Intermittent PTH after Prolonged Bisphosphonate Treatment Improves Bone Structure by Inducing Substantial New Bone Formation with Decoupled, Inhibited Bone Resorption in Ovariectomized Rats

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Introduction

Bisphosphonates (BP) are an osteoporosis treatment that acts to prevent bone loss by inhibiting bone resorption. While this adequately slows or stops further bone loss, it does not promote new bone formation. In addition, recent evidence has suggested that long-term bisphosphonate use may increase the risk of atypical femoral fractures.[1] Intermittent parathyroid hormone (PTH) is the only FDA approved anabolic agent, which promotes bone formation. Using PTH in conjunction with bisphosphonates would provide the maximum benefit to severely osteoporotic individuals, both inhibiting resorption and promoting bone formation. Early studies showed conflicting results regarding the efficacy of combined treatment of bisphosphonate and PTH therapy, leading to the hypothesis that PTH's anabolic effect may be dependent on prior resorption to initiate bone formation.[2,3] However, recent studies have shown a positive effect of combined or tandem BP and PTH therapies, attesting to PTH's anabolic effect in the absence of resorption.[4-7] Therefore, we hypothesized that PTH is able to act through a resorption-independent pathway to promote modeling-based bone formation. A better understanding of this pathway would be advantageous for both understanding combined PTH and bisphosphonate therapy, as well as designing new treatments which could more directly target modeling-based formation.

In this study, the efficacy of PTH following 12-weeks of alendronate (ALN, a BP) treatment was tested in ovariectomized (OVX) rats. While current methods of investigating bone remodeling over time are limited to cross-sectional animal studies or indirect biomarkers, the current study employed a novel *in vivo* imaging technique to assess bone formation and resorption rate simultaneously and longitudinally. This allowed for an evaluation of the coupling between bone resorption and formation. We hypothesized that prolonged ALN prior to PTH treatment does not blunt PTH's anabolic potential to activate new bone formation. Thus, PTH can be considered as a treatment for patients with a history of long-term BP treatment.

Methods

Animals: 30 female SD rats (n = 6/group) received surgery at 4-mo of age: 24 received a bilateral OVX, and 6 received a sham OVX surgery. The study began when all rats were 6-mo-old, after a 2-mo development of osteoporosis in the OVX rats. The treatment plan consisted of 2 phases (phase 1: weeks 0-12, and phase 2: weeks 12-16). The OVX rats were assigned to 4 groups: (1) a Veh group treated with saline for both phases; (2) an ALN group treated with ALN (28μg/kg 2x/wk) for both phases, (3) a Veh+PTH group treated with saline for phase 1, then switched to PTH (40 μg/kg 5x/wk) for phase 2, (4) an ALN+PTH group treated with ALN for phase 1, then PTH for phase 2 (Fig 1).

***In Vivo* μCT Scans:** The right proximal tibia of all rats were scanned (Scanco VivaCT40, 10.5 μm) at week 0 (2 mo post surgery), then weekly from weeks 11-16 corresponding with phase 2 (Fig 1). Trabecular microstructure of subsequent scan images was precisely aligned to baseline using an iterative registration method to identify the same volume of interest (VOI). Bone microstructure was analyzed for the same VOI for weeks 0, 12, 14, and 16.[8]

***In Vivo* Dynamic Histomorphometry:** A bone sub-volume ($1.575\times1.575\times1.05mm^3$) of each week in phase 2 was subtracted from the registered sub-volume of the previous week (weeks 11-15) to identify the newly formed bone voxels (green) and resorbed voxels (red) during each week (Fig 1 Right). New bone voxels were used to calculate the bone formation rate (BFR/BS), and lost voxels to calculate bone resorption rate (BRR/BS) weekly over phase 2.[9]

Femur 3-Point Bending: The right femoral midshaft was scanned *ex vivo* for evaluation

