Effects of local administration of osteocalcin on experimental tooth movement

Yasuhiro Kobayashi, DDS, DDSc; Hiroshi Takagi, DDS, DDSc; Hideaki Sakai, DDS, DDSc; Fumio Hashimoto, DDS, DDSc; Shiro Mataki, DDS, DDSc; Kazuhide Kobayashi DDS, DDSc; Yuzo Kato, DDS, DDSc

In orthodontic treatment, teeth are rearranged in response to applied mechanical forces that cause remodeling of the periodontal tissues.[1-3] When orthodontic force is applied to a tooth, the alveolar bone on the pressure side undergoes successive cycles of bone resorption and formation, while bone on the tension side predominantly undergoes continuous bone formation. Bone resorption is induced by a complex process. It implies the recruitment of osteoclast precursor cells to future resorption sites in which they differentiate into mature osteoclasts and adhere to the matrix. This is followed by the organization of the sealing zone and ruffled border. Various hormonal and local factors, including parathyroid hormone (PTH),[4] 1,25-dihydroxy vitamin D_3,[5] interleukin-1 (IL-1),[6] interleukin-6 (IL-6),[7] and prostaglandins,[8] are involved in this process.

In addition to these factors, some bone matrix proteins may also play an important role in bone remodeling.[9] For example, osteopontin and bone sialoprotein support the cell-matrix interactions of osteoclasts and may influence their activity.[10] Some investigators have reported that osteocalcin, also called Bone Gla Protein (BGP), shows chemotactic activity for human cells in the osteoclast lineage and rat osteosarcoma (ROS) cells in *in vitro* studies.[11-13] Additionally, osteocalcin-deficient bone particles from warfarin-treated rats are shown to be degraded to a lesser extent than normal bone particles when they are implanted subcutaneously into rats.[14] The subsequent experiments with hydroxyapatite particles revealed that pretreatment with osteocalcin stimulated the appearance of tartrate-resistant acid phosphatase (TRAPase)-positive multinuclear cells around these particles.[15] These

Abstract

The purpose of this study was to evaluate the effects of local administration of osteocalcin, a major noncollagenous bone matrix protein, on experimental tooth movement in rats. An orthodontic elastic band was inserted between the upper first and second molars, and the first molar was moved mesially. Purified osteocalcin (0 to 10 μg) in 20 μl of phosphate-buffered saline was injected into the region of the root bifurcation of the first molar daily for 4 days. Tooth movement increased significantly following the injections. Histological studies revealed that the injections markedly stimulated the appearance of osteoclasts on the pressured side of the alveolar bone surface. The results suggest that osteocalcin has an additive effect on the rate of orthodontic tooth movement through the enhancement of osteoclastogenesis on the pressured side.

Key Words

Osteocalcin • Tooth movement • Osteoclast recruitment

Submitted: August 1996 **Revised and accepted:** February 1997 Angle Orthod 1998;68(3):259-266.

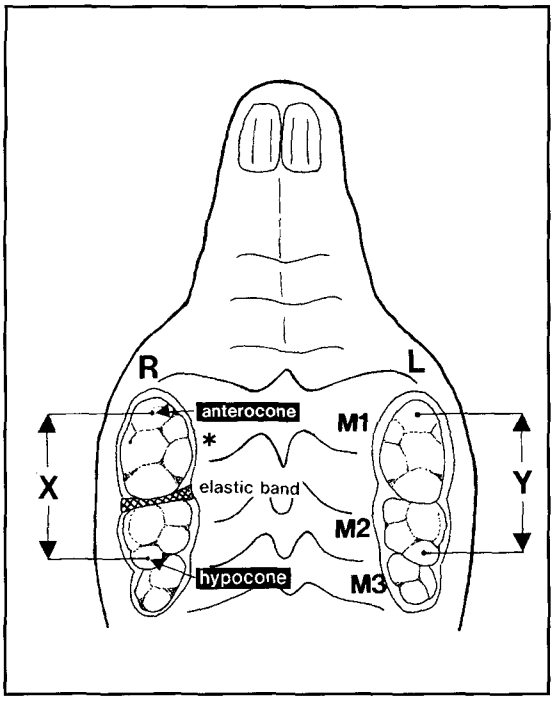

Figure 1
An orthodontic elastic band was inserted between the maxillary first and second molars on the right side. The asterisk indicates the site of injection. X and Y indicate the distances between the anterocone of the first molar and the hypocone of the second molar on the right side and the left side, respectively. These distances were measured on each plaster replica. The amount of tooth movement was obtained by subtracting the Y value from the X value.

Figure 1

reports suggest the possibility that osteocalcin functions as a matrix signal in the recruitment and differentiation of osteoclasts. However, the precise role of osteocalcin on the active bone remodeling process still needs to be elucidated.

