European Journal of Orthodontics 26 (2004) 469–473

European Journal of Orthodontics vol. 26 no. 5

Effects of local administration of clodronate on orthodontic tooth movement and root resorption in rats

Lin Liu*, Kaoru Igarashi**, Naoto Haruyama**, Shuichi Saeki**, Hisashi Shinoda*** and Hideo Mitani*

Divisions of *Orthodontics and Dentalfacial Orthopedics, **Oral Dysfunction Science and ***Pharmacology, Tohoku University Graduate School of Dentistry, Sendai, Japan

SUMMARY Clodronate, a non-N-containing bisphosphonate, strongly inhibits bone resorption and also has anti-inflammatory properties. The purpose of this study was to examine the effect of the local administration of clodronate on orthodontic tooth movement. Both the right and left upper first molars of 26 7-week-old male Wistar rats were moved buccally with a standardized expansion spring. Clodronate solution was injected into the sub-periosteum area adjacent to the left upper molar every third day during the experimental period. The right first molar served as the control.

Local injection of clodronate caused a significant ($P < 0.001$) and dose-dependent reduction in tooth movement in the rats. The number of osteoclasts on the clodronate-injected side was significantly less ($P < 0.01$) than on the control side. Local clodronate also inhibited root resorption incident to tooth movement. These results suggest that localized use of clodronate could be a useful therapeutic adjunct in orthodontic treatment.

Introduction

In orthodontic treatment, mechanically induced bone resorption is an essential step in tooth movement and, therefore, can be a major target for pharmacological intervention to enhance the treatment outcome. If undesirable tooth movement could be prevented with blockers of bone resorption, treatment would be less complex and more secure.

Bisphosphonates selectively inhibit osteoclasts, and have been used to treat various metabolic bone diseases associated with excessive bone resorption (Fleisch *et al.*, 2002). However, studies on the structure–activity relationships of these compounds indicate that their potency and mode of action vary depending on the side-chain on the carbon atom of the P–C–P bond, a common structure of bisphosphonates (Shinoda *et al.*, 1983; Rogers *et al.*, 2000; Dunford *et al.*, 2001). Recent investigations have revealed that bisphosphonates that contain a nitrogen atom in the side-chain (N-containing bisphosphonates) are more potent than those without a nitrogen atom (non-N-containing bisphosphonates), and that they can inhibit the production of isoprenoid compounds (farnesyl pyrophosphate and geranylgeranyl pyrophosphate) in the mevalonate pathway and, hence, prevent protein prenylation in osteoclasts (Dunford *et al.*, 2001). It has also been shown that non-N-containing bisphosphonates can be incorporated into ATP analogues and act by inhibiting protein synthesis and inducing apoptosis in osteoclasts (Rogers *et al.*, 2000; Frith *et al.*, 2001).

Clodronate is a non-N-containing bisphosphonate that contains two chlorine atoms in its side-chain. Due to its close structural similarity to pyrophosphate, this bisphosphonate, like etidronate and tiludronate, is known to inhibit osteoclast function by being incorporated metabolically into a non-hydrolysable ATP analogue (Rogers *et al.*, 2000). In addition to this anti-bone resorbing activity, it has been suggested that this compound also has anti-inflammatory activity (Elomaa *et al.*, 1992; Österman *et al.*, 1995; Richards *et al.*, 2001). The present study was undertaken to examine the effect of the local administration of clodronate on tooth movement in rats as the first step to determine future clinical application of this compound.

Materials and methods

The general condition and weight of each animal used in this study were monitored during the experimental period. The animals were treated in accordance with the guidelines for the use of experimental animals of the Animal Care and Use Committee of Tohoku University Graduate School of Dentistry.

Drug

Clodronate (dichloromethylene bisphosphonate disodium salts) was obtained from Proctor and Gamble Pharmaceuticals (Woods Corners Laboratories, Norwitch, New York, USA).

