Original Article

Effect of zoledronic acid on bone healing subsequent to mini-implant insertion

Sarandeep S. Huja[a]; Burçak Kaya[b]; X. Mo[c]; Andrew M. D'Atri[d]; Soledad A. Fernandez[e]

ABSTRACT

Objective: To examine remodeling in bone supporting mini-implants by comparing a no drug (ND) group with a group that received a potent intravenous bisphosphonate in a canine model.

Materials and Methods: Twelve skeletally mature (2- to 3-year-old) male dogs were divided into two groups. Seven dogs were administered 0.1 mg/kg/mo of zoledronic acid (ZA) for 16 weeks, while five age-matched dogs received no drug. Two mini-implants (Tomas, Dentaurum, Newton, Pa) were placed unilaterally in the maxilla and mandible (4 mini-implants per animal $\times$ 12 = 48). Serial fluorescent bone labels were administered in vivo. Postmortem, the bone blocks containing the mini-implants were harvested and used for histomorphometric analyses at two regions of interest (adjacent: within 1 mm of interface; distant: 1–4 mm from the interface) supporting the mini-implant. Data were analyzed using mixed models.

Results: In general, the ZA group had a significantly lower bone formation rate than the ND group ($P < .05$) for all jaws/regions except for the adjacent region in the maxilla, $P = .12$. Despite the reduction, mean intracortical remodeling in the ZA group ranged from 35%–42% per year in the implant adjacent bone. This rate is substantially higher than that reported for noninjured sites in the jaw.

Conclusions: Bone remodeling is typically elevated in implant supporting bone. After ZA administration, the healing response represented by elevated turnover in implant supporting bone was diminished but was not abolished. (*Angle Orthod.* 2011;81:363–369.)

KEY WORDS: Zoledronic acid; Mini-implant; Orthodontics; Histomorphometry; Osteonecrosis; Bone remodeling

INTRODUCTION

Since 2003, an uncommon but serious complication of bisphosphonate therapy has been documented. This entity has now been well described in the literature and is referred to as bisphosphonate-related osteonecrosis of the jaw (BRONJ).[1,2] Clinically, osteonecrosis of the jaw (ONJ) is characterized by the absence of normal bone healing after injury. Although a large number of risk factors such as diabetes, corticosteroids use, and smoking have been identified,[3] surgical injury such as tooth extraction is currently considered the primary event that precedes the development of ONJ. Similar to tooth extraction, mini-implant placement causes injury to the alveolar bone, and there have been increasing concerns regarding bone healing and implant success in patients who are receiving bisphosphonates.[4]

Adult orthodontic patients may have a positive history of bisphosphonate treatments for prevention or treatment of osteoporosis.[5] In addition, adults can require challenging treatments. These complex adult treatments may benefit from temporary anchorage devices, such as mini-implants. However, it is unknown if uneventful bone healing will occur in patients receiving bisphosphonate treatments or those with a history of bisphosphonate treatments subsequent to mini-implant placement.

A substantial body of information exists on bone remodeling in endosseous implant supporting bone.[6,7] Osteonal remodeling in implant supporting bone is

[a] Associate Professor, Division of Orthodontics, College of Dentistry, The Ohio State University, Columbus, Ohio.

[b] Assistant Professor, Department of Orthodontics, Faculty of Dentistry, Başkent University, Ankara, Turkey.

[c] Biostatistician, Center for Biostatistics, The Ohio State University, Columbus, Ohio.

[d] Research Assistant, Division of Orthodontics, College of Dentistry, The Ohio State University, Columbus, Ohio.

[e] Biostatistical Scientist, Center for Biostatistics, The Ohio State University, Columbus, Ohio.

Corresponding author: Sarandeep S. Huja, DDS, PhD, Division of Orthodontics, College of Dentistry, The Ohio State University, 4088 E Postle Hall, 305 W 12th Ave, Columbus, OH 43210 USA.

