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Giant Osteoclast Formation and Long-Term Oral Bisphosphonate Therapy

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Abstract

BACKGROUND—Bisphosphonates decrease bone resorption and are commonly used to treat or prevent osteoporosis. However, the effect of bisphosphonates on their target cells remains enigmatic, since in patients benefiting from therapy, little change, if any, has been observed in the number of osteoclasts, which are the cells responsible for bone resorption.

METHODS—We examined 51 bone-biopsy specimens obtained after a 3-year, double-blind, randomized, placebo-controlled, dose-ranging trial of oral alendronate to prevent bone resorption among healthy postmenopausal women 40 through 59 years of age. The patients were assigned to one of five groups: those receiving placebo for 3 years; alendronate at a dose of 1, 5, or 10 mg per day for 3 years; or alendronate at a dose of 20 mg per day for 2 years, followed by placebo for 1 year. Formalin-fixed, undecalcified planar sections were assessed by bone histomorphometric methods.

RESULTS—The number of osteoclasts was increased by a factor of 2.6 in patients receiving 10 mg of alendronate per day for 3 years as compared with the placebo group ($P < 0.01$). Moreover, the number of osteoclasts increased as the cumulative dose of the drug increased ($r = 0.50$, $P < 0.001$). Twenty-seven percent of these osteoclasts were giant cells with pyknotic nuclei that were adjacent to superficial resorption cavities. Furthermore, giant, hypernucleated, detached osteoclasts with 20 to 40 nuclei were found after alendronate treatment had been discontinued for 1 year. Of these large cells, 20 to 37% were apoptotic, according to both their morphologic features and positive findings from in situ end labeling.

CONCLUSIONS—Long-term alendronate treatment is associated with an increase in the number of osteoclasts, which include distinctive giant, hypernucleated, detached osteoclasts that are undergoing protracted apoptosis.

Bisphosphonates are used worldwide to prevent fractures in patients with osteoporosis.^{1–6} Treatment with these drugs decreases the rate of bone resorption and levels of biochemical markers of bone turnover and causes progressive increases in bone mineral density. The clinical efficacy of nitrogen-containing bisphosphonates is widely believed to result from their potent ability to decrease the number of osteoclasts by promoting their apoptosis.^{7–9} Once osteoclasts become apoptotic, they are usually quickly ingested by bone marrow

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phagocytes.¹⁰ However, enumeration of osteoclasts in specimens of cancellous bone (bone composed of multiple trabecular structures) obtained from patients treated with nitrogen-containing bisphosphonates shows surprisingly little, if any, change in the number of osteoclasts.^{11,12} This observation suggests that the mechanism by which these drugs work in vivo may differ from the current thinking. Heretofore, the discrepancy between the antifracture efficacy of bisphosphonates and the absence of any effect on the number of osteoclasts has been attributed to the imprecision of histomorphometric indexes of bone resorption.¹¹ Indeed, osteoclasts in normal persons occupy less than 1% of the cancellous perimeter, so that a decrease in their number might be difficult to detect.¹³ Alternative explanations for this discrepancy include a greater effect of the drugs on cortical than on cancellous bone, a bisphosphonate-induced decrease in the rate of bone resorption by osteoclasts, and nonadherence to long-term drug therapy.^{11,12,14}

Bisphosphonate-induced inhibition of bone resorption in vitro does not require apoptosis of osteoclasts.¹⁵ In addition, administration of bisphosphonates to beagles results in a higher rather than a lower number of osteoclasts owing to prolongation of the osteoclast lifespan.¹⁶ These observations suggested to us that nitrogen-containing bisphosphonates may inhibit bone resorption without decreasing the number of osteoclasts and may even increase the number of these cells in cancellous bone. To reconcile the paradoxical findings of these studies, we conducted an in-depth examination of osteoclast morphologic features in bone-biopsy specimens from women who had participated in a trial of alendronate for the prevention of postmenopausal osteoporosis.³

