inactive periods of 10 gf of orthodontic force in rats and found two main results regarding the amount of tooth displacement and root resorption. First, the tooth displacement for 14 days increased proportionately to the increase in the active period of orthodontic force application. Igarashi *et al.*[12]

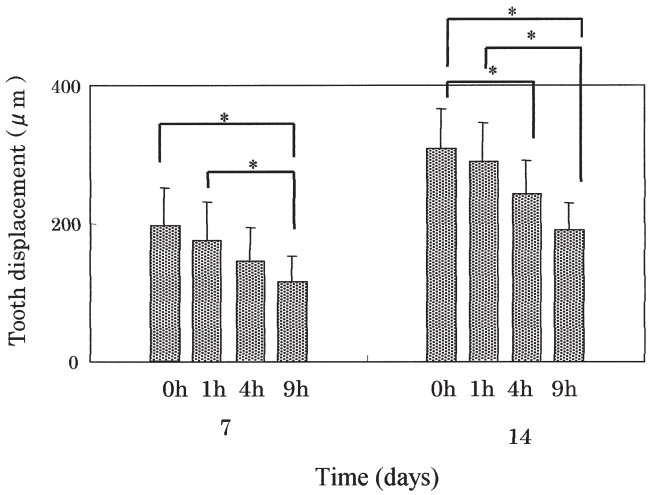

Fig. 7 The distance of tooth displacement on days 7 and 14 (mean ± SE). The distance of tooth displacement of the 0- and 1-hour groups showed significantly greater than that of the 9-hour group. *$P < 0.05$.

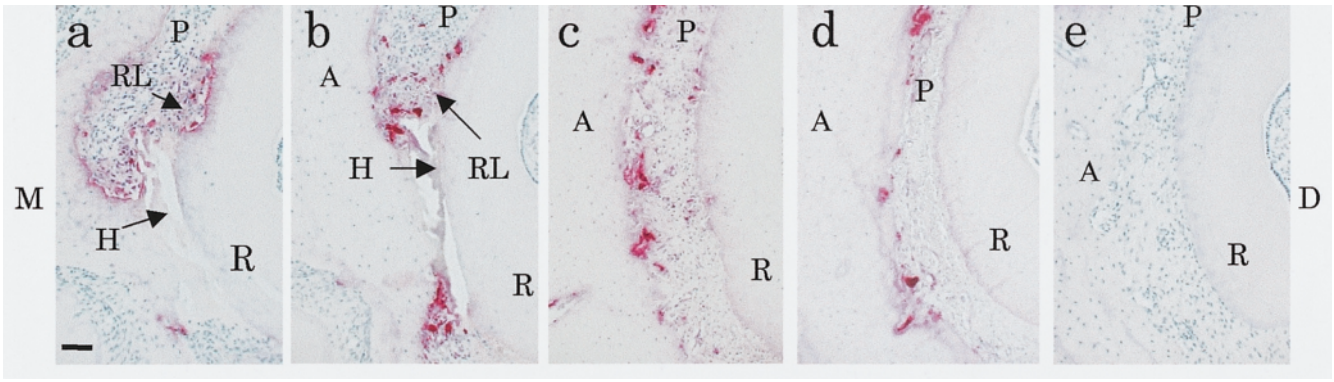

Fig. 8 The stained mesial compression sides of distobuccal roots of M1 in five groups on day 14. (a) 0-hour group; (b) 1-hour group; (c) 4-hour group; (d) 9-hour group; (e) control group. A hyalinized tissue and undermining bone resorption were found in the 0- and 1-hour groups (a,b). In contrast, little hyalinized tissue and undermining bone resorption were found in the 4- and 9-hour group (c,d). In the control group, TRAP-positive cells could not be recognized on the root surface (e). A: alveolar bone; R: root; P: periodontal ligament; RL: resorption lacuna; H: hyalinized tissue; M: mesial; D: distal. TRAP stain. Bar = 50 μm.