Accepted: September 2010. Submitted: July 2010.
Published Online: January 24, 2011

considered a healing mechanism in the short[8] term (within 3–6 months) and can remain elevated up to 2 years after placement.[9] In addition, studies suggest that the healing response in bone supporting mini-implants is very similar to that of endosseous dental implants.[10,11] The translational research finding of the above studies on osteonal remodeling at an implant device interface suggests that there is intense localized remodeling in the implant and mini-implant adjacent (within 1 mm of the interface) bone and that this remodeling rate decreases as a function of distance from the implant interface (eg, 1–4 mm).[9]

Zoledronic acid (ZA) is a potent nitrogen-containing bisphosphonate that is administered intravenously. Bisphosphonates bind with strong affinity to calcium in bone and can have a half life in bone of nearly 10 years.[12] Anecdotal claims of lack of a healing response in ZA-treated patients have led to concerns in providing restorative options such as implants. A few clinical studies have attempted to address the risk associated with placing dental implants in patients with a history of bisphosphonate treatment.[4,13] However, there is no study that examines histologic healing events subsequent to dental implant or mini-implant insertion on an animal model that has been exposed to zoledronic acid.

The purpose of this study was to characterize the short-term healing process after mini-implant placement in bisphosphonate-treated animals. We hypothesize that bone remodeling surrounding the mini-implants in the zoledronic acid group will be suppressed when compared with the no drug group.

MATERIALS AND METHODS

Institutional animal care and use committee approval was obtained. Twelve 2- to 3-year-old male dogs were obtained (T_0, Figure 1) from Marshall Farms, USA (North Rose, NY) and divided into two groups. In the treatment (ZA) group, seven dogs were administered zoledronic acid intravenously (0.1 mg/kg) once a month for a total of four doses (D_1 through D_4, Figure 1) over a 16-week period. The dosage was higher than that given to humans (0.06 mg/kg or approximately 4 mg/mo for a typical 70-kg healthy adult) but is safe for this animal model.[14] Five dogs received no drug (ND) treatment. It was anticipated that as ZA suppresses turnover, a larger sample size was required in that group.

One week after the completion of the drug treatment (T_7, Figure 1) both groups received self-drilling mini-implants (2 × 6 mm, Tomas, Dentaurum, Newton, Pa). At the time of surgery, each dog was sedated with acepromazine (2 mg), anesthetized with ketamine (100 mg)/diazepam (5 mg) intravenously, and intubated and maintained on isoflurane (2.0%–2.5%). The small screw-like device was placed in the interradicular region, apical to the bifurcation of the roots in the premolar and molar regions in both jaws unilaterally and in both groups of animals (Figure 2). The mini-implant placement side (right or left) was randomly determined in order to sample tissue from both sides of the jaw. No force was applied to the mini-implants during the duration of the study.

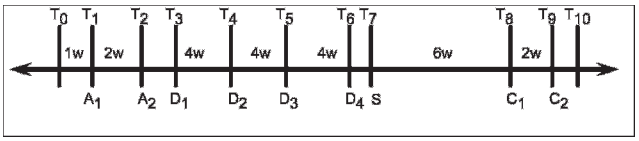

Figure 1. Schematic of timeline. T_0–T_{10} indicate events on the timeline. T_0 is arrival and 1 week is given for acclimatization, prior to first bone label, T_1–T_7 is surgical (S) mini-implant placement. T_{10} is time at harvest of tissue. The 1w, 2w, 4w, and 6w indicate the time in weeks between the time points. A_1 and A_2 indicate alizarin label, D_1 through D_4 is the drug administration (zoledronic acid). C_1 and C_2 represent the calcein labels. The only treatment difference between the two groups was the lack of any drug treatments in the ND group.

Two pairs of two different bone labels were administered to both groups of animals. These bone labels chelate calcium, thus any newly forming bone incorporates the label, and the bone is marked.[15] Alizarin (20 mg/kg, Sigma, St Louis, Mo) was administered starting 1 week post acclimatization (T_1, Figure 1); a second dose of the pair was administered 2 weeks later (T_2, Figure 1). Calcein (5 mg/kg, Sigma) was administered 6 weeks (T_8, Figure 1) and 8 weeks (T_9, Figure 1) post mini-implant insertion. The animals were killed (T_{10}) with an intravenous overdose of sodium pentobarbitone within 4–5 days after the second calcein (8 weeks post mini-implant insertion) label.

