Cyclic Treatment Regime Rescues PTH Withdrawal-Induced Bone Loss and Microarchitecture Deterioration

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Introduction

Osteoporosis in postmenopausal women and elderly men is a life-long chronic condition. Intermittent parathyroid hormone (PTH) is currently the only FDA-approved, anabolic agent for osteoporosis. In clinical practice, the recommended duration of PTH treatment is 18-24 months. Despite its potent effect of promoting new bone formation, bone mineral density rapidly decreases upon withdrawal from PTH treatment[1]. It has been recommended that anti-resorptive treatment should be applied upon discontinuation of PTH to prevent bone loss. However, recent reports of atypical subtrochanteric and femoral shaft fractures raised concerns on long-term use of bisphosphonates.

To maximize the efficacy of PTH, the first objective of this study was to uncover the mechanisms behind the adverse effect of PTH withdrawal in an ovariectomized (OVX) rat model. The second objective was to test the efficacy of a cyclic PTH treatment regime[2] on rescuing the PTH withdrawal effect.

Methods

Withdrawal study

27 female Sprague Dawley (SD) rats received bilateral OVX surgery at age 4 months. <u>μCT *Imaging*:</u> 15 rats were assigned to 2 groups: PTH (n = 6, PTH 40μg/kg 5x/wk for 3 weeks followed by saline for 9 weeks) and VEH (n = 9, saline for 12weeks). Sequential scans of proximal tibiae were performed by *in vivo* μCT (Scanco Medical) at 10.5 μm voxel size at weeks -4 (OVX surgery), 0, 3, 4, 5, 6, 8, 10, and 12. 3D image registration[3] was applied to identify the same volume of interest (VOI) in all scans before performing bone microstructure analysis. <u>*Bone histomorphometry:*</u> 12 rats were euthanized after 3 weeks of VEH (V3), 3 weeks of PTH (P3), 3 weeks of PTH followed by 1 week of VEH (P3V1), and 3 weeks of PTH follow by by 2 weeks of VEH (P3V2) treatments (n = 3/group) with the right tibiae harvested for methylmethacrylate (MMA) embedding. Five μm-thick longitudinal sections were stained with Goldner's trichrome to identify osteoblasts, osteoclasts, and bone surface. Osteoclast number and surface per bone surface (Oc.N/BS and Oc.S/BS) were quantified. <u>*Serum TRAP:*</u> In addition to V3, P3, P3V1, and P3V2, blood was collected at P3V9 and V3V9 from rats in the μCT experiment to determine serum TRAP 5b levels.

Cyclic treatment study

6 OVX rats were assigned to (1) cyclic PTH (n = 3, PTH for 3 weeks followed by saline for 3 weeks, 3 cycles) and VEH (n = 3, saline for 18 weeks). Trabecular bone microstructural analysis was applied to registered *in vivo* μCT scans of the proximal tibia at weeks -4, 0, 3, 4, 6, 9, 10, 12, 15, 16, and 18. Longitudinal comparisons were made using 2-way, repeated-measures ANOVA, adjusted for baseline values, and cross-sectional comparisons were made using 1-way ANOVA. Bonferroni corrections were applied to all *post hoc* tests.

Results

Withdrawal study

4 weeks post OVX, bone volume/total volume (BV/TV), trabecular thickness (Tb.Th), and trabecular number (Tb. N) decreased 50%, 13%, and 31% respectively, while structure model index (SMI) increased (all $p < 0.05$, Figure 1 A-D). Bone loss continued in VEH rats for 9 weeks. In contrast, 3 weeks of PTH treatment effectively slowed down the bone loss, causing no changes in BV/TV, Tb.N, or SMI, and a 35% increase in Tb.Th. At week 3, BV/TV and Tb.Th were 97% and 27% greater, and SMI was 30% lower in the PTH- *vs.* VEH-treated animals (all $p < 0.05$). Interestingly, Oc.N/BS and Oc.S/BS were 81% and 83% lower in PTH *vs.* VEH groups. 1 week after the withdrawal (week 4), BV/TV, Tb.Th, and SMI continued to show trends of improvement (Figure 1 ABD). Trends of bone deterioration appeared during the 2nd week of PTH withdrawal (week 5). These are consistent with bone histomorphometry results showing no change in Oc.N/BS and Oc.S/BS 1 week after withdrawal (P3V1, Figure 1 I-J). In contrast, Oc.N/BS and Oc.S/BS became 77% and 82% greater 2 weeks after withdrawal (P3V2) compared to P3 (Figure 1 I-J). Qualitative examination indicated increased number of osteoblasts on the bone surface at P3 compared to V3 (Figure 1 LM). At P3V1, there were no apparent changes in number of osteoblasts or osteoclasts. However, cell height of osteoblasts decreased (Figure 1N).

