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ALENDRONATE TREATMENT RESULTS IN SIMILAR LEVELS OF TRABECULAR BONE REMODELING IN THE FEMORAL NECK AND VERTEBRA

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Abstract

INTRODUCTION—Bone turnover suppression in sites that already have a low surface-based remodeling rate may lead to oversuppression that could have negative effects on the biomechanical properties of bone. The goal was to determine how alendronate suppresses bone turnover at sites with different surface-based remodeling rates.

METHODS—Dynamic histomorphometric parameters were assessed in trabecular bone of the femoral neck and lumbar vertebrae obtained from skeletally mature beagles treated with saline (1 ml/kg/day) or alendronate (ALN 0.2 or 1.0 mg/kg/day). The ALN0.2 and ALN1.0 doses approximate, on a milligram per kilogram basis, the clinical doses used for the treatment of postmenopausal osteoporosis and Paget's disease, respectively.

RESULTS—Alendronate treatment resulted in similar absolute levels of bone turnover in the femoral neck and vertebrae, although the femoral neck had 33% lower pre-treatment surface-based remodeling rate than the vertebra ($p < 0.05$). Additionally, the high dose of alendronate (ALN 1.0) suppressed bone turnover to similar absolute levels as the low dose of alendronate (ALN 0.2) in both sites.

CONCLUSIONS—Alendronate treatment may result in a lower limit of trabecular bone turnover suppression, suggesting that sites of low pre-treatment remodeling rate are not more susceptible to oversuppression than those of high pre-treatment remodeling rate.

Keywords

Anti-remodeling; Bisphosphonates; Animal models; Histomorphometry

INTRODUCTION

The hallmark of osteoporosis is an increase in activation frequency and marked imbalance in bone remodeling at the cellular level resulting in bone loss, with consequent increased susceptibility to fracture [1-5]. Numerous studies have shown that bisphosphonates are

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effective in preventing bone loss and reducing the incidence of osteoporotic bone fractures [6-11]. For example, Black *et al.* [6] have found that three years of treatment with alendronate increases lumbar spine BMD by about 6% and reduces vertebral fracture rate by about 50% [6]. Interestingly, alendronate treatment results in a 32% greater BMD increment in the lumbar spine than the hip, but reduces fracture risk at both sites by roughly the same degree [6]. This suggests that factors other than BMD play an important role in dictating the effects of bisphosphonates on preventing bone fractures. These factors may include the site specific biomechanical milieu, the propensity to fall or alterations in the bone tissue matrix properties. It has also been proposed that the pre-treatment rate of bone turnover contributes to the ability of bone to resist fracture [12-15].

Clinical studies have found that after adjusting for BMD, the biochemical markers of bone turnover can still predict fracture risk [12-15]. This finding is consistent with Riggs *et al*. [16] who demonstrated that suppression of bone turnover and an increase in vertebral BMD contribute equally to the propensity of bone to resist a vertebral fracture. Riggs *et al*. [16] proposed that the efficacy of the suppression of bone turnover rate in reducing the incidence of bone fractures could be explained by considering the pre-treatment turnover rate. The high turnover rate in the vertebra may exacerbate the effects of bone loss by accelerating the loss of trabecular connectivity and thickness [1,16,17,18,19]. Normalization of the bone turnover rate in the vertebra, therefore, reduces the effects of high turnover rate on the microarchitectural deterioration and improve bone's biomechanical properties even more than expected based on BMD alone [16,17,20]. However, the effects of turnover suppression on sites with lower pre-treatment rates may lead to oversuppression that could compromise the bone tissue matrix properties. As treatment with alendronate is extending beyond the first decade in some patients, long term suppression at sites that already have low turnover could have negative effects that may override the beneficial effects of bone turnover suppression on BMD and bone microarchitecture.

