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Administration of osteocalcin accelerates orthodontic tooth movement induced by a closed coil spring in rats

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SUMMARY The effect of local administration of osteocalcin (OC) on experimental tooth movement was examined in the rat. The maxillary first molar was first moved mesially with an initial tipping force of 30 g with a closed-coil spring anchored to the incisor for 10 days (n = 48). Three experimental groups (n = 8) were injected with purified rat OC at doses of 0.1, 1, and 10 μg, respectively. The injection into the palatal bifurcation site of the first molar was repeated daily. The control groups (n = 8) were injected with rat serum albumin (10 μg), phosphate buffered saline (PBS), or were not injected. Tooth movement was evaluated daily by measuring the inter-cuspal distance between the first and the second molars on a precise plaster model.

The cumulative tooth movement (mm) in the 1-μg OC-injected groups was significantly more than that in all of the control groups on day 9. The rate of tooth movement (mm/day) showed periodical elevation, with high values on days 1, 4, 7, and 9. Acceleration of tooth movement by OC was significant in the early experimental period. Subsequently, acceleration of early tooth movement by OC was histologically evaluated (n = 40). Each of four animals from the control (PBS, n = 20) and the experimental (1 μg OC, n = 20) groups was killed daily up to 5 days. A significantly larger number of osteoclasts accumulated on the mesial alveolar bone surface in the 1-μg OC-injected group on day 3 than that observed in control group. These results suggest that administration of OC accelerates orthodontic tooth movement due to enhancement of osteoclastogenesis on the pressure side, primarily in the early experimental period.

Introduction

Osteocalcin (OC) is the most abundant non-collagenous matrix protein in bone. It is composed of 46–50 amino acids, containing 2–3 γ-carboxyglutamic residues. Because of its strong capacity to associate with Ca^{2+} and hydroxyapatite, OC is a negative regulator for mineral apposition and bone formation (Hauschka *et al*., 1975; Price *et al*., 1976; Poser *et al*., 1980; Ducy *et al*., 1996). In addition, OC is proposed to be a chemo-attractant for progenitor/mature osteoclasts, based on the following findings:

(1) OC-deficient bone particles obtained from warfarin-treated rats are not degraded to the same extent as normal bone particles when they are implanted subcutaneously in rats (DeFranco *et al*., 1991);
(2) hydroxyapatite particles pre-treated with OC stimulate the appearance of tartrate-resistant acid phosphatase (TRAP)-positive multinuclear cells around the particle (Glowacki and Lian, 1987; Glowacki *et al*., 1991);
(3) OC has been shown to be a chemotactic for osteoclast precursor cells (Malone *et al*., 1982; Mundy and Poser, 1983) and osteoclast-like cells (Chenu *et al*., 1994).

Previously, the effect of topical administration of OC on experimental tooth movement

produced by placing a piece of elastic band between the first and second upper molar teeth in the rat, the so called 'Waldo's method' (Waldo and Rothblatt, 1954) was examined. It was found that pharmacological doses of OC had an additive effect on tooth movement (Kobayashi *et al*., 1998). Since the effect was well correlated to the augmentation of osteoclasts on the alveolar bone in the pressure side, it was strongly suggested that the OC promoted the recruitment of osteoclasts into the local bone remodelling site. Because the elasticity of the inserted elastic band was lost after 4 days, the observation could not be extended for longer than that period. Additionally, the method does not allow for light continuous force to avoid trauma to the periodontal tissues. Previously, the method of King *et al*. (1991) that used a closed-coil spring to obtain a light continuous force of sufficient duration was modified, and the behaviour of bone cells involved in orthodontic tooth movement was observed (Hashimoto *et al*., 1997, 1998; Kobayashi *et al*., 2000). Therefore, the aim of this experiment was to elucidate whether administration of OC continuously accelerates tooth movement due to the enhancement of osteoclastogenesis for an extended duration (10 days).

Materials and methods

Animals

A total of 88 male Wistar rats, 5 weeks old and with an average body weight of 125 ± 9.44 g (mean ± SD), were used in this study. They were fed ground laboratory chow (Oriental Yeast Co., Ltd. Japan), given tap water *ad libitum*, and housed in a room maintained at 25°C with a standard 12-hour light/dark cycle during the experimental period. The rats were weighed every day. All animal treatment procedures were ethically performed in compliance with Nagasaki University regulations.