Tooth movement in rats

Twenty-six male Wistar rats, 7 weeks old and weighing an average of 180 g, were used in this study. Both the right and left upper first molars of the animals were moved buccally with a standardized expansion spring, which initially generated an average force of 120 mN on each side. The expansive force was applied without adjustment for 3 weeks. Clodronate solution, 50 µl at a concentration of either 0 (saline), 2.5, 10 or 40 mM, was injected into the sub-periosteum area adjacent to the left upper first molar every third day during the experimental period under light ether anaesthesia. The right first molar served as the control, with an injection of 50 µl of saline into the corresponding area. The clodronate solutions were prepared by dissolving clodronate in distilled water; the pH was adjusted to 7.4 with NaOH and osmolarity was adjusted to 310 mOsm with NaCl. Tooth movement was measured according to a method described previously (Igarashi *et al.*, 1994). Briefly, the occlusal view of a precise plaster model of the right and left upper jaws was magnified ×10 with a profile projector and traced. The contours of the palatal cusps of the second and third molars of the 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 mesio-palatal cusps of the first molars before and after tooth movement was measured with sliding callipers. Data were expressed as either millimetres or as a percentage of the paired control value. The latter was calculated as: (tooth movement on the clodronate-treated side ×100)/ tooth movement on the control side.

Histological examinations

After 3 weeks of force application, the animals were killed under diethyl ether anaesthesia, the upper jaws were dissected, fixed in 4 per cent paraformaldehyde for 5 days, and then decalcified with 10 per cent EDTA solution (0.01 M phosphate-buffered saline, pH 7.4) for 5 weeks. The samples were dehydrated and embedded in paraffin wax. Periodontal tissues of the mesiobuccal root of the upper first molar were examined with a light microscope in transverse serial sections of the molars. Sections (5 µm thick) were stained for tartrate-resistant acid phosphatase (TRAP) with 0.16 mg/ml naphthol AS-MX phosphate and 1.4 mg/ml fast red violet LB salt in 0.1 M sodium acetate buffer (pH 5.0) containing 50 mM sodium tartrate, and counter-stained with toluidine blue. The mesiobuccal root area was divided into a pressure and a tension side, based on the mesiodistal axis of the root. The osteoclasts on the alveolar bone surface of the pressure side were counted. Osteoclasts were defined as multinucleated TRAP-positive cells on the bone surface or in bone-resorptive lacunae. The root-resorptive area was measured by image analysis (WinROOF, Mitani Shoji Co., Fukui, Japan) of the microscopic image that was fed directly to a television monitor with a CCD video camera at a magnification of ×850, according to a method described previously (Igarashi *et al.*, 1996). The data were expressed as square micrometres. For both the number of osteoclasts and the root-resorptive area, the values for 12 sections, selected at five-section intervals, were averaged for each animal.

Statistical analysis

All of the data were first subjected to one- or two-way analysis of variance (ANOVA). The dose–response data were then subjected to the Scheffe *F* multiple comparison test to identify differences between groups. The paired *t*-test was used to evaluate the significance of differences in tooth movement, osteoclast count and root resorption between the treated side and the contralateral control side. Statistical calculations were carried out with Microsoft Excel 98.

Results

The local administration of clodronate did not cause any appreciable inflammatory reactions at the injection site or any systemic side-effects, such as loss of appetite or change in body weight.

As shown in Figure 1a, tooth movement on the clodronate-injected side was significantly less than on the control side after 3 weeks of orthodontic force application. The percentage of the control value was 81, 65 and 56 at concentrations of 2.5, 10 and 40 mM, respectively. Thus, the inhibitory effect was dose-dependent. Figure 1b shows the time course of tooth movement in animals injected with clodronate (50 µl of 10 mM). There was a significant reduction in tooth movement on the treated side compared with the control side on days 14, 17 and 21.

Figure 2 shows the number of osteoclasts on the pressure side of the mesiobuccal roots of upper first molars on day 21. The number of osteoclasts on the clodronate (10 mM)-injected side was significantly less than on the control side. Figure 3 shows the area of root resorption on the pressure side of the mesiobuccal roots of upper first molars on day 21. The root-resorptive area on the clodronate (10 mM)-injected side was significantly smaller than on the control side.