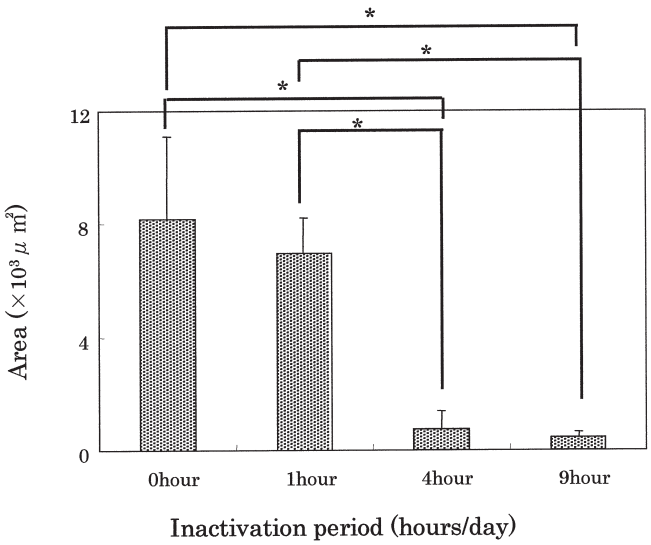

Fig. 9 Sectional area of root resorption lacuna (per section) on day 14. There was a significant difference in the area of root resorption lacuna between the 0-, 1-, and 4-hour group, and the 9-hour inactivation group. $*P < 0.05$.

found that the application of orthodontic forces for 12 hours per day during the rat's rest period moved teeth almost as much as full-time force, and the teeth did not move during the rat's active period at night. In our pilot study, the intermittent application of 2 gf, which seems to be a near to optimal force for continuous application, could not move the rat molar. Taken together with our results, the magnitude of orthodontic force, duration, and period of the day are mutually related, and appear to be involved in determining the efficiency of tooth movement obtained with an intermittent orthodontic force.

Furthermore, the results demonstrate that damage such as hyalinization of PDL and root resorption markedly decreased when the inactive period exceeded 4 hours per day. Root resorption lacunae measured in this experiment were related to the experimental tooth movement because no root resorption was seen on the mesial side of the root in the control group. In general, orthodontic root resorption is known to occur at the periphery of hyalinized tissue.[20–23] Root resorption involves differentiation, fusion, and activation of odontoclasts that are recruited and activated by increased production of local inflammatory cytokines in the periodontal ligament due to mechanical stress or necrosis of the PDL[24–28]. In this study, the 0-hour group, which received a continuous load of 10 gf, presented with root resorption, which we related to continuous mechanical stress and hyalinization of the periodontal ligament. Results from the 1-hour group were similar to those of the 0-hour group because the active period was almost the same and the inactive period was too short. By contrast, the 4- and 9-hour groups showed less root resorption because of a decrease in mechanical stress and hyalinized tissue, recovery of form and function of the blood vessels, reduction of cytokine production and subsequent odontoclast formation. The threshold for the appearance of hyalinized tissue and root resorption may occur in the 1- and 4-hour inactivation period.

Figure 10 shows the relation between tooth displacement and root resorption area to the inactivation periods. The amount of tooth displacement gradually decreased as the inactivation periods were increased, whereas the amount of root resorption decreased between the 1- and 4-hour inactivation period of ortho-