METHODS

STUDY POPULATION AND DESIGN

Transiliac bone-biopsy specimens were available from the trial by McClung et al., which was a 3-year (1994 to 1997), double-blind, randomized, placebo-controlled, dose-ranging trial of alendronate in 447 healthy postmenopausal women 40 through 59 years of age who had entered menopause 6 to 36 months before enrollment.³ The trial by McClung et al. was a prevention trial, and the bone mineral density of the patients was within 2 SD above or below normal peak adult values at the initiation of the trial. However, the bone mineral density of the patients at baseline was approximately 10% below the mean value for young adult women, and the bone mineral density in patients receiving placebo decreased by 3 to 4% at the spine, femoral neck, and trochanter during the trial. This result indicates that these women were having postmenopausal bone loss.³ Women who had disorders of bone and mineral metabolism, smoked more than 20 cigarettes per day, or consumed three or more alcoholic drinks per day were excluded. The patients were randomly assigned to one of five groups: those receiving placebo for 3 years; oral alendronate at a dose of 1, 5, or 10 mg per day for 3 years; or oral alendronate at a dose of 20 mg per day for 2 years, followed by placebo for 1 year. The original trial had shown that alendronate at a dose of 5 or 10 mg per day for 3 years or at a dose of 20 mg per day for 2 years, followed by placebo for 1 year, increased total body bone mineral density and bone mineral density at the lumbar spine, femoral neck, and trochanter by 1 to 4% from baseline. Patients receiving alendronate at a dose of 1 mg per day had a lower rate of loss of bone mineral density than those receiving placebo. Alendronate also decreased the levels of biochemical markers of bone turnover as compared with baseline.¹⁻³

After 3 years of treatment, 55 patients volunteered to undergo a transiliac bone biopsy to determine whether the drug had impaired skeletal mineralization, as had occurred with etidronate, a first-generation bisphosphonate.¹⁷ To label the bone for the measurement of the mineral appositional rate and the mineralizing perimeter, the patients were given oral tetracycline hydrochloride (250 mg four times per day) 19, 18, 7, and 6 days before the

biopsy. Thus, each patient underwent two periods of tetracycline labeling separated by an interval of approximately 2 weeks. Fifty-five biopsy specimens 7 mm in diameter were obtained; 51 of these included enough of the two cortices and the intervening cancellous bone to permit histomorphometric evaluation. All 15 centers that contributed specimens received approval from their institutional review boards, and all patients provided written informed consent. Additional approval for reexamining the specimens was obtained from the review board of the University of Arkansas for Medical Sciences, where the specimens were held and read centrally. Each specimen was coded so that the reader was unaware of the patient's study-group assignment. The finding of normal osteoid thickness and a decreased mineralizing surface (the length of double-tetracycline-labeled cancellous bone plus half the length of single-tetracycline-labeled cancellous bone) in the alendronate groups as compared with the placebo group has already been reported.³ To our knowledge, the rates of bone formation and mineral apposition and the number of osteoclasts have not been previously reported.

HISTOMORPHOMETRIC MEASUREMENTS

Within 24 hours after the biopsy, the specimens were fixed in iced Millonig's phosphate-buffered 10% formalin at pH 7.4 and sent by express mail to the University of Arkansas for Medical Sciences. At the university, the specimens were dehydrated in graded ethanol solutions and embedded undecalcified in methylmethacrylate.¹⁸ The tungsten-carbide D-profile rotary microtome blades (Delaware Diamond Knives) were sharpened every 2 weeks to maintain the quality of the sections. Longitudinal sections, 5 μm in thickness, were taken from one third and one half the depth of the specimens. Two sections from each depth were left unstained for examination of the tetracycline labels, and two sections were stained by a modification of the Masson technique to enhance nuclear detail.¹³ Additional sections were stained for tartrate-resistant acid phosphatase (TRAP).^{19,20} TRAP staining was performed to confirm that the abnormal cells were indeed osteoclasts, but TRAP staining impairs nuclear detail. Therefore, all osteoclast measurements were performed on the Masson-stained sections. The coverslips were compressed with 2-oz (approximately 60-g) fishing weights for 30 minutes to facilitate the production of smooth, planar sections free of distortion.