Appliance

The maxillary right first molar was moved mesially as described previously (Hashimoto *et al*., 1997, 1998). An Elgiloy closed coil spring (0.0056 × 0.022-inch No. 462–71, Rocky Mountain Morita Co., Tokyo, Japan) with an average length of 5.45 ± 0.13 mm (mean ± SD, n = 88) was inserted between the maxillary incisor and the right first molar under ether anaesthesia. To prevent detachment of the appliance, small cavities were made on the labial cervical surface of the incisors and also on the buccal and palatal surface of the first molar. One end of the spring was fixed to the molar with a clamp, which distorted a Co–Cr wire (0.016-inch, Green Elgiloy, Rocky Mountain Morita Co.) into a triangular form while the other was ligated to the incisor using a ligature wire. The initial activation of the spring was set at 0.74 mm, corresponding to a contractile force of 30 g. Figure 1 shows the force-activation relationship which was initially determined using a dial calliper (Mitsutoyo Co., Ltd., Tokyo, Japan) with a minimal graduation of 20 μm and a tension gauge (Tomy International Co., Tokyo, Japan). The initial force of 30 g was chosen to obtain the maximal effect for rat molar tooth movement according to a previous report (King *et al*., 1991).

Effect of OC on tooth movement

Forty-eight rats were divided into six groups of eight rats each. The rats in the three

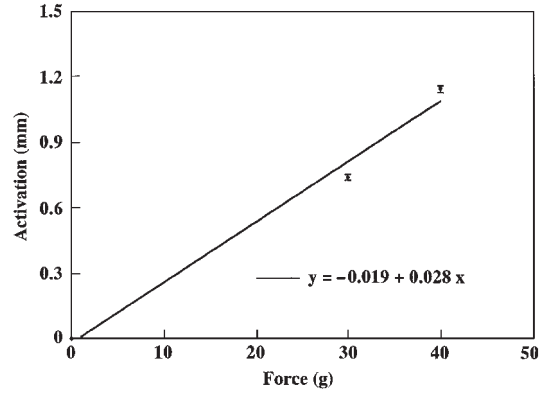

Figure 1 Force-activation relationship of the closed coil spring. Each spring was stretched at 30 and 40 g, respectively. The activation (mm) was determined by subtracting the initial length from the stretched length. Each value and vertical bar represents the mean ± SEM, n = 88. The spring was set at initial activation of 0.74 mm, corresponding to 30 g.

experimental groups were injected with purified rat OC (Kobayashi *et al*., 1998) at doses of 0.1, 1, or 10 μg dissolved in 20 μl of phosphate buffered saline (PBS), respectively. The rats in two of the three control groups were injected with rat serum albumin 10 μg in 20 μl of PBS, with equivalent molarity to 1 μg of OC in 20 μl of PBS, and with 20 μl of PBS alone. These solutions were first passed through a 0.22-μm filter to sterilize them. In the third control group, the injection was omitted to evaluate whether any effects were caused by the injection itself. Each solution was injected in the palatal subperiosteum adjacent to the furcation of the maxillary right first molar using a micro-syringe with a 27-gauge needle under ether anaesthesia, as described previously (Kobayashi *et al*., 1998). The first administration was given on the day that the orthodontic appliance was inserted and repeated daily until day 9.

A precise plaster model of the maxilla was prepared from an impression, made with silicone material (Exaflex, injection type, GC Dental Industrial Co., Tokyo, Japan) and dental stone (New Fujirock, GC Dental Industrial Co.). The setting expansion of the plaster according to the manufacturer's literature is 0.08 per cent. An impression was taken daily with an individual resin tray under light ether anaesthesia. Under a stereoscopic microscope, the distance between the anterocone of the first molar and the metacone of the second molar on each plaster model was measured with a dial calliper as described previously (Kobayashi *et al*., 1998). The individuals examining the plaster models were not informed as to which group was being evaluated. The increase of the inter-cuspal distance (mm) during the experimental period was defined as the amount of tooth movement. The error of measurement was found to be 0.024 mm when a single investigator measured 20 plaster models replicated from a single dry skull. Error was calculated using the formula Error = $(\sum d^2/2n)^{1/2}$, where d is the difference for each measurement from the mean value, and n = 20 (Houston, 1983).

The statistical differences among groups for the data at each time-point were evaluated using one-way analysis of variance (ANOVA). Fisher's protected least significant difference (PLSD) was used to identify differences between groups when ANOVA indicated that a significant difference ($P < 0.05$) existed.