Sample Preparation

The procedures for specimen harvesting, processing, and histomorphometric analyses have been described previously.[11] Briefly, the bone blocks of interest were cut out from the jaw bones with a band saw (Mar-Med Inc, Cleveland, Ohio) and placed immediately in 70% ethanol until further processing. The specimens were dehydrated in graded alcohols and embedded in methyl methacrylate. One unstained section (approximately 100 μm thick) was obtained for each mini-implant using a diamond wire saw (Delaware Diamond Knives, Wilmington, Del) under water lubrication. The bone-implant sections were mounted on glass slides with Eukitt (Electron Microscopy Sciences, Hatfield, Pa).

The bone implant sections were examined under epifluorescence (Olympus, BX51, Tokyo, Japan) at 100× with the group information being blinded to the

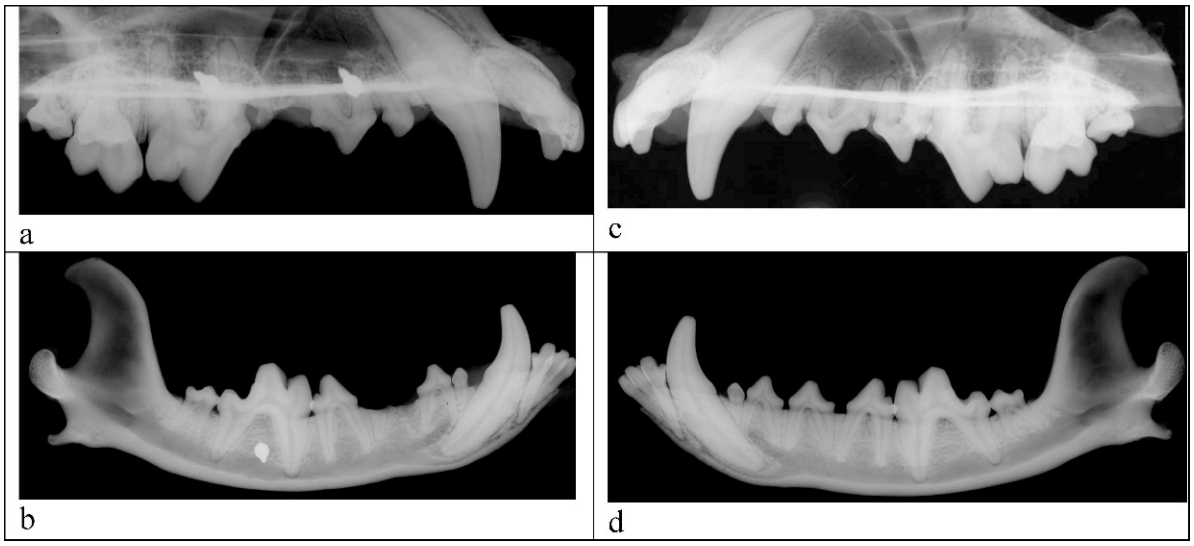

Figure 2. Faxitron images of the maxilla (a,c) and the mandible (b,d) at harvest. In this animal, mini-implants were placed on the right side (a,b) while no implants were placed on the left side (c,d). The mini-implants were placed in the interradicular regions of the second and fourth premolar in the maxilla. In the mandible, the mini-implants were placed in the interradicular region of the second premolar (note implant lost) and first molar.

single investigator. The investigator was calibrated prior to the start of data collection. Bone injury such as implant placement or extraction evokes a *localized* healing response that has been described as the regional acceleratory phenomena.[16] Thus, the bone surrounding the implant was divided into two localized regions for the purposes of data collection (Figure 3). Bone adjacent to the implant was defined as bone up to 1 mm from the implant interface, while the distant region was composed of bone 1–4 mm from the implant interface. The primary static and dynamic histomorphometric parameters were quantified using standard hit/intercept methods with the aid of a Merz grid (Figure 3) in both (adjacent/distant) regions of interest.[17] Primary histomorphometric parameters included: bone volume (BV), bone surface (BS), single labeled surface (sLS), double labeled surface (dLS), and interlabel thickness (Ir.L.Th.). From these measurements, the following secondary[18] histomorphometric variables were calculated: mineral apposition rate (MAR, μm/d, Ir.L.Th./interlabel time in days), mineralizing surface/bone surface (MS/BS, % of total bone surface, [dLS + sLS/2] × 100/BS), and bone formation rate (BFR/BV, %/y, MAR × [(dLS + sLS/2)/BV] × 100 × 365). The measurements were obtained from both sides of the implant and averaged, resulting in one measurement for each secondary variable/region/implant.