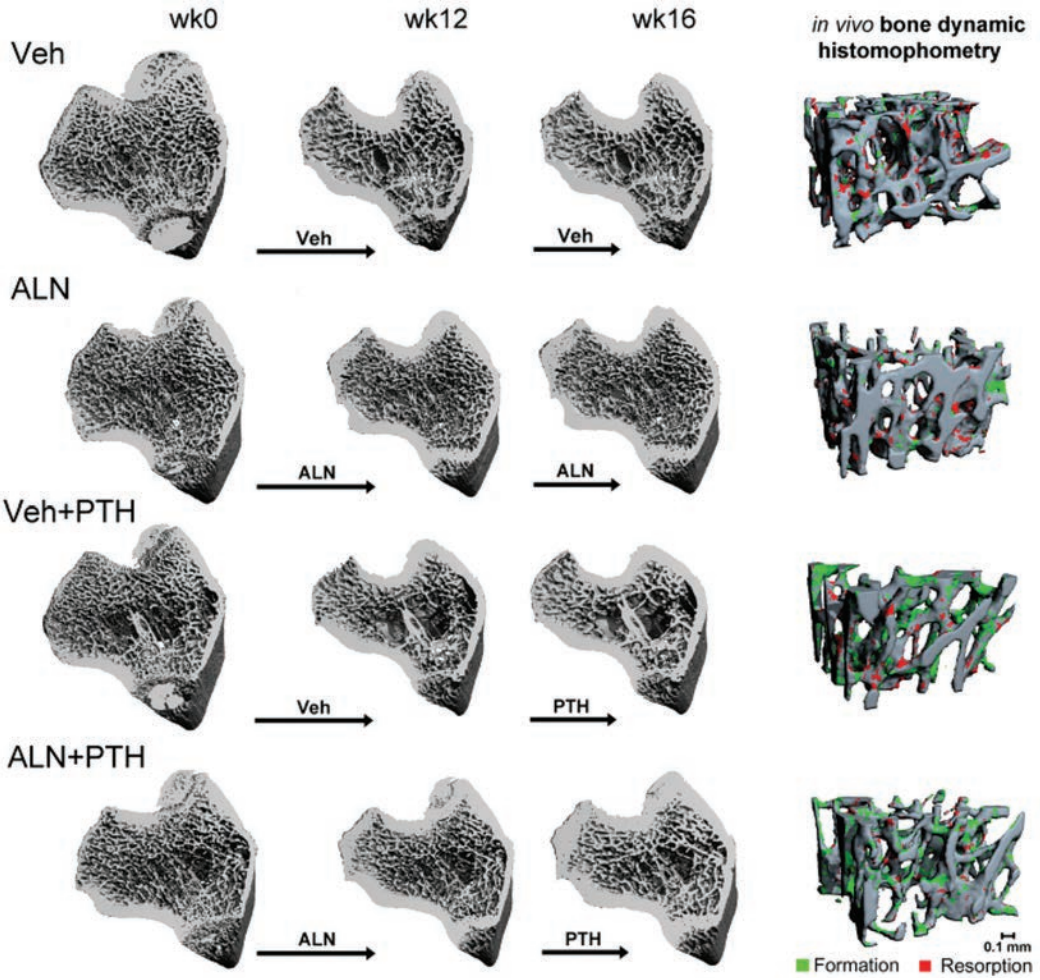

Figure 1. Left: Registered comparison of tibia bone segments at each timepoint with treatment indicated by the arrows between time points. Right: μCT-based *invivo* bone dynamic histomorphometry, green indicates areas of new bone (formation), and red indicates lost bone (resorption) during the final week of treatment.

of cortical thickness and polar moment of inertia. Then the femur was subjected to a 3-point bending test for evaluation of stiffness and elastic modulus.

Spine: Lumbar vertebra, L2 was scanned *ex vivo* (Scanco μCT35, 3.5 μm) for microstructural and tissue mineral density (TMD) analysis. TMD was calculated for bone tissue at trabecular surface layers (sTMD), and central bone tissue (cTMD).

Finite Element (FE) Analysis: The trabecular bone subvolume of weeks 12 and 16 were converted to voxel-based FE models to estimate axial stiffness under compression.

Statistical Analysis: Longitudinal measures were compared over time and between groups using repeated measures ANCOVA adjusted for baseline, and cross-sectional measures were compared using ANOVA.

Results

Tibia Microstructure: 2-mo after surgery, OVX resulted in an average BV/TV of 0.13, in contrast to 0.50 in the SHAM group at week 0. BV/TV continued to drop in the Veh and Veh+PTH to 0.06 by week 12 (Fig 2).ALN treatment effectively stabilized BV/TV for ALN and ALN+PTH groups. Switching to PTH resulted in a dramatic increase in BV/TV by week 16 compared to week 12 (40% and 42%) driven by increased Tb.Th (33% and 25%) in both Veh+PTH and ALN+PTH groups (Fig 2).