Histological examinations

Forty rats treated with the orthodontic apparatus described above were divided into two groups of 20 rats each. One group was injected with 1 μg of OC in 20 μl of PBS and the other with 20 μl of PBS for 5 days. Since, in the previous experiment, no differences in tooth movement were observed among the three control groups, only a PBS-injected group was used as a control in this histological examination. Four rats from each group were killed daily for 5 days after the start of the application of orthodontic force. The dissected maxilla was fixed with periodate-lysine-paraformaldehyde solution (0.01 M $NaIO_4$, 0.037 M PBS, 2 per cent paraformaldehyde, pH 7.2) at 4°C for 24 hours, followed by decalcification with 10 per cent ethylenediaminetetraacetic acid (EDTA)/15 per cent glycerol (pH 7.2) for 2 weeks at 4°C. The sample was then dehydrated in a graded series of ethanol and embedded in paraffin. The left maxillary dentition on day 1 from each group served as the sample without application of orthodontic force. Twenty serial horizontal sections, each 6 μm thick, were obtained from the level of the first molar root furcation. Every fifth section in the series was stained for tartrate-resistant acid phosphatase (TRAP) as described previously (Hashimoto *et al*., 1997). The sections were counterstained with toluidine blue. Some of the other sections were stained with hematoxylin-eosin to examine infiltration of inflammatory cells such as lymphocytes or polymorphonuclear cells. The sections were observed with a light microscope (Olympus BH-2, Tokyo, Japan).

In this experiment, osteoclasts were defined as TRAP-positive multinuclear cells on the bone surface. In a few sections, not only multinuclear cells but also mononuclear cells with strong TRAP activity on the bone surface were included in the count, since it was highly possible that cells in these sections were part of large multinuclear cells present on the bone. On a

vertical plane to the mesio-distal axis, the area around the mesio-palatal root of the maxillary first molar was divided into mesial and distal halves, and osteoclasts (TRAP-positive cells) in the mesial half were counted. The values in four sections were averaged for each rat.

The statistical differences between the OC-injected and the PBS-injected groups for the data at each time-point were evaluated using an unpaired Student's *t*-test ($P < 0.05$).

Results

Effect of local OC on animal condition

Throughout the experimental period, the local application of OC did not affect the growth of the rats (Figure 2). Moreover, the injection caused no apparent macroscopic inflammation of the mucosa at the site of injection (data not shown).

Effect of local OC on experimental tooth movement

Table 1 shows the cumulative amount of tooth movement on each day during the experimental period. In all groups, the distance between the anterocone of the first molar and the metacone of the second molar gradually increased during the experimental period. There were no significant differences among the three control groups throughout the experimental period, including the groups injected with rat serum albumin and without injection. On the other hand, the groups injected with OC doses of 0.1 and 1 μg showed greater tooth movement than the controls. Notably, tooth movement in the group injected with 1 μg of OC was significantly greater compared with the values in all control groups in the period from day 4 to 9 ($P < 0.05$ one-way ANOVA and Fisher's PLSD). In the group injected with 0.1 μg of OC, the values showed significant differences compared with some control values from day 3 to 10. Interestingly, the group injected with 10 μg of OC showed no significant differences from the controls except on day 7. On day 10, the amount of tooth movement in the rats injected with 0.1, 1, and 10 μg of OC was 147, 152, and 121 per cent, respectively, of the value in the group injected with PBS.

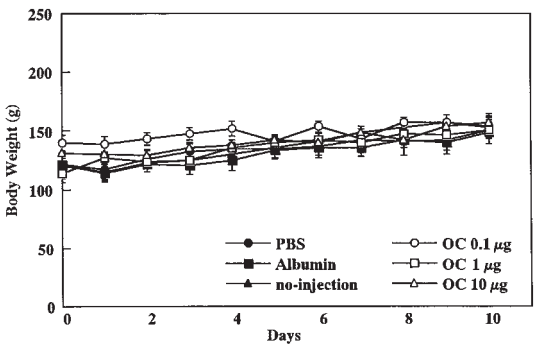

Figure 2 Mean body weight in each group. Each value and vertical bar represents the mean ± SEM, $n = 8$.

To further characterize the effect of OC on tooth movement, the changes in the rate of tooth movement (mm/day) was also determined. The pattern of the change in each group was periodical, but consistent for all groups, showing a high value on days 1, 4, 7, and 9 (Figure 3). The groups injected with OC showed a greater rate of tooth movement than the control groups in the early experimental period, although the differences were not statistically significant until day 3. On day 4, at the second peak of the rate, the values in the groups injected with 0.1 and 1 μg of OC reached their maxima. At this time-point, the group injected with 1 μg of OC showed a significantly higher rate compared with those in all control groups ($P < 0.05$ one-way ANOVA and Fisher's PLSD). In the subsequent period (from day 5 to 10), the effect of OC administration was not apparent and the OC-treated groups showed nearly the same levels as those in the control groups. On day 7, the value in the PBS-injected group was significantly lower when compared with those in all five other groups.

