ELSEVIER

Bone 39 (2006) 345–352

BONE

www.elsevier.com/locate/bone

Long-term risedronate treatment normalizes mineralization and continues to preserve trabecular architecture: Sequential triple biopsy studies with micro-computed tomography

B. Borah [a,*], T.E. Dufresne [a], E.L. Ritman [b], S.M. Jorgensen [b], S. Liu [a], P.A. Chmielewski [a], R.J. Phipps [a], Xiaojie Zhou [a], J.D. Sibonga [d], R.T. Turner [c]

[a] *Procter & Gamble Pharmaceuticals, Inc. Health Care Research Center, 8700 Mason Montgomery Road, Mason, OH 45040, USA*
[b] *Department of Physiology and Biomedical Engineering, Mayo Clinic College of Medicine, Rochester, MN 55905, USA*
[c] *Department of Nutrition and Exercise Sciences, Oregon State University, Corvallis, OR 97331, USA*
[d] *Bone Mineral Laboratory, Universities Space Research Association, Division of Space Life Sciences, Houston, TX 77058, USA*

Received 1 August 2005; revised 30 November 2005; accepted 13 January 2006
Available online 29 March 2006

Abstract

The objective of the study was to assess the time course of changes in bone mineralization and architecture using sequential triple biopsies from women with postmenopausal osteoporosis (PMO) who received long-term treatment with risedronate. Transiliac biopsies were obtained from the same subjects (n = 7) at baseline and after 3 and 5 years of treatment with 5 mg daily risedronate. Mineralization was measured using 3-dimensional (3D) micro-computed tomography (CT) with synchrotron radiation and was compared to levels in healthy premenopausal women (n = 12). Compared to the untreated PMO women at baseline, the premenopausal women had higher average mineralization (Avg-MIN) and peak mineralization (Peak-MIN) by 5.8% (P = 0.003) and 8.0% (P = 0.003), respectively, and lower ratio of low to high-mineralized bone volume (BMR-V) and surface area (BMR-S) by 73.3% (P = 0.005) and 61.7% (P = 0.003), respectively. Relative to baseline, 3 years of risedronate treatment significantly increased Avg-MIN (4.9 ± 1.1%, P = 0.016) and Peak-MIN (6.2 ± 1.5%, P = 0.016), and significantly decreased BMR-V (−68.4 ± 7.3%, P = 0.016) and BMR-S (−50.2 ± 5.7%, P = 0.016) in the PMO women. The changes were maintained at the same level when treatment was continued up to 5 years. These results are consistent with the significant reduction of turnover observed after 3 years of treatment and which was similarly maintained through 5 years of treatment. Risedronate restored the degree of mineralization and the ratios of low- to high-mineralized bone to premenopausal levels after 3 years of treatment, suggesting that treatment reduced bone turnover in PMO women to healthy premenopausal levels. Conventional micro-CT analysis further demonstrated that bone volume (BV/TV) and trabecular architecture did not change from baseline up to 5 years of treatment, suggesting that risedronate provided long-term preservation of trabecular architecture in the PMO women. Overall, risedronate provided sustained benefits on mineralization and architecture, two key determinants of bone strength, over 5 years lending support for its long-term efficacy in fracture risk reduction.

Keywords: Osteoporosis; Risedronate; Bone turnover; Mineralization; Architecture

Introduction

Bone strength and resistance to fracture are determined by structural and material properties including architecture, mineralization, and bone turnover [1,2]. Increased bone turnover can reduce bone strength and increase the risk of fracture by decreasing the degree of mineralization and disrupting the trabecular architecture [3,4]. Changes in mineralization can influence mechanical properties of bone tissue by altering stiffness and strength [5,6]. However, if bone is too highly mineralized due to a prolongation of secondary mineralization, the toughness can be reduced making bone brittle and more prone to fracture [5–7]. Thus, there is a trade-off between stiffness and toughness, and it

* Corresponding author.
E-mail address: borah.b@pg.com (B. Borah).

8756-3282/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bone.2006.01.161

seems likely that there is an optimal balance between the amount and distribution of low- and high-mineralized bone to maximize resistance to fracture. In addition to changes in mineralization, the deterioration of bone architecture is also a factor in the pathogenesis of osteoporotic fractures [8]. It has been shown previously that trabecular architecture contributes to bone strength, independent of bone density [9] and the relative contribution of architecture to bone strength increases as the bone mass decreases in osteoporosis [10,11].

Risedronate reduces the risk of radiographic vertebral fractures in PMO women over 1, 3, and 5 years based on prospective placebo-controlled clinical trials [12–14]. Post hoc analyses have shown that risedronate reduces the risk of nonvertebral and clinical vertebral fractures within 6 months of commencing treatment [14–16]. Reduction of bone turnover (assessed by biochemical markers) accounted for approximately two-thirds of this reduction in vertebral fracture risk over 3 years [17]. We have previously shown that 3 years of treatment with risedronate increased the degree of mineralization and preserved trabecular architecture in iliac crest biopsies [18,19]. These changes likely resulted from the reduction in bone turnover, and contributed to the fracture risk reduction seen after 3 years of treatment with risedronate. Most antiresorptive therapies, including risedronate, are chronic, and therefore, understanding the long-term effect of turnover reduction on mineralization and architecture is of clinical importance.