Statistical Analyses

Due to the hierarchical structure of the data, mixed models were used to analyze relationships between the histomorphometric variables for the groups (ZA and ND), skeletal sites (mandible, maxilla), and regions (adjacent and distant). Interaction effects were included in all models, and pairwise comparisons were performed when the interaction effects were signifi-

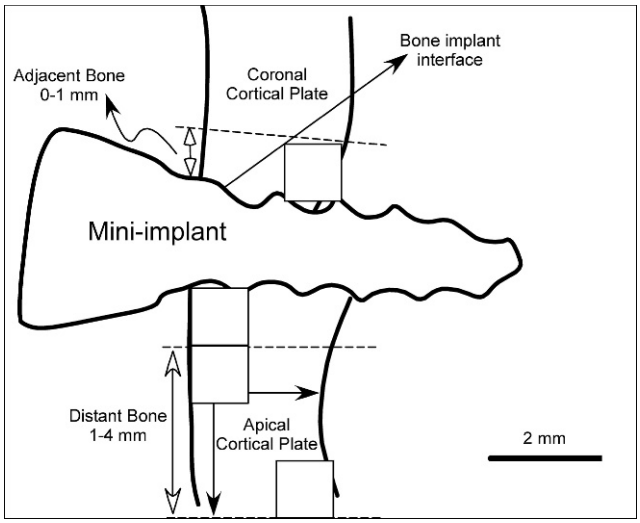

Figure 3. Schematic of histomorphometric quantification. The schema represents the mini-implant in the mandible. The implant supporting bone is divided into adjacent and distant regions. Adjacent bone boundary is defined as bone from the interface to the dotted line, which is approximately 1 mm from the interface. The distant bone extends from 1 mm from the interface to 4 mm from the interface. There are two sides to the implant, the coronal being towards the tooth and the apical towards the basal bone. The adjacent and distant bone on both sides of the implant was quantified with aid of the Merz grid which is represented by the square. Essentially all the bone in the coronal and the apical sides of the implant in both the adjacent and distant regions was analyzed by standard dynamic histomorphometric methods.

Table 1. Mean (SD) of Histomorphometric Parameter of Interest as Determined From Calcein Label for the Zoledronic (ZA) and No Treatment (ND) Groups.[a]

			MAR, μm/d		MS/BS, %		BFR, %/y	
Group	Bone	Region	Mean	SD	Mean	SD	Mean	SD
ND	Mandible	Adjacent	1.65	0.24	54.45	15.66	75.03	36.65
		Distant	1.76	0.25	48.51	7.42	61.38	19.38
	Maxilla	Adjacent	1.49	0.36	45.15	18.08	52.57	34.41
		Distant	1.57	0.27	33.20	24.50	37.59	28.3
ZA	Mandible	Adjacent	1.40	0.37	53.52	15.19	42.92	23.1
		Distant	1.36	0.22	30.79	8.07	17.84	8.9
	Maxilla	Adjacent	1.44	0.30	37.37	10.95	35.31	11.43
		Distant	1.47	0.44	15.75	10.64	9.46	7.93

[a] MAR indicates mineral apposition rate; MS/BS, mineralizing surface/bone surface; and BFR, bone formation rate.

cant. A random effect for dog and a fixed effect for the treatment group were included in the models.

RESULTS

A total of 48 mini-implants were placed in the 12 dogs, with 28 in the ZA group and 20 in the untreated group. Six mini-implants were lost or became loose in the ZA group compared with three in the ND group. All failed mini-implants except two were in the anterior site. Another two specimens could not be analyzed due to complications during processing and sectioning of the specimens. As a result, 37 mini-implant bone sections (21 in the ZA group, 16 in the no drug treatment group) were analyzed.

The analyses of the differences in MAR, MS/BS and BFR as determined from the calcein labels (C_1 and C_2, Figure 1) between the ND and ZA groups were our main focus and are discussed below.

Mineral Apposition Rate

The mean MAR ranged from 1.4–1.8 μm/d (Table 1). There were no differences ($P > .05$) in the maxillary or mandibular MAR between the adjacent and distant bone within the ND and within the ZA groups. When between group comparison was made, there were no differences in MAR with the one exception being the distant bone between the two groups ($P = .047$).