Kinetics of appearance of osteoclasts on pressure side

Figure 4 shows the microscopic appearance of the periodontal tissue of the mesio-palatal root of the right first molar, before the application of force. Numerous TRAP-positive osteoclasts

Table 1 Amount (mm) of tooth movement.

	Day				
	1	2	3	4	5
Control groups					
PBS	0.049 ± 0.017	0.074 ± 0.029	0.163 ± 0.032	0.177 ± 0.045	0.220 ± 0.041
Albumin	0.043 ± 0.022	0.069 ± 0.030	0.109 ± 0.021	0.129 ± 0.045	0.171 ± 0.030
No-Injection	0.051 ± 0.032	0.080 ± 0.048	0.091 ± 0.041	0.149 ± 0.050	0.213 ± 0.047
OC injected groups					
OC 0.1μg	0.109 ± 0.023	0.183 ± 0.033	0.240 ± 0.040[b,c]	0.280 ± 00.40[b,c]	0.337 ± 00.51[b]
OC 1μg	0.103 ± 0.045	0.146 ± 0.050[b]	0.243 ± 0.056[b,c]	0.329 ± 0.057[a,b,c]	0.347 ± 0.051[a,b,c,d]
OC 10μg	0.080 ± 0.031	0.126 ± 0.032	0.149 ± 0.037	0.217 ± 0.034	0.249 ± 0.033
	Day				
	6	7	8	9	10
Control groups					
PBS	0.286 ± 0.041	0.233 ± 0.065	0.303 ± 0.056	0.349 ± 0.060	0.394 ± 0.045
Albumin	0.226 ± 0.030	0.291 ± 0.028	0.351 ± 0.048	0.343 ± 0.037	0.443 ± 0.028
No-Injection	0.269 ± 0.046	0.309 ± 0.033	0.363 ± 0.037	0.406 ± 0.044	0.409 ± 0.022
OC injected groups					
OC 0.1μg	0.377 ± 0.057[b]	0.437 ± 0.063[a,b]	0.480 ± 0.052[a]	0.543 ± 0.060[a,b]	0.580 ± 0.062[a,c]
OC 1μg	0.437 ± 0.043[a,b,c,d]	0.477 ± 0.050[a,b,c]	0.520 ± 0.046[a,b,c]	0.574 ± 0.058[a,b,c]	0.600 ± 0.077[a,c]
OC 10μg	0.280 ± 0.065	0.383 ± 0.044[a]	0.434 ± 0.054	0.463 ± 0.063	0.477 ± 0.073

Each value (mm) represents the mean ± SEM, $n = 8$. When analysis of variance (ANOVA) indicated significant differences among the groups. Fisher's protected least significant difference (Fisher's PLSD) was employed to examine the difference between each pair of groups ($P < 0.05$). [a]Significantly higher than PBS-injected group; [b]significantly higher than albumin-injected group; [c]significantly higher than no-injection group; [d]significantly higher than 10 μg of OC-injected group.

were found on the distal alveolar bone surface (filled arrowheads in panel A). In contrast, few osteoclasts were found on the mesial side. Instead, many osteoblasts with abundant cytoplasm were aligned on the mesial alveolar bone surface (open arrowheads in panel B). These histological features demonstrated that bone resorption occurred on the distal side, while bone formation occurred on the mesial side, due to physiological distal movement of the first molar, the so-called distal drift (Sicher and Weinmann, 1944; Vignery and Baron, 1980).

When the molar was moved mesially by the orthodontic force, the remodelling phase of the alveolar bone around the tooth was markedly changed. Figure 5 shows the changes in the mesial periodontal tissue after application of orthodontic force for 1 and 3 days. Compression of the periodontal ligament (PDL) was found in these sections, but obvious necrosis or hyalinization of the PDL could not be observed in either the PBS- or OC-injected (1 μg) rats. Additionally, there was no difference between the control and OC-injected groups for infiltration of inflammatory cells such as polymorphonuclear cells or lymphocytes, as examined in sections stained with haematoxylin-eosin (data not shown). On day 1, a few TRAP-positive cells appeared on the alveolar bone surface in the OC-injected group (panel A). The cells positive for TRAP were frequently found in the sections obtained on day 3 (panel B). Most TRAP-positive cells on the bone surface were multinuclear, with typical osteoclastic features as shown at higher magnification (panel C). Identical findings were seen in the PBS-injected group (panels D, E, and F). These histological observations suggest that the area was predominantly bone resorptive.