***In Vivo* Dynamic Histomorphometry:** During phase 2, the Veh+PTH group had a dramatic increase in BFR/BS by week 14, which began to stabilize by week 16. The ALN+PTH group had a similar increase in BFR/BS which remained elevated throughout phase 2 (Fig 3a). BRR/BS was low in all treatment groups after the onset of PTH therapy.This was further confirmed by TRAP serum ELISA (Fig 3b). Fig 3c showed that SHAM and ALN treated groups had highly coupled remodeling, with similar BFR/BS and BRR/BS. Resorption outpaced formation for the Veh group. Bone resorption and formation were decoupled in both Veh+PTH and ALN+PTH groups as shown by a substantially greater BFR/BS than BRR/BS.

Femur: No difference was found in cortical structure or mechanical parameters between groups.

Spine Microstructure: Compared to the tibia, OVX resulted in less reduced vertebral BV/TV (0.21 in Veh *vs.* 0.33 in SHAM). All 3 treatments groups resulted in BV/TV that was

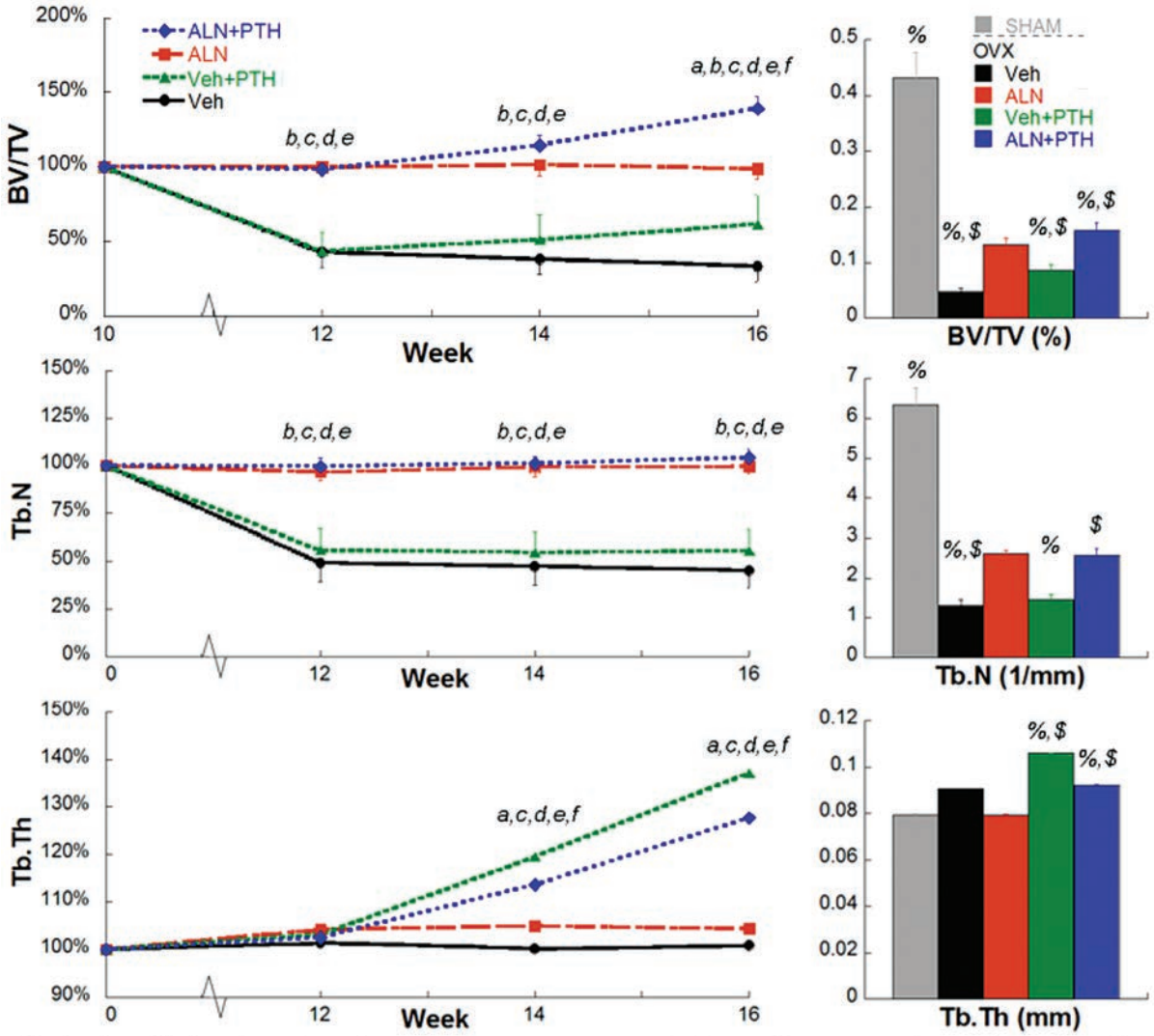

Figure 2. *Invivo* tibial trabecular microstructure parameters. Left panel: percent change from baseline, right panel: value at the study endpoint. Letters (left) indicate significant differences between groups: *a*: ALN to ALN+PTH, *b*: ALN to OVX, *c*: ALN to Veh+PTH, *d*: ALN+PTH to OVX, *e*: ALN+PTH to Veh+PTH, *f*. OVX to Veh+PTH. Symbols (right) indicate significant change from baseline (%) or week 12 (*$*).

not different from SHAM. The Veh+PTH had less Tb.N but greater Tb.Th than SHAM. Compared to SHAM, Tb.N was no different from the ALN and ALN+PTH groups, while Tb.Th was greater in the ALN+PTH group.

