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Reduced Cortical Bone Compositional Heterogeneity with Bisphosphonate Treatment in Postmenopausal Women with Intertrochanteric and Subtrochanteric Fractures

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Abstract

Reduction of bone turnover with bisphosphonate treatment alters bone mineral and matrix properties. Our objective was to investigate the effect of bisphosphonate treatment on bone tissue properties near fragility fracture sites in the proximal femur in postmenopausal women with osteoporosis. The mineral and collagen properties of cortico-cancellous biopsies from the proximal femur were compared in bisphosphonate-naïve (-BIS, n=20) and bisphosphonate-treated (+BIS, n=20, duration 7 ± 5 y) patients with intertrochanteric (IT) and subtrochanteric (ST) fractures using Fourier transform infrared imaging (FTIRI). The mean values of the FTIRI parameter distributions were similar across groups, but the widths of the parameter distributions tended to be reduced in the +BIS group relative to the -BIS group. Specifically, the distribution widths of the cortical collagen maturity and crystallinity were reduced in the +BIS group relative to those of the -BIS group by 28% (+BIS 0.45 ± 0.18 vs. -BIS 0.63 ± 0.28 , $p=0.03$) and 17% (+BIS 0.087 ± 0.012 vs. -BIS 0.104 ± 0.036 , $p=0.05$), respectively. When the tissue properties were examined as a function of fracture morphology within the +BIS group, the FTIR parameters were generally similar regardless of fracture morphology. However, the cortical mineral:matrix

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ratio was 8% greater in tissue from patients with atypical ST fractures (n =6) than that of patients with typical (IT or spiral ST) fractures (n=14) (Atypical 5.6 ± 0.3 vs. Typical 5.2 ± 0.5 , $p=0.03$). Thus, although the mean values of the FTIR properties were similar in both groups, the tissue in bisphosphonate-treated patients had a more uniform composition than that of bisphosphonate-naïve patients. The observed reductions in mineral and matrix heterogeneity may diminish tissue-level toughening mechanisms.

Keywords

Fourier transform infrared imaging; cortical bone; material properties; hip fracture; atypical subtrochanteric fracture

Introduction

Over the past three decades, bisphosphonates have become the primary pharmacologic treatment for postmenopausal osteoporosis. Bisphosphonate therapy typically reduces bone turnover, increases areal bone mineral density (BMD) assessed by dual energy x-ray absorptiometry, and reduces vertebral fracture incidence in osteoporotic patients.⁽¹⁻⁵⁾ Additionally, alendronate, risedronate, and zoledronate reduce vertebral, non-vertebral, and hip fracture incidence.⁽¹⁻³⁾ Bisphosphonate treatment generally produces modest increases in BMD that do not fully explain the observed reductions in fracture incidence, although the relationships between treatment-induced changes in BMD and fracture incidence vary with drug type and anatomic site.^(6,7) Therefore, factors other than BMD, including bone quality components such as bone tissue properties or microarchitecture, may also contribute to the anti-fracture efficacy of antiresorptive therapy.

On the tissue level, suppression of bone turnover with bisphosphonate treatment alters bone mineral and matrix properties. Treatment with alendronate for three years increased mean tissue mineral content and decreased the spatial heterogeneity of mineral properties of iliac crest tissue in healthy postmenopausal women without fractures⁽⁸⁾ and postmenopausal osteoporotic women⁽⁹⁾ relative to placebo-treated controls. Similarly, in a canine model, one year of alendronate or risedronate increased mean tissue mineral content and collagen maturity and decreased their spatial heterogeneity in the distal tibial cortices and trabeculae of intact beagles.⁽¹⁰⁾ The contribution of these changes in bone tissue composition to the treatment-induced reductions in fracture risk remains unknown.