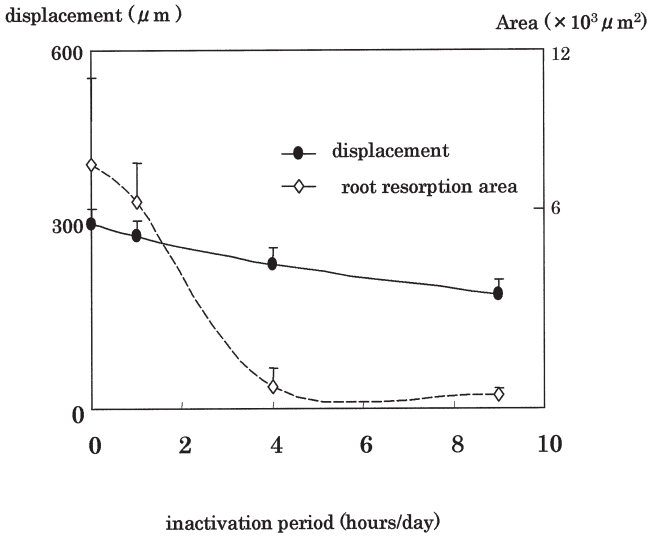

Fig. 10 Relationship between the root resorption area and the tooth displacement on day 14.

dontic force. Comprehensive analysis of this figure revealed that the optimal orthodontic force duration to move the rat M1 mesially with 10 gf comprised a 4-hour period of inactivation each day during the biological active period at night. The root surface area of the human maxillary first molar and canine teeth are 20 and 10 times larger than the rat M1.[29,30] If we extrapolate our findings to the clinical situation, if orthodontic forces equivalent to a 200 or 100 gf are used to move a first molar or canine, respectively, a 4-hour inactivation period during the human active period in daytime may reduce the risk of root resorption and move teeth effectively, without compromising tooth movement efficiency.

Acknowledgements

This research was supported in part by Grants-in-Aid for Scientific Research (No. 10771169, 12671988, 12771271, 19671989) from the Ministry of Education, Culture, Sports, Science and Technology, Japan. Part of this work was presented at the 60th Annual Meeting and 3rd International Congress of the Japanese Orthodontic Society (October 2001, Tokyo, Japan).

References

1. Storey E, Smith R. Force in orthodontics and its relation to tooth movement. *Aust J Dent* 1952; **56**: 11–18.
2. Burstone CH. The biomechanics of tooth movement. In Kraus BS, Riedel RA (Eds) *Vistas in Orthodontics*. Philadelphia: Lea & Febiger, 1962: 202–214.
3. King GJ, Fischlschweiger W. The effect of force magnitude on extractable bone resorptive activity and cemental cratering in orthodontic tooth movement. *J Dent Res* 1982; **61**: 775–779.
4. Kirino Y, Tsuchiya T, Kurihara S, Chiba M, Miura F. A study of tooth movement with super-elastic force. *J Jpn Orthod Soc* 1991; **50**: 315–324.
5. Kono H., Tsuchiya T, Warita H., Kubota M, Iida J, Mogi M, *et al*. A histological studies on tooth movement with super-elastic force (part.1). *J Jpn Orthod Soc* 1991; **50**: 126–136.
6. Warita H, Iida J, Yamaguchi S, Matsumoto Y, Fujita Y, Domon S, *et al*. A study on experimental tooth movement with Ni-Ti alloy orthodontic wires: comparison between light continuous force and light dissipating force. *J Jpn Orthod Soc* 1996; **55**: 515–527.
7. Kohno T, Matsumoto Y, Kanno Z, Warita H, Soma K. Experimental tooth movement under light orthodontic forces—rates of tooth movement and changes of the periodontium. *J Orthod* 2002; **29**: 125–132.
8. Reitan K. Some factors determining the evaluation of forces in orthodontic. *Am J Orthod* 1957; **43**: 32–45.
9. Reitan K. Effects of force magnitude and direction of tooth movement on different alveolar bone types. *Angle Orthod* 1964; **34**: 244–255.
10. Levander E, Malmgren O, Eliasson S. Evaluation of root resorption in relation to two orthodontic treatment regimes. A clinical experimental study. *Eur J Orthod* 1994; 16: 223–228
11. Rygh P. Orthodontic root resorption studied by electron microscopy. *Angle Orthod* 1977; **47**: 1–16.
12. Igarashi K, Miyoshi K, Shinoda H, Saeki S, Mitani H. Diurnal variation in tooth movement in response to orthodontic force in rats. *Am J Orthod Dentofac Orthop* 1998; **114**: 8–14.
13. Konoo T, Kim YJ, Gu GM, King GJ. intermittent force in orthodontic tooth movement. *J Dent Res* 2001; **80**: 457–460.
14. Kameyama T, Matsumoto Y, Warita H, Otsubo K, Soma K. A mechanical stress model applied to the rat periodontium. Using controlled magnitude and direction of orthodontic force with an absolute anchorage. *Oral Med Pathol* 2002; **7:** 1–7
15. Shinoda H, Stern PH. Diurnal rhythms in calcium transfer into bone calcium release from bone and bone resorbing activity in serum of rats. *Am J Physiol* 1992; **262**: R235–R240.
16. Simmons DJ, Menton DN, Russell JE, Smith R, Walker WV. Bone cell populations and histomorphometric correlates to function. *Anat Rec* 1988; **222**: 228–236.
17. Roberts WE, Klingler E, Mozsary PG. Circadian rhythm of mechanically mediated differentiation of osteoblasts. *Calcif Tissue Int* 1984; **36**: S62–S66.
18. Domon S, Shimokawa H, Matsumoto Y, Yamaguchi S, Soma K. *In situ* hybridization for matrix metalloproteinase-1 and cathepsin K in rat root-resorbing tissue induced by tooth movement. *Arch Oral Biol*, 1999; **44**: 907–915
19. Sringkarnboriboon S, Matsumoto Y, Soma K. Root resorption related to hypofunctional periodontium in experimental tooth movement. [Abstract]. *J Dent Res* 2001; **80**: 789.

