A Closer Look at the Immediate Trabeculae Response to Combined Parathyroid Hormone and Alendronate Treatment

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Introduction

Aging shifts bone remodeling toward a negative balance between bone formation and resorption, causing bone loss and increased fracture risk. Anti-resorptive agents are commonly used to inhibit bone resorption and stabilize bone mass. While they are effective to prevent further bone loss, there is also a great need for anabolic agents which can reverse bone deterioration and regain lost skeletal integrity. Parathyroid hormone (PTH) is the only FDA-approved anabolic treatment for osteoporosis, which greatly stimulates bone formation. Combined therapy of anti-resorptive treatments, such as alendronate (ALN), and PTH have been proposed and are expected to further increase bone mass. However, studies show conflicting results regarding the effectiveness of combined treatments: some have reported the addition of ALN to impair PTH function,[1] while others suggest an improvement over PTH monotherapy.[2-3] The first objective of this study is to document the immediate changes of individual trabecular structure due to PTH and combined therapy within 12 days using *in vivo* micro computed tomography (μCT). As PTH is typically prescribed for 1 to 2 years to osteoporotic patients, a treatment of 12 days for rats (approximately equivalent to one year of human life) may be more clinically relevant than long-term treatment studies on rats. The secondary purpose of this study was to gain insight into the mechanism of combined versus PTH treatments through a detailed tissue level analysis by using ultra-high resolution *ex vivo* μCT. We hypothesized that PTH and combined treatments would immediately enhance bone formation on the trabecular surface that can be detected by both the structural and tissue level analyses.

Methods

Seventeen 3-month-old SD rats were assigned to vehicle (Veh) (n=6), PTH (n=8), and combined PTH and ALN (COMBO) treatment (n=3) groups. The Veh group received saline while the PTH and COMBO groups received 80 μg/kg PTH (1-34) daily injections for 12 days. Every 3 days the COMBO group also received injections of 50 μg/kg ALN. The right tibia of each rat was scanned using an *in vivo* μCT system (VivaCT 40, Scanco Medical, 10.5 μm/voxel). A 4 mm region, distal to the proximal tibia growth plate, was scanned. The Veh group was scanned on day 0 and day 12 to minimize radiation exposure. In contrast, the PTH and COMBO rats were scanned every 4 days for 12 days, because PTH exerts a protective effect against radiation to allow multiple scans. This was further confirmed in the current study by a right-left tibia comparison at day 12, which revealed negligible effects of radiation for each group. In addition, the left tibia of each rat was scanned using *ex vivo* μCT (MicroCT 35, Scanco, Medical, 3.5μm/voxel) for high resolution tissue mineral density (Tb.TMD) analysis.

For the *in vivo* scans, a 2.5 mm region of trabecular bone at day 12 was chosen and then the corresponding VOI was accurately identified

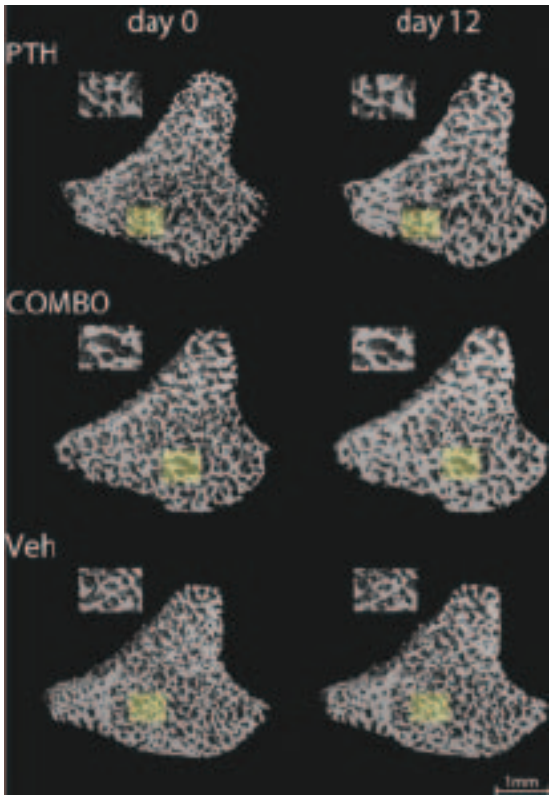

Figure 1. Registered trabecular structure at day 0 (Left) and day 12 (Right) of PTH, Combo, and Veh groups.