Mineralizing Surface/Bone Surface

The mean MS/BS ranged from 15.7% to 54.4% (Table 1). We examined for both within group and between group comparisons. For both the maxilla and mandible in the ZA group, the MS/BS was significantly ($P < .005$) higher in the adjacent than in the distant bone sites. Within the ND group, there was no gradient of MS/BS between the adjacent and distant bone sites in both the mandible ($P = .5$) and maxilla ($P = .08$). For the distant bone in both the mandible ($P = .04$) and maxilla ($P = .018$), a significantly higher MS/BS was detected in the ND group compared with the ZA group. However, this between group difference was not observed in the adjacent bone region in either the mandible ($P = .9$) or maxilla ($P = .27$).

Bone Formation Rate

In cortical bone, BFR represents the bone turnover rate or remodeling rate. The mean BFR ranged from 9.5% to 75% per year in the two groups (Table 1). In the ZA group, the BFR was significantly different between the adjacent and distant bone sites of the mandible ($P = .048$) and maxilla ($P = .026$). These differences between the bone regions based on proximity to the implant were not different for the ND group in both the mandible ($P = .29$) and maxilla ($P = .17$) (Table 1). When comparing BFR between the two groups, significant smaller measurements were seen in the ZA group at both regions in the mandible and at the distal region in the maxilla ($P < .05$). The same trend also existed at the adjacent region in the maxilla, although the difference was statistically insignificant ($P = .12$).

Since the study design required that each animal have two different labels, the differences in BFR between the alizarin and calcein labels at each of the two mini-implant regions (adjacent and distant) were also compared (Figure 4). At both regions (adjacent and distant), the BFR was significantly different ($P < .01$) for the ND and ZA animals, with the only exception being the distant region ($P > .05$) in the ZA group.

DISCUSSION

This study specifically addressed healing at mini-implant supporting bone sites in bisphosphonate-treated animals. A potent bisphosphonate, zoledronic acid, was administered intravenously, in dose and duration that is known to severely suppress bone turnover.[19,20] In this study, a local bone healing response of increased remodeling was evident after placement of mini-implants in a zoledronic acid treatment animal model. This finding suggests that in