In this study, we evaluated the changes in bone mineralization and architecture in postmenopausal women with osteoporosis (PMO) using sequential triple biopsies taken from the same subjects at baseline and after 3 and 5 years of treatment with risedronate. Our ability to delineate low- and high-mineralized bone fractions with high-resolution microcomputed tomography (micro-CT) with synchrotron radiation provided us with new insights into how the degree and distribution of mineralization are affected by long-term risedronate treatment and how they compare to levels in premenopausal women. The trabecular bone architecture was measured using bench-top micro-CT on the same triple biopsies.

Materials and methods

Experimental subjects

The bone biopsy specimens used for this analysis were collected from a cohort of women who participated in a 3-year, randomized, placebo-controlled clinical trial to evaluate the efficacy of risedronate on vertebral fractures in postmenopausal osteoporosis (Vertebral Efficacy with Risedronate Therapy, North American trial (VERT-NA)) [12,20,21] who were invited to continue on therapy for an additional 2 years. The women in this trial were postmenopausal (22.1 ± 7.5 years from menopause, Table 1) and required to have either 2 prevalent vertebral fractures at baseline or 1 prevalent vertebral fracture and a lumbar spine bone mineral density *T* score of −2 or less. Bone biopsies were performed at baseline and after treatment in women who were enrolled at a subset of study centers (see Acknowledgment). All studies were conducted according to the Declaration of Helsinki and approved by the local ethics committees or institutional review boards (IRB). Women received either risedronate 5 mg or placebo daily for up to 5 years along with 1000 mg elemental calcium daily in the form of calcium carbonate and vitamin D (as cholecalciferol, 500 IU/day) if deficient at baseline. The clinical efficacy endpoints of this study were vertebral and nonvertebral fractures and changes in bone mineral density; bone biopsies were collected and analyzed by standard histomorphometric techniques for bone safety evaluation [19,20]. For comparison, we also examined the transiliac biopsies from a younger group of premenopausal women (n = 12) [22].

Table 1
Baseline characteristics of PMO women in the risedronate group and premenopausal women

Characteristic	PMO	Premenopausal [22]
Age, years	68.5 (7.1)	36 (3)
Years since menopause	22.1 (7.5)	NA
Lumbar spine BMD [a] (g/cm^2)	0.79 (0.03)	1.30 (0.18)
Number of prevalent vertebral fractures	2.18 (0–7)	Not available

Values are mean (SD) for age, years, years since menopause, and BMD. Values are mean (range) for prevalent fractures.

[a] The lumbar spine BMDs of the PMO women were measured by dual X-ray absorpsiometry using Lunar (Lunar Corporation, Madison, Wis) or Hologic (Hologic Inc, Waltham, MA) densitometers and were analyzed at a central location (Department of Radiology, University of California, San Francisco). Standardized lumber spine BMD was calculated to correct for differences in instrumentation [12]. The BMD of the pre-menopausal women was measured by dual photon absorptiometry [22].

Biopsy specimens

We previously analyzed trabecular bone mineralization and architecture in 11 pairs of transiliac biopsies taken in this study at baseline and after 3 years of treatment with risedronate [18]. Biopsies were obtained at a site 2 cm below and 2 cm behind the anterior–superior iliac spinous process. A third biopsy was obtained from 8 of these 11 subjects after an additional 2 years of treatment with risedronate (5 years total). The protocol specified that the third biopsy should be taken from the same side as the baseline biopsy at least 2 cm away from the previous biopsy site. These biopsies were evaluated qualitatively for structural integrity by visual examination of the 3-dimensional (3D) micro-CT images. Biopsies were excluded if the cortical or the trabecular bone was broken or missing. Using this criterion, one biopsy of the eight 5-year biopsies was excluded from analysis. Thus, 7 patients had useable biopsies obtained sequentially at baseline, year 3, and year 5. All biopsies were embedded in polymethyl methacrylate. These biopsies represented a subset with greater than median baseline mineralizing surface, in which both mineralization and architecture were found to be more susceptible to changes in turnover, as previously reported [18,19]. The biopsies from the premenopausal women were collected for comparative histomorphometric studies at the Mayo Clinic Research Center. The study was approved by the IRB of Mayo Clinic and signed informed consent forms were obtained from all patients [22].

Measurement of mineralization parameters

Three-dimensional micro-CT with X-ray radiation from a synchrotron source (synchrotron-μCT) [23,24] was used to quantify bone mineralization [19]. The micro-CT scanner with the X2B beamline at the National Synchrotron Light Source (NSLS) at the Brookhaven National Laboratories was used to image the biopsies. The 3D images were collected at 4 μm isotropic voxel resolution. Scanning time was 3–4 h per specimen. After acquisition, the projections were reconstructed to form a 16-bit binary gray level image and bone was segmented from the plastic background using simple thresholding. Because the radiation is monochromatic, the gray level intensities (X-ray attenuation coefficients) of the Synchrotron-μCT images are used to quantify the material attenuation coefficient that is proportional to the concentration of the local mineral (calcium-containing apatite crystals) in bone,