In this study we used Waldo's rat experimental tooth movement model in which an elastic band is inserted between the maxillary first and the second molars.[1] We examined the effect of local administration of osteocalcin on the mesial movement of the first molar. Since the maxillary first molar undergoes physiological distal drift, the remodeling phase of the alveolar bone surface in the tooth socket drastically changes upon insertion of the elastic band, from resorption to formation on the distal side, and from formation to resorption on the mesial. Local administration of factors that modulate bone remodeling is expected to significantly influence tooth movement. The effects of the local administration of prostaglandin E and 1,25-dihydroxy vitamin D_3 on tooth movement and the remodeling of alveolar bone in experimental tooth movement models have been reported elsewhere.[22,23,25]

Materials and methods

Purification of rat osteocalcin

Rat osteocalcin was purified from formic acid extracts of rat cortical bone according to the procedure described by Hale,[16] with some modifications. Final purification was performed by high-performance liquid chromatography (HPLC) using a reversed phase C_{18} column (μ-Bondasphere, 19 mm x 300 mm, Waters Millipore Inc, Tokyo, Japan) with a linear gradient of 0-60% (v/v) acetonitrile in 0.1% trifluoroacetic acid. The purity of osteocalcin was analyzed by a reverse phase HPLC-photo diode array system (Japan Spectroscopic Co Ltd, Tokyo, Japan) on a C_{18} reverse phase analytical column (μ-Bondasphere, 5 mm x 150 mm). Peak detection was performed by measuring absorbency in the wavelength range between 195 and 650 nm. Furthermore, the N-terminal amino acid sequence of the purified protein was confirmed by automated Edman degradation (477A protein sequencer, Applied Biosystems Inc, Foster City, Calif). No detectable contamination with fatty acids, sugars, or collagen fragments was observed.

Experimental tooth movement

Thirty-three 5-week-old male Wistar rats, average body weight 159.2±7.0g (mean ± SD) were divided into 5 groups. The control group (n=9) received 20 μl phosphate-buffered saline (PBS) via injection; the 4 experimental groups (n=6 in each group) were injected with 0.01 μg, 0.1 μg, 1 μg and 10 μg osteocalcin/20 μl PBS, respectively. The rat osteocalcin was resolved in PBS at the desired concentration and sterilized with a 0.22 μm filter (Millipore). Twenty microliters of the solution was injected into the submucosal palatal area corresponding to the root furcation of the maxillary right first molar using a microsyringe (27-gauge) under ether anesthesia. The injection was repeated once a day from day 0 to day 3.

Orthodontic elastic bands (No 404-136, Unitek/3M, Monrovia, Calif) were randomly chosen, and their thicknesses were measured using an ocular micrometer with a minimal graduation of 10 μm under a microscope. An elastic band with an average thickness of 590 ± 31 (SD) μm was stretched and inserted in the interproximal space between the first and second molars of the right maxilla for 4 days according to the method outlined by Waldo and Rothblatt.[1] The elastic bands were checked every 12 hours under light ether anesthesia, for a total of 7 checks during the experimental period. About 3% of the bands fell out during each 12-hour period. In this event, a new band was inserted between the teeth.

The rats were treated in compliance with the ethics regulations of Nagasaki University. A standard diet for rodents (Oriental Yeast Co, Ltd, Japan) and water were provided *ad libitum*. The rats were housed in a room exposed to a natural light cycle and weighed every day during the experimental periods. Before the rats were sacrificed, blood samples were collected for chemical analyses. Total calcium content was

measured by an atomic absorption spectrophotometer.[17] Inorganic phosphorous content was measured by the ascorbic acid method.[18]

Measurement of tooth movement

To measure tooth movement, a plaster replica of the maxilla, including the molars, was made for each rat. The distance between the anterocone of the first molar and the hypocone of the second molar was measured on the cast under a stereoscope using dial calipers (Mitsutoyo Co, Ltd, Japan) with a minimal graduation of 0.02 mm. The amount of tooth movement was calculated by subtracting the intercanine distance on the control side (Y-value) from the distance on the experimental side (X-value), as shown in Figure 1. In order to improve the reliability of data, all the plaster replicas were coded and stored. Thus each plaster model was selected randomly.

