ORIGINAL ARTICLE

Effect of alendronate on orthodontic tooth movement in rats

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Introduction: Osteoclastic activity is required for orthodontic force to move teeth through alveolar bone. Bisphosphonates are drugs that inhibit osteoclast maturation, function, and survival. The aim of this study was to assess orthodontic tooth movement in rats receiving bisphosphonate treatment. **Methods:** Two groups of Sprague-Dawley rats were used. The rats in the treatment group received 7 mg per kilogram of body weight per week of alendronate sodium, and those in the control group received no drugs. A coil spring exerting a constant 50-g force was activated across the span from the central incisors to the first molar. As the first molar tipped mesially, a diastema between the first and second molars was created. Vinyl polysiloxane impressions were poured in die stone, and the diastema was measured indirectly with a charged-couple device microscope camera and Optimas software (Media Cybernetics, Newburyport, Mass). **Results:** Statistical analysis with repeated-measures analysis of variance showed less orthodontic tooth movement in the alendronate group compared with control group (0.06 vs 0.24 mm at 2 weeks, and 0.45 vs 1.06 mm at 4 weeks; $P = 0.0004$ for the alendronate vs control main effect). **Conclusions:** This study demonstrated an inhibitory effect of alendronate administration on orthodontic tooth movement in a rat model. (Am J Orthod Dentofacial Orthop 2009;136:843-7)

Although the exact cellular mechanisms involved in orthodontic tooth movement (OTM) are not fully understood, it is clear that osteoclastic activity required for resorption of bone is essential.[1,2] Therefore, any interference with the function of osteoclastic cells might result in decreased efficiency and effectiveness of orthodontic treatment. Several types of drugs can cause this sort of interference, and many have been studied by using various models.[3-8] Recently, a class of drugs called bisphosphonates (BPs) has begun to appear frequently in the dental literature because of its potential for clinical use and its potential for causing undesirable side effects.[9-15] BPs are known to suppress osteoclast-mediated bone resorption and are widely used in the treatment of skeletal disorders characterized by excessive bone resorption such as osteoporosis, Paget's disease, and some types of metastatic cancer.[16-18] In orthodontics, the use of BPs has been suggested as possible means to control relapse and even to generate "pharmacological anchorage."[7] Although the benefits of BPs in orthodontics might be realized in the future, the more immediate concerns to orthodontists are their potential side effects in relation to the clinical practice of orthodontics.[12-14] Chief among these potential side effects is a reduction in the rate of OTM.

BPs are structurally related to pyrophosphates, but instead of a central oxygen molecule, they have a characteristic phosphorus-carbon-phosphorus structure, which is essential for binding to hydroxyapatite.[18-21] Once a BP is taken up into the body, it is quickly redistributed to areas of increased bone turnover and subsequently incorporated into osteoclasts involved in bone resorption. There are 2 subclasses of BPs, distinguished by the type of side chains attached to the central carbon: nonamino and amino BPs.[21] The nonamino BPs have lower potency and inhibit osteoclast function via metabolism into toxic adenosine triphosphate metabolites. The amino BPs inhibit an enzyme of the mevalonate biosynthetic pathway called farnesyl pyrophosphate synthase, which in turn inhibits the enzymatic modification of small guanosine triphosphate-binding proteins in osteoclasts. This disrupts cytoskeletal function and intracellular signaling, which

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The authors report no commercial, proprietary, or financial interest in the products or companies described in this article.
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Submitted, November 2006; revised and accepted, November 2007.
0889-5406/$36.00

doi:10.1016/j.ajodo.2007.11.035

leads to impaired osteolytic activity and eventually to osteoclast apoptosis.[19-21]

Alendronate is an amino BP commonly used to treat osteoporosis.[22, 23] For example, in 2005, approximately 22.4 million prescriptions were filled for Fosamax (Merck, Whitehouse Station, NJ), making it the 19th most-prescribed drug in the United States.[24] Most of these prescriptions were for postmenopausal women undergoing treatment for osteoporosis. Because alendronate's mechanism of action is to decrease osteoclastic activity, it would seem logical this drug could interfere with the bone remodeling required for OTM—perhaps significantly reducing the rate and magnitude of OTM.[14,25,26]

The purpose of this study was to investigate the effect of alendronate on the magnitude of OTM in a rat model.

MATERIAL AND METHODS

Fifty Sprague-Dawley rats were randomly and evenly divided into 2 groups of 25 animals each: the untreated control group and the alendronate-treated experimental group. However, because of some appliance bond failures as a result of a slight variation in bonding technique compared with that of Miller et al,[27] each group was reduced to 11 rats each. The rats were acclimated for 2 weeks in plastic shoebox housings with a 12-hour alternating light and dark schedule. They were fed powdered rodent chow and distilled water ad libitum during this study. Approval was obtained from the institutional animal care and use committee of the University of Minnesota. At the end of this study, the rats were killed according to institutional guidelines.