The histomorphometric examination was performed with the use of a digitizer tablet (Osteo-Metrics) interfaced to a Zeiss Axioscope with a drawing-tube attachment, as previously described.^{13,17,18,21} The total number of osteoclasts on or adjacent to cancellous bone was expressed as the number per millimeter of cancellous bone perimeter. A giant osteoclast was defined as one having at least three of the following characteristics: more than eight nuclear profiles (two-dimensional images of a section taken through three-dimensional objects), detachment from bone, a shallow or absent resorption cavity, pyknotic nuclei, or the presence of other mononuclear cells interposed between the bone perimeter and the osteoclast. The osteoclasts of patients in the placebo group had fewer than three of these characteristics, but they infrequently showed some small degree of detachment or nuclear pyknosis. The osteoclasts were then further classified according to the number of nuclear profiles that could be counted in the 5- μm -thick sections; these sections contained only a small portion of the giant osteoclasts, which were 80 μm to more than 100 μm in thickness. A normal osteoclast is shown in Figure 1.

MEASUREMENT OF APOPTOSIS IN UNDECALCIFIED BONE SECTIONS

Apoptosis of osteoclasts was detected in additional sections taken from the blocks by in situ end labeling with the use of the Klenow FragEL DNA Fragmentation Detection Kit (Oncogene Research Products), as previously described.²² Osteoclasts positive for in situ end labeling also had morphologic changes that included discretely condensed chromatin, nuclear fragmentation, nuclear peripheral beading, and cell shrinkage. To be counted as

apoptotic, an osteoclast had to be stained by in situ end labeling and have at least three of the four morphologic criteria. For negative controls, we used archival specimens from patients with renal osteodystrophy who had abundant osteoclasts due to secondary hyperparathyroidism.¹⁸

STATISTICAL ANALYSIS

To evaluate group changes in the histomorphometric data, we used one-way analysis of variance, followed by Dunnett's multiple-comparison test.^{23,24} The data are reported as means and standard errors, unless otherwise indicated; P values of less than 0.05 were considered to indicate statistical significance. The total oral dose of alendronate was calculated by multiplying the daily dose by the number of days it was taken. Comparisons of interest were specified a priori. Consequently, when comparing groups with respect to the number of normal-appearing osteoclasts, we tested only the hypothesis that the number of such osteoclasts was higher in the group receiving 10 mg of alendronate per day (a commonly used dose) than in the placebo group. Pearson correlation coefficients were used to test for an association between the number of osteoclasts and the total amount of alendronate administered. Patients for whom treatment with alendronate was stopped after 2 years were excluded, although similar results were obtained when all patients were included. All cell counts, including zeroes, were included in the analyses. The residuals from a linear regression of the total number of osteoclasts against the total amount of alendronate administered passed the test of normality; this result supports the use of Pearson correlation coefficients, although similar results were obtained with the use of Spearman coefficients.

RESULTS

STANDARD HISTOMORPHOMETRIC FINDINGS

To establish that alendronate reduced bone turnover, as expected, we examined histomorphometric measurements of bone formation (Table 1). The rate of bone formation was diminished to the same extent in the groups receiving 1, 5, or 10 mg of alendronate per day ($P < 0.003$ for each comparison with the placebo group). However, in the group receiving alendronate at a dose of 20 mg per day for 2 years, followed by placebo for 1 year, the rate of bone formation was not significantly different from that in the group receiving placebo, a result indicating that the antiremodeling effect of the drug was reversed a year after cessation of therapy. Such a reversal had been suggested by the results of the original 3-year trial of alendronate,³ which showed a greater length of mineralizing surface in the group receiving alendronate at a dose of 20 mg per day for 2 years, followed by placebo for 1 year, than in the other alendronate groups. The reduction in bone formation was entirely due to a reduction in the tetracycline-labeled perimeter, rather than a reduction in the distance between the double tetracycline labels — an expression of the mineral appositional rate (Table 1).