Histochemical examinations

The animals were sacrificed by cardiac puncture under ether anesthesia on day 4. The maxilla was dissected out and fixed in a periodate lysine paraformaldehyde solution at 4°C for 6 hours. It was washed in PBS containing increasing amounts of glycerol, up to 15%, and then demineralized in 10% ethylenediaminetetraacetic acid (EDTA)/15% glycerol (pH 7.2) at 4°C for 4 weeks. The demineralized samples were washed in the same buffer containing increasing amounts of sucrose, up to 20%, and decreasing amounts of glycerol, to 0%. They were embedded in OCT compound. Forty transverse serial cryo-sections (6 μm) were obtained at the level of the first molar furcation to a depth of 240 μm by Cryostat (Frigocut-E, Reichert-Jung Co Ltd, Germany). Eight sections at 30 μm intervals were analyzed for TRAPase activity by Fast garnet GBC with naphtol AS-BI as a substrate in the presence of 50 mM L(+)-tartaric acid. The sections were then counterstained with 0.5% toluidine blue and observed under a light microscope (Axiophoto, Carl Zeiss Co, Ltd, Germany). The other sections were processed either for nonspecific acid phosphatase (ACPase) in which tartrate was omitted, or for hematoxylin-eosin staining. The mesiobuccal root area of the first molar was divided in half, one half being the pressure side and the other the tension side, in accordance with the direction of tooth movement. The number of osteoclasts on the alveolar bone surface on the pressure side was counted in each section. Osteoclasts were defined as TRAPase-positive multinuclear cells on the bone surface. The values from eight sections were averaged for each rat.

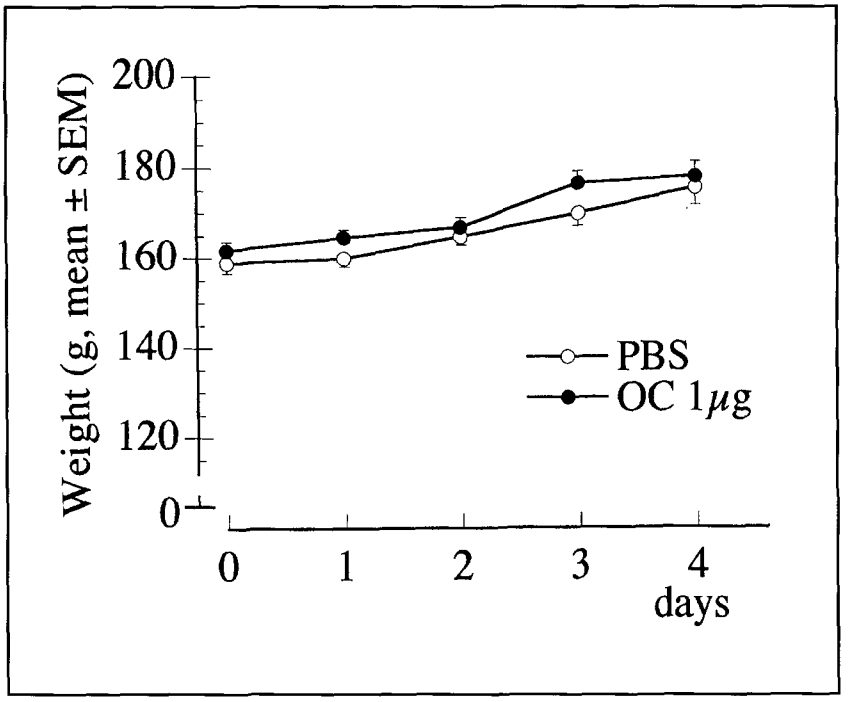

Figure 2

Contact microradiogram (CMR)

In order to evaluate the systemic effects of osteocalcin on the remodeling of limb bone, contact microradiograms (CMR) of the tibia were prepared. Briefly, the proximal parts of the tibiae were dissected and fixed as described above. These tibiae were dehydrated in ethanol and embedded in polyester resin. Ground sections (60 μm in thickness) of each tibia were made, and then the CMR of each tibia was obtained using Kodak 649-0 film with a soft X-ray generator (SOFRON SRO-M50, SOFRON Co, Ltd, Japan).

Statistical analysis

Values are expressed as mean ± standard error of the mean (SEM). Statistical differences among groups were evaluated using one-way analysis of variance (ANOVA). Fisher's protected least significant difference (Fisher's PLSD) was used to identify differences between groups when ANOVA indicated that a significant difference ($p<0.05$) existed.

Results

Animal condition

Throughout the experimental period, there were no significant differences in body weight between the control and the osteocalcin-injected rats. Body weight changes of the control and the 1 μg osteocalcin-injected rats are shown in Figure 2. The daily administration of osteocalcin or PBS caused no appreciable macroscopic changes in the mucosa at the site of injection during the experimental period (data not shown). To evalu-

Figure 2
Changes in body weight during the experimental period. There was no significant difference between the PBS-injected control (open circle) and 1 μg osteocalcin-injected (closed circle) rats during the experimental period. Values represent the mean ± SEM.

