European Journal of Orthodontics 31 (2009) 572–577
doi:10.1093/ejo/cjp068
Advance Access Publication 19 October 2009

Influence of bisphosphonates on orthodontic tooth movement in mice

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SUMMARY Mechanical stress such as orthodontic tooth movement induces osteoclastogenesis. Sometimes, excessive mechanical stress results in root resorption during orthodontic tooth movement. It has been reported that bisphosphonate inhibits osteoclastogenesis. Recently, there have been concerns for orthodontic patients receiving bisphosphonates. Thus, the aim of this study was to investigate the effect of bisphosphonates on orthodontic tooth movement and root resorption in mice.

A nickel-titanium (Ni-Ti) closed coil spring delivering a force of 10 g was inserted between the upper anterior alveolar bone and the first molar in 8-week-old male C57BL/6 mice. Bisphosphonate (2 μg/20 μl) was injected daily into a local site adjacent to the upper molar. After 12 days, the distance the tooth had moved was measured. The number of tartrate-resistant acid phosphatase (TRAP)-positive cells was counted as osteoclasts in histological sections. Root resorption was assessed by scanning electron microscopy. The data were analysed with a Student's *t*-test.

The orthodontic appliance increased the number of osteoclasts on the pressure side and mesial movement of the first molar. Bisphosphonates reduced the amount of tooth movement and the number of osteoclasts. In addition, they also reduced root resorption on the pressure side.

Bisphosphonates inhibit orthodontic tooth movement and prevent root resorption during orthodontic tooth movement in mice. These results suggest that bisphosphonates might have an inhibiting effect on root resorption during orthodontic tooth movement in humans and that they may interrupt tooth movement in orthodontic patients undergoing treatment, thus altering the outcome of treatment.

Introduction

Bone is commonly remodelled through resorption of old bone by osteoclasts and new bone formation by osteoblasts. The amount of bone tissue is determined by the balance between the rates of bone formation and bone resorption during physiological growth and skeletal remodelling. Mechanical stress such as orthodontic force also influences bone remodelling. Orthodontic tooth movement is achieved by the process of repeated alveolar bone resorption by osteoclasts on the pressure side and new bone formation by osteoblasts on the tension side (Storey, 1973).

Osteoclasts that are responsible for bone resorption in bone metabolism are multinucleated cells that originate from haematopoietic mononuclear precursor cells of the monocyte/macrophage lineage. Receptor activator of nuclear factor-κB ligand (RANKL; Anderson *et al.*, 1997; Udagawa *et al.*, 1999; Teitelbaum, 2000), also known as osteoclast differentiation factor (Yasuda *et al.*, 1998), tumour necrosis factor (TNF)-related activation-induced cytokine (Wong *et al.*, 1997; Wani *et al.*, 1999), and osteoprotegerin ligand (Lacey *et al.*, 1998), a member of the TNF receptor family, are crucial differentiation factors for osteoclasts. This has been demonstrated in RANKL-deficient mice, which have osteopetrosis caused by mature osteoclast deficiency (Kong *et al.*, 1999). It has been reported that TNF-α is able to induce the formation of osteoclastic cells *in vitro* and *in vivo* (Kitaura *et al.*, 2004, 2005). In rat tooth movement experiments, excessive orthodontic force induces the expression of TNF-α in periodontal tissues (Ogasawara *et al.*, 2004). In addition, orthodontic tooth movement increases the levels of TNF-α in the gingival sulcus in humans (Lowney *et al.*, 1995; Uematsu *et al.*, 1996). Recently, it has been reported that TNF-α plays an important role in mechanical loading-induced osteoclastogenesis and bone remodelling during orthodontic tooth movement (Yoshimatsu *et al.*, 2006; Kitaura *et al.*, 2008).

Bisphosphonates are used to treat bone metabolism disorders such as osteoporosis, Paget's disease, and bone metastasis. Bisphosphonates bind strongly to the bone mineral hydroxyapatite (Jung *et al.*, 1973) and inhibit bone resorption. They target calcified tissues, in which they are internalized selectively by bone-resorbing osteoclasts (Sato *et al.*, 1991; Frith *et al.*, 2001). Once internalized, bisphosphonates inhibit the ability of osteoclasts to resorb bone by mechanisms that interfere with cytoskeletal organization and formation of the ruffled border, and this leads to cell death by apoptosis (Rogers, 2003; Roelofs *et al.*, 2006).