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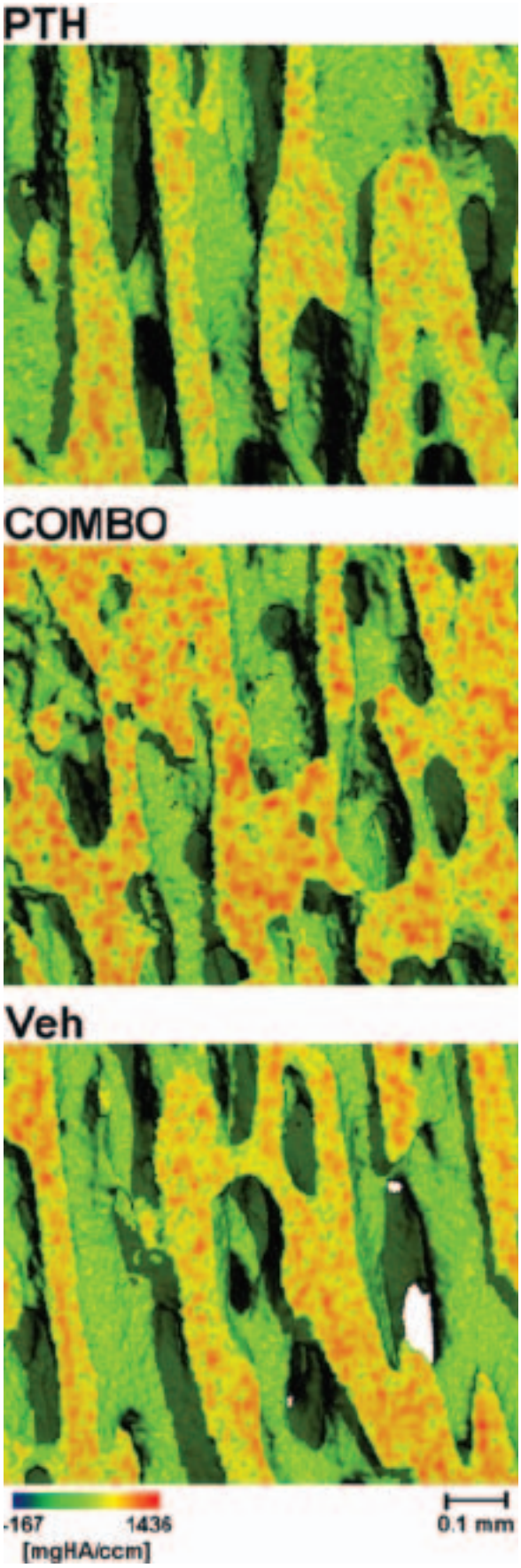

Figure 2. Trabecular tissue mineralization distribution.

in the trabecular bone images of day 0, 4, and 8 scans (Fig. 1) by using mutual information-based 3D image registration software (ITK, NLM). Variables of interest included bone volume fraction (BV/TV), structure model index (SMI), trabecular number (Tb.N*), thickness (Tb.Th*), and spacing (Tb.Sp*). Customized microstructural finite element analysis (μFEA) software was used to calculate the trabecular stiffness (Tb.Stiff). In addition, both the mean Tb.TMD and the spatial