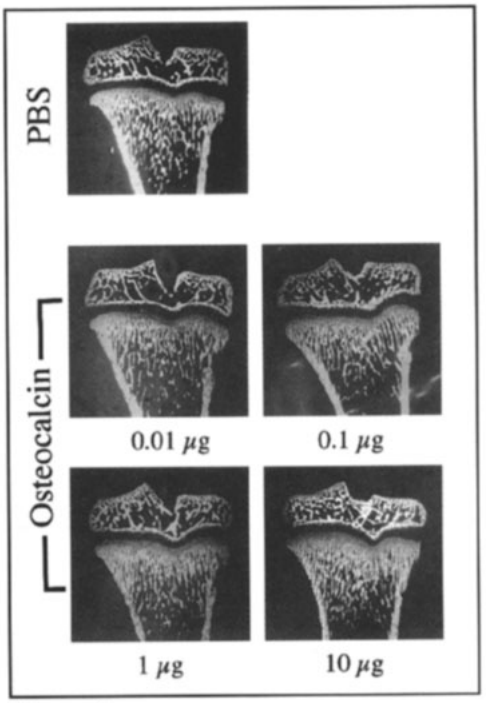

Figure 3

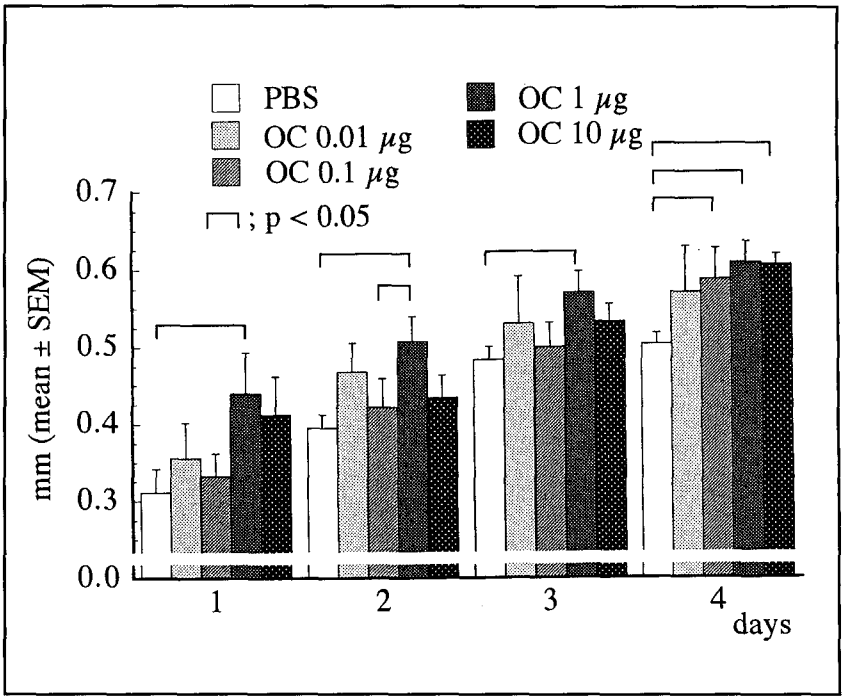

Figure 4

Table 1
Effect of osteocalcin on total calcium and inorganic phosphorous content in plasma

	n	Ca (mg/dl) mean ± SEM	Pi (mg/dl) mean ± SEM
PBS	9	10.8 ± 0.7	11.4 ± 0.7
OC 10 ng	6	10.9 ± 0.6	10.6 ± 0.5
OC 100 ng	6	11.2 ± 0.6	11.3 ± 0.7
OC 1 μg	6	11.3 ± 0.5	11.6 ± 0.9
OC 10 μg	6	10.6 ± 0.5	11.8 ± 1.3

Serum was collected before the rats were sacrificed.
PBS: phosphate-buffered saline. OC:osteocalcin.
No significant difference in Ca and Pi content between pairs of these groups.

Figure 3
Contact microradiograms (CMR) of the proximal part of the tibia in PBS-injected control and osteocalcin-injected rats. There was no marked difference between the groups in the trabecular bone mass or in the thickness of the growth plate.

Figure 4
Changes in the cumulative amount of tooth movement (mm). The amount of tooth movement was evaluated by measuring the intercuspal distance between the first and the second molars on the precise plaster replica of the maxilla of each rat. Values represent the mean ± SEM, n=9 for control and n=6 for each experimental group. ⊓ $p<0.05$: significant difference between the two groups (by one-way ANOVA and Fisher's PLSD).

ate the effects of osteocalcin injections on systemic bone growth, CMR of the proximal part of the tibiae were obtained. There were no apparent differences in the structure of cortical bone, cancellous bone, primary spongiosa, or growth plate of the tibia between the control and the osteocalcin-injected rats (Figure 3). Moreover, the osteocalcin injections did not affect the total calcium or inorganic phosphorus content of the plasma during the experimental period (Table 1).

Tooth movement

One day after insertion of the elastic band between the first and second molars, 1 μg of osteocalcin caused a significant increase in tooth movement, as shown in Figure 4. The 1 μg osteocalcin-injected group showed significant tooth movement throughout the observation period ($p<0.05$). In the 10 μg osteocalcin-injected group, tooth movement tended to increase, but the increase was statistically insignificant up to day 3. Repeated administration of osteocalcin (0.1 to 10 μg) resulted in significant tooth movement on day 4 ($p<0.05$). The maximal amount of tooth movement was observed in the 1 μg osteocalcin-injected group.