Spine TMD: cTMD was higher in the SHAM than all treatment groups. cTMD of the Veh+PTH group was 7% and 4% lower compared to SHAM and Veh. sTMD was significantly reduced in the Veh+PTH (6%) and ALN+PTH (4%) compared to ALN.

Tibia Stiffness: The trabecular bone stiffness did not change during phase 2 in the SHAM, Veh or ALN groups. Stiffness improved in both Veh+PTH (97%) and ALN+PTH (114%) groups beyond that of all other groups.

Discussion

The results of this study clearly demonstrate the efficacy of PTH following BP therapy for stimulating new bone formation. In healthy bone, resorption and formation are coupled and balanced to sustain bone mass. Their uncoupling due to OVX results in resorption outpacing formation, and subsequent trabecular bone loss which compromises the mechanical competence of the bone. ALN treatment effectively re-couples this balance, preventing additional bone loss. PTH, however, uncouples the balance in favor of formation, resulting in thickened bone and greater structural stiffness. Resorption activities after ALN, Veh+PTH, and ALN+PTH treatments were not different and remained significantly lower than those of Veh and Sham. While we may have expected a higher BRR/BS or TRAP in the Veh compared to Sham group, the resorption-formation balance of OVX had been re-adjusted to the slower formation rate 6 months after OVX. In summary, our investigation across multiple skeletal sites suggests that ALN followed by PTH is a viable treatment strategy to maintain and improve bone quality by stimulating substantial new bone formation.