Alendronate sodium in crystalline form was obtained from Fisher Scientific International (Pittsburgh, Pa) in vials of the same lot number, each containing 100 mg of material. The contents of each vial were dissolved in 10 mL of sterile water to obtain a solution containing 10 mg per milliliter of alendronate sodium. To completely dissolve the alendronate into the water, each container was vortexed for 60 seconds. Then the alendronate solution was stored in a refrigerator until use.

Alendronate was administered at 1-week intervals for 5 weeks to the experimental group. Timing of alendronate administration was such that the rats received 2 doses before appliance activation and then once a week for the rest of the study. Alendronate was administered at a dosage of 7 mg per kilogram of body weight per week. This dosage was designed to approximate a common dosage used in humans to prevent or treat osteoporosis and correlates well with studies that demonstrated that in rats this amount appears to be a threshold dose that elicits a biologic effect.[25,26] Furthermore, oral bioavailability of alendronate has been shown to be similar for rats and humans.[27] A gavage technique with the drug delivered directly into the animal's stomach was used rather than standard oral administration to achieve greater accuracy in dosing.

Anesthesia was induced in both control and experimental groups in conjunction with placement of the orthodontic appliance and for each diastema measurement. A combination of 50 mg per kilogram of ketamine and 10 mg per kilogram of xylazine was injected intraperitoneally. Fifteen minutes after administration of the initial dose, the animals were evaluated for adequate anesthesia, and a subsequent dose of 150 μL of sodium pentobarbital was given if needed to obtain adequate anesthesia. This resulted in adequate anesthesia for 30 to 45 minutes.

The rats were anesthetized as described above and held in position with an animal holding board. With a clamp to hold the mouth open, the cheek on the working side (animal's right side) was retracted with a modified mixing spatula. After roughening the occlusal surface with a bur in a slow-speed handpiece, all accessible surfaces of the maxillary first molar were etched with 37% phosphoric acid for 30 seconds and rinsed for 15 seconds. The tooth was then thoroughly dried under suction with an air syringe. A thin coat of 3M Transbond XT primer (3M Unitek, Monrovia, Calif) was applied and light cured, after which Tetric-Flow flowable composite (Ivoclar Vivadent, Amherst, NY) was bonded to the primer layer to fill in the grooves on the occlusal surface of the molar. Next, a modified cleat was bonded to the occlusal surface with Transbond XT composite and light cured. A Sentalloy closed coil spring (GAC International, Bohemia, NY) 3 mm in length was attached to the cleat and stretched to the maxillary incisors. This coil spring was designed to apply a constant tipping force of 50 g to the maxillary first molar. A wire tie was used to secure the spring to the cervical area of the incisors near the gingiva. Flowable composite was placed over the molar cleat and latch assembly to prevent dislodgement of the coil spring. To inhibit eruption of the incisors, stainless steel pedodontic crowns (3M ESPE Dental Products, St. Paul, Minn) were placed over the incisors and cemented with Ultra Band-Lok (GAC International) (Fig 1, *A*).

At the 2-week and 4-week time-points, the rats were again anesthetized, and impressions of the diastema distal to the maxillary first molar were made using vinyl polysiloxane (Imprint II, 3M ESPE Dental Products) impression material (Fig 1, *B*). After

