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Research paper

Effects of ageing, prolonged estrogen deficiency and zoledronate on bone tissue mineral distribution

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ABSTRACT

The quantity and distribution of bone tissue mineral are key determinants of bone strength. Recent research revealed altered mineral distribution within sheep femora following estrogen deficiency. Rapid increases in bone remodeling occur at the onset of estrogen deficiency and abate over time. Therefore, altered tissue mineralization might be a transient characteristic of osteoporosis. Bisphosphonates reduce fracture incidence by 40–60% but increases in bone mineral density are insufficient to explain such changes. In this study the hypotheses that bone tissue mineralization is altered over prolonged estrogen depletion and bisphosphonate treatment were tested. Quantitative backscattered imaging (qBEI) was used to quantify bone mineral density distribution (BMDD) parameters (mean, FWHM) in trabeculae from the proximal femora of an ovariectomized sheep model that underwent estrogen deficiency for 31 months, an ovariectomized group administered with Zoledronic acid and age-matched controls. To assess the effects of normal ageing and prolonged estrogen deficiency, data were compared to BMDD data from sheep that were estrogen deficient for 12 months and age-matched controls. This study reports that normal ageing increases mean mineralization and mineral heterogeneity at a trabecular level. In contrast, prolonged estrogen deficiency leads to significantly decreased mean mineralization and further exacerbates increases in mineral heterogeneity. Interestingly, ZOL treatment of OVX sheep significantly reduced tissue mineral variability, both at a trabecular level and between femoral regions. Together, these findings indicate that ZOL treatment acts to reverse the increased mineral heterogeneity occurring during estrogen deficiency, which may contribute to its capacity to reduce osteoporotic fractures.

1. Introduction

One of several bone quality characteristics known to govern the mechanical strength of bone is the quantity of mineral together with its distribution within the bone tissue matrix (Currey, 1984; Follet et al., 2004). The degree of bone mineralization is influenced by the frequency of bone remodeling and mineral deposition rates. Increases in the degree of bone tissue

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mineralization are associated with a significant enhancement of overall bone strength (Vose and Kubala, 1959). However, beyond approximately 66% mineralization, further elevations in mineral content lead to brittleness and decreased bone mechanical strength (Bonfield and Clark, 1973; Currey, 1969). There are conflicting observations of altered bone mineralization as a consequence of ageing. Studies on healthy human bone, which found increased mineralization with ageing (Currey et al., 1996; Vajda and Bloebaum, 1999), indicate a material level alteration in tissue properties with ageing, whilst others demonstrate no correlation (Bloebaum et al., 2004; Roschger et al., 1998).

Estrogen deficiency, occurring following the menopause, has been established as the primary causative factor in postmenopausal osteoporosis. A marked increase in bone turnover rates occurs (Balena et al., 1993) and leads to low bone mass and strength, depleted bone architecture and increased fracture risk (Cummings and Melton, 2002). Previous studies quantifying trabecular bone mineralization have found increases (Boyde et al., 1998; Busse et al., 2009; McNamara et al., 2006), decreases (Gadeleta et al., 2000; Yao et al., 2007), whilst others present no or only slight alterations in mineral content (Brennan et al., 2011; Ciarelli et al., 2003) as a consequence of estrogen deficiency. These discrepancies may be explained by the duration of estrogen depletion under investigation, as it has been shown that after the onset of estrogen deficiency, biological and structural alterations occur immediately, but these responses wane over time (Binkley et al., 1998; Smith et al., 2003). Supporting evidence in primates has shown that bone mass deterioration and increases in bone turnover markers stabilize at about 9 months post-ovariectomy (Binkley et al., 1998). A further study on ovariectomized monkeys noted a rapid phase of bone loss lasting approximately 8–12 months following ovariectomy, and by 16 months post-ovariectomy, a trend toward normalization of markers and stabilization of bone mass (Smith et al., 2003). However, the impact of such time dependent changes in remodeling and bone mass on bone tissue mineralization has not been elucidated. Recent studies reveal increased mineral heterogeneity within trabeculae, as well as alterations in mineral distribution along the intertrochanteric fracture line, the most common osteoporotic fracture site, in the proximal femur of sheep following 12 months of estrogen deficiency (Brennan et al., 2011). However, whether these changes are sustained with prolonged estrogen deficiency is unknown.