HISTOMORPHOMETRIC FEATURES OF CANCELLOUS OSTEOCLASTS

The total number of osteoclasts per millimeter of cancellous perimeter among patients receiving alendronate at a dose of 1 mg or 5 mg per day was not significantly different from that among patients receiving placebo. However, the total number of osteoclasts was higher by a factor of 2.6 in the group receiving 10 mg of alendronate per day for 3 years than in the placebo group ($P < 0.01$) (Fig. 2A). In addition, the total number of osteoclasts was correlated with the total amount of alendronate administered during the 3 years ($r = 0.50$, $P < 0.001$) (Fig. 2C). Furthermore, in the group receiving 10 mg of alendronate per day for 3 years, 27% of the osteoclasts were giants with pyknotic nuclei that were adjacent to superficial resorption cavities with mononuclear cells between the osteoclast and the bone perimeter; this finding proves that the detachment was not due to tissue shrinkage during histologic

preparation (Fig. 2B and 3). The number of giant osteoclasts was significantly greater in the group receiving 10 mg of alendronate per day than in the group receiving 1 mg or in the group receiving 5 mg of alendronate per day ($P < 0.001$). No patients in the placebo group had giant osteoclasts. Moreover, the number of giant osteoclasts was correlated with the cumulative alendronate dose ($r = 0.55$, $P < 0.001$) (Fig. 2C). All the osteoclasts stained positive for TRAP, and the intensity of staining was similar in the placebo and the alendronate groups (Fig. 3C and 3D). Giant osteoclasts were also found in the group receiving 20 mg of alendronate per day for 2 years, followed by placebo for 1 year, even though treatment with the drug had been stopped for 1 year (Fig. 3A). However, not all patients had abnormal osteoclasts; for example, in the group receiving 10 mg of alendronate per day, only 56% had abnormal osteoclasts (Table 1). It was remarkable that there were more normal-appearing osteoclasts in the patients receiving 10 mg of alendronate per day than in the patients in the placebo group ($P < 0.03$), and the number of normal-appearing osteoclasts was correlated with the total amount of alendronate administered ($r = 0.40$, $P < 0.01$) (Table 1 and Fig. 2C).

In the placebo group, the number of osteoclast nuclear profiles ranged from two to eight. In the alendronate groups, giant osteoclasts had up to 40 nuclear profiles, and 20 to 40 nuclear profiles were present even in the group in which treatment with alendronate had been discontinued for 1 year (Fig. 3A and 4A). Furthermore, 20 to 37% of the giant osteoclasts (0.7 to 8.0% of the total number of osteoclasts) had in situ end labeling, nuclear condensation, fragmentation, and peripheral beading indicative of apoptosis (Fig. 3E). Staining by in situ end labeling was restricted to the giant osteoclasts, and all nuclei in the cells with in situ end labeling were apoptotic. Apoptosis of osteoclasts was not seen in all patients, and in those patients with apoptosis of osteoclasts, not all the osteoclasts were affected. However, the mean prevalence of osteoclast apoptosis was 30% in the group receiving 10 mg of alendronate per day and 26% in the group receiving 20 mg of alendronate per day for 2 years, followed by placebo for 1 year (Fig. 4B).

DISCUSSION

In early postmenopausal women, treatment with alendronate for 3 years decreased biochemical markers and histomorphometric indexes of bone turnover and simultaneously increased bone mineral density.³ Our study indicates that these changes were accompanied by a dose-dependent increase in the number of osteoclasts. Approximately one third of these osteoclasts were giant and detached. Although a significantly higher number of normal-appearing osteoclasts was present in the group receiving 10 mg of alendronate per day than in the placebo group, the decrease in biochemical markers and the increase in bone mineral density suggest that even the normal-appearing osteoclasts resorbed bone poorly. Normally, the number of osteoclasts is a faithful index of bone resorption,²¹ but clearly, this is not true after treatment with alendronate. Furthermore, the treatment gave rise to a distinctive giant, hypernucleated, detached osteoclast, with a seemingly protracted duration of apoptosis. The duration of apoptosis in these cells is difficult to determine, because the histologic distinction between a rare but long-lasting event and a frequent but brief event is not usually possible. However, the acquisition of up to 40 nuclei noted in both the group receiving 10 mg of alendronate per day for 3 years and the group receiving 20 mg of alendronate per day for 2 years, followed by placebo for 1 year, would be expected to take some time.