Furthermore, the effects of long-term bisphosphonate treatment on bone quality and the optimal treatment duration for bisphosphonates in the setting of postmenopausal osteoporosis are incompletely characterized. The safety and efficacy of alendronate is generally maintained with treatment durations as long as 10 years.^(11,12) Nevertheless, increasing evidence specifically linking long-term bisphosphonate treatment to a rare atypical subtrochanteric fracture morphology has emerged over the past decade.⁽¹³⁾ The discovery of this association suggests that bisphosphonate use may alter bone quality and fracture resistance in a subset of patients and has highlighted the need for improved understanding of the effects of prolonged suppression of bone turnover on bone tissue properties.

In this investigation we focus on the effects of bisphosphonates on bone tissue properties in women with postmenopausal osteoporosis. Our objective was to investigate the effect of bisphosphonate treatment on bone tissue properties near fragility fracture sites in the proximal femur in postmenopausal women. We hypothesized that bisphosphonate-treated tissue would be characterized by increased mean mineral content and collagen maturity and reduced spatial heterogeneity of these properties.

Methods

Postmenopausal women with low-energy intertrochanteric and subtrochanteric femoral fractures scheduled for open reduction and internal fixation using a cephalomedullary device were considered for inclusion in the study. Patients with the following conditions or medications were excluded: prior fragility fracture; high-energy traumatic fracture (e.g., motor vehicle accident or fall from height greater than standing height); metabolic bone diseases other than osteoporosis (Paget's disease, osteogenesis imperfecta, osteomalacia); renal or hepatic failure; hyperparathyroidism; bone metastasis, active malignancy with or without documented metastasis; and medications other than bisphosphonates known to affect bone metabolism (anticonvulsants, aromatase inhibitors, glucocorticoids, raloxifene, teriparatide) as well as bisphosphonates given at doses larger than those recommended for postmenopausal osteoporosis. Allocation to treatment groups was based on patient-reported bisphosphonate use. Patients were allocated to the bisphosphonate-naïve group (-BIS, n=20) if they had no history of bisphosphonate use; all others were allocated to the bisphosphonate group (+BIS, n=20, duration 7 ± 5 y) (Table 1). Preoperative radiographs were evaluated in a blinded fashion to classify the fractures as intertrochanteric (IT), typical spiral subtrochanteric (typical ST), or atypical subtrochanteric (atypical ST) as defined by the major features outlined in the American Society of Bone and Mineral Research task force report on atypical subtrochanteric and diaphyseal femoral fractures.⁽¹³⁾ When fracture morphologies were categorized as Typical or Atypical for the purpose of subsequent statistical analyses, Typical fractures comprised IT or typical ST morphologies, and Atypical fractures comprised atypical ST morphology. All procedures were approved by the Institutional Review Boards of the Hospital for Special Surgery and New York-Presbyterian Hospital.

During fracture repair, 8-mm-diameter cylindrical cortico-cancellous bone biopsies were removed from the lateral aspect of the proximal femur, at the insertion site for the spiral blade of the cephalomedullary device. The specimens were dehydrated in graded ethanols and embedded in poly methyl methacrylate (PMMA). Undecalcified 1- μ m-thick sections were cut in the transverse plane to facilitate analysis of both cortical and trabecular tissue in the same sections. While most biopsies contained both cortical and trabecular bone, several specimens had no trabeculae due to natural variation in the extent to which cancellous bone extends distally to the femoral diaphysis. Specifically, 8 +BIS biopsies (5 IT, 3 Atypical) and 11 -BIS biopsies lacked trabeculae while 2 +BIS biopsies (1 IT, 1 Atypical) and 1 -BIS lacked detectable cortex. For each of the 40 biopsies, 3 non-consecutive sections spaced a minimum of 10 μ m apart were microtomed from the center of the core and placed on BaF₂ windows (Spectral Systems, Hopewell Junction, NY, USA) for Fourier transform infrared imaging (FTIRI). For each section, three cortical and three trabecular FTIR imaging regions

(64×80 pixels², 400×500 μm^2) were selected to span the entire cortical and trabecular regions within each section with approximately even spacing.

