Tooth movement and changes in periodontal tissue in response to orthodontic force in rats vary depending on the time of day the force is applied

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SUMMARY The purpose of this study was to investigate whether there are any differences in tooth movement or in the response of periodontal tissue to orthodontic force when the force is applied at different times of the day. One hundred 6-week-old male Wistar rats were divided into one control group without force application and three experimental groups based on the time of day the force was applied to the upper first molars. Animals in the whole-day group received force continuously throughout the experimental period, while animals in the light- and dark-period groups received force only during the light (07:00–19:00) or dark period (19:00–07:00), respectively. Tooth movement was measured using the occlusal view of a precise plaster model with a profile projector. Periodontal tissues were evaluated histologically.

The time course of tooth movement varied among the groups. Tooth movement over 21 days in the whole-day and light-period groups was about twice that as in the dark-period group. The formation of new bone on the tension side in the whole-day and light-period groups was more than twice that as in the dark-period group. On the pressure side, more osteoclasts appeared on the alveolar bone in the whole-day and light-period groups than in the dark-period group. The light-period group showed less extensive hyalinization of the periodontal ligament (PDL) than the whole-day group. The area of root resorption on day 21 also varied among the groups. Interference by masticatory forces did not seem to be a principal cause of the decreased tooth movement in the dark-period group. These results indicate that there are considerable variations in tooth movement and in the response of periodontal tissue to orthodontic force when the force is applied at different times of the day in rats. The results suggest that diurnal rhythms in bone metabolism have important implications in orthodontic treatment.

Introduction

Diurnal variation is an essential feature of bone physiology. Various bone metabolic markers including plasma calcium and phosphate (Milhaud et al., 1972; Shinoda and Seto, 1989), calciotropic hormones (Jubiz et al., 1972; Hirsch and Hagaman, 1982), osteocalcin (Gundberg et al., 1985; Greenspan et al., 1997), and serum and urinary markers of collagen formation and degradation (Hassager et al., 1992; Bollen et al., 1996) have been shown to fluctuate according to diurnal rhythms. Diurnal variations have also been found in various activities related to bone formation and bone resorption, such as the proliferation of osteogenic cells (Oudet and Petrovic, 1978; Roberts et al., 1979, 1984; Simmons, 1992), matrix formation (Simmons, 1992; Saeki, 1995), osteoclast-bone surface contact (Simmons et al., 1988), prostanoid secretion in bone (Yosipovitch et al., 1995), and local enzyme activities (Petrovic et al., 1984; Stutzmann and...
Petrovic, 1984). Since biological responses to orthodontic treatment inevitably include mechanically-induced bone modelling and remodelling, it is reasonable to assume that these changes in response to orthodontic external forces may vary depending on the time of day such forces are applied.

A diurnal variation in tooth movement in response to orthodontic force in rats was recently demonstrated (Igarashi et al., 1998). More tooth movement was achieved in rats where orthodontic force was applied during the day than in rats which received the force during the night. However, that study was only preliminary, and the cause and cellular mechanisms which induce this variation were not clarified. The effects of the timing of the application of force on the integrity of periodontal tissues are also not known. In orthodontic treatment, since teeth must be moved safely as well as efficiently, it is important to compare the possible adverse effects of various types of force application. With these questions in mind, the present study was undertaken to evaluate in detail whether there are any differences in the response of periodontal tissue to orthodontic force, as well as in tooth movement, when the force is applied at different times of the day.

Materials and methods

Experimental design

A total of 100 male Wistar rats (Japan SLC Co., Shizuoka, Japan), 6 weeks old and weighing an average of 117 g, were used in this study. The animals were adapted to a 12/12-hour light/dark cycle (with light from 07:00 to 19:00) for 2 weeks in a room at 25°C and 55 per cent humidity. They were fed a laboratory diet (Funabashi Farms Co., Funabashi, Japan) and de-ionized water ad libitum.

A uniform standardized expansive spring, made of 0.012-inch nickel-titanium wire (Rocky Mountain Morita Corp., Tokyo, Japan), was placed in each animal’s mouth between the right and left upper first molars. The spring initially generated an average expansive force of 165 mN on each side and was retained in the mouth by its own expansive force. The method has been described previously in detail (Igarashi et al., 1994).