Several studies have investigated the influence of bisphosphonates in rat models of orthodontic tooth movement (Adachi *et al.*, 1994; Igarashi *et al.*, 1994; Kim *et al.*, 1999; Liu *et al.*, 2004). Recent advances in molecular biology techniques have provided opportunities for the use of various gene-mutated mice, including those with genes that regulate bone metabolism. The use of these mice in tooth movement experiments can be advantageous for exploring the molecular mechanisms that underlie tooth movement and mechanical loading-induced bone changes. However, mouse models for orthodontic tooth movement have been lacking. For this reason, no study has investigated the effect of bisphosphonates in mice during orthodontic tooth movement. In order to resolve this problem, a mouse model of orthodontic tooth movement was established using a nickel-titanium (Ni-Ti) closed coil spring inserted between the upper incisor and upper first molar (Yoshimatsu *et al.*, 2006).

Root resorption is often observed as an undesirable side-effect of orthodontic treatment. It is a serious problem for most clinicians, but it is still not resolved. At present, root resorption is thought to be caused by odontoclasts, similar to bone resorption by osteoclasts. Root resorption, which is dependent on odontoclast activity, may be prevented by bisphosphonates. Thus, in the present study, the effect of bisphosphonates on orthodontic tooth movement and root resorption in a mouse model was investigated.

Materials and methods

All experiments were undertaken in accordance with the 1989 Guidelines for Animal Experimentation of Nagasaki University.

Animals

Eight-week-old male C57BL/6 mice were obtained from Japan SLC, Inc., Shizuoka, Japan. The animals were fed a granular diet (Oriental Yeast, Tokyo, Japan) to prevent them from exerting an excessive chewing force. During the experiment, the mice were kept in cages in a room maintained at 25°C with a 12- to 24-hour light/dark cycle and their body weight and health were checked daily.

Experimental tooth movement

The mice were anaesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg) during setting and adjusting of the orthodontic appliance (Yoshimatsu *et al.*, 2006). The appliance comprised a Ni-Ti closed coil spring. The diameter of the Ni-Ti wire was 0.15 mm and of the coil 0.9 mm. The appliance was inserted between the upper anterior alveolar bone and the upper left first molar and was fixed with a 0.1 mm stainless steel wire. According to the manufacturers, the force level of the coil spring after activation is approximately 10 g. A bisphosphonate solution (2 μg/20 μl) was injected daily into a local site adjacent to an upper molar during the experimental period. Phosphate-buffered saline (PBS) was used as the control. The left maxillary molar in each mouse served as the experimental tooth movement and the right maxillary molar as the control.

Measurement of tooth movement

The amount of tooth movement after application of the appliance was measured on alternate days until day 12. The mice were then killed with a perfusion of 10 mM PBS to remove their blood and then perfused and immersed in 4 per cent paraformaldehyde for fixation. After removing the maxillae, an individual tray was placed on the maxillary teeth to obtain impressions using an injection type dental hydrophilic vinyl polysiloxane impression material (Exafine, GC Co., Tokyo, Japan). The amount of tooth movement was evaluated by measuring the distance between the first and second molars in the impression under a stereoscopic microscope (VH-7000; Keyence, Osaka, Japan).

Histological preparation

After fixation, the maxillae were demineralized in 10 per cent ethylenediaminetetraacetic acid for 10 days at 4°C. Paraffin-embedded samples were sectioned at 4 μm. Vertical and horizontal sections of the first molar region were prepared. The vertical sections were used for general histological examination after staining with haematoxylin and eosin. The root length between the bifurcation surface and the apical end of a mouse first molar approximately 600 μm. This suggests that approximately 300 μm of the root from the bifurcation surface at the mesial side is the pressure side during tooth movement. In addition, the alveolar crest is about 80 μm away from the bifurcation surface. Thus, horizontal sections from five levels of the root: 100, 140, 180, 220, and 260 μm away from the bifurcation surface were prepared. These sections were used to count the number of osteoclasts after staining for tartrate-resistant acid phosphatase (TRAP) activity. For TRAP staining, the sections were incubated in acetate buffer (pH 5.0) containing naphthol AS-MX phosphate (Sigma, St Louis, Missouri, USA), Fast Red Violet LB salt (Sigma), and 50 mM sodium tartrate. The sections were counterstained with haematoxylin. The number of TRAP-positive osteoclasts was expressed as the mean from the five sections obtained from the five levels of the root in each mouse.