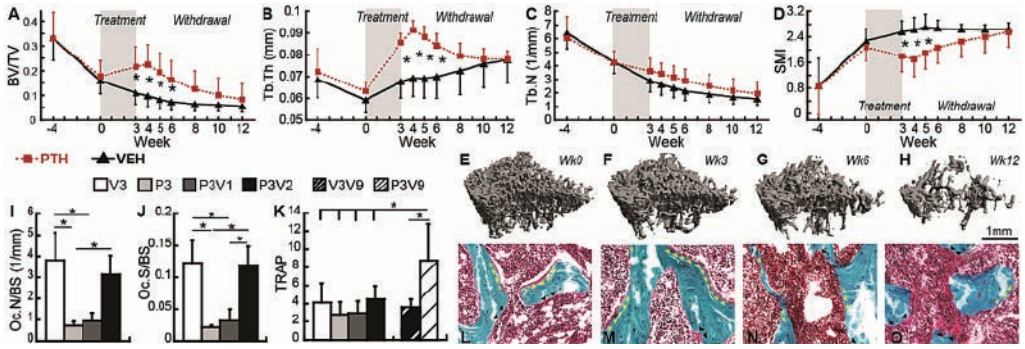

Figure 1. (A-D) % changes in trabecular bone microstructure measurements in PTH and VEH groups. **(E-H)** 3D rendering of trabecular bone microarchitecture at week 0, week 3 (end of PTH treatment). and week 6 and 9 (3 and 6 weeks after discontinuation of PTH) in a same rat. **(I-K)** Osteoclast activities were compared among rats with 3-week VEH (V3) and PTH (P3) treatments, and 3-week PTH treatment followed by 1 and 2 weeks withdrawal (P3V1 and P3V2). Serum TRAP was also measured for PTH rats at 9 week after discontinuation of PTH treatment (P3V9) and for the corresonding VEH rats (V3 V9). **(L-O)** Osteoblasts (yellow arrows) and osteoclasts (black arrows) were shown in Goldner's Trichrome staining of trabecular bone sections at (L) V3, (M) P3, (N) P3V1, and (0) P3V2.
*indicates significant difference between PTH and VEH groups ($p < 0.05$).

At P3V2, cell height of osteoblasts continued to decrease to a similar level as bone lining cells (Figure 1O). 3 weeks after PTH withdrawal (week 6), BV/TV and Tb.Th were still 123% and 20% greater in PTH *vs.* VEH groups, respectively. However, treatment benefit by PTH diminished 5 weeks after withdrawal (week 8). 9 weeks after withdrawal, serum TRAP levels were 59% greater than that in VEH group (Figure 1K).

Cyclic treatment study

Cyclic treatment regime efficiently maintained the benefit of PTH treatment in BV/TV and Tb.N, and further increased Tb.Th (Figure 2). Trends of increase in BV/TV and Tb.Th were observed 1 week after PTH withdrawal in all 3 cycles. Compared to VEH group, BV/TV and Tb.Th were significantly greater in PTH group after 3-week withdrawal in all 3 cycles.

Discussion

Significant bone loss and bone microarchitecture deterioration occurred in OVX animals in response to discontinuation of PTH treatment. Intriguingly, there is a continuous anabolic window during the first week withdrawal from PTH in OVX rats during which no significant change occurred in number of osteoclasts while the height of osteoblasts started to decrease. In contrast, osteoclast number and surface increased 2 weeks after withdrawal from PTH.The morphology of osteoblasts continued to change and became difficult to differentiate from bone lining cells. Changes in bone cells in week 2 after withdrawal led to a decline in bone mass and bone microarchitecture. 9 weeks after withdrawal, the benefits of PTH treatment completely diminished, with no difference in any trabecular bone parameters between PTH and VEH groups. However, serum TRAP analysis indicated more than double the number of active osteoclasts in rats after 9-week withdrawal as compared to VEH rats, potentially leading to more rapid bone loss after long-term PTH withdrawal. Lastly, the continuous anabolic window upon early withdrawal allowed the cyclic treatment regime to efficiently maintain and improve upon the PTH treatment benefit on bone.

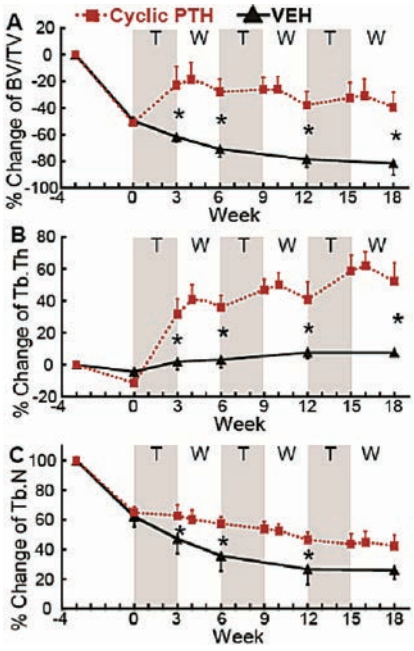

Figure 2. (A-C) % changes in trabecular bone microstructure measurements in cyclic PTH and VEH groups.

Significance

This study demonstrates a continuous anabolic window upon early withdrawal from PTH, which offers a new mechanism in support of the cyclic administration regime of PTH to maximize the total duration and efficacy of treatment.

References

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