Enhanced Individual Trabecular Repair and its Mechanical Implications in PTH and Alendronate Treated Rat Tibial Bone

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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. 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. Despite conflicting results in the literature,[1,2,3] previous work in our lab has shown that combined PTH and ALN therapy results in an additive effect, with enhanced bone volume fraction beyond that of PTH or ALN monotherapy.[4] This additive effect was driven by preferential plate thickening, resulting in an increase in the relative number of plate- versus rod-like structures.[4] The current study was aimed at identifying the mechanism through which plate-structure was enhanced, and the resulting implications on the mechanical behavior of the trabecular bone. A novel *in vivo* imaging technique allowed the precise alignment of subsequent scans in such a way that changes in each individual trabecula can be explored. By viewing the individual trabecular dynamics (ITD), we may gain insight into the mechanisms of combined PTH and ALN therapy. In addition, using computational modeling, this study aimed to determine the mechanical implications of combined therapy when compared to monotherapy. We hypothesized that combined treatment would result in enhanced structural repair and strength than PTH or ALN monotherapy. Furthermore, we expected that a small percent increase in bone volume by structural fortification would result in a significant strength enhancement.

Methods

24 3-mo-old SD rats were assigned to vehicle (Veh) (n=5), PTH (daily 60 μg/kg s.c. injections, n=6), ALN (50 μg/kg s.c. injections every 3 days, n=6), and PTH+ALN (both PTH and ALN treatments, n=7) treatment groups. *In vivo* μCT scans (VivaCT 40, Scanco Medical, 10.5 μm/voxel) were performed at baseline and after 12 days of treatment. A 1.575x1.575x1.05 mm^3 cube of trabecular bone was precisely registered between time points using several iterations of 3D image registration.

Individual Trabecular Dynamics (ITD) Analysis

Each registered and thresholded pair of baseline and follow-up images of trabecular bone was subjected to ITD analysis. Sites of structural deterioration (rod disconnection or plate perforation) and structural repair (rod connection or plate perforation filling) were identified and the percent occurrence was calculated as the number of occurrences of the above structural changes normalized by the total number of trabeculae analyzed (468 trabeculae/sample on average).

Finite Element Analysis (FEA)

Registered baseline and follow-up scans were converted to voxel FE models and subjected to axial compression tests using a customized software. An additional model was generated based on the baseline image and ITD analysis where only enhancements due to thickening were present, rather than structural repair identified by ITD. Stiffness was calculated for the baseline models, the models with enhancement due to thickening, and the models with both thickening and structural repair.

Statistical Analysis

All ITD and FEA measurements were compared using a one-way ANOVA between treatment groups. The relative increases in bone volume (BV) and stiffness due to structural repair or thickening were compared using a one-way ANOVA between groups.

Results

ITD

The tracking of individual trabecular structures' connectivity over the course of