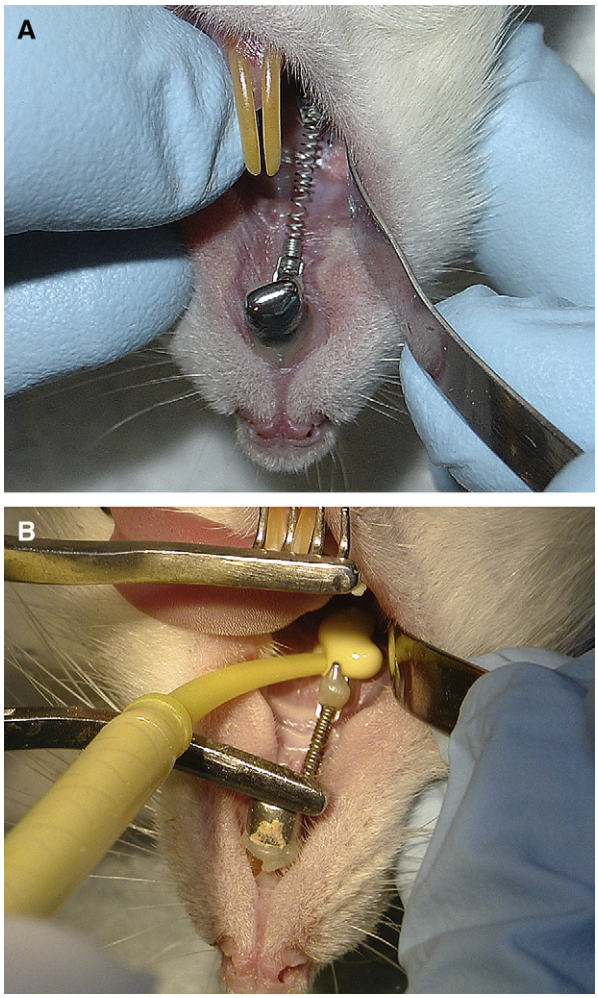

Fig 1. A, Initial placement of activated coil spring extending from the central incisors to the maxillary first molar; **B,** vinyl polysiloxane impression of diastema between the maxillary first and second molars.

waiting 4 minutes to allow the material to adequately polymerize, the impression was removed and inspected to ensure that the diastema was adequately captured. The impressions were poured using an improved die stone (Fujirock II, GC Europe, Leuven, Germany) that was vacuum-mixed under 25 psi for 30 seconds on a vibrating surface. The poured stone was allowed to set for 24 hours before separation from the impression.

Next, an image of the diastema was captured using a Navitar macro zoom 18-108 lens (Rochester, NY) attached to a CCD camera (MTI 3, Leeds Precision, Minneapolis, Minn). Optimas software (Media Cybernetics, Newburyport, Mass) was used to measure the diastema on the captured images with a calibrated ruler algorithm.

Statistical analysis

For the statistical analysis, we used repeated-measures analysis of variance (ANOVA): a rat was a subject, and the repeated measures were the diastema sizes at 2 and 4 weeks. Diastema size showed a clear tendency for variation to increase as the average diastema increased; this is inconsistent with the assumptions of ANOVA. The usual remedy—supported in this case by so-called diagnostics such as residual plots and the box-Cox procedure—is to analyze the logarithm of diastema size instead of the diastema size itself. However, some diastemas were measured as 0 mm, and the logarithm of zero is not defined. Thus, for testing differences between groups, the dependent variable was the common logarithm of diastema size plus 0.23 mm, with 0.23 mm as the 2.5th percentile of the positive diastemas. For summaries of diastema sizes, we give averages of actual diastemas and their standard deviations.

RESULTS

Each group consisted of 11 rats with intact appliances. At 2 weeks, 10 diastema measurements were taken from each group (1 impression from each group was unreadable) for a total of 20 measurements. At 4 weeks, once again 10 diastema measurements were taken from each group (1 rat from each group had been eliminated because of debonding of the appliance) for a total of 20 measurements. Therefore, 10 measurements were collected at each time-point for both groups (40 measurements), and these were used for statistical analysis.

In the control group, the mean diastema measurements were 0.24 mm (SD, 0.16) at 2 weeks and 1.06 mm (SD, 0.33) at 4 weeks. The alendronate group had mean diastema measurements of 0.06 mm (SD, 0.13) at 2 weeks and 0.45 mm (SD, 0.38) at 4 weeks (Fig 2). The standard deviations, describing variations between rats, were greater for the groups with larger average mean diastemas. For this and other reasons indicated in the statistical methods section, the common logarithm (log to base 10) of diastema measurement was used as the dependent variable in the repeated-measures ANOVA testing for differences between groups.

In the repeated-measures ANOVA, the diastemas were significantly larger in the control group than in the alendronate group (group main effect, averaged over the 2 time points, $P = 0.0004$). Also, the diastemas were larger at 4 weeks than at 2 weeks (time main effect, averaged over the 2 groups, $P < 0.0001$). The diastemas in the alendronate group fell short of those in the control group in percentage terms, by somewhat less at 4 weeks (0.45 and 1.06 mm, respectively, or 42%) than at 2

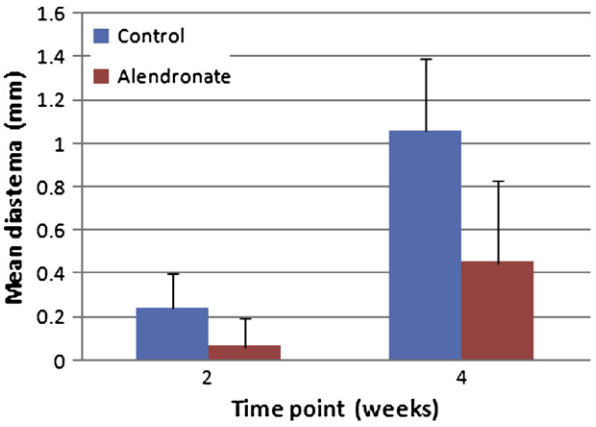

Fig 2. Comparison of orthodontic tooth movement in the control group vs the experimental (alendronate) group showing mean diastemas (SD) at 2 and 4 weeks. OTM in the alendronate group was significantly lower than in the control group ($P = 0.0004$ for the group main effect).