Discussion

The present results demonstrate that clodronate strongly inhibits orthodontic tooth movement. Tooth movement on the control side exhibited three typical

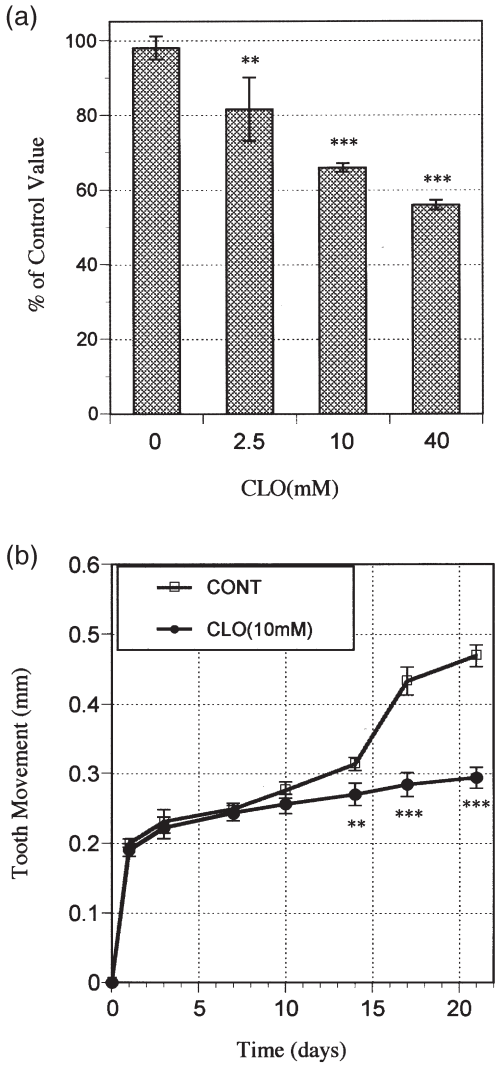

Figure 1 The effects of clodronate (CLO) on tooth movement in rats. (a) Dose-dependent inhibition of tooth movement by CLO. Each column and bar represents the mean ± standard error of the mean (SEM). The effect of treatment was significant ($P < 0.001$ one-way ANOVA). $^{**}P < 0.01$ and $^{***}P < 0.001$ versus the value in animals injected only with saline (Scheffe F multiple comparison test). (b) The time course of tooth movement on the CLO (10 mM)-injected side and the control side. Each point and bar represents the mean ± SEM ($n = 7$). The effects of both treatment and time were significant ($P < 0.001$ two-way ANOVA). CONT = control. $^{**}P < 0.01$ and $^{***}P < 0.001$ versus the control side (paired t-test).