Paper No: 0031
2014 Annual Meeting
Orthopaedic Research Society
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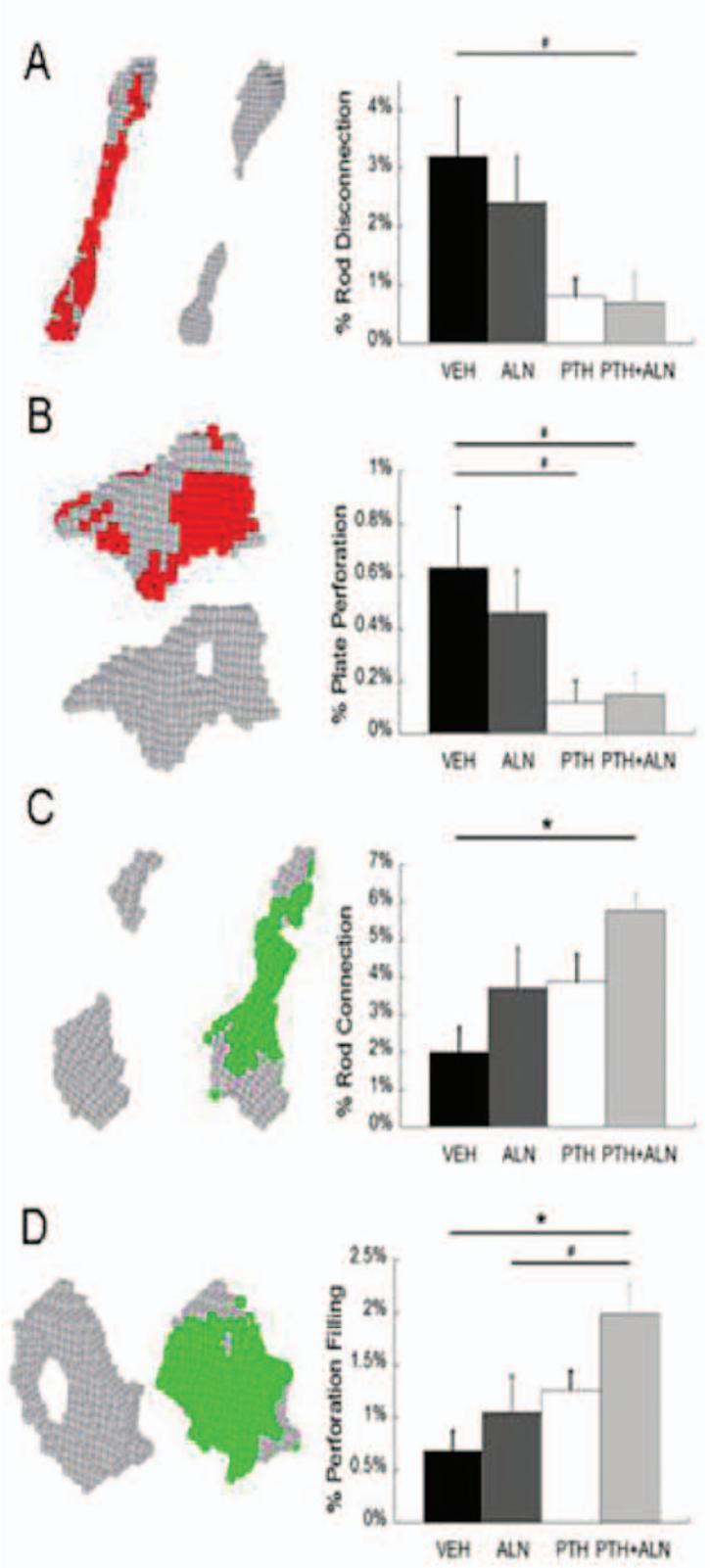

Figure 1. Comparison of percent occurrences of (A) rod disconnection, (B) plate perforation, (C) rod connection and (D) perforation filling. Red = Bone loss, and Green = new bone. *Significant differences ($p<0.05$) # trend differences ($p<0.1$).

treatment indicated several rod disconnections and plate perforations even in the Veh-treated trabeculae (3.2% and 0.6% of the total trabeculae respectively). In contrast, this was balanced by similar structural repair with 2.0% connected rods and 0.7% filled plates. In the ALN-treated group this balance was tipped towards structural repair with 3.7% connected rods, and 1.0% filled plates. Interestingly, the PTH-treated group had a similar amount of structural repair (3.9% connected rods and 1.3% filled plates) to that of the ALN group. In addition, the PTH group tended to have a lower incidence of plate perforation (0.1%, $p<0.1$) compared to the Veh group. The combined PTH+ALN-treated group tended to have similarly reduced rod disconnection (0.7%) and plate perforation (0.1%, $p<0.1$), and displayed significant structural repair beyond that of the Veh group (5.8% connected rods, and 2.0% filled plates), resulting in the greatest net gain in connectivity by both structural fortification and increased protection against lost connectivity (Figure 1).