Measurement of root resorption

After the experimental period, measurement of root resorption was performed according to previously described methods (Gonzales *et al.*, 2008). Briefly, the upper first molar, including its surrounding bone, was cut as a whole block, followed by careful removal of the alveolar bone to avoid any root surface damage. The resected molar was

submerged in 1 per cent sodium hypochlorite for 10 minutes to eliminate periodontal ligament remnants. The mesial side of the distobuccal root was observed by scanning electron microscopy (TM-1000; Hitachi, Tokyo, Japan). The ratio of the root resorption area was calculated.

Statistical analysis

Statistical analysis of the data was performed using a Student's *t*-test.

Results

Bisphosphonates inhibited orthodontic tooth movement

Figure 1 shows the amount of tooth displacement. After 12 days of tooth movement, the first and the second molar distances were increased to about 126 ± 60 μm. The distance was significantly reduced to 61 ± 18 μm by injection of bisphosphonates (2 μg). This effect was dose dependent (Figure 1B).

Bisphosphonates decreased osteoclast formation during orthodontic tooth movement

To identify osteoclasts histologically, TRAP staining was performed on transverse histological sections of the distobuccal root. Figure 2 summarizes the histology of TRAP staining before and after orthodontic tooth movement, with or without bisphosphonate administration. Before tooth movement, TRAP activity was observed on the alveolar bone surface at the distal region of the periodontium, while no activity was noted in the mesial region. In contrast, after tooth movement, the mesial region of the periodontium was compressed and strong TRAP activity was observed. However, in the bisphosphonate-treated group, TRAP activity was weak at the mesial region of the periodontium (Figure 2A). The number of TRAP-positive osteoclasts was also reduced in the bisphosphonate-treated group, compared with that after tooth movement without bisphosphonate (Figure 2B).

Bisphosphonates decreased root resorption during orthodontic tooth movement

Figure 3A shows scanning electron micrographs of the mesial side of the distobuccal root of the upper first molars. Before tooth movement, most of the roots exhibited areas covered by undamaged cementum with a characteristic smooth surface. In contrast, after tooth movement, root resorption was observed. The ratio of the root resorption area reached approximately 13 per cent. However, in the bisphosphonate-treated group, most root resorption was prevented (Figure 3C).