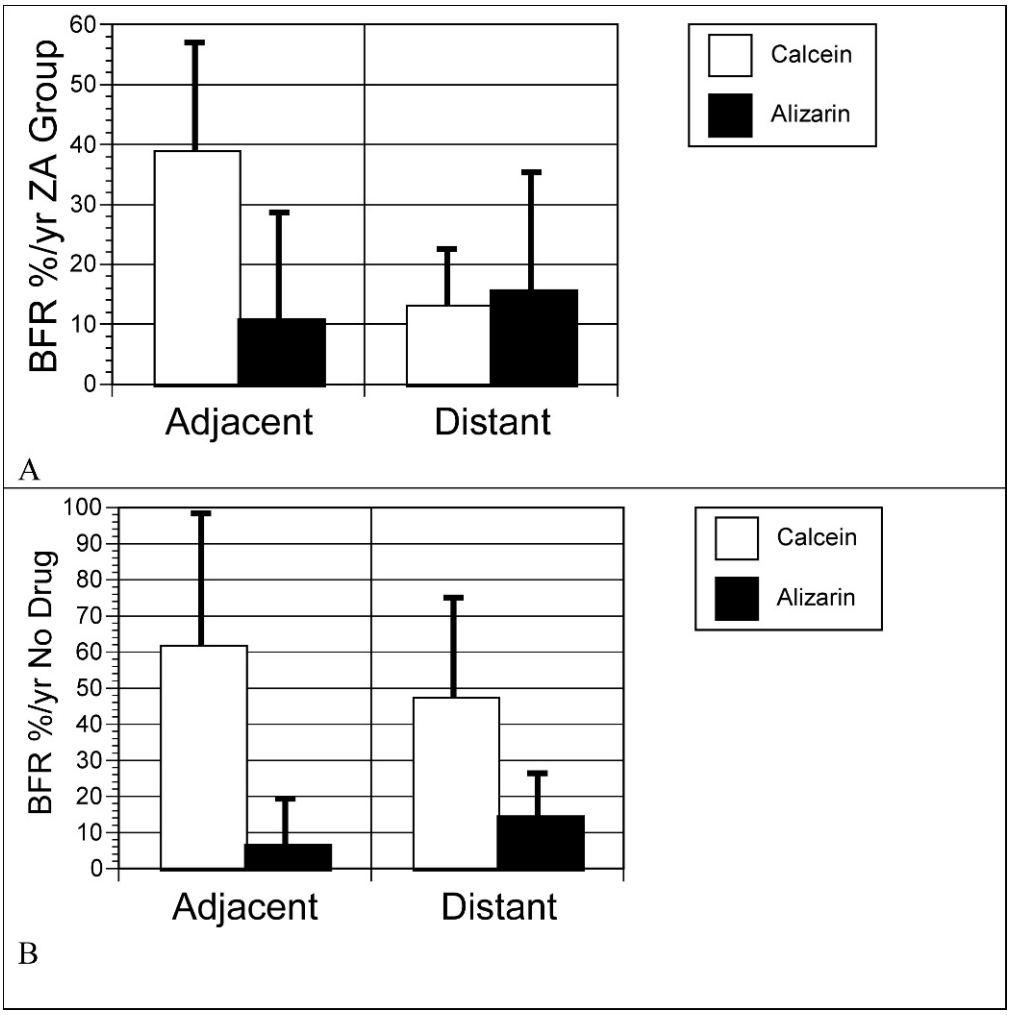

Figure 4. Comparison of alizarin and calcein labeled bone formation rate (BFR, % per year, mean, SD) in the (A) ZA and (B) ND groups for mini-implant adjacent (near) and distant bone. There is a significant ($P < .01$) increase in BFR in both groups except for the distant bone in the ZA group ($P > .05$). The healing response results in increase in calcein label in the ZA group distant bone but not to the extent in the ND group. Note that scales of the bar graphs in A and B are different.

the short term a certain degree of remodeling can occur after injury to the bone due to mini-implant placement, even in a bisphosphonate-treated animal.

In this study, a relatively aged canine model that demonstrated secondary intracortical osteonal remodeling was selected. At 33 months, male canines are approximately 2 years beyond skeletal maturity. An appropriately aged animal model is important because remodeling decreases with age in the jaws of these animals.[21] For instance, the remodeling rates in the alveolar process of the mandible may dramatically decrease from 36% per year in a 1- to 2-year-old dog[22] to only 3% in a 10-year-old dog.[21] While extrapolation of results from a canine model to humans is to be conducted with caution, both humans and canines demonstrate intracortical remodeling, and canines have been used extensively in the past to study bone remodeling[23] and dental implant healing.[24]

During clinical use, it has been reported that approximately 10% to 20% of the mini-implants can become loose.[25,26] In this study, 21% of the mini-implants failed in the ZA group in contrast to a 15% failure rate in the ND group. Unloaded implants can have a higher failure rate than those receiving orthodontic loads.[27] In addition, loading alters the biologic healing response.[28] While we report the failure rate, the primary focus of the study was to examine the undisturbed (no load) histologic healing response in animals in the ND and ZA groups.

Some patients may be denied treatment because of concerns of complications in patients receiving bisphosphonates. Understanding of the healing response is thus critical information for clinicians. A major finding of this study is that the bone remodeling rate was significantly lower in the ZA group when compared with the same mini-implant placement injury in the ND

group. Specifically, bone adjacent (<1 mm) and distant (1–4 mm) regions around the mini-implant had BFRs of approximately 30%–40% and approximately 40%–45% lower in the ZA group than in the ND group, respectively. Importantly, the BFR in the ZA group varied between 9.5% and 43.0% per year. This finding is contrary to some suggestions that no or minimal healing is possible in bisphosphonate-treated bone. In terms of absolute values, it is important to note that the mean BFR of the ZA group in the implant adjacent bone was as high as 43% per year in the mandible and 37% per year in the maxilla. These results are notable, given the mean physiologic cortical BFR values in the alveolar bone obtained by evaluating multiple sections from the anterior, middle, and posterior regions in 1- to 2-year-old canines range between 19% in the maxilla and 36% in the mandible.[22]