Forty animals were used for both the measurement of tooth movement and histological examinations. Thirty of these 40 animals were divided into three experimental groups (10 animals in each group) based on the time of day the force was applied. Animals in the whole-day group received force continuously throughout the experimental period of 21 days, while animals in the light- and dark-period groups received force only during the light (07:00–19:00) or dark period (19:00–07:00), respectively. The other 10 animals served as a control group and were maintained for 21 days without force application. For the animals in the light- and dark-period groups, the appliances were set or removed at 07:00 and 19:00 every day under light ether anaesthesia. The animals in the control group and whole-day group received the anaesthetic at 07:00 and 19:00 every day. The body weights and food intake of the animals were monitored throughout the experimental period. After the experiment, the animals were killed for histological examinations on day 21.

Fifty animals were used only for the histological examinations. Forty-five of the 50 animals were divided into the whole-day, light- and dark-period groups (15 animals in each group) as described above. The animals in each group were killed on days 3, 7, and 14 (five animals, respectively) after the application of force. The other five animals served as a day 0 control and were killed on day 0.

In a separate experiment, 10 animals were used. Since rats consume most of their daily food during the dark period, and since masticatory forces could interfere with tooth movement induced by orthodontic force, the effect of masticatory mechanical forces on tooth movement during the dark period were examined. In this experiment, the force was applied to all of the animals only during the dark period (19:00–07:00), but half of them were fed powdered food containing the same components as ordinary solid food. The grain size of the powdered food was smaller than 0.75 mm in diameter. Tooth movement over 21 days was compared between the two groups.
The animals were treated ethically following the guidelines for the use of experimental animals promulgated by the Animal Care and Use Committee of Tohoku University Graduate School of Dentistry.

Measurement of tooth movement

In the experimental groups, an expansive spring was placed in each animal's mouth, and the right and left upper first molars were moved buccally. The expansive force was applied without adjustment during the experimental period. Movement of the upper first molars was measured on days 0, 1, 3, 7, 10, 14, 17, and 21 after the application of the expansive force according to a previously described method (Adachi et al., 1994). Briefly, a precise impression of the upper jaw was taken with silicone materials immediately after removal of the spring. A tracing of the occlusal view of a precise plaster model of the right and left upper jaw was then magnified ×10 with a profile projector. The contours of the palatal cusps of the second and third molars of these tracings were then superimposed on those of the second and third molars on tracings from a pre-treatment plaster model. The distance between the crests of the mesiopalatal cusps of the first molars before and after tooth movement was measured with sliding callipers. Values for the right and left first molars were added together for each animal. The error of the measurement was 0.01 mm when 20 randomly selected samples were measured twice by a single investigator in a blind test. Errors were calculated as $E = \sqrt{\Sigma d^2/2n}$ ($E$: Error; $d$: difference between two measurements; $n$: number of samples).

Histological procedure

During the experiment, the rats were injected with 4 mg Pb/kg of nitrilotriacetato lead (NTA-Pb) at 7-day intervals (on days 0, 7, 14, and/or 21) to label the bone chronologically (Asoda et al., 1982; Shinoda and Okada, 1988), and killed 6 hours after the last injection. After the experiment, the animals were killed under pentobarbital anaesthesia and the maxillary bones, including the molars, were dissected. The specimens were fixed with 4 per cent paraformaldehyde in 0.01 mol/l phosphate buffer (pH 7.4) for 3 days at 4°C, and then decalcified in 10 per cent EDTA solution with 5 per cent Na$_2$S for 45 days at room temperature. They were then dehydrated in a graded series of ethanol and embedded in paraffin. Periodontal tissues of the mesiobuccal root of the upper first molar were examined with a light microscope in serial cross-sections of the molars at a bifurcation level. Sections (7 µm-thick) were first gold-plated with 0.1 per cent HAuCl$_4$ to make the lead-labelled lines visible, and then stained with haematoxylin and eosin. This method has been described in detail previously (Igarashi et al., 1998).