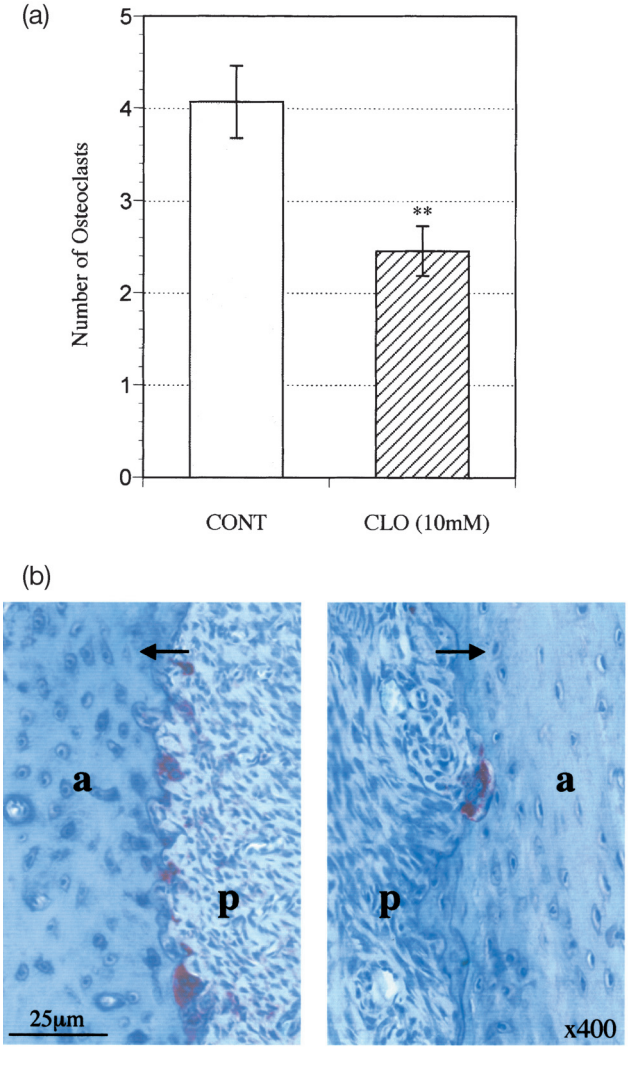

Figure 2 The effects of clodronate (CLO) on osteoclasts induced by orthodontic mechanical stress. (a) The number of osteoclasts on the pressure side of the mesiobuccal roots of the upper first molars on day 21. The values for 12 sections, selected at five-section intervals, were averaged for each sample. Each column and bar represents the mean ± standard error of the mean ($n = 7$). CONT = control. $^{**}P < 0.01$ versus CONT (paired t-test). (b) Periodontal tissues on the pressure side of the mesiobuccal roots of the upper first molars on day 21. (Left) control side; (right) CLO (10 mM)-injected side. Scale bar = 25 μm (magnification ×400). a, alveolar bone; p, periodontal ligament. Arrows indicate the direction of force.

phases, i.e. a phase of rapid movement, a lag phase, and a phase of progressive movement (Roberts, 1994). The local administration of clodronate caused a significant reduction in tooth movement in the third phase, which is considered to be due to bone resorption by osteoclasts. This indicates that clodronate inhibits bone resorption induced by orthodontic mechanical stress. The results are comparable with those obtained in animals injected with N-containing bisphosphonates such as alendronate (Igarashi *et al.*, 1994) and risedronate (Adachi *et al.*, 1994).

As the number of osteoclasts decreased at the clodronate-injected side, clodronate may have either inhibited the recruitment of osteoclasts or promoted osteoclast apoptosis, or both (Rogers *et al.*, 2000). Osteoclast apoptosis is considered to be a major mechanism of action for the inhibition of bone resorption by this bisphosphonate (Halasy-Nagy *et al.*, 2001). However, as other mechanisms cannot be excluded, an *in vitro* study is currently being undertaken.

Clodronate is an anti-resorptive agent, and is widely used in the treatment of metabolic bone disease