The aim of this study was to determine whether alendronate treatment suppresses bone turnover more, on either an absolute or percentage basis, in sites with lower surface-based pre-treatment remodeling rates. Greater suppression in sites of low pre-treatment remodeling rate could suggest that these sites are more susceptible to oversuppression than those of high pre-treatment remodeling rate. The turnover rates in the trabecular bone of the vertebra and femoral neck of beagles treated with alendronate for 3 years were compared, as previous data have shown that, in trabecular bone, the femoral neck has a lower pre-treatment remodeling rate than the vertebra [21,22].

MATERIALS AND METHODS

Animals

Thirty-six intact female beagles (~ 1 year old) were purchased from Marshall Farms USA (North Rose, NY) and LBL (Reelsville, IN). On arrival, skeletal maturity was confirmed using radiographs to assess closure of the proximal tibia and lumbar vertebra growth plates. Dogs were housed two per cage at the Indiana University School of Medicine's AALAC-accredited facility, under environmentally controlled conditions, with free access to dry canine chow and water. All procedures were in accordance with NIH guidelines and approved by the Indiana University School of Medicine Animal Care and Use Committee.

Experimental design

Following two weeks of acclimatization, the animals were treated for either 1 year (N = 12) with saline (1ml/kg/day) or 3 years (N = 24) with alendronate. The group of dogs treated with saline was designated as the pre-treatment control group. The dogs treated with alendronate

were assigned to one of two treatment groups (N =12/group): 1) Alendronate (ALN0.2, 0.2 mg/kg/day); or 2) Alendronate (ALN1.0, 1.0 mg/kg/day). The ALN0.2 and ALN1.0 doses approximate, on a milligram per kilogram basis, the clinical doses used for the treatment of postmenopausal osteoporosis and Paget's disease, respectively. Alendronate sodium (Merck and Co., Inc) was dissolved in saline and administered orally, by a syringe, each morning after an overnight fast and at least 2 h prior to feeding. Prior to sacrifice, the animals were injected intravenously with calcein (0.20 ml/kg) using a 2–12–2–5 day labeling schedule. Due to a scheduling error, a 2–5–2–5 day labeling schedule was used for some of the pre-treatment control animals (N = 3). Two animals in the three-year ALN0.2 group developed hernias, one of which progressed to the point that the animal was euthanized early (month 34 of treatment); the data obtained from this dog were included in all analyses. All other animals completed the study without complication. Animals were euthanized by an overdose of sodium pentobarbital (0.22 mg/kg Beuthanasia-D Special, IV). After death, the right femoral neck and the second lumbar vertebrae were dissected and fixed in 10% neutral buffered formalin for histology.

Histology

Section preparation procedure and histomorphometric data of the second lumbar vertebrae were previously published [23,24]. The femoral neck specimens were stained in basic fuchsin (1 %) dissolved in increasing concentrations and embedded in methyl metacrylate [24]. Two transverse sections (80 - 100 μm) from each specimen were cut using a diamond wire saw (Histosaw; Delaware Diamond Knives). The basic fuchsin staining was used to identify microdamage for a separate investigation.

Histological measurements were performed in trabecular bone using a semiautomatic analysis system (Bioquant OSTEO 7.20.10, Bioquant Image Analysis Co.) attached to a microscope equipped with an ultraviolet light source (Nikon Optiphot 2 microscope, Nikon). An approximately 20 mm^2 region of interest was examined from each specimen. Primary variables of interest included single- and double label perimeter (sL.Pm, dL.Pm), bone perimeter (B.Pm) and interlabel width (Ir.L.Wi). From these variables, the following dynamic histomorphometric parameters were calculated: mineralizing surface (MS/BS = 100 × [0.5 × sL.Pm + dL.Pm]/ B.Pm; %), mineral apposition rate (MAR = Ir.L.Wi/d; μm/day; d is the labeling period in days), and bone formation rate (BFR/BS = MAR × MS/BS × 3.65; $\mu m^3/\mu m^2$/year). One femoral neck specimen in the ALN1.0 group did not have double label and was assigned a value of 0.3 μm/ day for MAR [25]. All variables were measured and calculated in accordance with ASBMR recommended standards [26].