The alizarin labels (A_1 and A_2, in Figure 1) were given prior to the administration of the zoledronic drug (T_3), and the calcein (C_1 and C_2 in Figure 1) approximately 6–7 months after the first alizarin label. By comparing the two labels, alizarin and calcein can provide an estimate of change in bone remodeling that is attributed to the drug primarily in the ZA group and due to age in the ND group. The alizarin marks the remodeling rate in bone prior to the mini-implant placement, and thus represents noninjured bone. However, comparisons of the alizarin and calcein data must consider the following two caveats. As the time period between the labels increases, there is an increased probability of losing[29] the first label (alizarin). Whether the first label is resorbed or replaced by the second label (calcein), an underestimation of the measurement could occur at the site of the original (alizarin) labels. Another consideration is that bone remodeling decreases with age. Thus, even within the ZA group, the change in amount of bone labels and the resulting remodeling rate cannot be attributed solely to the drug. In summary, we examined the ZA effect in two ways. First, we compared the calcein labels between the ZA and the ND groups. Second, we examined the within animal change in remodeling by comparing the alizarin and calcein labels. Interestingly, similar conclusions were gained by both data analysis methods, further strengthening the findings of this study.

Whether our results can be extrapolated to a clinical situation, in which the mini-implants are loaded for an extended duration in humans, is unclear. Further studies are required to evaluate the sustainability of the bone response for the duration of clinical use of the mini-implant in appropriately designed studies, and to ascertain whether the bone remodeling response would be adequate to provide clinical success of the mini-implant for the entire duration of its use.

CONCLUSIONS

- ZA suppresses, but does not abolish, bone remodeling in mini-implant supporting bone.
- Osteonal bone remodeling suggestive of a partial healing response occurs after injury to the bone even in an animal treated with a high-dose potent bisphosphonate.

ACKNOWLEDGMENTS

Funding from the College of Dentistry at the Ohio State University, American Association of Orthodontist Foundation, and Delta Dental is acknowledged. The biostatistics for the project described was also supported by Award UL1RR025755 from the National Center for Research Resources. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Research Resources or the National Institutes of Health. The authors are grateful to Dr Zongyang Sun for reviewing this manuscript and for his valuable suggestions.

REFERENCES

1. Bamias A, Kastritis E, Bamia C, et al. Osteonecrosis of the jaw in cancer after treatment with bisphosphonates: incidence and risk factors. *J Clin Oncol*. 2005;23:8580–8587.
2. Migliorati CA, Siegel MA, Elting LS. Bisphosphonate-associated osteonecrosis: a long-term complication of bisphosphonate treatment. *Lancet Oncol*. 2006;7:508–514.
3. Vahtsevanos K, Kyrgidis A, Verrou E, et al. Longitudinal cohort study of risk factors in cancer patients of bisphosphonate-related osteonecrosis of the jaw. *J Clin Oncol*. 2009;27:5356–5362.
4. Madrid C, Sanz M. What impact do systemically administrated bisphosphonates have on oral implant therapy? A systematic review. *Clin Oral Implants Res*. 2009;20(suppl 4):87–95.
5. Zahrowski JJ. Bisphosphonate treatment: an orthodontic concern calling for a proactive approach. *Am J Orthod Dentofacial Orthop*. 2007;131:311–320.
6. Roberts EW, Poon LC, Smith RK. Interface histology of rigid endosseous implants. *J Oral Implantol*. 1986;12:406–416.
7. Huja SS, Katona TR, Burr DB, Garetto LP, Roberts WE. Microdamage adjacent to endosseous implants. *Bone*. 1999;25:217–222.
8. Hoshaw SJ, Fyhrie DP, Schaffler MB. The effect of implant insertion and design on bone microdamage. In: Davidovitch Z, ed. *The Biological Mechanisms of Tooth Eruption, Resorption and Replacement by Implants*. Boston, Mass: Harvard Society for the Advancement of Orthodontics; 1994:735–741.
9. Garetto LP, Chen J, Parr JA, Roberts WE. Remodeling dynamics of bone supporting rigidly fixed titanium implants: a histomorphometric comparison in four species including humans. *Implant Dent*. 1995;4:235–243.
10. Huja SS. Biologic parameter that determine success of screws used in orthodontics to supplement anchorage. In: McNamara JA Jr, ed. *Implant Anchorage in Orthodontics*. Ann Arbor, Mich: 31st Annual Moyers Symposium; 2005:177–188.
11. Huja SS, Rao J, Struckhoff JA, Beck FM, Litsky AS. Biomechanical and histomorphometric analyses of mono-