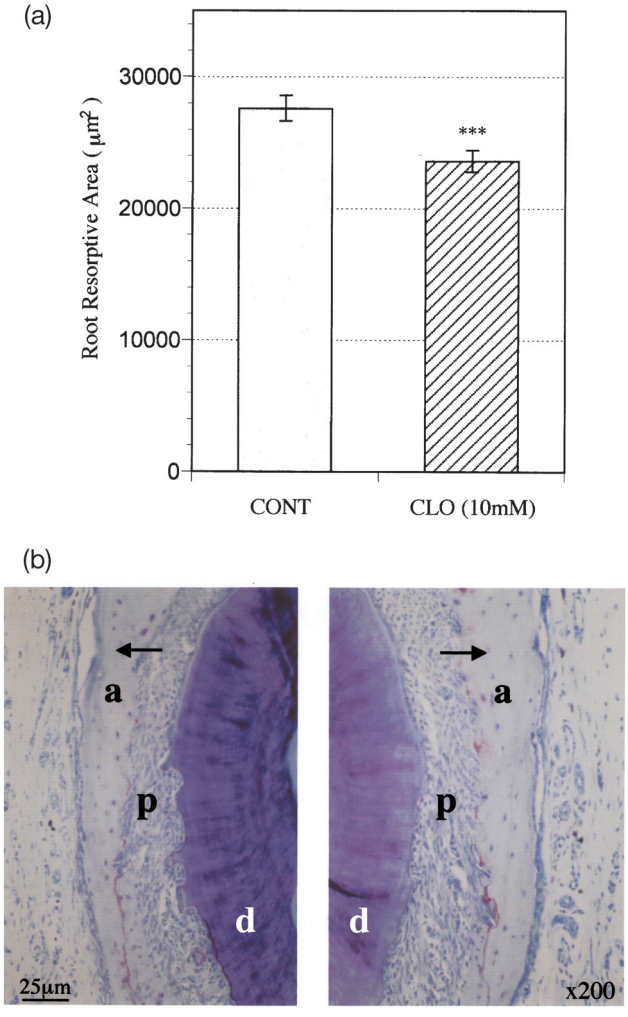

Figure 3 The effect of clodronate (CLO) on root resorption incident to orthodontic tooth movement. (a) The area of root resorption on the pressure side of the mesiobuccal roots of the upper first molars on day 21. The values for 12 sections, selected at five-section intervals, were averaged for each sample. Each column and bar represents the mean ± standard error of the mean (n = 7). CONT = control. ***P < 0.001 versus CONT (paired t-test). (b) Periodontal tissues on the pressure side of the mesiobuccal roots of the upper first molars on day 21. (Left) control side; (right) CLO (10 mM)-injected side. Scale bar = 25 μm (magnification ×200). a, alveolar bone; d, dentine; p, periodontal ligament. Arrows indicate the direction of force.

(Plosker and Goa, 1994). Although clodronate is not part of the new class of bisphosphonates and is less potent than N-containing bisphosphonates (Fleisch, 2000), it can be considered a potential anti-inflammatory drug. Clodronate has been shown to suppress signs of inflammation in arthritic rats (Österman *et al.*, 1995; Richards *et al.*, 2001) and to relieve bone pain in patients with metastatic prostate cancer (Elomaa *et al.*, 1992). Furthermore, clodronate has been shown to inhibit the production or release of pro-inflammatory molecules such as interleukin-1 beta (Pennanen *et al.*, 1995; Makkönen *et al.*, 1999), interleukin-6 (Mönkkönen *et al.*, 1994; Pennanen *et al.*, 1995; Giuliani *et al.*, 1998; Makkönen *et al.*, 1999), tumour necrosis factor alpha (Mönkkönen *et al.*, 1994; Pennanen *et al.*, 1995; Makkönen *et al.*, 1999), nitric oxide (Makkönen *et al.*, 1996, 1999) and prostaglandin E_2 (Felix *et al.*, 1981; Igarashi *et al.*, 1997) in macrophages and/or osteoblastic cells. As these inflammatory cytokines and mediators play important roles in the biological response to orthodontic mechanical stimulation (Yamasaki *et al.*, 1980; Chumbley and Tuncay, 1986; Saito *et al.*, 1991; Lowney *et al.*, 1995; Zhou *et al.*, 1997; Iwasaki *et al.*, 2001; Alhashimi *et al.*, 2001; Shirazi *et al.*, 2002; Hayashi *et al.*, 2002), including adverse reactions such as pain (Ngan *et al.*, 1994) and root resorption (Brudvik and Rygh, 1991), clodronate could be a more desirable drug for the control of orthodontic tooth movement than other N-containing bisphosphonates, some of which are rather pro-inflammatory (Mönkkönen *et al.*, 1998; Makkönen *et al.*, 1999; Pecherstorfer *et al.*, 2000). Although the present results show that local clodronate inhibits root resorption incident to tooth movement, further studies are necessary to determine whether clodronate is also useful for preventing inflammatory reactions to orthodontic treatment.