FTIR images over the spectral range $800\text{--}2000$ cm^{-1} were collected at a spectral resolution of 4 cm^{-1} and a spatial resolution of 6.25 μm using an infrared imaging system (Spotlight 300, PerkinElmer Instruments, Waltham, MA, USA). Background (BaF₂ window only). Following background subtraction and baseline correction, the PMMA spectral contribution to the bone spectra was subtracted using chemical imaging software (ISys, Malvern Instruments, Worcestershire, UK). The infrared spectrum at each pixel was analyzed to determine the following parameters:⁽¹⁴⁾ the mineral:matrix ratio (area ratio of the phosphate ν_1 and amide I peaks), which characterizes tissue mineral content; the carbonate:phosphate ratio (area ratio of the carbonate and phosphate ν_1 peaks), which characterizes the extent of carbonate substitution into the mineral lattice; the collagen maturity (XLR, intensity ratio of 1660 cm^{-1} and 1690 cm^{-1} bands), which is related to the ratio of nonreducible to reducible collagen crosslinks;⁽¹⁵⁾ and the mineral crystallinity (XST, intensity ratio of 1030 cm^{-1} and 1020 cm^{-1} bands), which is related to crystal size and perfection assessed by x-ray diffraction.⁽¹⁶⁾

The FTIR parameter values calculated at each of the bone pixels within each image yielded a distribution of values for each FTIR parameter. Each distribution was characterized by the mean of the distribution to assess average composition and the full width at half maximum (FWHM) of the Gaussian curve fit to the distribution to assess compositional heterogeneity. For each parameter, the mean and FWHM values for each of the images were averaged to obtain mean and FWHM values for each specimen. In total, for each sample 8 outcomes were assessed separately for cortical and trabecular bone: mean mineral:matrix, FWHM mineral:matrix, mean carbonate:phosphate, FWHM carbonate:phosphate, mean XLR, FWHM XLR, mean XST, and FWHM XST. The outcome variables for each group were compared with t-tests with significance levels of 0.05. As a secondary analysis within the +BIS group, Mann-Whitney U tests with significance levels of 0.05 were used to compare the properties of tissue from patients with Typical or Atypical fracture morphologies.

Results

In the +BIS group, patients had been taking alendronate, risedronate, and ibandronate, or sequential combinations thereof, for an average duration of 7.0 ± 4.8 years (mean $\pm$ SD) (Table 1). When fracture morphology was evaluated, patients in the -BIS group had 19 IT, 1 typical ST, and 0 atypical ST fractures; patients in the +BIS group had 13 IT, 1 typical ST, and 6 atypical ST fractures (Table 1). Within the +BIS group, patients with typical fractures had been taking bisphosphonates for 6.6 ± 5.1 years, while those with atypical fractures had been taking bisphosphonates for 7.8 ± 2.6 years. Patients in the -BIS group were 7% younger than those in the +BIS group (-BIS 80 ± 11 years vs. +BIS 87 ± 6 years, $p=0.04$). In the +BIS group, the patients with atypical fractures tended to be younger than patients with typical fractures, although this trend did not reach statistical significance (Typical 82 ± 11 vs. Atypical 75 ± 10 years, $p=0.11$).

