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Alendronate and PTH Combination Therapy Stimulates Bone Formation While Inhibiting Bone Resorption Activities in the Rat Tibia: A Longitudinal, In Vivo, Dynamic Bone Histomorphometry Study

Introduction

Osteoporotic bone loss is characterized by a shift in bone remodeling such that resorption outpaces formation. Treatments improve bone mass by increasing net formation: anti-resorptive drugs such as alendronate (ALN) block osteoclast activity, while anabolic agents such as PTH increase bone remodeling, with a greater effect on formation. Although these drugs are widely used, their role in modulating formation and resorption is not fully understood, due in part to technical limitations in the ability to longitudinally assess bone remodeling. Importantly, it is not known whether or not PTH-induced bone formation is independent of resorption, resulting in controversy over the effectiveness of combination therapies that use both PTH and ALN.^{1,2} Although formation can be assessed using dynamic histology, this technique is destructive, imprecise, and cannot assess bone resorption. Methods for improved quantification of bone formation and resorption have been developed,^{3,4} but are limited by their destructive nature or low temporal resolution, making it difficult to monitor drug effects over clinically relevant treatment times. We developed an *in vivo* dynamic bone histomorphometry technique for rat tibiae, and applied this method to longitudinally track changes in bone resorption and formation as a result of treatment with ALN, PTH, or combination therapy. We hypothesized that adding ALN to PTH treatment would inhibit bone resorption activities while maintaining the elevated bone formation activities induced by PTH treatment, thus resulting in an additive, beneficial effect on trabecular bone.

Methods

Animal Protocol

3 month-old, female, Sprague Dawley rats were assigned to Veh (n=5), PTH (n=8), ALN (n=6), and PTH+ALN (n=7) treatment groups (IACUC approved). Starting on day 0, rats were treated with daily 60 µg/kg PTH (PTH and PTH+ALN), 50 µg/kg alendronate every three

days (ALN and PTH+ALN), or daily saline (Veh) through subcutaneous injections over 12 days. Calcein was injected on days 3 and 11 to allow for dynamic histomorphometry. The right proximal tibia of each rat was scanned by µCT at 10.5µm resolution on days -8, 0, and 12 (vivaCT 40, Scanco Medical, Brüttisellen, Switzerland). Rats treated with PTH and PTH+ALN had an additional scan at day 4, as our previous study suggested that PTH can prevent radiation damage.⁵ All rats were sacrificed on day 12, and serum and tibiae were harvested from a subset of rats to measure standard histological parameters of bone formation, measure osteoblast and osteoclast surface (Ob.S/BS and Oc.S/BS), and assess serum resorption marker TRAP.

Image Processing

µCT images from day -8 to 0 and day 0 to 12 (day 4 to 12 in PTH and PTH+ALN groups) were registered using mutual-information-based, 3D image registration software (ITK, NLM) to precisely align the trabeculae in the secondary spongiosa. Following registration, images were Gaussian filtered, thresholded, and subtracted to obtain a map of the locations of bone formation and resorption. Formation and resorption parameters analogous to histology-based measurements were calculated as follows: bone formation rate (BFR/BS) = volume of bone formed/(# of days*bone surface), bone resorption rate (BRR/BS) = volume of bone resorbed/(# of days*bone surface), mineral apposition rate (MAR) = thickness of formed bone/# of days, and mineral erosion rate (MER) = thickness of resorbed bone/# of days.

Statistics

Correlations between µCT- and histology-based measures of BFR/BS and MAR and between µCT-based BRR/BS and serum levels of TRAP were assessed using linear regression. Comparisons among treatment groups and over time were made using a two-way ANOVA with Bonferroni correction (NCSS, LCC, Kaysville UT).

Results

The locations of bone formation identified through μ CT yielded excellent agreement with calcein-labeled regions identified through histology, and μ CT-based measures of BFR/BS ($r=0.78$), MAR ($r=0.60$), and BRR/BS ($r=0.91$) correlated strongly with histology-based measures of bone formation and serum levels of TRAP (Figure 1). The longitudinal design of this study allowed us to assess drug effects within each rat over time as well as compare bone formation/resorption parameters among treatment groups. Over the treatment period, changes in bone volume fraction (BV/TV) indicated an additive effect of combination therapy over treatment with PTH or ALN alone (Figure 2). Measurements derived through *in vivo* dynamic bone histomorphometry demonstrated an increase of 220% in BFR/BS and 30% in MAR over time in specimens treated with PTH and an increase of 378% in BFR/BS and 37% in MAR in rats treated with PTH+ALN ($p<0.05$, Figure 2). In specimens treated with PTH, MER increased 27% ($p<0.05$), while specimens treated with ALN and PTH+ALN showed 37% decreased MER ($p<0.05$) and 65% decreased BRR/BS ($p<0.05$), respectively (Figure 2). At the end of the treatment period, both CT-based BFR/BS and histology-based osteoblast surface were elevated in rats treated with PTH and PTH+ALN as compared to those treated with Veh and ALN (Figure 3, $p<0.05$). CT-based BRR/BS indicated that resorption