When the clinical application of clodronate is considered, it would be desirable to minimize possible systemic effects of the local administration of this drug. Further studies are also necessary in this respect.

Conclusions

The results of the present study suggest that the localized use of clodronate could be a beneficial therapeutic adjunct for orthodontic treatment. Bisphosphonates such as clodronate that have anti-inflammatory properties may also be helpful in the treatment of increased bone resorption associated with inflammatory diseases such as rheumatoid arthritis and periodontitis.

Address for correspondence

Kaoru Igarashi
Division of Oral Dysfunction Science
Department of Oral Health and Development Sciences
Tohoku University Graduate School of Dentistry
4-1 Seiryo-machi
Aoba-ku
Sendai 980-8575
Japan

Acknowledgements

We wish to thank Dr M. Ohtsuka-Isoya for her valuable advice. This research was supported in part by a Grant-in-Aid for Scientific Research (C) from the Ministry of Education, Science, Sports, and Culture of Japan (no. 13672138).

References

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

Alhashimi N, Frithiof L, Brudvik P, Bakhiet M 2001 Orthodontic tooth movement and *de novo* synthesis of proinflammatory cytokines. American Journal of Orthodontics and Dentofacial Orthopedics 119: 307–312

Brudvik P, Rygh P 1991 Root resorption after local injection of prostaglandin E_2 during experimental tooth movement. European Journal of Orthodontics 13: 255–263

Chumbley A B, Tuncay O C 1986 The effect of indomethacin (an aspirin-like drug) on the rate of orthodontic tooth movement. American Journal of Orthodontics 89: 312–314

Dunford J E *et al.* 2001 Structure–activity relationships for inhibition of farnesyl diphosphate synthase *in vitro* and inhibition of bone resorption *in vivo* by nitrogen-containing bisphosphonates. Journal of Pharmacology and Experimental Therapeutics 296: 235–242

Elomaa I *et al.* 1992 Effects of oral clodronate on bone pain. A controlled study in patients with metastic prostatic cancer. International Urology and Nephrology 24: 159–166

Felix R, Bettex J D, Fleisch H 1981 Effect of diphosphonates on the synthesis of prostaglandins in cultured calvaria cells. Calcified Tissue International 33: 549–552

Fleisch H 2000 Bisphosphonates in bone disease: from the laboratory to the patient, 4th edn. Academic Press, San Diego, pp. 40–41

Fleisch H, Reszka A, Rodan G, Rogers M 2002 Bisphosphonates, mechanisms of action. In: Bilezikian J P, Raisz L G, Rodan G A (eds) Principles of bone biology, 2nd edn. Academic Press, San Diego, pp. 1361–1385

Frith J C, Mönkkönen J, Auriola S, Mönkkönen H, Rogers M J 2001 The molecular mechanism of action of the antiresorptive and antiinflammatory drug clodronate. Arthritis and Rheumatism 44: 2201–2210

Giuliani N, Pedrazzoni M, Passeri G, Girasole G 1998 Bisphosphonates inhibit IL-6 production by human osteoblast-like cells. Scandinavian Journal of Rheumatology 27: 38–41

Halasy-Nagy J M, Rodan G A, Reszka A A 2001 Inhibition of bone resorption by alendronate and risedronate does not require osteoclast apoptosis. Bone 29: 553–559

Hayashi K, Igarashi K, Miyoshi K, Shinoda H, Mitani H 2002 Involvement of nitric oxide in orthodontic tooth movement in rats. American Journal of Orthodontics and Dentofacial Orthopedics 122: 306–309

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

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, Hirafuji M, Adachi H, Shinoda H, Mitani H 1997 Effects of bisphosphonates on alkaline phosphatase activity, mineralization, and prostaglandin E_2 synthesis in the clonal osteoblast-like cell line MC3T3-E1. Prostaglandins, Leukotrienes and Essential Fatty Acids 56: 121–125