Histological observation

In order to determine whether the additive effect of osteocalcin on tooth movement was related to the enhancement of osteoclast recruitment and/or differentiation on the alveolar bone surface, we evaluated the number of osteoclasts by counting TRAPase-positive multinuclear cells. TRAPase is one of the best charac-

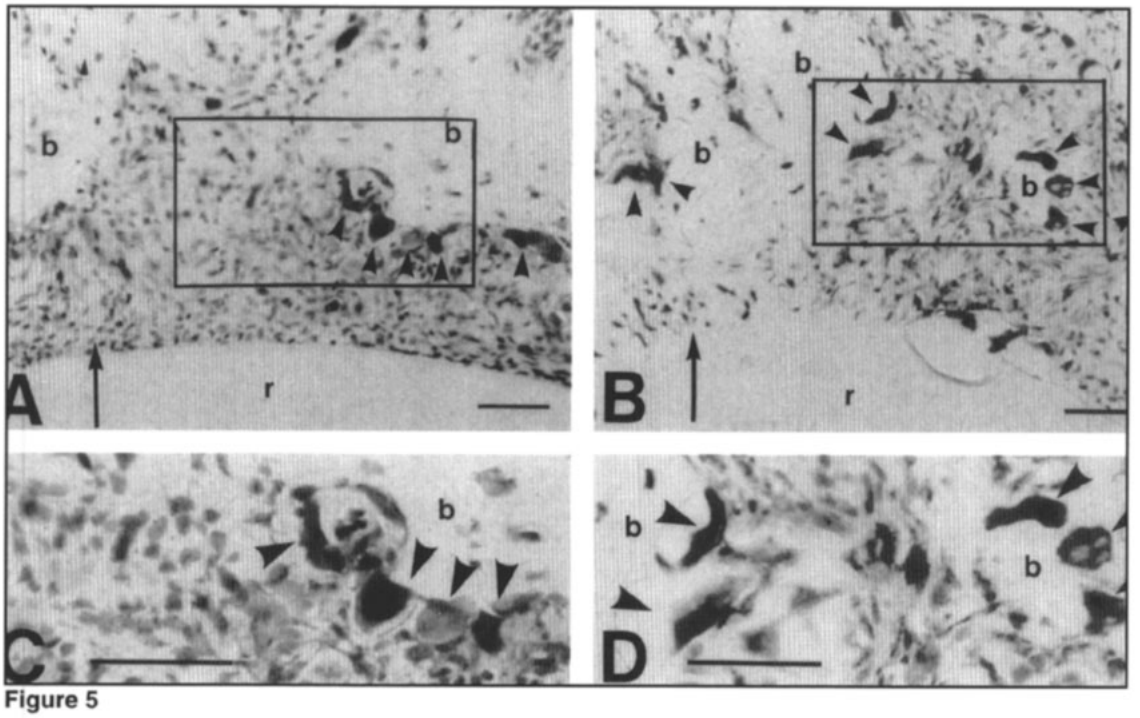

Figure 5

Figure 5A-D
Periodontal tissues on the pressured side of the mesiobuccal root of maxillary first molars 4 days after elastic band insertion in a control rat (A), and a 1 μg osteocalcin-injected rat (B). C and D show higher magnification of the boxed areas (bars = 50 μm.) Many TRAPase-positive multinuclear cells were found on the alveolar bone surface of osteocalcin-injected rats compared with the controls. Pointers indicate TRAPase-positive multinuclear cells. Large arrows indicate direction of tooth movement. b=alveolar bone; r=mesiobuccal root of maxillary first molar.

terized and requisite criteria for identifying osteoclasts.[19,20] In the 1 μg osteocalcin-injected rats, numerous osteoclasts were found on the alveolar bone surface of the pressure-side tooth socket and in the adjacent marrow space, whereas fewer osteoclasts were observed on the alveolar bone surface of the pressure-side for the control rats (Figure 5). The increase in the number of osteoclasts in the osteocalcin-injected groups (0.1 to 10 μg) was significant (Figure 6). Under this experimental condition, the highest number of osteoclasts was observed in the rats administered 1 μg osteocalcin/day, in accordance with the greatest effect on tooth movement.