Evaluation of new bone formation, hyalinization and root resorption (Figure 1)

The mesiobuccal root area was divided into a pressure and a tension side based on the mesio-distal axis of the root. The formation of new bone was evaluated by measuring the area between the first and most recent lead-labelled lines on the tension side. The extent of

![Figure 1](image-url)
hyalinization of periodontal ligament (PDL, surface length of the root facing the hyalinized PDL) and root-resorptive area were measured on the pressure side of the root. The former was expressed as a percentage of the entire surface length of the root on the pressure side. Hyalinization was defined as a homogeneous cell-free degenerative change of PDL. These measurements were made by analysing a microscopic image that was fed directly to a high-resolution monitor with a CCD video camera. Either NIH-image version 1.60b7/fat or Win ROOF version 3.04 (Mitani Co., Japan) was used for the image analyses. The values on the right and left sides in each section were first averaged. Values for five sections, which were selected at three-section intervals, were then averaged for each animal. The errors of the measurements were determined to be \(0.046 \times 10^4\) \(\text{mm}^2\) for the areas and 3.89 per cent for the extent of hyalinization. They were calculated using the same formula as in the measurement of tooth movement.

**Count of osteoclasts**

The osteoclasts on the alveolar bone surface were counted on the pressure side of the mesiobuccal roots of the upper first molar. Osteoclasts were defined as multi-nucleated eosinophilic cells on the bone surface or in bone-resorptive lacunae. The number of osteoclasts on the right and left sides in each section was first averaged. Values for five sections, which were selected at three-section intervals, were then averaged for each animal. The error of the measurement was determined to be 0.04 per area.

**Statistics**

The data were subjected to either an analysis of variance (ANOVA) followed by Tukey’s test or an unpaired t-test. \(P < 0.05\) was considered a significant difference.

**Results**

There were no significant differences in either body weight or food intake among the four groups during the experimental period. The animals in all four groups consumed an average of 92 per cent (ranging from 84 to 99 per cent) of their daily food during the dark period.

Figure 2 shows the time course of tooth movement in animals in the three experimental groups. There were no differences in tooth movement within the first 3 days among the three groups. Thereafter, the time course of tooth movement varied among the groups. Tooth movement in the whole-day group exhibited three typical phases, i.e., a phase of initial rapid movement, a lag phase, and a phase of progressive movement. On the other hand, tooth movement in the light-period group continued to increase without an apparent lag period until day 21, and was significantly greater than that even in the whole-day group on days 7, 10, and 14. In contrast, tooth movement in the dark-period group almost stopped after day 10, and was significantly less than that in the light-period group on days 7, 10, 14, 17, and 21. Tooth movement on day 21 in the light-period group was about twice that as in the dark-period group. There was no significant difference between the light-period group and the whole-day group. Tooth movement in the control group, which received no force application, was \(0.02 \pm 0.01\) mm.

![Figure 2](image-url)
Mean tooth movement (mean ± SEM) on day 21 in animals which received the force only during the dark period and were fed normal solid food and in animals which received the same force application but were fed powdered food was 0.58 ± 0.08 and 0.65 ± 0.09 mm, respectively. There was no significant difference between the two groups.

Chronological bone labelling with NTA-Pb revealed that the formation of new bone occurred on the tension side in the three experimental groups, and continued to increase during the experimental period (Figures 3 and 4). However, significantly less bone was formed in the dark-period group than in the whole-day and light-period groups. The mean bone-formative area at 21 days in the light-period group was more than twice that as in the dark-period group. There was no difference between the light-period group and the whole-day group.