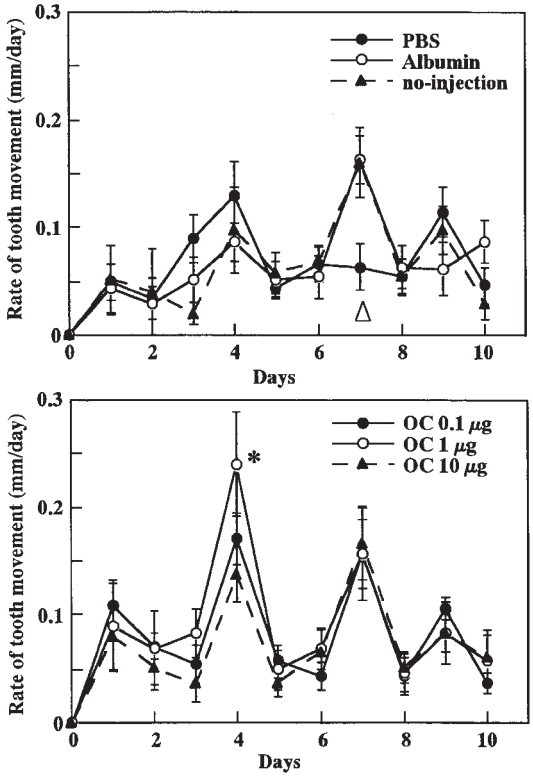

Figure 3 Rate of tooth movement during the experimental period. The rate of tooth movement was defined as the increase of the inter-cuspal length per day (mm/day). Each value and vertical bar represent the mean ± SEM, $n = 8$. The asterisk (*) indicates a significant difference compared with all control groups by ANOVA and Fisher's PLSD ($P < 0.05$). The open arrowhead indicates a significant difference compared to all other groups by ANOVA and Fisher's PLSD ($P < 0.05$).

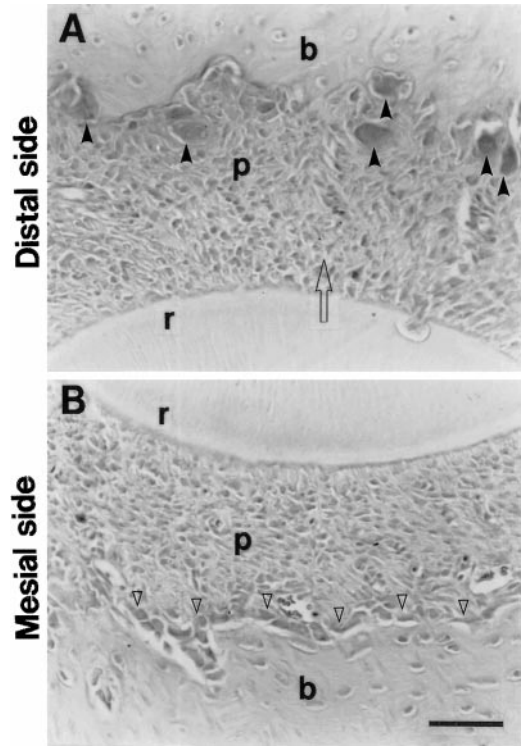

Figure 4 Microscopic appearance of the periodontal tissue around the mesio-palatal root of the maxillary first molar before application of orthodontic force on the distal side (A) and the mesial side (B). Numerous TRAP-positive cells (arrowheads) were found on the distal alveolar bone surface. In contrast, cuboidal osteoblasts (open arrowheads), instead of TRAP-positive cells, tightly aligned on the mesial alveolar bone surface. r, Root; p, periodontal ligament; b, alveolar bone. Open arrow indicates the direction of the physiological tooth movement (distal drift). Bar = 50 μm.

The OC-injected rats showed a greater number of osteoclasts on the mesial alveolar bone surface compared with the control rats (Figure 6). In the control rats, the number of osteoclasts was increased from 4.3 ± 3.2 (mean ± SE) on day 1 to 10 ± 1.8 on day 2, and remained nearly stable on subsequent days. On the other hand, the number in the OC-injected rats increased from 6.5 ± 1.8 on day 1, to 16.3 ± 0.3 on day 3. Unpaired Student's *t*-test ($P < 0.05$) indicated that the number of osteoclasts in the OC-injected rats was significantly higher than in the control rats on day 3. In both groups, the number of osteoclasts decreased on day 4.