Differences in tissue composition were evident in FTIR images of bone tissue from the +BIS and -BIS groups. While the mean values of the FTIR parameter distributions differed minimally across groups, the widths of the parameter distributions tended to be reduced in the +BIS group (Fig. 1). When the FTIR parameter means were examined, the tissue properties were similar across treatment groups for all of the parameters examined in cortical and trabecular bone (Fig. 2a) although there was a trend toward reduced mean trabecular collagen maturity in the +BIS group relative to the -BIS group that did not reach statistical significance (-9%, +BIS 3.67 ± 0.36 vs. -BIS 4.02 ± 0.45 , $p=0.06$). In contrast, the distributions of cortical tissue properties tended to be narrower in the +BIS group (Fig. 2b). Specifically, the distribution widths of the cortical collagen maturity and crystallinity were reduced in the +BIS group relative to those of the -BIS group by 28% (+BIS 0.45 ± 0.18 vs. -BIS 0.63 ± 0.28 , $p=0.03$) and 17% (+BIS 0.087 ± 0.012 vs. -BIS 0.104 ± 0.036 , $p=0.05$), respectively (Fig. 2b). Although reductions in the widths of the cortical and trabecular mineral:matrix distributions were evident (Fig. 2b), these differences did not reach statistical significance. The distribution widths of all other cortical and trabecular properties were not different across groups.

When FTIR properties were examined as a function of fracture morphology within the +BIS group, the properties of tissue from patients with atypical fractures generally fell within the range of values of the corresponding properties of tissue from patients with typical fractures (Figure 3). However, the cortical mineral:matrix distribution mean was 8% greater in tissue from +BIS patients with atypical fractures than that of +BIS patients with typical fractures (Atypical 5.6 ± 0.3 vs. Typical 5.2 ± 0.5 , $p=0.03$). The trabecular XST distribution width was 80% greater in tissue from +BIS patients with atypical fractures compared to that of +BIS patients with typical fractures (Atypical 0.18 ± 0.03 vs. Typical 0.10 ± 0.02 , $p=0.01$). No other FTIR properties were different between the +BIS Atypical and +BIS Typical subgroups.

Discussion

The composition of cortical tissue near fracture sites in the proximal femur differed in bisphosphonate-treated postmenopausal women relative to bisphosphonate-naïve controls. While the mean tissue properties were similar across treatment groups, the distributions of the cortical tissue properties were narrower in the +BIS group. In particular, the distributions of cortical collagen maturity and crystal perfection were respectively 28% and 17% narrower in the +BIS group relative to those of the -BIS group. Thus, in postmenopausal women with proximal femoral fractures, the tissue in bisphosphonate-treated patients had a more uniform composition than that of bisphosphonate-naïve patients.

The effects observed here are consistent with previous reports of reduced mineral and matrix heterogeneity with bisphosphonate treatment in humans and in animal models. In postmenopausal osteoporotic women, three years of alendronate treatment narrowed the bone mineral density distributions of iliac crest tissue characterized by backscattered electron imaging by ~15% relative to those of untreated controls.⁽⁹⁾ Similarly, three years of alendronate treatment increased mean tissue mineral content and decreased the spatial heterogeneity of mineral properties of iliac crest tissue from postmenopausal women without

fractures assessed by FTIRI.⁽⁸⁾ In a canine model of bisphosphonate treatment of intact (estrogen-replete) skeletally mature beagles, one year of alendronate or risedronate treatment increased the mean mineral:matrix ratio and collagen maturity and decreased the heterogeneity of these parameters in the cortical and trabecular tissue of the distal tibia assessed by FTIRI.⁽¹⁰⁾ Because compositional heterogeneity arises from remodeling activity, narrower distributions of tissue properties are consistent with the expected effects of antiresorptive therapy. Although normative data for bone turnover at the human subtrochanteric cortex are unavailable, the analogous rate of turnover in the femoral cortex of estrogen-replete 5-year-old beagles is approximately 1%/year.^(13,17) Turnover rates at the subtrochanteric cortex may be increased with estrogen deficiency in the setting of postmenopausal osteoporosis; however, absolute rates are expected to be low, suggesting that relatively long treatment durations such as those encountered in the current study (mean: 7 years) would be required to effect compositional changes at this site.

