The Effects of Estrogen Loss on the Vascular Porosity in Rat Cortical Bone

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INTRODUCTION

To date, the ways in which variations in the vascular porosity and blood supply contribute to changes in bone health and communication between cells have not been clearly explained. While it is established that estrogen deficiency produces bone loss by modifying bone architecture, there has been little analysis related to changes in cortical microporosity due to estrogen loss.

Our recent high-resolution micro-CT studies show that the cortical vascular porosity and average vascular canal diameter increase in the tibia metaphysis of ovariectomized (OVX) rats (Figure 1). The present study further investigated changes in the vascular porosity in the rat OVX model of osteoporosis by using histological techniques to quantify several parameters, including osteoclast resorption, vascular canal and blood vessel diameter, and vascular canal density.

METHODOLOGY

Permission for this study was granted by the IACUC. Thirty-six female Sprague Dawley rats (20 week old, Harlan Laboratories) were divided into two groups. The OVX group (n=18) underwent bilateral ovariectomy, and the SHAM group (n=18) underwent sham surgery. The animals were acclimatized for one week after surgery and fed ad libitum. The OVX group was then pair-fed to the average food intake of the SHAM group. Six animals from both groups were sacrificed at three time points post-surgery: one, two, and six weeks. Harvested tibiae were cut ~2 mm below the proximal growth plate and at the diaphyseal midpoint, and fixed in 10% phosphate-buffered formalin for 48 hours.

Osteoclast Resorption: The tibia metaphysis and mid-diaphysis were decalcified in 20% EDTA for one week, and embedded in paraffin wax. Longitudinal sections were cut (7 μm thick) and stained with Tartrate-Resistant Acid Phosphatase (TRAP). The percentage of vascular canals with TRAP activity was quantified in the anterior region of the metaphysis and mid-diaphysis using a Nikon Microphot-FXA microscope and Bioquant software.

Vascular Canal and Blood Vessel Morphology Assessment: A proximal tibia from each animal was embedded in PMMA and whole cross-sections were cut (7 μm thick) from the metaphysis ~2 mm below the growth plate. The sections were stained with Goldner’s Trichrome. The number of cross-sectional vascular canals and blood vessels within each canal were determined in the anterior region of the metaphysis and quantified per unit area. Vascular canal and blood vessel diameter were also measured. The smallest cross-sectional diameter was measured for both canal and vessel. All measurements were averaged for three sections per animal. The prevalence of unmineralized matrix surrounding vascular canals was also quantified for the 6-week time point (Figure 2).

Statistical Analysis: TRAP (osteoclast) activity, vascular canal density, vascular canal diameter, blood vessel diameter, and number of blood vessels per canal were assessed using two-way ANOVA with the two factors being treatment (OVX vs. SHAM) and time post-surgery. Bonferroni post-test analyses were performed with a significance level of p < 0.05.

RESULTS

Osteoclast resorption as assessed through TRAP enzyme activity was minimal, and there were no significant differences between SHAM and OVX with respect to anatomical site (metaphysis vs. mid-diaphysis) or time post-surgery. There were no differences in the vascular canal density at all time points (Table 1). Additionally, the average number of blood vessels per canal was ~1 for all groups. There were no differences in average vascular canal diameter or average blood vessel diameter between SHAM and OVX groups (Figure 3). There was also no significant difference between SHAM and OVX in the percentage of vascular canals surrounded by unmineralized matrix at 6-weeks post-OVX (Table 2).

DISCUSSION

The low level of TRAP activity suggests that osteoclast resorption may not be occurring in the vascular pores.

No increases were seen in the cortical vascular canal diameter at one, two, or six weeks post-OVX, in contrast to our previous micro-CT measurements, which demonstrated a 48% increase in canal diameter in the proximal tibia metaphysis six weeks post-OVX. A potential explanation could be attributed to micro-CT thresholding, which may have separated unmineralized osteoid from mineralized bone such that lower mineralized bone would have been segmented as “non-bone,” thus exhibiting a larger canal diameter than was actually present. While there were no significant differences in the prevalence of unmineralized matrix between groups at the 6-week time point, the trend was that the OVX group had more canals surrounded by unmineralized matrix. Further assessment of local vascular porosity changes are being performed using fluorescent labeling to quantify mineral apposition rate to better identify the time course of changes in mineralization in the vascular canals. Additional work is also being done to link any mineralization changes in the vascular porosity to alterations in the osteocyte lacunar-canalicular porosity that occur in the estrogen-deficient condition.

SIGNIFICANCE

Quantifying vascular-related changes due to estrogen deficiency will help to understand the development and progression of postmenopausal osteoporosis.

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REFERENCES