weeks (0.06 and 0.24 mm, respectively, or 25%). However, this difference between 2 and 4 weeks was not statistically significant (group-by-time interaction, $P = 0.26$). If the alendronate and the control groups are compared separately at 2 and 4 weeks using contrasts in the context of the ANOVA, they test significantly different at both times ($P = 0.019$ at 2 weeks; $P = 0.0009$ at 4 weeks).

DISCUSSION

The results of this study clearly show an inhibitory effect of alendronate administration on the magnitude of OTM in rats. This inhibitory effect is most likely due to disruption of osteoclast function and survival at sites of periodontal ligament compression where resorption of bone is required for OTM to occur. As alendronate is ingested, it is redistributed to bone and particularly to areas of increased bone turnover. Once alendronate is taken up into osteoclasts, the mevalonate biosynthetic pathway is disrupted, thus inhibiting the enzymatic modification of proteins essential for cellular function and survival—particularly those involved in cytoskeletal function. Without proper cytoskeletal function, osteoclasts can no longer form a ruffled border or perform their role in the resorption and hydrolysis of the bone matrix. Eventually, these inactivated osteoclasts undergo apoptosis. Thus, a significant reduction in the resorptive activity of osteoclasts as a result of inhibition by alendronate could cause less and slower OTM.

Although the inhibitory effect of alendronate was significant at the dosage used in this study, this dose is just beyond the threshold needed to produce an observable biologic effect.[28,29] It is a dose that—based on body-mass conversion factors—approximates a common dosage taken by human patients to prevent osteoporosis. Much higher doses of amino BPs similar to alendronate or other amino BPs of greater potency are used to treat conditions such as metastatic cancer. BPs can also be administered intravenously; this dramatically increases their bioavailability and thus their effects.[28] In these situations, the potential for inhibition of OTM might be significantly increased. Furthermore, although the rats in this study received regular doses of alendronate over a relatively short time, patients taking BPs for the treatment of chronic conditions such as osteoporosis generally take them over a much longer period. An even more pronounced effect might be seen in these situations.[30,31] With the extremely long half-life of BPs—particularly in humans—their biologic effects can continue long after the patient has stopped taking the drug.

These results support and expand on published studies that have shown a reduction in orthodontic tooth movement with BP administration. Igarashi et al[7] demonstrated a significant decrease in tooth movement in rats receiving local injections of alendronate over 3 weeks. Liu et al[4] administered clodronate (a non-nitrogen–containing BP) by local injection to rats; it caused a dose-dependent reduction in OTM. Administration of pamidronate (a nitrogen-containing BP similar to alendronate) over 8 days resulted in a statistical trend toward reduced OTM in mice in a study by Keles et al.[32] Kim et al[33] showed a reduction in orthodontic relapse after experimental movement of rat molars with intravenous administration of pamidronate.

The possibility of reduced OTM due to BP administration reinforces the need to consider BPs with regard to patient health histories, treatment planning, and informed consent, as suggested by Zahrowski[12] and Rinchuse et al.[14] Furthermore, staying informed regarding current drugs used to treat bone disorders as recommended by Zahrowski[12] is important because of changes and advances in the treatment of these disorders. For example, administration of recombinant osteoprotegerin was evaluated recently as a possible pharmacologic means of decreasing osteoclastic activity and has also been shown to be a potent inhibitor of OTM in animal studies.[32,34] Development of a human monoclonal antibody that targets a molecule critical for osteoclastic activity (RANKL) is well underway and could see widespread use soon.[35]

As patients with increasingly complex health histories and medical profiles continue to seek orthodontic

treatment, orthodontists must remain vigilant and informed about these medications so that they can provide optimal care.[3,25,36-38]

CONCLUSIONS

1. Administration of alendronate inhibits OTM in rats by 75% at 2 weeks and 58% at 4 weeks.
2. Orthodontists should inform their patients who are currently taking or who have recently taken BPs that treatment time could be prolonged and treatment results might be compromised.

Although more studies concerning the potential interaction of BPs with orthodontic treatment are needed, these data suggest potentially negative effects with which the orthodontist should be familiar.

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