In contrast to prior studies of bisphosphonate treatment in humans and animals without fragility fractures, no differences in the mean mineral:matrix ratio or collagen maturity were observed in the current study. The observed trend toward slightly lower mean trabecular collagen maturity in the +BIS group relative to the -BIS group was small in magnitude (-9%), nonsignificant ($p=0.06$), and derived from the smaller subset of biopsies in which trabeculae were present ($n=12$ +BIS, $n=9$ -BIS). Mean tissue properties were therefore essentially similar in both groups. Elevated mineral:matrix ratio and collagen maturity have previously been identified as predictors of fracture risk in untreated tissue,⁽¹⁸⁾ and these parameters may not have differed in the groups examined here because both groups comprised patients with fragility fractures.

The effects of altered bone tissue compositional heterogeneity on bone strength or fracture risk have not been examined directly. Nevertheless, reduced heterogeneity is associated with fragility: osteoporotic bone has narrower distributions of mineral:matrix ratio, collagen maturity, and mineral crystallinity relative to normal bone;⁽¹⁴⁾ and iliac crest tissue of women with vertebral fractures has smaller coefficients of variation of tissue mineral density than that of non-fracture controls.⁽¹⁹⁾ Tissue heterogeneity may contribute to mechanical integrity because spatial variations in tissue material properties reduce local stresses and strains in microscale computational models of trabecular bone⁽²⁰⁾ and enhance ductility and energy dissipation in nanoscale computational models of cortical bone.⁽²¹⁾ Thus, reductions in compositional heterogeneity may diminish tissue-level toughening mechanisms.

When FTIR properties of tissue from patients within the +BIS group with atypical and typical fractures were compared, tissue properties were generally similar across groups. However, the mean mineral content was 8% greater in the cortices of +BIS patients with atypical fractures than those of +BIS patients with typical fractures. Greater local mineral content in the cortices of patients with atypical fractures is expected to strengthen bone but also to embrittle it^(22,23) and is consistent with the transverse fracture patterns characteristic of brittle materials seen in atypical fractures of the subtrochanteric cortex.⁽¹³⁾ The trabecular crystallinity distribution widths were also 80% greater in tissue from +BIS patients with atypical fractures compared to those of +BIS patients with typical fractures, although the

clinical significance of these differing trabecular properties is unclear because atypical fractures occur in the cortex.

Some inherent limitations and strengths arise from design of the current study. Because this study included only osteoporotic postmenopausal women with fragility fractures, it cannot address the extent to which the observed differences in bone tissue properties across treatment groups contribute to fracture risk. The observational, retrospective study design precluded randomization of patients to treatment groups and allows the possibility for some underlying differences between treatment groups, including differences in age (+7% in +BIS) or BMD (data not available). We relied on patient-reported drug treatment histories. Finally, because atypical fractures are rare (~8.4/10,000 patient-years for > 2 years BIS use⁽²⁴⁾), this study was not powered to assess differences in tissue properties in tissue from patients with typical versus atypical fractures; rather we included these comparisons as a secondary analysis. The generalizability of these comparisons is limited by the small number of biopsies from patients with atypical fractures (6 total, of which 6 had cortical bone and 3 had trabecular bone in the biopsy). Because we made an effort to maximize collection of biopsies from patients with atypical fractures, the proportion of patients with atypical fractures (15%) is greater in our sample than in study populations for large clinical trials of bisphosphonates (<4%).⁽²⁵⁾

Nevertheless, data on tissue compositional properties from patients treated with durations of bisphosphonates greater than three years are scarce, and the data from the current study provide insight into the tissue properties of this population of patients, as well the first spectroscopic characterization of tissue from patients with atypical fractures. This is also one of the first studies to our knowledge to examine bone tissue composition near fragility fracture sites in the proximal femur, as opposed to the more commonly analyzed iliac crest, a site that rarely fractures and may differ compositionally from other anatomic sites, including the subtrochanteric femur.^(26,27) Finally, bone tissue was characterized using FTIRI, a technique with validated outcomes that contribute to fracture risk independently of BMD in untreated tissue.⁽¹⁸⁾