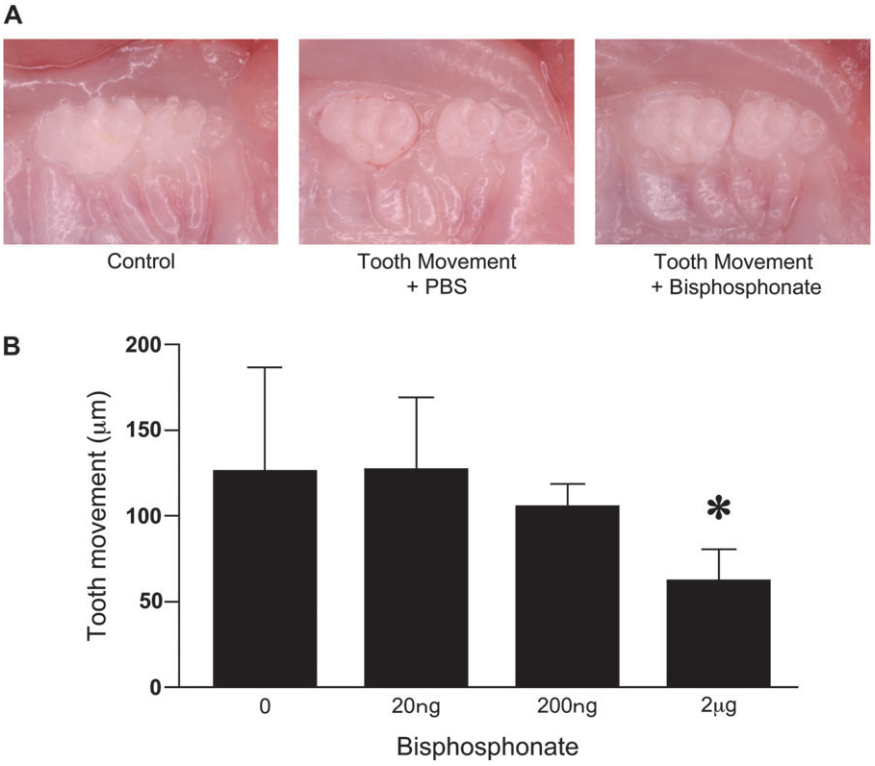

Figure 1 (A) Photograph showing tooth movement in mice with daily administration of phosphate-buffered saline (PBS) or bisphosphonates and in the controls. (B) The first and the second molar distances during orthodontic tooth movement. Dose-dependent inhibition of tooth movement by bisphosphonates. Four mice were used in each group. *$P < 0.05$ versus bisphosphonate 0 group.

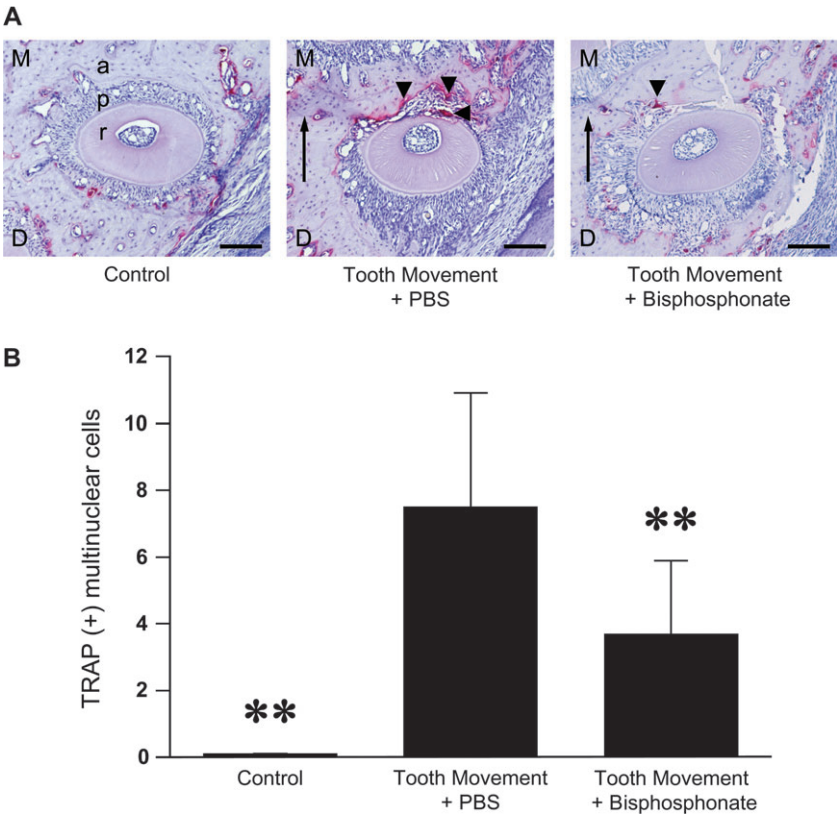

Figure 2 (A) Tartrate-resistant acid phosphatase (TRAP)-stained horizontal section. The black arrows indicates the direction of orthodontic force and the black arrowheads TRAP-positive cells: mesial side (M), distal side (D), alveolar bone (a), periodontal space (p), and root (r). Scale bar = 100 μm. (B) Number of TRAP-positive cells on the pressure side of the distobuccal root of the first molar during orthodontic tooth movement. Four mice were used in each group. $^{**}P < 0.01$ versus tooth movement + phosphate-buffered saline (PBS) group.

Discussion

There have been a limited number of studies on animal models of tooth movement with administration of bisphosphonates. It has previously been reported that bisphosphonates cause a reduction in orthodontic tooth movement (Adachi *et al.*, 1994; Igarashi *et al.*, 1994; Kim *et al.*, 1999; Liu *et al.*, 2004). The present results were similar to those of the earlier studies. However, all previous research was carried out on rats. This is believed to be the first study using mice to investigate the effect of bisphosphonates on orthodontic tooth movement.

Bisphosphonates bind strongly to hydroxyapatite in bone (Jung *et al.*, 1973) and are selectively internalized into mature osteoclasts (Sato *et al.*, 1991; Frith *et al.*, 2001). Osteoclasts then lose their bone-resorbing activity and are induced to apoptosis. The present results demonstrate that local administration of bisphosphonates causes a significant reduction in orthodontic tooth movement and osteoclast formation. Tooth movement resulted from resorption of alveolar bone by osteoclasts that were induced on the pressure side by mechanical stress. These results suggest that tooth movement was prevented by reduced bone resorption by apoptotic osteoclasts.