FEA

There were significant increases in BV over time for all treatment groups (6.6% ALN, 25.5% PTH, and 35.8% PTH+ALN), which correspond to significant increases in stiffness over baseline (14.8% ALN, 46.6% PTH, and 87.4% PTH+ALN) (Figure 2). For all treatment groups, increases in BV due to thickening were far greater than those due to structural repairs, with 5.1% vs. 1.5% in ALN group, 23.9% vs. 1.7% in the PTH group, and 33.5% vs. 2.3% in the PTH+ALN group. Trabecular thickening itself caused 10.3%, 41.0%, and 75.1% increases in stiffness in the ALN, PTH, and PTH+ALN

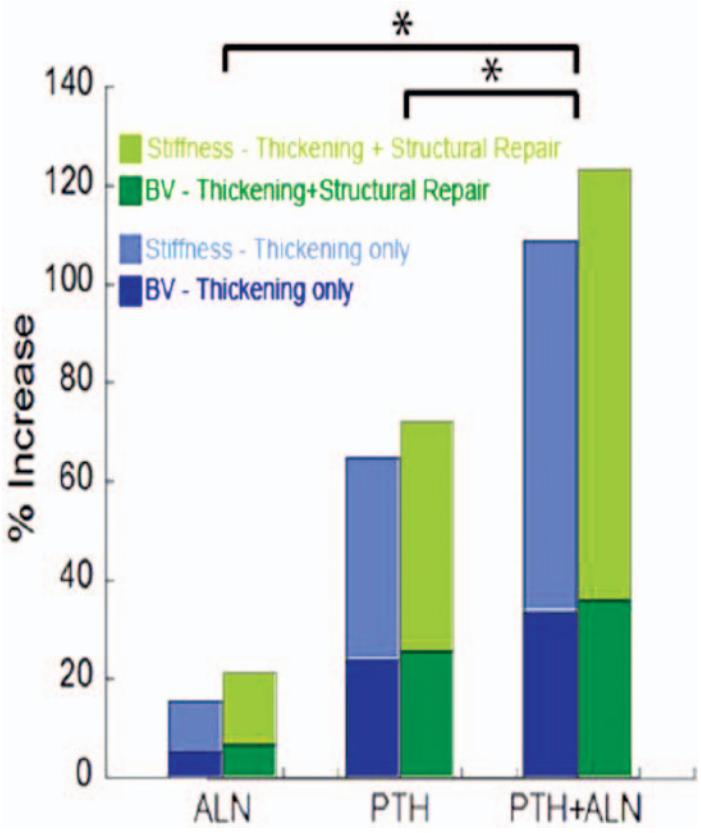

Figure 2. Comparison of relative increases in bone volume and stiffness due to thickening, and both, thickening and structural enhancements for each treatment group. Black* indicates differences in relative increases in stiffness due to structure alone between treatment groups ($p<0.05$).

groups, respectively. Moreover, the trabecular structural repair led to an additional improvement in stiffness, with the highest in PTH+ALN (by an additional 12.4%), which was significantly greater than either PTH (5.6%) or ALN (4.5%).

Discussion

Higher structural repair resulted in substantial increases in stiffness in the combined therapy group after only 12 days of treatment. The ITD results indicate similar amounts of structural repair in both monotherapies. The combined treatment group had enhanced structural repair, as well as reduced structural deterioration for both plate and rod structures, yielding the greatest net gain in trabecular integrity. In combination with our previous work, this suggests that combined therapy can more effectively repair damaged trabeculae despite a similar bone formation rate as those treated with PTH. The relative increase in bone volume due to structural repair did not differ between the treatment groups; however, the relative increase in stiffness in the combined therapy group was significantly higher than either monotherapy group, demonstrating the functional significance of structural repair. In conclusion, enhanced structural repair and reduced structural deterioration allow combined therapy to further improve the stiffness of the trabecular bone. This increased stiffness may help to enhance bone quality, and ultimately improve the bone's resistance to fractures.

Significance

Combined PTH and ALN therapy has the potential to more efficiently rescue trabecular structural deterioration and enhance structural repair over either monotherapy. Although associated with a minimal amount of new bone volume, rod connection and plate filling result in a significant increase in trabecular bone stiffness. Through such structural repair mechanisms, combination therapy showed an advantage in improving bone strength over either monotherapy.

Acknowledgments

NIH/NIAMSP30AR050950

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