The increase in the number of normal-appearing osteoclasts as well as in the number of giant, hypernucleated, detached, apoptotic osteoclasts after long-term treatment with alendronate may seem counterintuitive, but we propose that both findings could be explained if nitrogen-containing bisphosphonate therapy prolongs osteoclast lifespan¹⁶ as

well as the duration of the DNA-fragmentation phase of apoptosis that is detected by in situ end labeling. Nitrogen-containing bisphosphonates inhibit bone resorption by interfering with farnesyl pyrophosphate synthase and the mevalonate-to-cholesterol pathway, resulting in the loss of small guanosine triphosphate-binding proteins required to form the ruffled border of the osteoclasts (i.e., the microvillus-lined bone interface required for resorption)^{7–9,15} (Fig. 1). The loss of the guanosine triphosphate-binding proteins leads to cytoskeletal disruption, loss of osteoclast orientation, and detachment of the osteoclasts from the bone perimeter (Fig. 3). However, osteoclasts are produced by the fusion of mononuclear precursors to form polykaryons, a process necessary to maintain resorption efficiency.^{7–9,25,26} Osteoclasts normally acquire up to eight nuclei before dying by apoptosis, probably as a result of exposure to the high extracellular concentration of calcium that occurs during bone resorption.^{10,27} Other substances are also liberated during bone resorption, but the evidence that released calcium is an important signal for osteoclast death is strong.²⁸ Inhibition of bone resorption by nitrogen-containing bisphosphonates would effectively diminish the signal for death that results from calcium release during resorption, thereby prolonging osteoclast lifespan and allowing time for fusion of osteoclasts with additional mononuclear progenitors. Bisphosphonate administration and the resultant increased accumulation of mononuclear cells may then lengthen the duration of osteoclast apoptosis and thus prolong the period of its detection by in situ end labeling.

Increases in the number and size of osteoclasts and in the number of their nuclear profiles are also features of Albers-Schönberg disease or autosomal dominant osteopetrosis type II, a disorder in which the osteoclasts fail to erode bone because of mutations in the *CLCN7* gene, which encodes a chloride channel required for acid production and bone resorption.²⁹ These dysfunctional osteoclasts are numerous and large, and they contain up to 15 nuclei.^{30,31} In addition, in Paget's disease, bone resorption is extensive and hypernucleated osteoclasts are abundant, but the resorptive capacity of individual osteoclasts may be decreased by as much as 65%.³² On the basis of these findings, we hypothesize that interference with the resorptive capacity of osteoclasts (whether that interference is a result of bisphosphonate administration or inherited, as in Albers-Schönberg disease or Paget's disease), rather than the induction of apoptosis, is the cause of the appearance of giant hypernucleated osteoclasts. Removal of the calcium signal for apoptosis prolongs the lifespan of osteoclasts and permits excessive fusion of osteoclasts with progenitor cells, thus explaining the presence of these giant, hypernucleated osteoclasts in several clinical situations.

In our study, giant, hypernucleated, detached osteoclasts were present even 1 year after discontinuation of high-dose alendronate. However, the cumulative dose of alendronate during the 3-year trial was highest in the group receiving 20 mg of alendronate per day for 2 years, followed by placebo for 1 year (Fig. 4B). Despite the presence of giant osteoclasts and the recent concern that long-term bisphosphonate therapy causes prolonged suppression of bone turnover,^{33–35} our observations show that the rate of bone formation in the group receiving 20 mg of alendronate per day for 2 years, followed by placebo for 1 year, was equivalent to that found in the group receiving placebo, a result suggesting that discontinuation of therapy reverses the reduction in bone formation. When the group receiving 20 mg of alendronate per day was switched to placebo after 2 years, the suppression of the biochemical markers of bone turnover was partially attenuated, a result indicating that it is the current dosage, rather than the cumulative dose, that determines the reduction in bone formation. However, the effects of bisphosphonates on osteoclasts may persist after the discontinuation of therapy. In agreement with this interpretation, a 10-year study showed that after 5 years of treatment with alendronate, followed by 5 years of placebo, the levels of bone resorption markers increased but were still below those at baseline.³⁶