Statistics

The differences in the histomorphometric parameters among the groups (Pre-treatment control; ALN0.2; ALN1.0) within each site were examined using one-way analysis of variance (ANOVA) tests following Anderson-Darling normality tests. When a significant overall F value ($p < 0.05$) was present, differences between individual group means were compared using Fisher's protected least-significant difference (PLSD) posthoc tests. To compare the differences in the histomorphometric parameters between sites within each group, paired t-tests were used following Anderson-Darling normality tests. For those variables failing the normality test, nonparametric tests (Kruskal-Wallis or Wilcoxon signed rank test) were used. For all tests, $p < 0.05$ was considered statistically significant. MINITAB 15 software (Minitab, Inc.) was used for all the statistical analyses.

RESULTS

Bone formation rate (surface-based remodeling rate) in the pre-treatment control animals was significantly lower in the femoral neck ($p < 0.05$; Fig. 1) compared to the vertebra, due to both lower MAR ($p < 0.05$; Table 1) and MS/BS ($p < 0.05$; Table 1) in the femoral neck.

Following three years of treatment, BFR/BS was significantly lower in both the femoral neck and vertebra compared to pre-treatment control ($p < 0.05$; Fig. 1). The absolute levels of BFR/BS after alendronate treatment were similar between the femoral neck and vertebra (Fig. 1). This suppression of bone formation rate was achieved by lower MAR ($p < 0.05$; Table 1) and MS/BS ($p < 0.05$; Table 1) compared to pre-treatment control. However, the similarity in BFR/BS between sites was a result of different trends in MAR and MS/BS between the femoral neck and vertebra. The MAR at the femoral neck was significantly less than the vertebra after treatment with either dose of alendronate ($p < 0.05$; Table 1). MS/BS was, however, lower (non-significantly) in the vertebra (Table 1) such that there were no differences in BFR/BS between the femoral neck and vertebra. Also, there was no difference in any bone formation parameter between the two doses of ALN at either site (Fig. 1 and Table 1).

DISCUSSION

Following three years of alendronate treatment, the absolute levels of bone turnover in the trabecular bone of the femoral neck and vertebra were similar, even though the pre-treatment surface-based remodeling rate was significantly lower (-33%) in the femoral neck than the vertebra. In addition, the high dose of alendronate (ALN 1.0) suppressed bone turnover to similar absolute levels as the low dose of alendronate (ALN 0.2) in both sites. These findings imply that a lower limit for bone turnover may exist, beyond which bone turnover cannot be suppressed further with the clinical doses of alendronate. This in turn suggests that compared to sites of high pre-treatment remodeling rate, sites of low pre-treatment remodeling rate are no more susceptible to oversuppression.

When an anti-resorptive agent is administered, there is an increase in bone density due to suppression of the activation of new remodeling sites, but with continued bone formation in resorptive cavities excavated by pre-treatment remodeling cycles [27]. Here, we show that irrespective of the pre-treatment remodeling rate, the end-point point of bone turnover may be similar suggesting that those sites with higher pre-treatment rates will experience a greater percentage reduction in bone turnover, but not a greater absolute suppression. A greater percentage suppression of bone turnover would in turn lead to a greater absolute increase in BMD. This is consistent with Gonnelli *et al*. [28] who found that treatment with alendronate results in a greater BMD increment in patients with high pre-treatment turnover rate compared to patients with low pre-treatment turnover rate.

The data reported here indicate that treatment with alendronate will suppress but will not abolish bone turnover. Bone turnover will be reduced to a non-zero limit and it will reach this limit regardless of the pre-treatment remodeling rate or the treatment dose. In agreement with these observations, clinical trials have demonstrated that in postmenopausal women treated up to 10 years with 5 mg or 10 mg of alendronate, bone turnover reaches a non-zero steady state after about 12 months of treatment [29,9]. More importantly, both doses suppress bone turnover to a similar limit [29,9]. Our findings also suggest that no oversuppression of bone turnover will be expected in patients on alendronate with low pre-treatment turnover rate because bone turnover will reach the same lower limit regardless of the pre-treatment turnover rate, or the drug dosage.