was lower in rats treated with PTH+ALN ($p<0.05$), and tended to be lower in rats treated with ALN ($p<0.1$) than in PTH-treated rats, while histology-based osteoclast surface showed no difference among groups.

Discussion

In this study we developed an *in vivo* dynamic bone histomorphometry technique to monitor changes in bone remodeling activities in response to anti-resorptive and anabolic treatment. The strong correlations between μ CT-based and traditional measures of bone remodeling, and the qualitative agreement between μ CT- and histology-based localization of bone formation sites, indicate that the *in vivo* dynamic bone histomorphometry technique developed in this study allows for accurate assessment of bone formation and resorption. Application of this method resulted in a non-invasive, 3D evaluation of the bone formation and resorption events taking place as a result of treatment with PTH, ALN, or combined PTH+ALN. Additionally, the longitudinal nature of this technique allows for pre-treatment measurements, so that each rat can serve as its own control. Compared to pre-treatment, rats had decreased bone resorption rate and increased bone formation and mineral apposition rates during the period of treatment with PTH+ALN, which partially explains the additive effect of combined therapy with

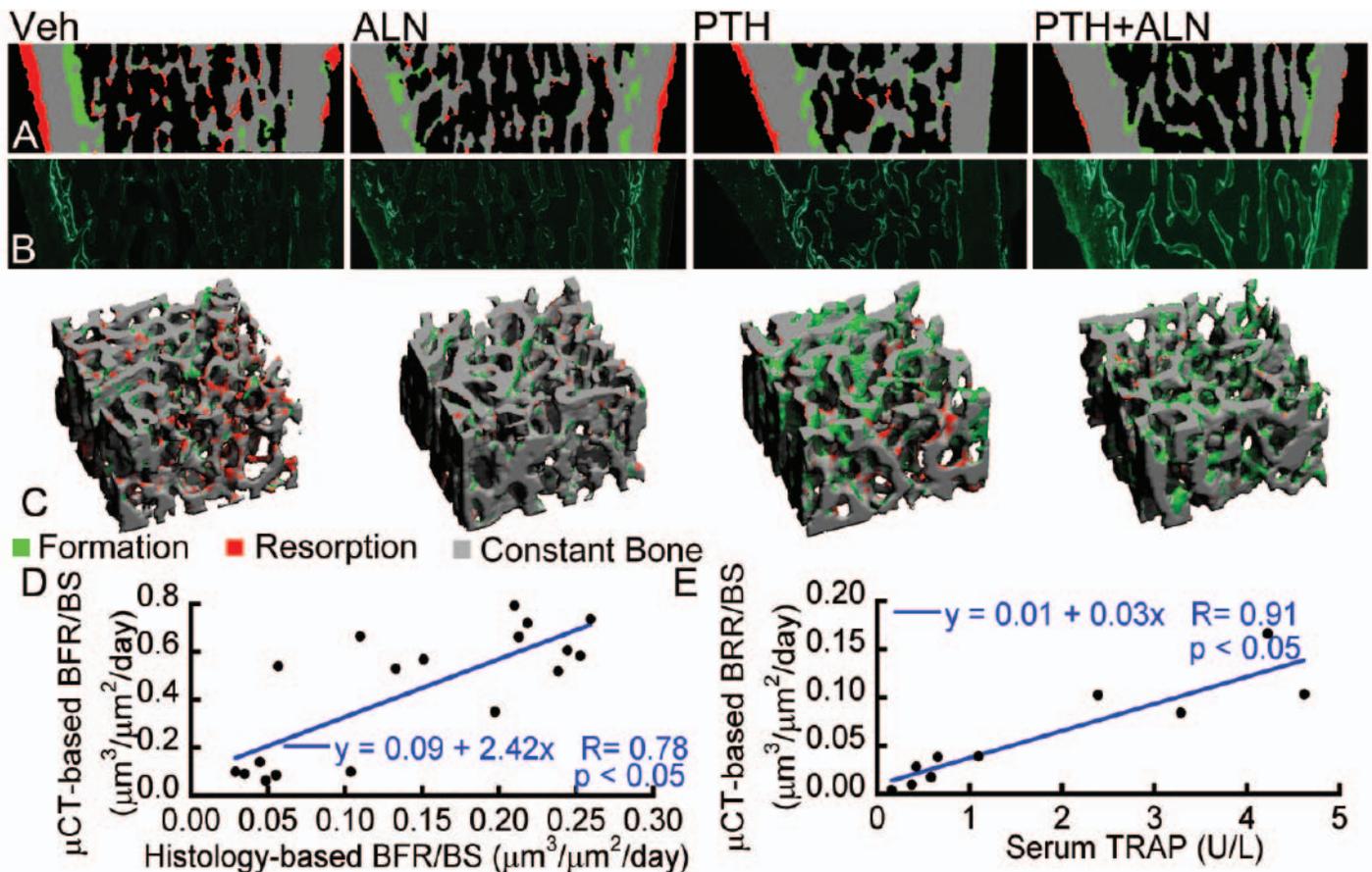


Figure 1. Validation of μ CT method: Paired comparisons were made of (A) *in vivo*, μ CT-based bone formation/resorption map to (B) calcein-labeled histology section for a representative Veh, ALN, PTH and PTH+ALN tibia. (C) A 3D rendering of bone resorption and formation within the analyzed trabecular region is also shown. Significant correlations were found (D) between μ CT- and histology based measure of BFR/BS and (E) between μ CT-based BRR/BS and serum TRAP.

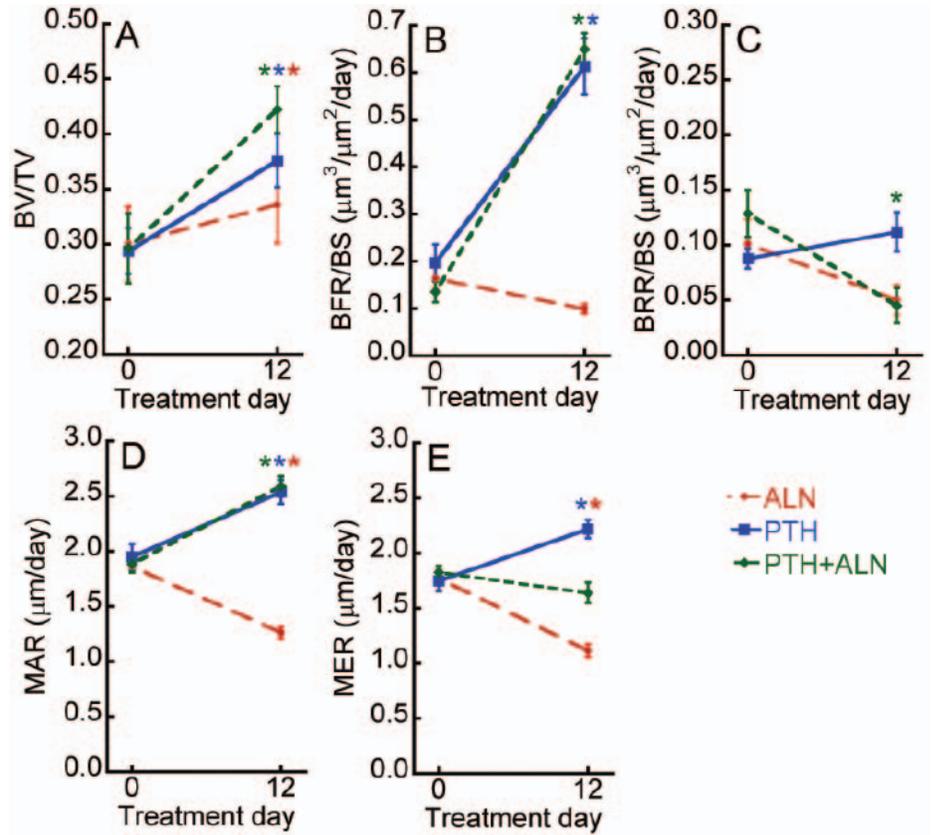


Figure 2. Effects of ALN, PTH, and PTH+ALN treatment on (A) BV/TV, (B) BFR/BS, (C) BRR/BS, (D) MAR, and E MER. *:significant differences from day 0 (p<0.05).