Figure 5 shows the time course of changes in the number of osteoclasts on the pressure side in the three experimental groups. The patterns in the three groups were similar. However, the number of osteoclasts in the dark-period group was less than in the light-period and whole-day groups throughout the experimental period. There was no significant difference in either the
pattern or number of osteoclasts between the light-period group and the whole-day group. The details of the time-course changes in the number of osteoclasts together with histological changes (Figure 6) in these groups were as follows. On day 0, there were almost no osteoclasts on the alveolar bone surface. The number of osteoclasts started to increase after the application of force. On day 3, hyalinization of the PDL, which is usually followed by active undermining bone resorption, was observed on the pressure side in the whole-day and light-period groups, and several osteoclasts appeared on the periphery of degenerative PDLs in these groups. In the dark-period group, however, hyalinization of the PDL was not as evident as in the whole-day and light-period groups, and only a few osteoclasts were present on the bone surface. On day 7, hyalinization of the PDL became far-reaching, and subsequent undermining alveolar bone resorption with many osteoclasts in large bone-resorptive lacunae appeared in the whole-day and light-period groups. Furthermore, consistent with the greater tooth movement in the light-period group than in the whole-day group from day 7 to 14 (Figure 2), hyalinization of the PDL was less extensive in the light-period group than in the whole-day group (Figure 7). In contrast to the above findings in the whole-day and light-period groups, undermining bone

**Figure 5** Time course of changes in the number of osteoclasts on the pressure side of the mesiobuccal roots of the upper first molar in rats in the three experimental groups during the 3-week experimental period. Each point represents the mean ± SEM (n = 5–7). $P < 0.001$ for treatment and $P < 0.001$ for time by two-way ANOVA. **$P < 0.01$** versus the light-period group by Tukey’s test.

**Figure 6** Photomicrographs of the pressure side of the mesiobuccal root of the upper first molars in rats in the three experimental groups 7 days after initiation of the application of force. (A) Whole-day group. (B) Light-period group. (C) Dark-period group. Bar = 50 μm (original magnification, ×200). Arrowheads: bone resorptive lacunae. *Hyalinization of the PDL.
resorption was limited, and direct bone resorption with several small resorptive lacunae was predominant in the dark-period group, indicating less bone resorption than in the whole-day and light-period groups. For all three groups, the number of osteoclasts reached a maximum on day 7 and decreased thereafter. On day 14, elimination of the hyalinized tissue by phagocytic cells along with active bone resorption was observed in the whole-day and light-period groups. On day 21, bone resorption was almost complete and the PDL was being regenerated in these two groups. On these days, appreciable histological differences were not observed between the whole-day and light-period groups. In the dark-period group, however, no histological features, which would indicate active bone resorption, were evident after day 14.

After day 7, resorption of the cementum and dentine was noted on the root surface on the pressure side in all three experimental groups. Figure 8 shows the total area of root resorption on the pressure side in the three groups. The amount of root resorption varied among the groups. The dark-period group showed significantly less root resorption than the whole-day and light-period groups.

The histological features on day 21 in the control group, which did not receive force application, were entirely different from those in the animals that received force application. Although the tooth movement in the control group was negligible in the bucco-palatal direction, significant bone modelling changes appeared, probably due to physiological movement of molars in the distal direction. Namely, moderate bone formation occurred on the mesial side of the PDL, while several bone resorptive lacunae were observed on the distal side. These changes were not observed in the experimental groups. Therefore, the histological results in the control group were not compared with those in the experimental groups.

The differences in tooth movement and in the response of periodontal tissue to orthodontic force among the three experimental groups are summarized in Table 1.

Discussion

The present results demonstrate that the response of periodontal tissues to orthodontic force varies with the time of day the force is applied. Histomorphometric examinations revealed that
new bone formation on the tension side in the light-period group was more than twice that of the dark-period group, and more osteoclasts appeared on the pressure side in the light-period group than in the dark-period group during the experimental period. On day 21, tooth movement in the light-period group was about twice that as in the dark-period group. Since spontaneous physiological tooth movement in the bucco-palatal direction during the experimental period was negligible, these values indicate actual tooth movement induced by orthodontic force. Therefore, the tooth movement in response to orthodontic force in rats that received orthodontic force during the light period was about twice that as in the rats that received force during the dark period.

There is considerable evidence to show that both bone formation and bone resorption are more active in the environmental light period than in the environmental dark period in rats. Various events such as the proliferation of osteogenic cells (Oudet and Petrovic, 1978; Roberts et al., 1979, 1984), matrix formation (Simmons, 1992; Saeki, 1995), calcium release from bone (Shinoda and Stern, 1992), and osteoclast-bone surface contact (Simmons et al., 1988) are maximal during daytime, when the animal is resting, and minimal at night, when it is active. In the present study, both the amount of new bone formed on the tension side and the number of osteoclasts on the pressure side were greater in the light-period group than in the dark-period group. This implies that periodontal tissues respond more when orthodontic force is applied during the light period, in which both physiological bone formation and bone resorption are active.