A study by Odvina *et al* [30] showed the absence of single or double-tetracycline labels in cancellous bone biopsies obtained from four patients treated with alendronate. Three of these

patients were, however, also administered estrogen or glucocorticoids. The co-treatment of alendronate with estrogen or glucocorticoids may have augmented the effects of alendronate on bone turnover leading to the complete absence of bone formation.

Two different approaches have been proposed in the literature to calculate mineral apposition rate (MAR) in the absence of double-labeled surfaces. Foldes *et al*. [25] proposed that in patients with missing double labels MAR be given a low value, reflecting the fact that formation was occurring, but very slowly. They suggested a lower biological limit of 0.3 μm/day. On the other hand, to be consistent with other bisphosphonate clinical trials [31-33], Recker *et al* [34] chose not to assign a specific missing value for MAR when no double-label was observed, and to consider these values as missing values (i.e., no value was assigned and BFR were not calculated from these data, reducing sample size for both MAR and BFR). This has the potential to lower the average turnover rate in the bisphosphonate treated group, and represents a bias. In the current study, only one specimen did not show the presence of double labeling. Using either approach for analyzing this specimen yielded the same conclusions. We elected to assign the value of 0.3 μm/day to this specimen.

Our findings should be interpreted with various limitations in mind. We considered measurements made from placebo-treated 2 year old dogs to represent the pre-treatment control values, even though alendronate treatment was initiated in 1 year old dogs. The reason for this is that this investigation was not part of the original experimental design of our dog study [23,24] and so no baseline control measurements were made in 1 year dogs. This only affects the calculated percentage reduction in turnover rate in the treated groups compared to the pre-treatment controls and does not affect the absolute calculated values for turnover in any way. Moreover, it reduces the normal age-related decline in activation frequency, providing a more stable baseline value. Another limitation of this study is the use of intact, non-ovariectomized, beagle dogs. It is also important to note that a large dose of alendronate, higher than the clinical doses, could suppress bone turnover beyond the lower limit observed here. However, there is no clinical rationale for such large doses of alendronate, and so those were not investigated here.

In conclusion, this study demonstrates that both doses of alendronate result in similar absolute levels of bone turnover in the trabecular bone of the femoral neck and vertebra, even though the femoral neck has significantly lower pre-treatment surface-based remodeling rate than the vertebra. This implies that sites with low pre-treatment remodeling rate are no more susceptible to oversuppression than those with higher pre-treatment remodeling rate. A better understanding of the relationship between bisphosphonates and bone turnover can provide better insight into how these agents can be most effectively and safely used in different patient populations.

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References

1. Parfitt AM, Mathews CH, Villanueva AR, et al. Relationships between surface, volume, and thickness of iliac trabecular bone in aging and in osteoporosis. Implications for the microanatomic and cellular mechanisms of bone loss. J Clin Invest 1983;72:1396–1409. [PubMed: 6630513]
2. Manolagas SC, Jilka RL. Bone marrow, cytokines, and bone remodeling. Emerging insights into the pathophysiology of osteoporosis. N Engl J Med 1995;332:305–311. [PubMed: 7816067]