cortical screws at placement and 6 weeks postinsertion. *J Oral Implantol*. 2006;32:110–116.
12. Kimmel DB. Mechanism of action, pharmacokinetic and pharmacodynamic profile, and clinical applications of nitrogen-containing bisphosphonates. *J Dent Res*. 2007;86: 1022–1033.
13. Starck WJ, Epker BN. Failure of osseointegrated dental implants after diphosphonate therapy for osteoporosis: a case report. *Int J Oral Maxillofac Implants*. 1995;10:74–78.
14. Fan TM, de Lorimier LP, Garrett LD, Lacoste HI. The bone biologic effects of zoledronate in healthy dogs and dogs with malignant osteolysis. *J Vet Intern Med*. 2008;22:380–387.
15. Frost HM. Tetracycline-based histological analysis of bone remodeling. *Calcif Tissue Res*. 1969;3:211–237.
16. Frost HM. The regional acceleratory phenomenon: a review. *Henry Ford Hosp Med J*. 1983;31:3–9.
17. Merz WA, Schenk RK. A quantitative histological study on bone formation in human cancellous bone. *Acta Anat (Basel)*. 1970;76:1–15.
18. Parfitt A, Drezner M, Glorieux F, et al. Bone histomorphometry: standardization of nomenclature, symbols, and units. Report of the ASBMR Histomorphometry Nomenclature Committee. *J Bone Miner Res*. 1987;2:595–610.
19. Allen MR, Burr DB. Mandible matrix necrosis in beagle dogs after 3 years of daily oral bisphosphonate treatment. *J Oral Maxillofac Surg*. 2008;66:987–994.
20. Allen MR, Kubek DJ, Burr DB. Cancer treatment dosing regimens of zoledronic acid result in near complete suppression of mandible intra-cortical bone remodeling in beagle dogs. *J Bone Miner Res*. 2010;25:98–105.
21. Tricker ND, Dixon RB, Garetto LP. Cortical bone turnover and mineral apposition in dentate bone mandible. In: Garetto LP, Turner CH, Duncan RL, Burr DB, eds. *Bridging the Gap Between Dental and Orthopaedic Implants*. Indianapolis, Ind: School of Dentistry Indiana University; 2002:226–227.
22. Huja SS, Fernandez SA, Hill KJ, Li Y. Remodeling dynamics in the alveolar process in skeletally mature dogs. *Anat Rec A Discov Mol Cell Evol Biol*. 2006;288:1243–1249.
23. Allen MR, Follet H, Khurana M, Sato M, Burr DB. Antiremodeling agents influence osteoblast activity differently in modeling and remodeling sites of canine rib. *Calcif Tissue Int*. 2006;79:255–261.
24. Brunski JB, Moccia AF Jr, Pollack SR, Korostoff E, Trachtenberg DI. The influence of functional use of endosseous dental implants on the tissue-implant interface. I. Histological aspects. *J Dent Res*. 1979;50:1953–1969.
25. Luzi C, Verna C, Melsen B. A prospective clinical investigation of the failure rate of immediately loaded mini-implants used for orthodontic anchorage. *Prog Orthod*. 2007;8:192–201.
26. Park HS, Jeong SH, Kwon OW. Factors affecting the clinical success of screw implants used as orthodontic anchorage. *Am J Orthod Dentofacial Orthop*. 2006;130:18–25.
27. Deguchi T, Takano-Yamamoto T, Kanomi R, Hartsfield JK Jr, Roberts WE, Garetto LP. The use of small titanium screws for orthodontic anchorage. *J Dent Res*. 2003;82: 377–381.
28. Roberts WE, Smith RK, Zilberman Y, Mozsary PG, Smith RS. Osseous adaptation to continuous loading of rigid endosseous implants. *Am J Orthod*. 1984;86:95–111.
29. Martin RB. Label escape theory revisited: the effects of resting periods and section thickness. *Bone*. 1989;10: 255–264.