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Changes in intracortical microporosities induced by pharmaceutical treatment of osteoporosis as detected by high resolution micro-CT

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ABSTRACT

Bone's microporosities play important biologic and mechanical roles. Here, we quantified 3D changes in cortical osteocyte-lacunae and other small porosities induced by estrogen withdrawal and two different osteoporosis treatments. Unlike 2D measurements, these data collected via synchrotron radiation-based μCT describe the size and 3D spatial distribution of a large number of porous structures. Six-month old female Sprague–Dawley rats were separated into four groups of age-matched controls, untreated OVX, OVX treated with PTH, and OVX treated with Alendronate (ALN). Intracortical microporosity of the medial quadrant of the femoral diaphysis was quantified at endosteal, intracortical, and periosteal regions of the samples, allowing the quantification of osteocyte lacunae that were formed primarily before versus after the start of treatment. Across the overall thickness of the medial cortex, lacunar volume fraction (Lc.V/TV) was significantly lower in ALN treated rats compared to PTH. In the endosteal region, average osteocyte lacunar volume (<Lc.V>) of untreated OVX rats was significantly lower than in age-matched controls, indicating a decrease in osteocyte lacunar size in bone formed on the endosteal surface after estrogen withdrawal. The effect of treatment (OVX, ALN, PTH) on the number of lacunae per tissue volume (Lc.N/TV) was dependent on the specific location within the cortex (endosteal, intracortical, periosteal). In both the endosteal and intracortical regions, Lc.N/TV was significantly lower in ALN than in untreated OVX, suggesting a site-specific effect in osteocyte lacuna density with ALN treatment. There also were a significantly greater number of small pores (5–100 μm^3 in volume) in the endosteal region for PTH compared to ALN. The mechanical impact of this altered microporosity structure is unknown, but might serve to enhance, rather than deteriorate bone strength with PTH treatment, as smaller osteocyte lacunae may be better able to absorb shear forces than larger lacunae. Together, these data demonstrate that current treatments of osteoporosis can alter the number, size, and distribution of microporosities in cortical rat lamellar bone.

1. Introduction

In addition to decreases in the quantity of tissue present in the skeleton, inherent defects in the material can also result in an increased susceptibility to fracture. Current FDA approved pharmaceutical treatments of osteoporosis focus on retaining or improving bone mass by targeting the activity of osteoclasts (resorption) or osteoblasts (formation). Both anti-resorptive (e.g., bisphosphonates) [1,2] and anabolic (e.g., intermittent parathyroid hormone, PTH) [3,4] treatments for musculoskeletal diseases may influence not only bone's quantity but also its quality, ultimately improving (or compromising) bone's ability to resist load [5,6]. However, little is known about how osteoporosis treatments affect the immediate environment of the most abundant cell type in bone, the osteocytes.

Osteocytes are the longest living cells in the skeleton and perform multiple functions associated with the regulation of bone remodeling. Osteocytes are thought to contribute to the regulation of bone remodeling in response to mechanical and micro-environmental changes by signaling to osteoblasts and osteoclasts [7–12]. In addition, mechanical and biochemical stimuli may cause osteocytes to directly contribute to the modulation of bone quality and quantity by directly remodeling its surrounding environment [13,14]. Therefore, these multi-purpose cells have potential as therapeutic targets for osteoporosis [15–17].

While the density, distribution, and geometry of osteocyte lacunae provide a time history of the 3D development of its surrounding

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mineral matrix, quantification of these variables is not entirely straightforward. Previous studies have reported changes in osteocyte lacunae and the cells that they contain. Evidence of osteocytic osteolysis in mice includes enlarged lacunae [18] and reduced radiodensity [19], suggestive of the ability of osteocytes to remodel its surroundings. However, current evidence is largely histological and radiographical, and thus limited by two dimensional methods. Irregular morphology, variability in spacing, and lack of consistent orientation of osteocyte lacunae are just a few of the technical challenges when using 2D measurements [20,21]. Most techniques to describe lacunar networks are either technically challenging or do not meet the required spatial resolution [22]. Therefore, advanced methods are necessary to understand the effect of 3D lacunar structures on bone quality.

The current standard evaluation of bone mass and architecture in small animal models is based on micro-computed tomography (μCT). Although desktop μCT scanners can provide critical information on the geometry of cortical and trabecular bone, at least in bones from small animals, small (<10 μm) porosities within the tissue typically cannot be detected. Switching from a local X-ray source to a synchrotron source provides the brightness, collimation, and micro-focused beam needed to increase both the signal-to-noise ratio and spatial resolution and to allow for determining the size and geometry of osteocyte lacunae and vascular channels at about 1 μm resolution [21,23,24] in bones from rodents [25] to humans [22].