3. Meunier PJ, Roux C, Seeman E, et al. The effects of strontium ranelate on the risk of vertebral fracture in women with postmenopausal osteoporosis. N Engl J Med Jan 2004;350:459–68.
4. Gass M, Dawson-Hughes B. Preventing Osteoporosis-Related Fractures: An Overview. Am J Med 2006;119(4 Suppl 1):S3–S11. [PubMed: 16563939]
5. Henriksen K, Tanko LB, Qvist P, et al. Assessment of osteoclast number and function: application in the development of new and improved treatment modalities for bone diseases. Osteoporos Int 2007;18:681–685. [PubMed: 17124552]
6. Black DM, Cummings SR, Karpf DB, et al. Randomised trial of effect of alendronate on risk of fracture in women with existing vertebral fractures. Fracture Intervention Trial Research Group. Lancet 1996;348:1535–1541. [PubMed: 8950879]
7. Cummings SR, Black DM, Thompson DE, et al. Effect of alendronate on risk of fracture in women with low bone density but without vertebral fractures: results from the Fracture Intervention Trial. JAMA 1998;280:2077–2082. [PubMed: 9875874]
8. Greenspan SL, Harris ST, Bone H, et al. Bisphosphonates: safety and efficacy in the treatment and prevention of osteoporosis. Am Fam Physician 2000;61:2731–2736. [PubMed: 10821153]
9. Bone HG, Hosking D, Devogelaer JP, et al. Alendronate Phase III Osteoporosis Treatment Study Group. Ten years' experience with alendronate for osteoporosis in postmenopausal women. N Engl J Med 2004;12:1189–1199. [PubMed: 15028823]
10. Papapoulos SE, Quandt SA, Liberman UA, et al. Meta-analysis of the efficacy of alendronate for the prevention of hip fractures in postmenopausal women. Osteoporos Int 2005;16:468–474. [PubMed: 15448985]
11. Rosen CJ, Hochberg MC, Bonnick SL, et al. Treatment with once-weekly alendronate 70 mg compared with once-weekly risedronate 35 mg in women with postmenopausal osteoporosis: a randomized double-blind study. J Bone Miner Res 2005;20:141–51. [PubMed: 15619680]
12. Garnero P, Hausherr E, Chapuy MC, et al. Markers of bone resorption predict hip fracture in elderly women: the EPIDOS Prospective Study. J Bone Miner Res 1996;11:1531–1538. [PubMed: 8889854]
13. Melton LJ 3rd, Khosla S, Atkinson EJ, et al. Relationship of bone turnover to bone density and fractures. J Bone Miner Res 1997;12:1083–1091. [PubMed: 9200008]
14. Sarkar S, Reginster JY, Crans GG, et al. Relationship between changes in biochemical markers of bone turnover and BMD to predict vertebral fracture risk. J Bone Miner Res 2004;19:394–401. [PubMed: 15040827]
15. Bauer DC, Black DM, Garnero P, et al. Change in bone turnover and hip, non-spine, and vertebral fracture in alendronate-treated women: the fracture intervention trial. J Bone Miner Res 2004;19:1250–1258. [PubMed: 15231011]
16. Riggs BL, Melton LJ 3rd, O'Fallon WM. Drug therapy for vertebral fractures in osteoporosis: evidence that decreases in bone turnover and increases in bone mass both determine antifracture efficacy. Bone 1996;18(3 Suppl):197S–201S. [PubMed: 8777088]
17. Riggs BL, Melton LJ 3rd. Bone turnover matters: the raloxifene treatment paradox of dramatic decreases in vertebral fractures without commensurate increases in bone density. J Bone Miner Res 2002;17:11–14. [PubMed: 11771656]
18. Akhter MP, Lappe JM, Davies KM, et al. Transmenopausal changes in the trabecular bone structure. Bone 2007;41:111–116. [PubMed: 17499038]
19. Chavassieux P, Seeman E, Delmas PD. Insights into material and structural basis of bone fragility from diseases associated with fractures: how determinants of the biomechanical properties of bone are compromised by disease. Endocr Rev 2007;28:151–164. [PubMed: 17200084]
20. Borah B, Dufresne TE, Ritman EL, et al. Long-term risedronate treatment normalizes mineralization and continues to preserve trabecular architecture: sequential triple biopsy studies with micro-computed tomography. Bone 2006;39:345–352. [PubMed: 16571382]
21. Forwood MR, Burr DB, Takano Y, et al. Risedronate treatment does not increase microdamage in the canine femoral neck. Bone 1995;16:643–650. [PubMed: 7669441]
22. Mashiba T, Hui S, Turner CH, et al. Bone remodeling at the iliac crest can predict the changes in remodeling dynamics, microdamage accumulation, and mechanical properties in the lumbar vertebrae of dogs. Calcif Tissue Int 2005;77:180–5. [PubMed: 16265598]