The effect of OC in the augmentation of osteoclasts was limited to the pressure side. As shown in Figure 7, no TRAP-positive osteoclasts were detected in the distal alveolar bone surface (tension side) after application of orthodontic force for 3 days, in both OC-injected and control rats. At the same site, numerous cuboidal osteoblasts were found on the bone surface.

Discussion

Recently, it was reported that topical administration of OC accelerated tooth movement for 4 days following elastic band insertion between

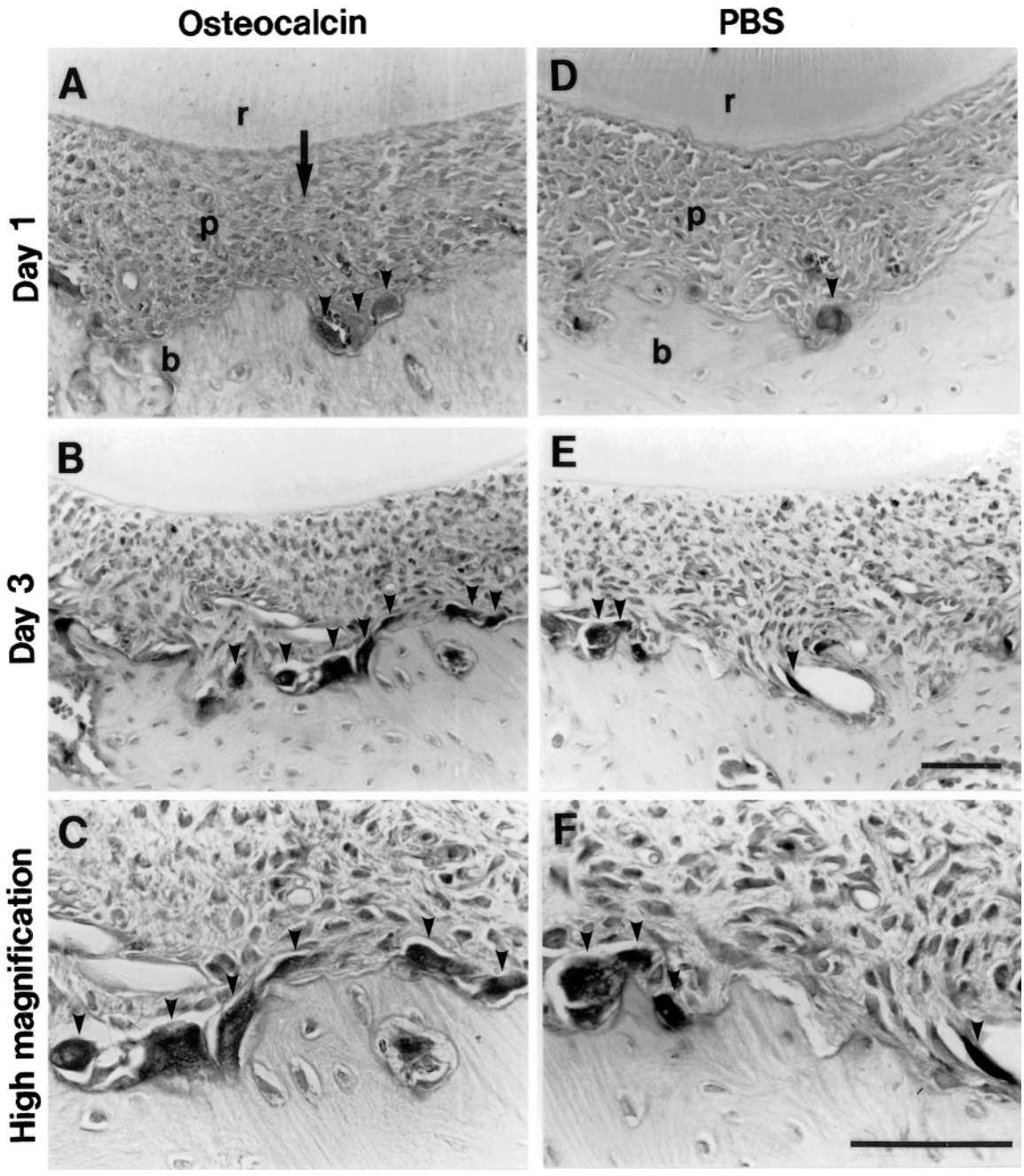

Figure 5 Histological changes in the mesial periodontal tissue after application of orthodontic force. (A, B) Appearance in OC-injected group on days 1 and 3, respectively. (C) Higher magnification of B. (D, E) Appearance in PBS-injected (control) group on days 1 and 3, respectively. (F) Higher magnification of E. A few TRAP-positive cells (arrowheads) appeared on day 1, and the number was increased in both groups on day 3. Arrow indicates the direction of tooth movement. r, Root; p, periodontal ligament; b, alveolar bone. Bar = 50 μm.