Like other diurnal rhythmic phenomena in various physiological processes, skeletal diurnal rhythms have been suggested to be under endocrine control. For example, it has been shown that in rats, rhythms of DNA and collagen synthesis in bone and cartilage depend on serum corticosterone and parathyroid hormone, respectively (Simmons, 1992). In humans, the rhythm of serum osteocalcin level is controlled by serum cortisol (Nielsen et al., 1992). Thus, rhythms of cell proliferation and matrix formation in bone and cartilage appear to be regulated by these hormones. Recently, it has been shown that diurnal rhythms of serum bone-resorbing activity in both rats and humans are controlled by undefined serum factor(s) (Shinoda and Stern, 1992; Lakatos et al., 1995). Therefore, the observed variations in tissue response and tooth movement may also be caused by hormonal rhythms.

In the present experiments, teeth were subjected to masticatory forces, as well as orthodontic force. Since masticatory forces could interfere with tooth movement induced by orthodontic force, the diurnal variation in masticatory function may have influenced the results. In fact, the masticatory activities of the animals were greater during the dark period than the light period. However, even though masticatory forces were decreased by feeding the animals powdered food (Kiliaridis and Shyu, 1988), there was no significant increase in tooth movement as compared with animals fed normal solid food. Therefore, the diurnal variation in

<table>
<thead>
<tr>
<th>Groups</th>
<th>Tooth movement (day 21)</th>
<th>Bone formation (day 21)</th>
<th>Osteoclasts (day 7)</th>
<th>Hyalinization (day 7)</th>
<th>Root resorption (day 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole-day</td>
<td>100</td>
<td>100</td>
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<tr>
<td>Light-period</td>
<td>97</td>
<td>96</td>
<td>90</td>
<td>79</td>
<td>84</td>
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<tr>
<td>Dark-period</td>
<td>46</td>
<td>42</td>
<td>65</td>
<td>11</td>
<td>61</td>
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Each value represents percentage of the mean value to that of the whole-day group.
masticatory activity is not likely to be a principal cause of the observed diurnal variation in tooth movement.

In the present study, there was no significant difference in either new bone formation on the tension side, the number of osteoclasts on the pressure side, or total tooth movement between the light-period group and the whole-day group. This indicates that the intermittent application of force for 12 hours (07:00–19:00) is as effective as continuous force application. Furthermore, the present results clearly demonstrate that the course of tooth movement as well as the amount of movement varies depending on the time of force application. As shown in Figure 2, the course of tooth movement in the light-period group had no apparent lag phase, in contrast to the whole-day group. In addition, tooth movement in the light-period group was significantly greater than that in the whole-day group on days 7, 10, and 14. These results are supported by the histological finding, which showed less extensive hyalinization of the PDL in the light-period group than in the whole-day group. It is generally accepted that the degeneration of periodontal tissues, such as hyalinization of the PDL, should be minimized in orthodontic tooth movement, and that intermittent forces would have a less adverse effect on periodontal tissue and local circulation than continuous forces (Stutzmann and Petrovic, 1984; Reitan and Rygh, 1994). Therefore, at least under the present experimental conditions, the intermittent application of force during the light period resulted in the most satisfactory tooth movement among the present three treatment regimens (Table 1). The above results suggest that more effective tooth movement with less damage to periodontal tissues could be achieved by applying orthodontic force at certain times of day, probably during the resting time of animals.

Conclusions

The present study demonstrated that there are considerable variations in the response of periodontal tissue to orthodontic force and in the resultant tooth movement when the force is applied at different times of the day in rats. If this is also true in humans, these variations should be taken into consideration when planning treatment. The results suggest that diurnal rhythms in bone metabolism have important implications in orthodontic treatment.

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Acknowledgements

This study was supported in part by a Grant-in-Aid for Scientific Research (B) from the Ministry of Education, Science, Sports, and Culture of Japan (No. 09470465).

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