23. Allen MR, Burr DB. Three years of alendronate treatment results in similar levels of vertebral microdamage as after one year of treatment. J Bone Miner Res 2007;22:1759–1765. [PubMed: 17663638]
24. Allen MR, Iwata K, Phipps R, et al. Alterations in canine vertebral bone turnover, microdamage accumulation, and biomechanical properties following 1-year treatment with clinical treatment doses of risedronate or alendronate. Bone 2006;39:872–879. [PubMed: 16765660]
25. Foldes J, Shih MS, Parfitt AM. Frequency distributions of tetracycline-based measurements: implications for the interpretation of bone formation indices in the absence of double-labeled surfaces. J Bone Miner Res 1990;5:1063–7. [PubMed: 2080717]
26. Parfitt AM, Drezner MK, Glorieux FH. Bone histomorphometry: standardization of nomenclature, symbols, and units. Report of the ASBMR Histomorphometry Nomenclature Committee. J Bone Miner Res 1987;2:595–610. [PubMed: 3455637]
27. Seeman E. Is a change in bone mineral density a sensitive and specific surrogate of anti-fracture efficacy? Bone 2007;41:308–317. [PubMed: 17644058]
28. Gonnelli S, Cepollaro C, Pondrelli C, et al. Bone turnover and the response to alendronate treatment in postmenopausal osteoporosis. Calcif Tissue Int 1999;65:359–364. [PubMed: 10541760]
29. Tucci JR, Tonino RP, Emkey RD, et al. Effect of three years of oral alendronate treatment in postmenopausal women with osteoporosis. Am J Med 1996;101:488–501. [PubMed: 8948272]
30. Odvina CV, Zerwekh JE, Rao DS, et al. Severely suppressed bone turnover: a potential complication of alendronate therapy. J Clin Endocrinol Metab 2005;90:1294–1301. [PubMed: 15598694]
31. Chavassieux PM, Arlot ME, Reda C, et al. Histomorphometric assessment of the long-term effects of alendronate on bone quality and remodeling in patients with osteoporosis. J Clin Invest 1997;100:1475–1480. [PubMed: 9294113]
32. Recker RR, Weinstein RS, Chesnut CH 3rd, et al. Histomorphometric evaluation of daily and intermittent oral ibandronate in women with postmenopausal osteoporosis: results from the BONE study. Osteoporos Int 2004;15:231–237. [PubMed: 14727011]
33. Eriksen EF, Melsen F, Sod E, et al. Effects of long-term risedronate on bone quality and bone turnover in women with postmenopausal osteoporosis. Bone 2002;31:620–625. [PubMed: 12477578]
34. Recker RR, Delmas PD, Halse J, et al. Effects of intravenous zoledronic acid once yearly on bone remodeling and bone structure. J Bone Miner Res 2008;23:6–16. [PubMed: 17892374]