the first and second molar teeth, the so-called Waldo's method (Kobayashi *et al*., 1998). The method was convenient to investigate the nature of orthodontic tooth movement; however, the orthodontic force exerted by the elastic band was intermittent for only a short period and did not allow precise force control. Hence, in the present investigation, the coil-spring method was adopted to obtain a continuous precise initial force for orthodontic tooth movement during a significantly long duration (King *et al*., 1991; Hashimoto *et al*., 1997). The results demonstrate that the local application of OC accelerates orthodontic tooth movement induced by a coil spring for 10 days. In addition, when 1 μg OC was injected daily, the maximum amount of tooth movement observed is consistent with previous findings (Kobayashi *et al*., 1998). The effect of OC does not seem to be a secondary effect induced by the injection itself or by the injection of protein, since no significant difference was found among the control groups. Thus, these studies suggest that OC may be an effective agent for facilitating orthodontic tooth movement regardless of the orthodontic force systems used, e.g. intermittent or continuous force.

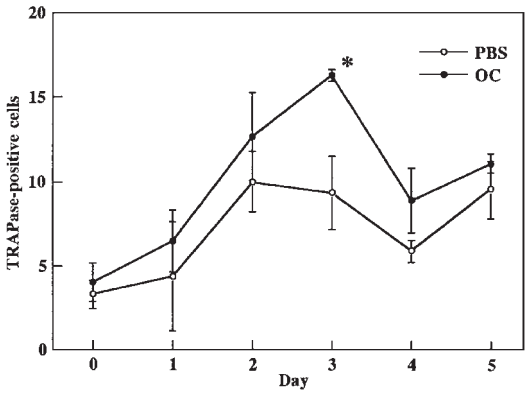

Figure 6 Changes in the number of TRAP-positive cells on the mesial alveolar bone surface of the mesio-palatal root of the maxillary first molar. The number of TRAP-positive cells was counted in the mesial half as described in the materials and methods section. Each value represents the mean ± SEM, $n = 4$. The asterisk (*) indicates that a significant difference from the control group was identified by unpaired t-test ($P < 0.05$).

In the rate of tooth movement (mm/day), the pattern of the change in each group was periodic, showing a high value on days 1, 4, 7, and 9 (Figure 3). This is considered to be attributable to the sequential process of tooth movement as follows:

(1) the deformation of the PDL reflects an initial movement;

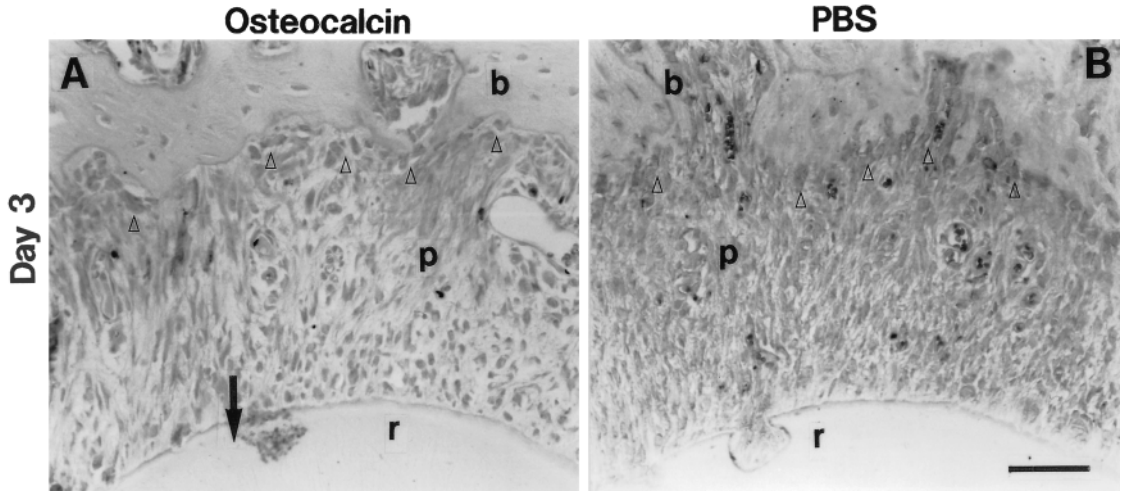

Figure 7 Distal periodontal tissue of the mesio-palatal root on day 3 after application of orthodontic force. A, OC-injected group. B, PBS-injected (control) group. TRAP-positive cells have disappeared and cuboidal osteoblasts (open arrowheads) lined on the alveolar bone surface in both groups. r, Root; p, periodontal ligament; b, alveolar bone. Arrow indicates the direction of orthodontic tooth movement. Bar = 50 μm.