Discussion

This is the first time that topical administration of osteocalcin has been shown to stimulate the appearance of osteoclasts in orthodontic tooth movement. It is likely that the increased number of osteoclasts caused tooth movement to accelerate. A maximal effect was observed when 1 μg of osteocalcin in 20 μl PBS, corresponding to 8.3 μM, was applied daily. At the higher dose of osteocalcin, 10 μg in 20 μl PBS, decreased tooth movement was observed, as well as fewer osteoclasts. Such a bell-shaped dose-response is comparable to the chemotactic activity of osteocalcin to human osteoclast-like cells[21] or to human peripheral monocytes.[11] Using elastic bands, we could extend the experimental period no longer than 4 days because of the decay of elasticity thereafter. Hence, at this time, we have no evi-

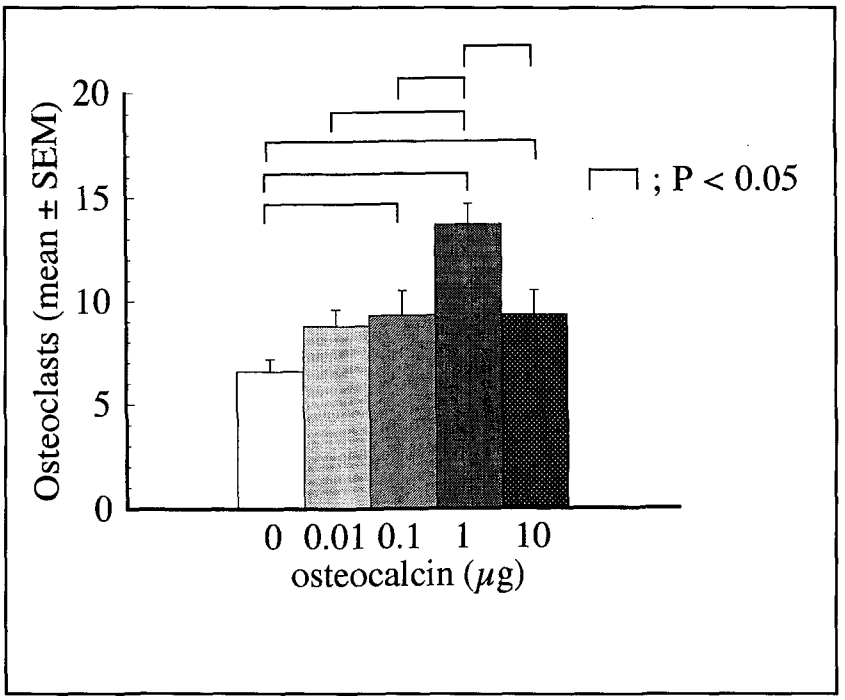

Figure 6
The number of osteoclasts on the pressured side of the mesiobuccal root of the maxillary first molars of the control and osteocalcin-injected groups. Values for 8 sections were averaged for each rat and represented as mean ± SEM, n=9 for control and n=6 for each experimental group. ⊓ $p<0.05$: significant difference between the two groups (by one-way ANOVA and Fisher's PLSD).

dence as to whether osteocalcin exerts an acceleration effect on tooth movement when mechanical force is applied to the tooth for more than 4 days.

The effects of osteocalcin did not seem to be secondary effects of inflammation or immunological reaction evoked by the protein injection; we observed no difference between the control and osteocalcin-injected rats in the infiltration of inflammatory cells, such as polymorphonuclear cells or lymphocytes in sections stained with hematoxylin-eosin (data not shown). The effects of osteocalcin on cells may be mediated by a putative cell surface receptor molecule, since osteocalcin can induce a rise in intracellular calcium levels via release of calcium from thapsigargin-sensitive calcium stores in cells.[21] However, the identity of the receptor molecule for osteocalcin is not known and needs to be elucidated.

Local administration of 1,25-dihydroxy vitamin D_3, or prostaglandin E has also been shown to accelerate experimental tooth movement.[22,23] The effects of these chemicals on osteoclast recruitment are different from that of osteocalcin. Both chemicals have been shown to act as potent inducers of bone resorption *in vitro* and *in vivo*.[24] Repeated injections of prostaglandin E1 in rat alveolar bone have been shown to produce marked changes in bone morphology with increased resorption at the site where the drug was infiltrated.[25] Administration of 1,25-dihydroxy vitamin D_3 has been reported to induce enlarged osteoclasts on the bone surface.[26,27] In contrast, local administration of osteocalcin itself had no appreciable effect on intact bone surface. We noted that osteocalcin induced mature osteoclasts on the pressure side only, not on the tension side, during orthodontic tooth movement. Although the injected osteocalcin can act as a chemoattractant for osteoclast precursors at sites where the protein is released, the microenvironment on the pressure side is also important for the maturation of osteoclasts into the mature phenotype with TRAPase-activity and multinuclei. Using the *in vitro* murine long-term bone marrow culture system, Liggett et al. demonstrated that osteocalcin itself has little or no effect on the formation of TRAPase-positive multinuclear osteoclast-like cells. While osteocalcin significantly enhances osteoclast-like cell formation in the presence of macrophage colony stimulating factor (M-CSF) and granulocyte/macrophage colony stimulating factor (GM-CSF) in the same system,[28] these CSFs are known to be produced by various cell types, including fibroblasts[29] and osteoblasts,[30,31] and their production is greatly stimulated by IL-1α and β and tumor necrosis factor alpha (TNFα) in fibroblasts.[29,32,33] Since IL-1α and β are produced on the pressure side in response to orthodontic force,[34] CSF may also be produced on the pressure side as a result of increased IL-1. Therefore, it is likely that osteocalcin promoted the osteoclastogensis in concert with the CSF.