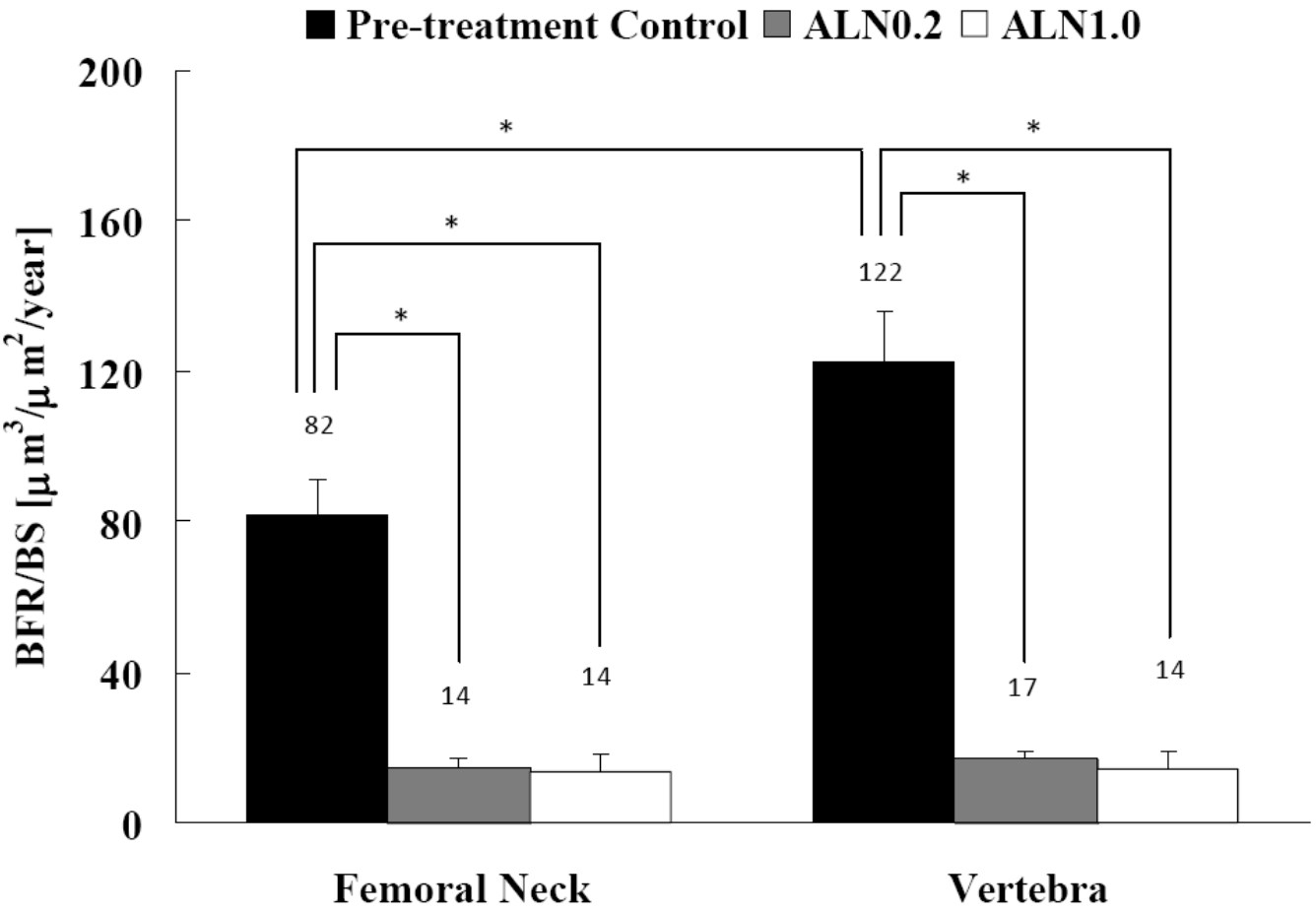

Figure 1.

Trabecular bone formation rate (BFR/BS) at the femoral neck and vertebra following three years of alendronate treatment. Three years of alendronate treatment resulted in similar bone formation rates between the femoral neck and vertebra. Numbers above bars represent the actual BFR/BS in each group. "*" indicates $p < 0.05$. Data are presented as + SE mean.

Table 1

Trabecular bone histomorphometry of the femoral neck and vertebra following three years of alendronate treatment.

	Femoral Neck			
	Pre-treatment Control	**Alendronate 0.2 mg/kg/day**	**Alendronate 1 mg/kg/day**	**p Value**
MAR [μm/day]	1.24 ± 0.08	0.81 ± 0.06*	0.81 ± 0.07*	<0.001
MS/BS %	17.88 ± 1.52	4.62 ± 0.65*	4.12 ± 1.22*	<0.001
	Vertebra			
	Pre-treatment Control	***Alendronate 0.2 mg/kg/day***	***Alendronate 1 mg/kg/day***	***p Value***
MAR [μm/day]	1.56 ± 0.09	1.13 ± 0.06*	1.11 ± 0.07*	<0.001
MS/BS %	21.34 ± 1.83	4.15 ± 0.51*	3.30 ± 0.92*	< 0.001

MAR, mineral apposition rate; MS/BS, mineralizing surface per unit bone surface.

* indicates $p < 0.05$ vs. Pre-treatment Control.

Data are presented as ± SE mean.