Earlier studies found that administration of bisphosphonates to animals^{16,37–39} and to children with osteogenesis imperfecta⁴⁰ or bisphosphonate induced osteopetrosis³³ increased the number of enlarged osteoclasts. Similar findings were reported in three abstracts describing treatment given to adults with osteoporosis.^{14,41,42} However, in a head-to-head study in rats, giant osteoclasts with excessive numbers of nuclei were noted after the administration of nitrogen-containing bisphosphonates but not after the administration of etidronate or clodronate, bisphosphonates that lack an amino group and are metabolized into toxic ATP analogues that cause rapid cell death.^{15,39} In contrast to treatment with nitrogen-containing bisphosphonate, replacement therapy with estrogens or androgens does not cause the production of giant osteoclasts and does reduce osteoclast number²⁰ as a result of direct proapoptotic effects of these agents on osteoclasts.⁴³

In conclusion, we counted and characterized the features of alendronate-induced giant osteoclasts in bone-biopsy specimens that had been obtained as part of a placebo-controlled, doseranging clinical trial. A greater total number of osteoclasts in the group receiving 10 mg of alendronate per day than in the group receiving placebo and the appearance of giant, hypernucleated, detached, apoptotic osteoclasts despite decreased resorption after long-term oral therapy with nitrogen-containing bisphosphonates may have important clinical implications. Although such cells may have no direct pathophysiologic effects on the patient, these cells contain TRAP and might release it or other factors affecting bone metabolism. Awareness of the possible presence of such cells may be important, because their presence could lead to a mistaken diagnosis of Paget's disease or hyperparathyroidism.^{42,44} However, in these two conditions, osteoid and osteoblasts are abundant, whereas after long-term oral therapy with nitrogen-containing bisphosphonates, reductions in osteoid, osteoblasts, and bone formation are expected.

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Figure 1. Normal Multinucleated Osteoclast Tightly Adherent to Bone from a Patient Receiving Placebo

The interface with the bone perimeter is marked by a ruffled or striated border of microvilli (white arrow), which is best visualized by electron microscopy but is demonstrated here by the dark-red-stained border. The osteoclast–bone interface creates a unique extracellular microenvironment, ideal for bone resorption. The lysosome- rich vacuolar zone (black arrow), composed of acidified vesicles containing cathepsin K and matrix metalloproteinases, is distinctly visualized. Polarized away from the bone surface are six nuclei with prominent nucleoli. This photograph was made with the use of a Zeiss 1.6× Optovar and a 100× oil immersion Plan-Apochromat lens with a 1.40 numeric aperture.

Figure 2. Numbers of Osteoclasts and Giant Detached Osteoclasts after Long-Term Alendronate Therapy

The women were assigned to one of five groups: those receiving placebo for 3 years; alendronate at a dose of 1, 5, or 10 mg per day for 3 years; or alendronate at a dose of 20 mg per day for 2 years, followed by placebo for 1 year. Panel A shows the mean ($\pm$ SE) total number of osteoclasts and Panel B the number of giant detached osteoclasts per millimeter of cancellous bone perimeter for the five groups. Panel C shows the total number of osteoclasts, the number of normal osteoclasts, and the number of giant detached osteoclasts in relation to the cumulative dose of alendronate administered during the 3 years of the trial (the daily dose multiplied by the number of days it was administered). The relationship between each line and the cumulative dose of alendronate is statistically significant ($P < 0.01$ to $P < 0.001$).

Figure 3. Giant Hypernucleated Osteoclasts after Alendronate Therapy in Postmenopausal Women

Panel A shows a detached osteoclast containing more than 30 nuclei in a patient who received 20 mg of alendronate per day for 2 years, followed by 1 year of placebo. Panel B shows an osteoclast from a patient who received 5 mg of alendronate per day for 3 years. The resorption cavity is superficial, and the hypernucleated osteoclast is detached from the bone perimeter, with mononuclear cells (arrow) interposed between the osteoclast and the bone. The nuclei of this osteoclast are pyknotic, but the adjacent marrow nuclei are perfectly preserved, demonstrating the absence of processing artifacts. Panel C shows an osteoclast from a patient who received 10 mg of alendronate per day for 3 years. Positive cytoplasmic red granular tartrate-resistant acid phosphatase (TRAP) staining was noted in all alendronate groups. The nuclei of this detached osteoclast are dispersed throughout the cell rather than polarized away from the bone surface, as is normally expected, and the lysosomal zone adjacent to the bone is absent. Panel D shows an osteoclast from a patient receiving placebo. TRAP staining in the placebo group was the same as in the alendronate groups. Note that the normal osteoclast is tightly adherent to the bone and that TRAP staining impairs nuclear detail. Panel E shows black staining by nuclear in situ end labeling in an osteoclast from a patient who received 10 mg of alendronate per day for 3 years. Morphologic changes of apoptosis are also present, including nuclear condensation, fragmentation, and peripheral beading. Panel F shows a control osteoclast without staining from in situ end labeling from a patient with renal osteodystrophy.