Our data also contribute to the identification of a number of bone quality factors that are altered with bisphosphonate treatment.⁽²⁸⁾ By reducing bone turnover, antiresorptive treatment tends to increase the mean values and decrease the widths of the tissue mineral content and collagen maturity distributions.⁽⁸⁻¹⁰⁾ In addition to alterations in enzymatic cross-links, reductions in turnover allow accumulation of nonenzymatic advanced glycation end products in the collagen matrix,⁽²⁹⁻³¹⁾ which is associated with reduced postyield energy absorption.^(31,32) Finally, microdamage accumulation has been observed in canine models using doses of bisphosphonates equivalent to those used for postmenopausal osteoporosis.^(33,34) Taken together, these changes may reduce fracture risk with moderate suppression of bone turnover, consistent with clinical experience for 3-5 years of bisphosphonate use at doses recommended for postmenopausal osteoporosis. However, suppression of turnover with high doses or long durations of antiresorptive treatment may lead to a loss of toughness that may reduce the tissue's intrinsic resistance to fracture. Embrittlement processes that occur with bisphosphonate treatment may be especially

important in the context of atypical fractures, which are characterized by brittle fracture morphologies.⁽¹³⁾

Currently bisphosphonates remain safe and effective for a wide range of patients, but the effects of their prolonged use on bone quality require further study. Characterization of the relationships among bone turnover, material properties, and structural properties will help to optimize pharmaceutical treatment type, timing, and duration to normalize bone turnover and minimize fracture risk.

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Figure 1. Representative FTIR images and associated pixel histograms with Gaussian fits of (a) collagen maturity and (b) crystallinity for bisphosphonate-naïve (-BIS) and bisphosphonate-treated (+BIS) cortical bone. The mean and the full width at half maximum (FWHM) values of the Gaussian curves are indicated on each histogram.

Figure 2. Cortical and trabecular FTIR properties for bisphosphonate-naïve (-BIS) and bisphosphonate-treated (+BIS) groups reported as image pixel distribution (a) means and (b) full widths at half maximum (FWHM). Error bars indicate standard deviations. FTIR properties are mineral:matrix ratio (MM), carbonate:phosphate ratio (CP), collagen maturity (XLR), and crystallinity (XST); * $p < 0.05$ vs. -BIS by t-test.

Figure 3.

FTIR properties of cortical and trabecular bone from bisphosphonate-naïve (-BIS) and bisphosphonate-treated (+BIS) groups reported as image pixel distribution (a) means and (b) full widths at half maximum (FWHM) and plotted according to fracture morphology: typical (intertrochanteric or spiral subtrochanteric) or atypical subtrochanteric⁽¹³⁾. # p < 0.05 vs. Typical by Mann-Whitney U test.

Table 1

Patient characteristics for bisphosphonate-naïve (-BIS) and bisphosphonate-treated (+BIS) groups reported as mean $\pm$ SD where applicable.

	Bisphosphonate-naïve (-BIS)	Bisphosphonate-treated (+BIS)
Number	20	20
% Female	100	100
Fracture Morphology	19 Intertrochanteric 1 Typical spiral subtrochanteric 0 Atypical subtrochanteric	13 Intertrochanteric 1 Typical spiral subtrochanteric 6 Atypical subtrochanteric
Age (years)	87 $\pm$ 6	80 $\pm$ 11 * Typical 82 $\pm$ 11 Atypical 75 $\pm$ 10
Bisphosphonate treatment duration (years)	0.0 $\pm$ 0.0	7.0 $\pm$ 4.8 Typical 6.6 $\pm$ 5.1 Atypical 7.8 $\pm$ 2.6
Bisphosphonate treatment type		12 Alendronate 4 Risedronate 1 Ibandronate 2 Sequential alendronate, risedronate 1 Sequential alendronate, ibandronate

* p < 0.05 vs. -BIS by t-test.