Recently, Chung *et al.* (2008) showed that, during application of a force of 10 g to teeth using a Ni-Ti closed coil spring in mice, root-resorbing osteoclasts and root resorption were observed at the pressure side during orthodontic tooth movement. Root-resorbing osteoclasts and root resorption at the pressure side was also observed in the present study in histological preparations and scanning electron micrographs. Excessive orthodontic force might be the cause of root resorption during orthodontic tooth movement (Harris *et al.*, 2006). These results suggest that the orthodontic force used in these studies was excessive. The mouse experimental model might be able to be applied as a model of root resorption caused by orthodontic tooth movement. In a rat orthodontic tooth movement model, bisphosphonates inhibited root resorption caused by orthodontic tooth movement (Alatli *et al.*, 1996; Igarashi *et al.*, 1996; Liu *et al.*, 2004). In the present study, bisphosphonates also inhibited root resorption in mice. The results indicate that bisphosphonates induce apoptosis of odontoclasts in the same way as in osteoclasts. This suggests that localized bisphosphonate might be useful for the prevention

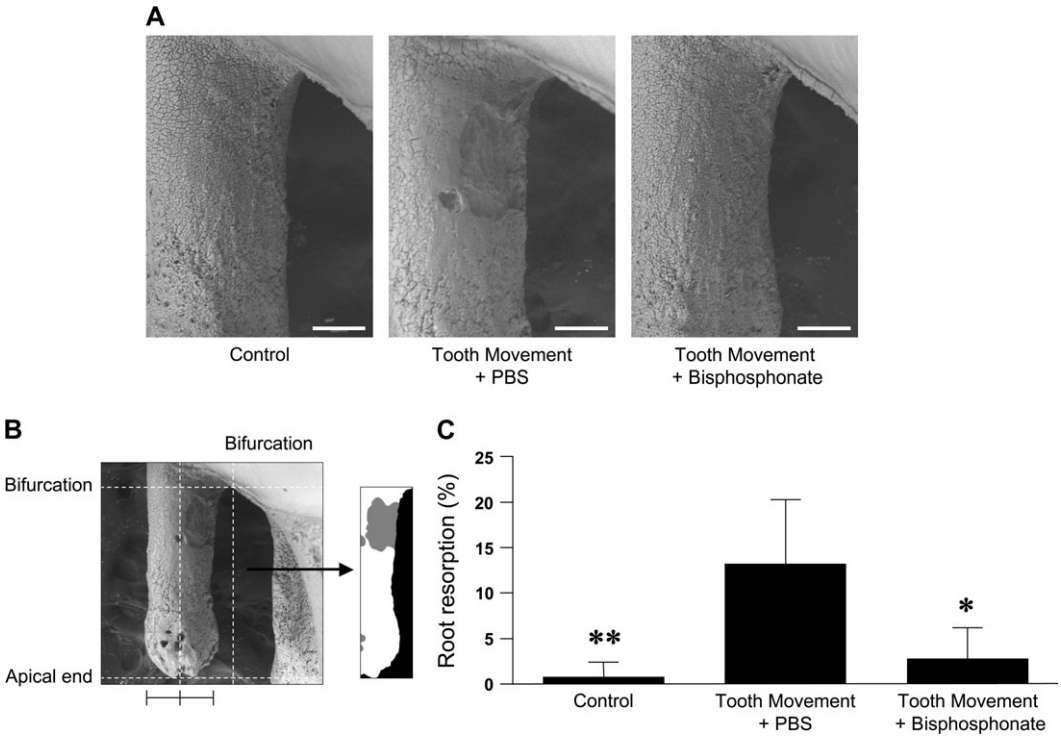

Figure 3 (A) Effects of bisphosphonates on root resorption caused by orthodontic tooth movement. Scale bar = 100 μm. (B) Evaluation of root resorption area by scanning electron microscopy on the pressure side of the mesial side of the distobuccal root of the first molar during orthodontic tooth movement. White area, non-resorption; grey area, resorption; root resorption area; percentage of grey area/(grey area + white area). (C) Ratio of root resorption area. Four mice were used in each group. $*P < 0.05$, $**P < 0.01$ versus tooth movement + phosphate-buffered saline (PBS) group.

of root resorption during orthodontic tooth movement. However, bisphosphonate inhibits orthodontic tooth movement at the same time. Further studies are necessary in this area.