Iwasaki L R, Haack J E, Nickel J C, Reinhardt R A, Petro T M 2001 Human interleukin-1 beta and interleukin-1 receptor antagonist secretion and velocity of tooth movement. Archives of Oral Biology 46: 185–189

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

Makkönen N, Hirvonen M R, Teräväinen T, Savolainen K, Mönkkönen J 1996 Different effects of three bisphosphonates on nitric oxide production by RAW 264 macrophage-like cells *in vitro*. Journal of Pharmacology and Experimental Therapeutics 277: 1097–1102

Makkönen N *et al.* 1999 Contrasting effects of alendronate and clodronate on RAW 264 macrophages: the role of a bisphosphonate metabolite. European Journal of Pharmaceutical Sciences 8: 109–118

Mönkkönen J, Pennanen N, Lapinjoki S, Urtti A 1994 Clodronate (dichloromethylene bisphosphonate) inhibits LPS-stimulated IL-6 and TNF production by RAW 264 cells. Life Sciences 54: PL229–234

Mönkkönen J, Similä J, Rogers M J 1998 Effects of tiludronate and ibandronate on the secretion of proinflammatory cytokines and nitric oxide from macrophages *in vitro*. Life Sciences 62: PL95–102

Ngan P, Wilson S, Shanfeld J, Amini H 1994 The effect of ibuprofen on the level of discomfort in patients undergoing orthodontic treatment. American Journal of Orthodontics and Dentofacial Orthopedics 106: 88–95

Österman T, Kippo K, Lauren L, Hannuniemi R, Sellman R 1995 Effect of clodronate on established collagen-induced arthritis in rats. Inflammation Research 44: 258–263

Pecherstorfer M *et al.* 2000 Effect of first treatment with aminobisphosphonates pamidronate and ibandronate on circulating lymphocyte subpopulations. Journal of Bone and Mineral Research 15: 147–154

Pennanen N, Lapinjoki S, Urtti A, Mönkkönen J 1995 Effect of liposomal and free bisphosphonates on the IL-1 beta, IL-6 and TNF alpha secretion from RAW 264 cells *in vitro*. Pharmaceutical Research 12: 916–922

Plosker G L, Goa K L 1994 Clodronate: a review of its pharmacological properties and therapeutic efficacy in resorptive bone disease. Drugs 47: 945–982

Richards P J, Williams B D, Williams A S 2001 Suppression of chronic streptococcal cell wall-induced arthritis in Lewis rats by liposomal clodronate. Rheumatology 40: 978–987

Roberts W E 1994 Bone physiology, metabolism, and biomechanics in orthodontic practice. In: Graber T M, Vanarsdall, Jr R L (eds) Orthodontics, current principles and techniques, 2nd edn. Mosby, St. Louis, pp. 193–234

Rogers M J *et al.* 2000 Cellular and molecular mechanisms of action of bisphosphonates. Cancer 88: 2961–2978

Saito M, Saito S, Ngan P W, Shanfeld J, Davidovitch Z 1991 Interleukin 1 beta and prostaglandin E are involved in the response of periodontal cells to mechanical stress *in vivo* and *in vitro*. American Journal of Orthodontics and Dentofacial Orthopedics 99: 226–240

Shinoda H, Adamek G, Felix R, Fleisch H, Schenk R, Hagan P 1983 Structure–activity relationships of various bisphosphonates. Calcified Tissue International 35: 87–99

Shirazi M, Nilforoushan D, Alghasi H, Dehpour A-R 2002 The role of nitric oxide in orthodontic tooth movement in rats. Angle Orthodontist 72: 211–215

Yamasaki K, Miura F, Suda T 1980 Prostaglandin as a mediator of bone resorption induced by experimental tooth movement in rats. Journal of Dental Research 59: 1635–1642

Zhou D, Hughes B, King G J 1997 Histomorphometric and biochemical study of osteoclasts at orthodontic compression sites in the rat during indomethacin inhibition. Archives of Oral Biology 42: 717–726