Therapeutic use of osteocalcin can be considered in clinical orthodontic treatment so that the effects of the protein limit the local bone remodeling. Mundy et al. reported that the COOH-terminal penta peptide derived from bovine osteocalcin is a chemoattractant for cells in osteoclastic lineage. If such a peptide possesses the capacity to support osteoclast maturation to the same extent as does mature protein, then the use of this peptide would be practical for orthodontic therapy.

Conclusions

The local administration of rat osteocalcin stimulated the appearance of osteoclasts on the alveolar bone surface of the pressured side during orthodontic tooth movement in rats. This resulted in increased tooth movement.

Acknowledgment

The authors thank Dr. N. Yoshida (Nagasaki University) for his comments on this manuscript. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan

Author Address

Yasuhiro Kobayashi
Department of Orthodontics
Nagasaki University School of Dentistry
1-7-1 Sakamoto, Nagasaki 852-8588 Japan.
Telephone: +81-95-849-7669
Fax: +81-95-849-7670
E-mail: h-sakai@net.nagasaki-u.ac.jp

Y. Kobayashi, assistant professor, Department of Orthodontics, Nagasaki University School of Dentistry.

H. Takagi, orthodontist, Takagi Dental Office, Omura, Nagasaki.

H. Sakai, associate professor, Department of Pharmacology, Nagasaki University School of Dentistry.

F. Hashimoto, research fellow, Department of Orthodontics, Nagasaki University School of Dentistry.

S. Mataki, associate professor, Department of Oral Diagnosis and General Dentistry, Faculty of Dentistry, Tokyo Medical and Dental University.

K. Kobayashi, professor, Department of Orthodontics, Nagasaki University School of Dentistry.

Y. Kato, professor, Department of Pharmacology, Nagasaki University School of Dentistry.

References

1. Waldo CM, Rothblatt JM. Histologic response to tooth movement in the laboratory rat. Procedure and preliminary observations. J Dent Res 1954;33:481-486.
2. Storey E. The nature of tooth movement. Am J Orthod 1973;63:292-314.
3. King GJ, Theims S. Chemical mediation of bone resorption induced by tooth movement in the rat. Arch Oral Biol 1979;24:811-815.
4. Raisz LG, Trummel CL, Simmons H. Induction of bone resorption in tissue culture: prolonged response after brief exposure to parathyroid hormone or 25-hydroxycholecalciferol. Endocrinology 1972;90:744-751.
5. Raisz LG, Trummel CL, Holick MF, DeLuca HF. 1,25 Dihydroxycholecalciferol: a potent stimulator of bone resorption in tissue culture. Science 1972;175: 768-769.
6. Heath JK, Saklatvala J, Meikle MC, Atkinson SJ, Reynolds JT. Pig Interleukin-1 (catabolin) is a potent stimulator of bone resorption in vitro. Calcif Tissue Int 1985;37: 95-97.
7. Ishimi Y, Miyaura C, Jin CH, Akatu T, Abe T, Nakamura Y, et al. IL-6 is produced by osteoblasts and induces bone resorption. J Immunol 1990;145:3297-3303.
8. Klein DC, Raisz LG. Prostaglandins: stimulation of bone resorption in tissue culture. Endocrinology 1970;86:1436-1440.
9. Robey PG, Fedaeko NS, Hefferan TE, Bianco P, Vetter UK, Gresik W, et al. Structure and molecular regulation of bone matrix proteins. J Bone Miner Res 1993;S483-S487.
10. Miyauchi A, Alvarez J, Greenfield EM, Teti A, Grano M, Colucci S, et al. Recognition of osteopontin and related peptides by alpha$_v$beta$_3$ integrin stimulates immediate cell signals in osteoclasts J Biol Chem 1991;266: 20369-20374.
11. Malone JD, Teitelbaum SL, Griffin RM, Senior RM, Kahn AJ. Recruitment of osteoclast precursors by purified bone matrix constituents. J Cell Biol 1982;92:227-230.
12. Mundy GR, Poser JW. Chemotactic activity of the gamma-carboxyglutamic acid containing protein in bone. Calcif Tissue Int 1983;35:164-168.
13. Lucas PA, Caplan AI. Chemotactic response of embryonic limb bud mesenchymal cells and muscle-derived fibroblasts to transforming growth factor beta. Connect Tissue Res 1988;18: 1-7.
14. DeFranco DJ, Glowacki J, Cox KA, Lian JB. Normal bone particles are preferentially resorbed in the presence of osteocalcin-deficient bone particles in vivo. Calcif Tissue Int 1991;49: 43-50.
15. Glowacki J, Rey C, Glimcher MJ, Cox KA, Lian JB. A role for osteocalcin in osteoclast differentiation. J Cellular Biochem 1991;45:292-302.
16. Hale JE, Williamson MK, Price PA. Carboxyl-terminal proteolytic processing of matrix gla protein. J Biol Chem 1991; 266: 211145-211149.
17. Trudeau DL, Freier EF. Determination of calcium in urine and serum by atomic absorption spectrophotometry (ASS). Clin Chem 1967;13:101-104.
18. Chen PS Jr , Toribara TY and Waner H. Microdetermination of phosphorous. Anal Chem 1956;28:1756-1758.
19. Baron R, Neff L, Tran Van P, Nefussi J-R, Vignery A. Kinetic, cytochemical identification of osteoclast precursors and their differentiation into multinucleated osteoclasts. Am J Pathol 1986;122:363-378.
20. Anderson GN, Marks SC Jr. Tartrate-resistant acid phosphatase as a cytochemical marker for osteoclasts. J Histochem Cytochem 1989;37:115-117.
21. Chenu C, Colucci S, Grano M, Zigrino P, Barattolo R, Zambonin G, et al. Osteocalcin induces chemotaxis, secretion of matrix proteins, and calcium mediated intracellular signaling in human osteoclast-like cells. J Cell Biol 1994;127:1149-1158.
22. Collins MK, Sinclair PM. The local use of vitamin D to increase the rate of orthodontic tooth movement. Am J Orthod Dentofac Orthop 1988;94:278-284.
23. Yamasaki K, Miura F, Suda T. Prostagrandins as a mediators of bone resorption induced by experimental tooth movement in rats. J Dent Res 1980;59:1635-1642.
24. Mundy GR, Roodman GD. Osteoclast ontogeny and function. In: Peck WA, ed. Bone and mineral research 5. Amsterdam: Elsevier, 1987; 209-279.
25. Goodson JM, Mcclatchy K, Revell C. Prostaglandin-induced resorption of the adult rat calvarium. J Dent Res 1972;33:670-677.
26. Holtrop ME, Raisz LG. Comparison of the effect of 1, 25 dihydrocholecalciferol, prostaglandin E_2 and osteoclast-activating factor with parathyroid hormone on the ultrastructure of osteoclasts in culture long bones of fetal rats. Calcif Tissue Int 1979;29:201-205.