Bisphosphonates are widely used to treat bone metabolic disorders. Recently, there have been reports of jaw osteonecrosis in dental patients receiving intravenous bisphosphonate (Marx, 2003; Migliorati, 2003; Ruggiero *et al.*, 2004). Dental surgical procedures are regarded as one of the risk factors associated with the development of osteonecrosis. In the present study, where bisphosphonate was applied locally, no osteonecrosis was observed. However, a causal relationship between bisphosphonate therapy and osteonecrosis of the jaws has not been established and further studies are necessary. In a recent investigation of orthodontic treatment of two patients who were taking bisphosphonates, both showed impeded tooth movement (Rinchuse *et al.*, 2007). The present results support the possibility of inhibition of orthodontic tooth movement in patients taking bisphosphonates.

Conclusion

Bisphosphonates decreased osteoclast formation induced by orthodontic force, which inhibited tooth movement in mice. Bisphosphonates also prevented root resorption associated with orthodontic tooth movement. These results suggest that bisphosphonate might be useful for control of orthodontic tooth movement and as a candidate inhibitor of root resorption during orthodontic tooth movement. On the other hand, it is possible that orthodontic tooth movement in patients taking bisphosphonates might be arrested.

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Funding

Grant for Scientific Research from the Ministry of Education, Science and Culture, Japan; the President's discretionary fund of Nagasaki University.

Acknowledgements

The bisphosphonate (alendronate sodium hydrate) was a gift from Merck & Co., Whitehouse Station, New Jersey, USA.

References

Adachi H, Igarashi K, Mitani H, Shinoda H 1994 Effects of topical administration of a bisphosphonate (risedronate) on orthodontic tooth movements in rats. Journal of Dental Research 73: 1478–1486

Alatli I, Hellsing E, Hammarström L 1996 Orthodontically induced root resorption in rat molars after 1-hydroxyethylidene-1,1-bisphosphonate injection. Acta Odontologica Scandinavica 54: 102–108

Anderson D M *et al.* 1997 A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. Nature 390: 175–179

Chung C J *et al.* 2008 OPN deficiency suppresses appearance of odontoclastic cells and resorption of the tooth root induced by experimental force application. Journal of Cellular Physiology 214: 614–620

Frith J C, Monkkonen J, Auriola S, Monkkonen H, Rogers M J 2001 The molecular mechanism of action of the antiresorptive and antiinflammatory drug clodronate: evidence for the formation *in vivo* of a metabolite that inhibits bone resorption and causes osteoclast and macrophage apoptosis. Arthritis and Rheumatism 44: 2201–2210

Gonzales C, Hotokezaka H, Yoshimatsu M, Yozgatian J H, Darendeliler M A, Yoshida N 2008 Force magnitude and duration effects on amount of tooth movement and root resorption in the rat molar. Angle Orthodontist 78: 502–509

Harris D A, Jones A S, Darendeliler M A 2006 Physical properties of root cementum: part 8. Volumetric analysis of root resorption craters after application of controlled intrusive light and heavy orthodontic forces: a microcomputed tomography scan study. American Journal of Orthodontics and Dentofacial Orthopedics 130: 639–647

Igarashi K, Adachi H, Mitani H, Shinoda H 1996 Inhibitory effect of the topical administration of a bisphosphonate (risedronate) on root resorption incident to orthodontic tooth movement in rats. Journal of Dental Research 75: 1644–1649

Igarashi K, Mitani H, Adachi H, Shinoda H 1994 Anchorage and retentive effects of a bisphosphonate (AHBuBP) on tooth movements in rats. American Journal of Orthodontics and Dentofacial Orthopedics 106: 279–289

Jung A, Bisaz S, Fleisch H 1973 The binding of pyrophosphate and two diphosphonates by hydroxyapatite crystals. Calcified Tissue Research 11: 269–280

Kim T W, Yoshida Y, Yokoya K, Sasaki T 1999 An ultrastructural study of the effects of bisphosphonate administration on osteoclastic bone resorption during relapse of experimentally moved rat molars. American Journal of Orthodontics and Dentofacial Orthopedics 115: 645–653