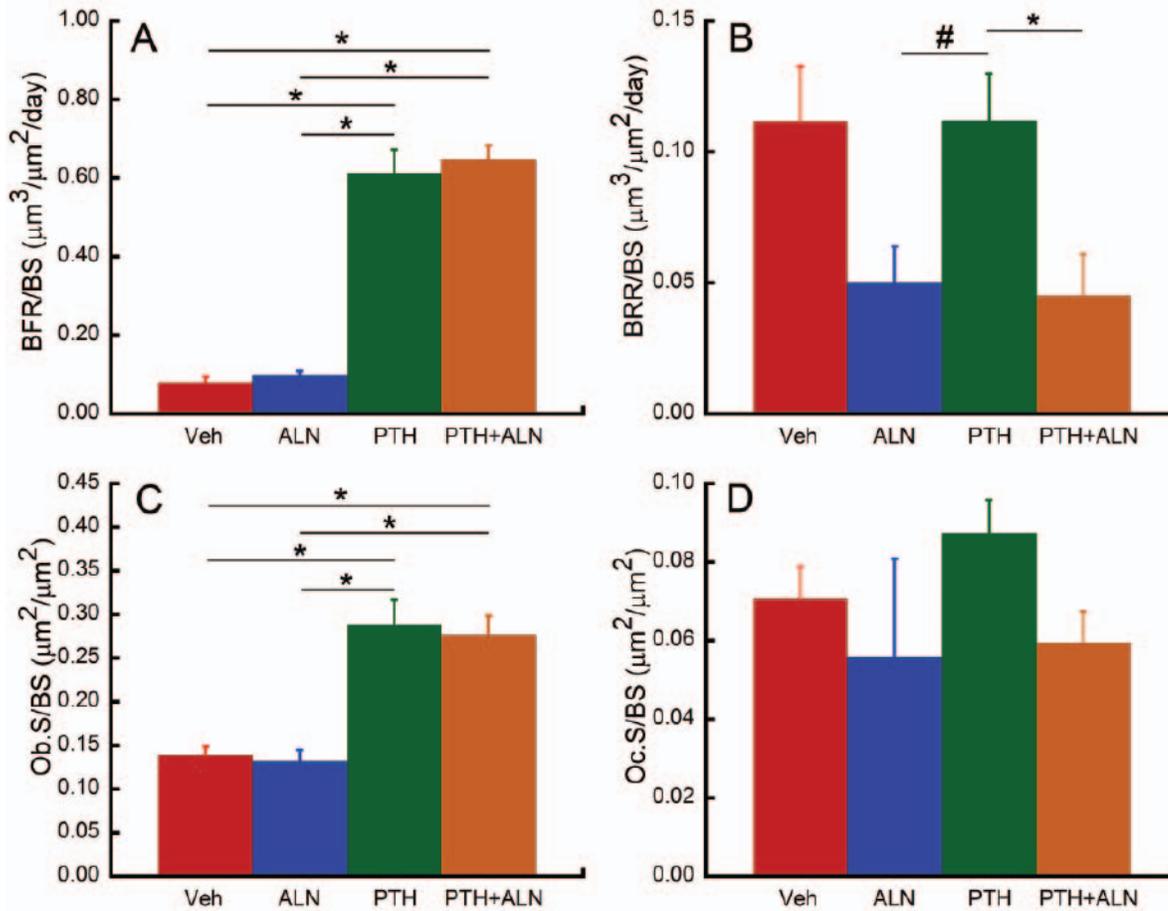


Figure 3. Cross-sectional comparison of CT-based (A) BFR/BS and (B) BRR/BS and histology-based (C) Ob.S/BS and (D) Oc.S/BS among the four groups after 12 days of treatment *: p<0.05, #: p<0.1.

PTH+ALN over monotherapy. This provides *in vivo*, direct evidence, suggesting that PTH's anabolic effect on stimulating bone formation is independent of resorption. In clinical applications, this suggests that co-treatment of PTH with an anti-resorptive is beneficial as the anti-resorptive may enhance the anabolic effect of PTH.

Significance

Non-invasive, longitudinal assessment of bone formation and resorption would allow for precise measurements of changes in bone remodeling, resulting in an improved understanding of the mechanisms of bone disorders and drug treatments. Application of this technique elucidated the effects of combination therapy of PTH and an anti-resorptive, suggesting that the anabolic effect of PTH is independent of resorption and thus combination therapy may provide greater improvements in bone quality over monotherapy.

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