In recent years, therapeutic options for osteoporosis have increased considerably and presently biphosphonates are the most widely used pharmaceutical for this purpose. Bisphosphonates act to inhibit bone resorption by selective absorption to bone mineral surfaces and subsequent internalization by osteoclasts, where they interfere with osteoclast function (Rodan and Reszka, 2002). They reduce bone turnover by decreasing activation frequency and markers of bone remodeling and thereby prolong the secondary mineralization of bone (Balena et al., 1993). Bisphosphonate treatment has been shown to increase bone mineral density (BMD), mechanical strength, and reduce the incidence of bone fractures (Balena et al., 1993). Zoledronic acid is a third generation, nitrogen containing bisphosphonate (NBP). NBPs perturb the cytoskeleton necessary for maintaining the ruffled border that facilitates osteoclastic bone resorption (Rodan and Reszka, 2002). Zoledronic acid has been shown to prevent bone loss, suppress bone resorption, increase bone mineral density and trabecular bone volume in postmenopausal women (Leal et al., 2010; Reid et al., 2002) and animal models of osteoporosis (Glatt, 2001). It also inhibited fractures of the vertebrae and the hip in clinical trials (Black et al., 2007). In general, a decrease in one standard deviation in BMD doubles fracture risk (Riggs, 2002), however, there is increasing evidence demonstrating that the small increases in BMD (2–9%) (Ettinger et al., 1999; Liberman et al., 1995) with anti-resorptive agents can only explain a portion of the associated significant reduction in fracture incidence (40–60%) (Black et al., 1996; Ettinger et al., 1999; Liberman et al., 1995; McClung et al., 2001). Indeed, it has been reported that changes in BMD with Raloxifene accounted for only 4% of the observed reduction in fracture risk (Sarkar et al., 2002). With Zoledronic acid treatment, the degree of bone mineralization is the most important factor for increasing bone strength (Yao et al., 2007). However, whether Zoledronate treatment counteracts the increased mineral heterogeneity occurring during estrogen deficiency (Brennan et al., 2011), thus contributing to reduced fracture incidence, has yet to be delineated.

In this study, quantitative backscattered imaging (qBEI) was used to quantify bone mineral density distributions (BMDD) within trabeculae from proximal femora of an ovine model of osteoporosis that underwent estrogen deficiency for 31 months, an ovariectomized group administered with Zoledronic acid and aged-matched controls. The hypotheses that (1) bone tissue mineralization is altered during normal ageing and (2) bone tissue mineralization is altered over the course of prolonged estrogen deficiency were investigated by comparing BMDD parameters with previously reported data from sheep that underwent estrogen deficiency for the shorter duration of 12 months and their aged matched counterparts (Brennan et al., 2011). Furthermore, the hypothesis that (3) Zoledronic acid treatment alters the distribution of bone mineral within the proximal femur was investigated.

2. Materials and methods

2.1. *Bone samples*

In this study, bone tissue originated from the ovine osteoporosis model described previously (Brennan et al., 2011, 2009). Briefly, skeletally mature mixed breed ewes were randomly assigned into either a control group (CON) or a cohort that underwent ovariectomy to induce estrogen deficiency (OVX). All surgery was performed following ethical approval and under an animal license, granted by the Irish Department of Health. Animals were maintained at pasture where feeding and activity levels were the same for both groups. Twenty months post-ovariectomy, four OVX animals were randomly assigned to a Zoledronic acid (Novartis Pharma, Basel, Switzerland) treated group (ZOL). Each animal received a 5 mg dose of Zoledronic acid in 100 ml of saline infused over 30 min via an indwelling jugular catheter per week for 5 weeks. Animals were either sacrificed at 12 months or 31 months post-ovariectomy and bones were harvested and frozen at −20 °C. Trabecular bone tissue of