Figure 4. Number of Osteoclast Nuclear Profiles (Panel A) and Prevalence of Osteoclast Apoptosis as Determined by Morphologic Features and Staining by in Situ End Labeling (Panel B) after Long-Term Alendronate Therapy

The cumulative dose of alendronate was calculated by multiplying the daily dose by the number of days the dose was taken (e.g., 10 mg per day $\times$ 365 days $\times$ 3 years = 10,950 mg). Data are represented as means $\pm$ SE.

Table 1
Bone Histomorphometric Features after Long-Term Treatment with Alendronate.*

Feature	Alendronate, 1 mg/day for 3 Yr (N = 8)	Alendronate, 5 mg/day for 3 Yr (N = 11)	Alendronate, 10 mg/day for 3 Yr (N = 9)	Alendronate, 20 mg/day for 2 Yr, then Placebo for 1 Yr (N = 8)	Placebo for 3 Yr (N = 15)
Bone-formation rate — $\mu\text{m}^2/\mu\text{m}/\text{yr}$					
Mean	$0.01 \pm 0.01^{\dagger}$	$0.02 \pm 0.01^{\dagger}$	$0.02 \pm 0.01^{\dagger}$	0.04 ± 0.01	0.05 ± 0.01
Median	0.01	0.02	0.02	0.04	0.05
5th to 95th percentile	0.01–0.02	0.01–0.05	0.01–0.03	0.01–0.06	0.01–0.09
Mineral appositional rate — $\mu\text{m}/\text{day}$					
Mean	0.78 ± 0.03	0.78 ± 0.05	0.77 ± 0.04	0.73 ± 0.07	0.73 ± 0.04
Median	0.77	0.73	0.79	0.70	0.73
5th to 95th percentile	0.63–0.89	0.63–1.15	0.60–0.92	0.51–1.06	0.53–1.06
Double-tetracycline-labeled perimeter — %					
Mean	$1.3 \pm 0.2^{\ddagger}$	$1.6 \pm 0.4^{\ddagger}$	$1.1 \pm 0.2^{\ddagger}$	2.9 ± 0.8	4.3 ± 0.7
Median	1.3	1.3	0.9	2.0	4.1
5th to 95th percentile	0.5–2.3	0.3–3.9	0.1–2.4	0.6–6.2	0.5–10.1
Single-tetracycline-labeled perimeter — %					
Mean	1.2 ± 0.3	2.1 ± 0.9	2.1 ± 0.4	3.7 ± 1.2	4.1 ± 1.4
Median	1.2	0.7	1.9	2.5	2.7
5th to 95th percentile	0.0–2.2	0.1–8.9	0.1–3.8	0.8–10.1	0.6–21.1
Osteoclasts with normal morphologic features — no./mm					
Mean	0.02 ± 0.01	0.08 ± 0.02	$0.10 \pm 0.02^{\ddagger}$	0.06 ± 0.02	0.05 ± 0.01

Feature	Alendronate, 1 mg/day for 3 Yr (N = 8)	Alendronate, 5 mg/day for 3 Yr (N = 11)	Alendronate, 10 mg/day for 3 Yr (N = 9)	Alendronate, 20 mg/day for 2 Yr, then Placebo for 1 Yr (N = 8)	Placebo for 3 Yr (N = 15)
Median	0.01	0.08	0.13	0.06	0.05
5th to 95th percentile	0.00–0.06	0.00–0.20	0.00–0.16	0.00–0.14	0.00–0.12
Patients with abnormal osteoclasts — no. (%)	2 (25)	5 (45)	5 (56)	2 (25)	0

* Plus-minus values are means $\pm$ SE.

[†] P<0.003 for the comparison with placebo.

[‡] P<0.03 for the comparison with placebo.