(2) the establishment of conditions enabling tissue remodelling and modelling reflects a delay in movement;
(3) a rapid movement occurs associated with tissue turnover until reduction of the applied force occurs (King *et al*., 1991).

In this experiment, OC-injected groups showed a greater tooth movement rate than the control groups in the early experimental period. On day 4, at the second peak of the movement rate, the values in the groups injected with 0.1 and 1 μg of OC reached their maxima.

To assess the effect in the early phase of tooth movement, the kinetics of the appearance of TRAP-positive cells, putative osteoclasts and their precursor cells were examined (Baron *et al*., 1986; Marks and Grolman, 1987) on the pressure side. The number of TRAP-positive cells on the mesial alveolar bone surface in the OC-injected groups reached its maximum and was significantly higher than that in the control group on day 3, which was 1 day before the maximum rate of tooth movement occurred. These results suggest that OC has an effect on induction of initial bone resorption, specifically on the appearance of osteoclasts in the future bone resorbing site, in the tooth movement process, since OC has been shown to be a chemoattractant for osteoclast precursor cells (Malone *et al*., 1982; Mundy and Poser, 1983) and osteoclast like cells (Chenu *et al*., 1994). In contrast to the early phase of the experiment, OC did not show an additive effect on the rate of tooth movement at days 7 and 9. Previously, it has been reported that the chemotactic activity of OC for human peripheral monocytes, osteoclast precursor cells, showed a bell-shaped dose-response curve. In addition, the serum OC level increased in the later phase of tooth movement in rats (King and Keeling, 1995). It is considered that the increase of serum OC associated with tooth movement may diminish the effect of exogenous OC in the later period of the present study. In spite of the reduction of the effect in the later period, total tooth movement in the OC injected groups was markedly augmented by the end of the experiment. Conversely, the application of OC only in the early period may be sufficient to accelerate tooth movement. Assuming that a single administration of OC in the early period of tooth movement is effective, therapeutic use of OC may be advantageous to the patient and the orthodontist.

Interestingly, the additive effect of OC on the appearance of TRAP-positive cells was limited to the pressure side in this orthodontic tooth movement model. As shown in Figure 7, no TRAP-positive osteoclasts were detected in the distal alveolar bone surface (tension side) after application of orthodontic force for 3 days in the OC-injected and control rats. At this site, numerous cuboidal osteoblasts were found on the bone surface, indicating that this side showed bone formation activity. It has previously been reported that an identical change in the tension side was observed using immunohistochemical and histochemical methods using the same tooth movement model (Hashimoto *et al*., 1997, 1998; Kobayashi *et al*., 2000). Thus, it is suggested that new bone formation in the tension side coincident to orthodontic tooth movement may not be affected by local application of pharmacological doses of OC. Previously, expression of transforming growth factor (TGF)-β was found to be markedly enhanced on the tension side (Nagai *et al*., 1999; Kobayashi *et al*., 2000). TGF-β had a negative role for osteoclastogenesis and such a factor produced in the tension side may minimize the effect of OC. Applied OC may have promoted the osteoclastogenesis in co-operation with other cytokines in the pressure side. In fact, OC has been shown to significantly enhance the formation of TRAP-positive multinuclear osteoclast-like cells in the presence of macrophage colony-stimulation factor (M-CSF) and granulocyte-macrophage colony-stimulating factor using the *in vitro* murine bone marrow culture system, but not in the absence of these factors (Liggett *et al*., 1994). M-CSF has been shown to be a crucial factor in osteoclastogenesis. No osteoclasts could be detected in M-CSF deficient mice (Wiktor-Jedrzejczak *et al*., 1982; Yoshida *et al*., 1990) and osteoclastogenesis was restored by the administration of M-CSF (Felix *et al*., 1990; Kodama *et al*., 1991). Thus, in the early phase of tooth movement, OC might possibly act as a chemoattractant for osteoclast

precursor cells and as an enhancer of maturation of multinuclear osteoclasts on the pressure side, where bone resorbing factors are present, giving rise to an appropriate micro-environment for osteoclastogenesis.

Conclusions

The local application of osteocalcin accelerated the rate of tooth movement (mm/day) in the early experimental period and increased the total amount of tooth movement. Histological examination revealed that this acceleration of tooth movement was caused by enhanced recruitment of osteoclasts.

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