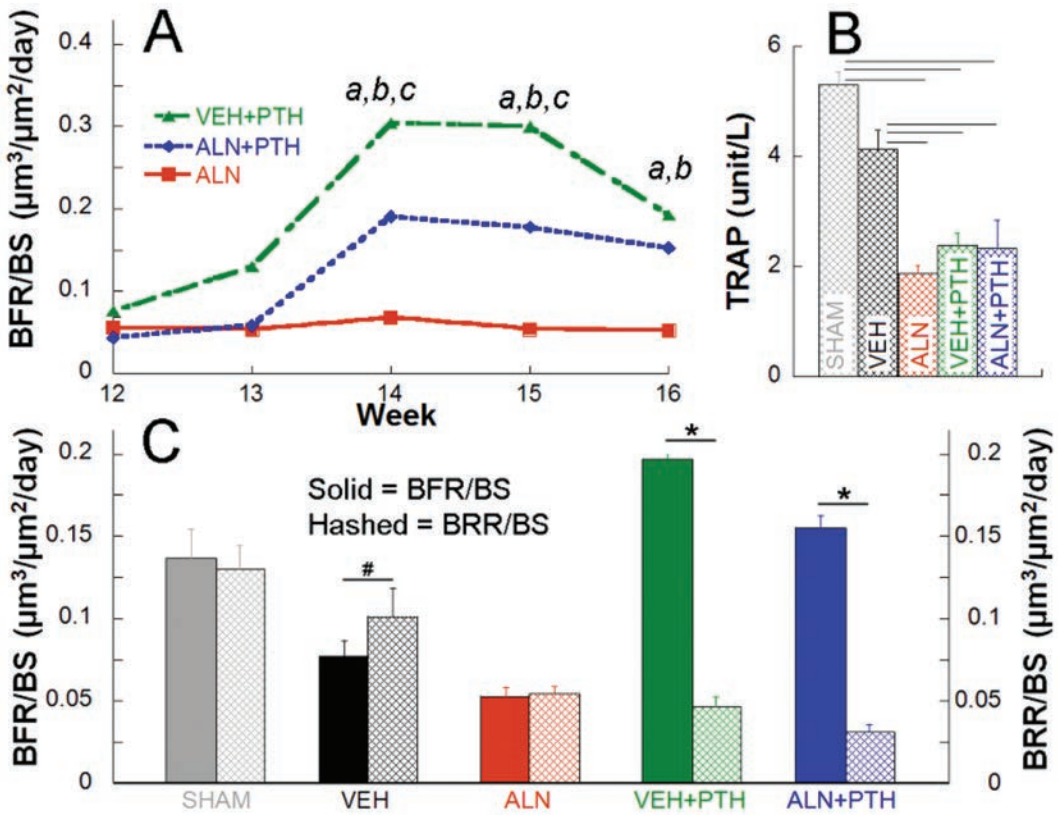

Figure 3. (A) *Invivo* dynamic histomorphometry, BFR/BS week 12-16. Letters indicate differences between groups, *a*: ALN to ALN+PTH, *b*: ALN to Veh+PTH, *c*: ALN+PTH to Veh+PTH. (B) Serum TRAP concentration, bars indicate differences between groups. (C) *Invivo* dynamic histomorphometry at week 16. Left axis: BFR/BS (solid), right axis: BRR/BS (hashed). *indicates differences between formation and resorption,# indicates a trend difference ($p < 0.1$).

Significance

Long term ALN therapy following OVX can be further augmented by subsequent PTH treatment. This increases the BFR/BS to thicken the existing trabeculae while inhibiting bone resorption. The novel *in vivo* dynamic histomorphometry analysis provides direct evidence for PTH's anabolic effect in the absence of bone resorption, further confirming its capacity for modeling-based bone formation.

Acknowledgments

Penn Center for Musculoskeletal Diseases (PCMD) NIH/NIAMSP30AR050950, NIH/NIAMS T32-AR007132, University of Pennsylvania Institute on Aging (IOA) Pilot Award.

References

1. Seraphim A, et al. Do bisphosphonates cause femoral insufficiency fractures? *J Orthopaed Traumatol*, 13(4):171-7 (2012).

2. Black DM, et al. The effects of parathyroid hormone and alendronate alone or in combination in postmenopausal osteoporosis. *N Engl J Med*, 349:1207-15 (2003).

3. Wu X, et al. Inhibition of Sca-1-positive skeletal stem cell recruitment by alendronate blunts the anabolic effects of parathyroid hormone on bone remodeling. *Stem Cell*, 7:571-80 (2010).

4. Cosman F, et al. Effects of intravenous zoledronic acid plus subcutaneous teriparatide in postmenopausal osteoporosis. *JBMR*, 26:503-11 (2011).

5. Muschitz C, et al. Antiresorptives overlapping ongoing teriparatide treatment result in additional increases in bone mineral density. *JBMR*, 28:196-205 (2013).

6. Schafer AL, et al. Postmenopausal women treated with combination parathyroid hormone and ibandronate demonstrate different microstructural changes at the radius vs. tibia: the PTH and Ibandronate Combination Study (PICS). *Osteoporosis Int*, 24(10):2591-601 (2013).

7. Altman AR, et al. A closer look at the immediate trabecula response to combined parathyroid hormone and alendronate treatment. *Bone*, 61:149-57 (2014).

8. Lan S, et al. 3D image registration is critical to ensure accurate detection of longitudinal changes in trabecular bonedensity, microstructure, and stiffness measurements in rat tibiae by in vivo microcomputed tomography (μCT). *Bone*, 56(1):83-90 (2013).

9. Schulte FA, et al. In vivo micro-computed tomography allows direct three-dimensional quantification of both bone formation and bone resorption parameters using time-lapsed imaging. *Bone*, 48(3):433-42 (2011).