20. Rygh P. Ultrastructural cellular reactions in pressure zones of rat molar periodontium incident to orthodontic tooth movement. *Acta Odontol Scand* 1972; **81**: 467–480.

21. Brudvik P, Rygh P. The initial phase of orthodontic root resorption incident to local compression of the periodontal ligament. *Eur J Orthod* 1993; **15**: 249–63.

22. Brezniak N, Wasserstein A. Root resorption after orthodontic treatment: part 1. Literature review. *Am J Orthod Dentofac Orthop* 1993; **103**: 62–66.

23. Brezniak N, Wasserstein A. Root resorption after orthodontic treatment: part 2. Literature review. *Am J Orthod Dentofac Orthop* 1993; **103**: 138–146.

24. Brudvik P, Rygh P. Root resorption after local injection of prostaglandin E2 during experimental tooth movement. *Eur J Orthod* 1991; **13**: 255–263.

25. Birkedal-Hansen H. Role of cytokines and inflammatory mediators in tissue destruction. *J Periodont Res* 1993; **28**: 500–510.

26. Murakami S, Takayama S, Ikezawa K, Shimabukuro Y, Kitamura M, Nozaki T, *et al*. Regeneration of periodontal tissues by basic fibroblast growth factor. *J Periodont Res* 1999; **34**: 425–30.

27. Wu YM, Richards DW, Rowe DJ. Production of matrix-degrading enzymes and inhibition of osteoclast-like cell differentiation by fibroblast-like cells from the periodontal ligament of human primary teeth. *J Dent Res* 1999; **78**: 681–689.

28. Kimoto S, Matsuzawa M, Matsubara S, Komatsu T, Uchimura N, Kawase T, *et al*. Cytokine secretion of periodontal ligament fibroblasts derived from human deciduous teeth: effect of mechanical stress on the secretion of transforming growth factor-beta 1 and macrophage colony stimulating factor. *J Periodontal Res* 1999; **34**: 235–243.

29. Sato T, Iida J, Kurihara S. A histological investigation on the periodontal tissue changes during molar depression in rats. *J Jpn Orthod Soc* 1984; **46**: 361–372.

30. Proffit WR. The biological basis of orthodontic therapy. In: Rudolph P (Ed.) *Contemporary Orthodontics*. London: Mosby, 1999: 309.