Here we combined conventional desktop μCT with high-resolution synchrotron radiation-based μCT to quantify changes in macro- and microscopic cortical bone structural properties induced by two different osteoporosis treatments in the ovariectomized (OVX) rat. Results from pharmaceutical interventions in the OVX rat model, such as bisphosphonates or PTH, generally match the effects of these agents in clinical trials in women with post-menopausal osteoporosis. In rats, bisphosphonates including the FDA-approved Alendronate [26], have been used successfully to stem the erosion of bone and restore bone strength by inhibiting bone cell activity and thus bone turnover [2]. In contrast, PTH injections, an anabolic treatment of osteoporosis, result in an increase in new bone formation by stimulating bone remodeling [27]. Little is known on whether these pharmaceutical treatments may modulate bone's internal structure. Here, we hypothesized that estrogen withdrawal will result in an increase in microporosities and that PTH treatment will induce subtle increases to the size and distribution of intracortical microporosities that previously may have gone undetected. Further, it was expected that Alendronate treatment will reduce the size and distribution of intracortical microporosities. Results from this study can offer critical information on how hormonal changes and current drug treatments alter a measure of matrix quality and, ultimately, may provide insight into why drug treatments have shown variable clinical effectiveness across different studies [3,26,28–30].

2. Materials and methods

2.1. Experimental design

Six-month old female Sprague–Dawley rats were separated into either age-matched control (Ctrl), untreated OVX, OVX treated with subcutaneous injections of parathyroid hormone 1–34 (PTH, 15 μg/kg/d), or OVX treated with subcutaneous injections of Alendronate (ALN, 100 μg/kg/2×/wk). Rats in the OVX groups were ovariectomized at 5 months of age (4 weeks prior to starting the experimental protocol). To standardize environmental conditions, all rats were allowed access to a standard rodent chow (Purina Rodent Chow 5001) and water ad libitum, subjected to a 12-h light:dark cycle, and raised in individual cages in the same room. All rats were sacrificed at 12 months of age (n = 6/group). The doses used in this study were derived from recent studies in rats [31–33]. They represent the therapeutic equivalent for the clinical treatment of osteoporosis extrapolated to the rat model [34,35] and previously were shown to have differential effects on bone quantity and quality [2]. All procedures were approved by the Stony Brook Institutional Animal Care and Use Committee.

The ovariectomized rat is currently the most studied animal model of post-menopausal osteoporosis. The focus of this study was to examine changes to specific aspects of bone quality during estrogen withdrawal and pharmaceutical treatment. The effects of OVX on rat long bones are well documented. The withdrawal of estrogen causes a rapid increase in bone turnover associated with a substantial decrease in bone mass [36–39]. Further, these previous studies did not find any effect of sham OVX surgery on bone mass. Therefore, no sham-surgery rats were included here. Treatment was administered for six months, a duration which ostensibly produces relatively large variation in bone quality parameters between groups [40,41].

Previous studies indicated that changes in bone morphology of OVX Sprague–Dawley rats treated with Alendronate and PTH for 2 months focus on trabecular rather than cortical bone [39,42–44]. Nevertheless, the cortical mid-diaphysis was chosen for the analysis in this study because in OVX rats, the contribution of cortical bone to whole-bone mechanical properties is greater than that of trabecular bone [45], allowing subtle changes in mineral density [46–49] or porosity [25] to have a large impact on bone's mechanical properties. Such bone quality consequences of osteoporosis treatments may have previously been ignored because of insufficient μCT resolution.

2.2. Macroscopic morphology via conventional desktop μCT

After sacrifice, the left femoral diaphyses were preserved in 70% EtOH and scanned at a resolution of 36 μm (55 kV energy, 145 μA intensity, 300 ms integration time) using a desktop μCT scanner (μCT40; Scanco Medical AG, SUI) as described previously [50]. Mineral density was calibrated in units of mg HA/cm^3 using a standard hydroxyapatite phantom (Scanco Medical AG). Cortical bone morphology and mineral composition including cortical thickness (Ct.Th), area (Ct.Ar), polar moment of inertia (J), and tissue mineral density (TMD) were determined for a 1.8-mm long volume of interest (VOI) at the mid-diaphysis.

2.3. Intracortical micro-porosity via synchrotron radiation μCT

After scanning the femurs by conventional desktop μCT, longitudinal bone strips were cut from the quartered diaphysis (Fig. 1) using a low-speed diamond wafer blade (South Bay Technology Inc.). Strips were 1 mm × ~600 μm × 5 mm. To assess treatment-induced changes in intracortical microporosity, medial strips were scanned via high resolution, synchrotron radiation-based μCT at beamline 2-BM at the Advanced Photon Source (APS) at Argonne National Laboratory.

Stacks of images were acquired at a photon energy of 20.98 keV along the length of each cortical strip (image plane perpendicular to long axis of femur). The image stacks were generated by filtered back-projection from sets of 1500 projection images. Each projection image was exposed for 450 ms and the angular rotation between each image was 0.12°. The reconstructed datasets consisted of approximately 2048 slices with a 2048×2048 in-plane data matrix, yielding a 750 nm isotropic voxel size. Typical scan time was 25 min per specimen.

At the selected resolution, both large pores such as vascular canals and small pores such as osteocyte lacunae were readily visible (Fig. 2). The high signal-to-noise ratio and high resolution of the synchrotron μCT provided clear boundaries between anatomical features for the algorithm, facilitating the segmentation of the images even in the presence of differences in tissue density. A sinogram-based algorithm removed ring artifacts which arose from defects on the scintillator of the optical system. Minimal noise within the