Kitaura H *et al.* 2004 Marrow stromal cells and osteoclast precursors differentially contribute to TNF-alpha-induced osteoclastogenesis *in vivo*. Journal of Immunology 173: 4838–4846

Kitaura H, Zhou P, Kim H J, Novack D V, Ross F P, Teitelbaum S L 2005 M-CSF mediates TNF-induced inflammatory osteolysis. Journal of Clinical Investigation 115: 3418–3427

Kitaura H *et al.* 2008 An anti-c-Fms antibody inhibits orthodontic tooth movement. Journal of Dental Research 87: 396–400

Kong Y Y *et al.* 1999 OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. Nature 397: 315–323

Lacey D L *et al.* 1998 Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 93: 165–176

Liu L, Igarashi K, Haruyama N, Saeki S, Shinoda H, Mitani H 2004 Effects of local administration of clodronate on orthodontic tooth movement and root resorption in rats. European Journal of Orthodontics 26: 469–473

Lowney J J, Norton L A, Shafer D M, Rossomando E F 1995 Orthodontic forces increase tumor necrosis factor alpha in the human gingival sulcus. American Journal of Orthodontics and Dentofacial Orthopedics 108: 519–524

Marx R E 2003 Pamidronate (Aredia) and zoledronate (Zometa) induced avascular necrosis of the jaws: a growing epidemic. Journal of Oral and Maxillofacial Surgery 61: 1115–1117

Migliorati C A 2003 Bisphosphanates and oral cavity avascular bone necrosis. Journal of Clinical Oncology 21: 4253–4254

Ogasawara T *et al.* 2004 *In situ* expression of RANKL, RANK, osteoprotegerin and cytokines in osteoclasts of rat periodontal tissue. Journal of Periodontal Research 39: 42–49

Rinchuse D J, Sosovicka M F, Robison J M, Pendleton R 2007 Orthodontic treatment of patients using bisphosphonates: a report of 2 cases. American Journal of Orthodontics and Dentofacial Orthopedics 131: 321–326

Roelofs A J, Thompson K, Gordon S, Rogers M J 2006 Molecular mechanisms of action of bisphosphonates: current status. Clinical Cancer Research 12: 6222s–6230s

Rogers M J 2003 New insights into the molecular mechanisms of action of bisphosphonates. Current Pharmaceutical Design 9: 2643–2658

Ruggiero S L, Mehrotra B, Rosenberg T J, Engroff S L 2004 Osteonecrosis of the jaws associated with the use of bisphosphonates: a review of 63 cases. Journal of Oral and Maxillofacial Surgery 62: 527–534

Sato M *et al.* 1991 Bisphosphonate action. Alendronate localization in rat bone and effects on osteoclast ultrastructure. Journal of Clinical Investigation 88: 2095–2105

Storey E 1973 The nature of tooth movement. American Journal of Orthodontics 63: 292–314

Teitelbaum S L 2000 Bone resorption by osteoclasts. Science 289: 1504–1508

Udagawa N *et al.* 1999 Osteoblasts/stromal cells stimulate osteoclast activation through expression of osteoclast differentiation factor/RANKL but not macrophage colony-stimulating factor: receptor activator of NF-kappa B ligand. Bone 25: 517–523

Uematsu S, Mogi M, Deguchi T 1996 Interleukin (IL)-1 beta, IL-6, tumor necrosis factor-alpha, epidermal growth factor, and beta 2-microglobulin levels are elevated in gingival crevicular fluid during human orthodontic tooth movement. Journal of Dental Research 75: 562–567

Wani M R, Fuller K, Kim N S, Choi Y, Chambers T 1999 Prostaglandin E2 cooperates with TRANCE in osteoclast induction from hemopoietic precursors: synergistic activation of differentiation, cell spreading and fusion. Endocrinology 140: 1927–1935

Wong B R *et al.* 1997 TRANCE is a novel ligand of the tumor necrosis factor receptor family that activates c-Jun N-terminal kinase in T cells. Journal of Biological Chemistry 272: 25190–25194

Yasuda H *et al.* 1998 Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proceedings of the National Academy of Sciences of the United States of America 95: 3597–3602

Yoshimatsu M *et al.* 2006 Experimental model of tooth movement by orthodontic force in mice and its application to tumor necrosis factor receptor-deficient mice. Journal of Bone and Mineral Metabolism 24: 20–27