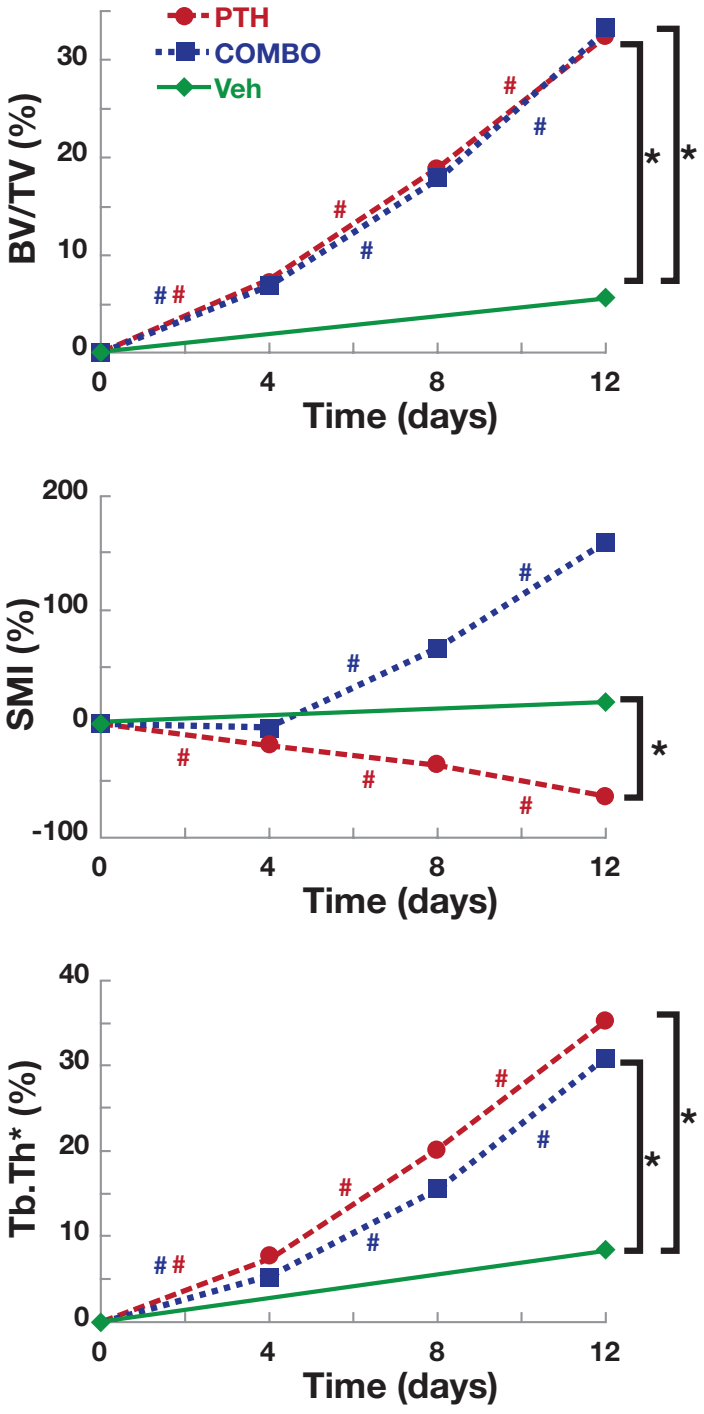

Figure 3. % change of BV/TV, Tb.Th*, and SMI over 12 days. # indicates difference over time and * difference between groups.

variation of tissue mineralization (Tb.TMDcv) were calculated based on a 1 mm^3 trabecular bone volume of the left tibia scanned at 3.5 μm voxel size (Fig 2).

All variables of interest were compared between time points within groups, and across time, using the %change at day 12 between groups. Student's t-tests were used for all comparisons, with $p<0.05$ considered a significant difference.

Results

After only 4 days, significant improvements in bone structure were detected in both the PTH and COMBO groups and the improvements persisted through day 12 (Fig 1 & 3). In contrast, no change was detected in Veh group after 12 days. Both treatment groups showed significant improvements over all time points in BV/TV and Tb.Th*, indicating substantial bone formation over the 12 day period. Tb.Sp* showed a 6% reduction in the COMBO group, which was significantly different from the Veh group after 12 days. Tb.N* showed no difference between groups or over time. SMI suggests a 158% increase in rod-like structures in the COMBO treatment group, and a 63% increase in plate-like structures in the PTH treatment group (Fig 3). μFEA revealed 68% increases in Tb.Stiff in both treatment groups and no change in the Veh group over 12 days.

By the end of the 12 day treatment, COMBO group had a 4% higher Tb.TMD compared to the PTH group while Tb.TMD in the PTH group was 2.4% lower than the Veh group (Fig 2). The Tb.TMDcv was different among all groups, with the highest variation in the COMBO group (29%), followed by the PTH group (27%), than the Veh group (25%), indicating a more heterogeneous trabecular mineralization in the treatment groups. Qualitative analysis of Tb.TMD distribution revealed new bone formation with less mineralized tissue on trabecular surface in the PTH group, but highly mineralized bone throughout in the COMBO group, even compared to the Veh (Fig 2).

Discussion

By using registered *in vivo* μCT imaging we demonstrated an immediate response in trabecular bone microstructure to PTH and combined PTH and ALN treatment starting as early as 4 days after treatment initiation. Over 12 days, continuous increases in BV/TV of both treatment groups were primarily caused by thickened trabeculae, which would suggest excessive formation of new bone on the trabecular surface. However, the Tb.TMD analysis, based on ultra-high resolution *ex vivo* μCT, revealed new anomalies in tissue mineralization. While this study is consistent with previous works which identified increased Tb.TMD from ALN treatment,[4] we did not expect a significant increase after only 12 days. In addition, in the COMBO group we would have expected the thickened trabeculae to have less mineralized tissue at the bone surface as seen in the PTH group. However, our data reveal higher mineralization throughout, including at the bone surface, due to COMBO treatment. Future investigations of the involvement of other types of cells, such as osteocytes, or changes in the biochemical environment of bone may help us to explain this paradox.

Significance

To our knowledge, this is the first study to examine immediate changes in trabecular structure, stiffness, and tissue mineralization in response to PTH and combined PTH and ALN therapies. Although no additional improvement was found in bone volume, structure, or mechanical stiffness by combined treatment over PTH alone, adding ALN may increase the treatment efficacy by reducing the mineralization lag time and osteoid maturation time.

References

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