27. Holtrop ME, Cox KA, Clark MB, Holick MF, Anast CS. 1, 25 dihydrocholecalciferol stimulates osteoclasts in rat bones in the absence of parathyroid hormone. Endocrinology 1981;108: 2293-2301.
28. Liggett WH, Lian JB, Greenberger JS, Glowacki J. Osteocalcin promotes differentiation of osteoclast progenitors from murine long-term bone marrow culture. J Cellular Biochem 1994;55:190-199.
29. Falkenburg JH, Harrington MA, de Paus RA, Walsh WK, Daub R, Landegent JE, et al. Differential transcriptional and posttranslational regulation of gene expression of the colony-stimulating factors by interleukin-1 and fetal bovine serum in murine fibroblasts. Blood 1991;78:658-665.
30. Felix R, Cecchini MG, Hofstetter W, Guenther HL, Fleisch H. Production of granulocyte-macrophage (GM-CSF) and granulocyte colony stimulating factor (G-CSF) by rat clonal osteoblastic cell line population CRP 10/30 and immortalized cell line IRC10/30-myc1 stimulated by tumor necrosis factor alpha. Endocrinology 1991;128:661-667.
31. Takahashi N, Udagawa N, Akatu T, Tanaka H, Isogai Y, Suda T. Deficiency of osteoclasts in osteopetrotic mice is due to a defect in the local microenvironment provided by osteoblastic cells. Endocrinology 1991;128:1792-1796.
32. Pang G, Couch L, Batey R, Clancy R, Cripps A. GM-CSF, IL-1 alpha, IL-1 beta, Il-6, Il-8, IL-10, ICAM-1 and VCAM-1 gene expression and cytokine production in human duodenal fibroblasts stimulated with lipopolysaccharide, IL-1 alpha and TNF-alpha. Clin Exp Immunol 1994;96:437-443.
33. Hamilton JA, Filonzi EL, Ianches G. Regulation of macrophage colony-stimulating factor production in cultured human synovial fibroblasts. Growth Factors 1993;9:157-165.
34. Davidovitch Z, Nicolay OF, Ngan PW and Shanfeld JL. Neurotransmitters, cytokines, and the control of alveolar bone remodeling in orthodontics. Dent Clin North Am 1988;32:411-435.
35. Takano-Yamamoto T, Kawakami M. Kobayashi Y. Yamashiro T, Sakuda M. The effect of local application of 1,25-dihydroxycholecalciferol on osteoclast numbers in orthodontically treated rats. J Dent Res 1992;71:53-59.