PTH ENHANCES AND SUSTAINS MECHANICAL RESPONSIVENESS OF TRABECULAR BONE

Chi Hyun Kim, Byron M. Gaing, Mei Lin Chan, Erica Takai, Hua Zhou*, Dietrich von Stechow, Ralph Müller**, David W. Dempster*, and X. Edward Guo**

Bone Bioengineering Laboratory
Department of Biomedical Engineering
Columbia University
New York, NY 10027

* Regional Bone Center
Helen Hayes Hospital
West Haverstraw, NY 10993

** Orthopedic Biomechanics Laboratory
Beth Israel Deaconess Medical Center and
Harvard Medical School
Boston, MA 02215

INTRODUCTION

Trabecular bone adapts in response to its mechanical and hormonal environment and quantifying the mechanism of bone adaptation is necessary to understand the etiology of age-related fractures, develop optimal designs for total joint replacements, and study bone loss due to space flight. It has been suggested that bone loses its sensitivity to mechanical loads with age and this loss of sensitivity may lead to the lack of success of exercise in adults [1,2]. Parathyroid hormone (PTH) has been shown to be responsible for the mechanical responsiveness of bone [3]. With the recent U.S. Food and Drug Administration (FDA) approval of PTH, an anabolic agent, as a treatment method for osteoporosis, it is important to examine the effect of interaction between mechanical and PTH stimulation in increasing and sustaining the mechanical responsiveness of trabecular bone.

In this study, we examined the effect of PTH and mechanical loading on trabecular bone adaptation using an *in vivo* rat tail vertebra model combined with histomorphometry and 3D micro-CT based finite element analysis (FEA) [4,5]. Specifically, the dynamic bone formation indices were quantified in the overall trabecular bone region and the tissue level bone responses were directly correlated to the tissue level mechanical environment after 1, 2, and 4 weeks of combined treatment.

MATERIALS AND METHODS

Two-hundred-six 9-month-old male Sprague-Dawley rats (Harlan, IN) of approximately 500 g were randomly assigned to 21 treatment groups (7 treatment types X 3 treatment durations; N=8-12 per group). The 7 treatment types included 1) control, 2) vehicle+0N, 3) PTH+0N, 4) vehicle+50N, 5) PTH+50N, 6) vehicle+100N, and 7) PTH+100N. The 3 treatment durations consisted of 1) 1 week, 2) 2 weeks, and 3) 4 weeks. Under general anesthesia, threaded K-wires (Zimmer, IN) were inserted into the seventh (C7) and ninth (C9) caudal vertebra of all animals except those assigned to control groups [4]. Animals assigned for PTH treatment received *s.c.* 15 μg/kg/day of rat-PTH 1-34 (Bachem, CA) prepared in 1 mM acetic acid and those assigned for vehicle treatment received similar amounts of 1 mM acetic acid. Under general anesthesia, the eighth caudal vertebra (C8) of loaded animals were subjected to 5 minutes of compressive loading of either 50 N or 100 N using a sinusoidal waveform at 1 Hz using a custom-built loading device [4]. 0 N loaded animals were also placed on the loading device for the same period of time as the loaded animals with only the actuator turned off. All treatments were initiated 3 days post-surgery and performed 5 days per week. The K-wires were clamped immobilizing the eighth caudal vertebra (C8) for the entire duration of the experiment except during daily mechanical loading. All animals were double labeled *i.p.* with 20 mg/kg calcein and sacrificed 24 hours after the final treatment.

The sixth caudal vertebra (C6) and C8 from each animal were harvested immediately after sacrifice. The C8s from the animals assigned to mechanical loading treatment were scanned using a micro-CT system at 34 μm resolution. Each image was converted into a 3D specimen-specific model through voxel-to-element conversion for FEA [4]. Boundary conditions simulated either 50 N or 100 N uniaxial compressive loading, which corresponded to the *in vivo* load level. The trabecular tissue modulus was assumed to be 18 GPa and the Poisson's ratio 0.3 [6]. Tissue level strain energy densities (SED) were extracted for local trabecular regions (proximal, center, distal) of the vertebrae that corresponded to sections analyzed through bone histomorphometry [4].

The C6s and C8s from all animals were embedded undecalcified in methylmethacrylate and serial sections were cut using a microtome (Leica, Germany). 4 μm sections were stained with Goldner's Trichrome to determine the trabecular bone surface (BS) and 8 μm sections were used to quantify the labeled surface (LS) and mineral apposition rate (MAR) in the trabecular bone using bone histomorphometry software (Osteometrics, GA). Labeled surface percentage (LS/BS) and MAR were determined for the overall trabecular region as well as for the three local regions (proximal, center, distal). LS/BS and MAR of C8 from each specimen were normalized by LS/BS and MAR of the corresponding C6. The normalized values were then multiplied by the average LS/BS (or MAR) of C6s from each treatment group. Bone formation rate (BFR)

was calculated by multiplying the normalized LS/BS with the normalized MAR. Three sections were analyzed per specimen.

The overall trabecular bone responses to different types of treatment were assessed by analysis of variance (ANOVA) followed by the post-hoc Fisher's least significant difference test. The dynamic bone formation activities within each local trabecular bone region were correlated to the local mechanical microenvironments using a linear regression for non-PTH treated groups (V+0, V+50, and V+100 groups of each treatment duration pooled together) and PTH treated groups (PTH+0, PTH+50, and PTH+100 groups of each treatment duration pooled together). Significance was assumed at $p<0.05$. All statistics were performed using SYSTAT (SPSS Science, Chicago, IL).

RESULTS

The overall trabecular bone response showed significant increases in LS/BS, MAR, and BFR (LS/BS and MAR results not shown) with either mechanical loading or PTH treatment for 1 week compared to control or V+0 (Fig. 1). However, the level diminished after 2 weeks and was not different from control groups by the fourth week. Interestingly, when PTH was combined with mechanical loading (PTH+50 and PTH+100), significant increases in the dynamic bone formation indices were present and the level was sustained even after 4 weeks.

Significant direct correlations were established between the tissue level mechanical environment and the bone formation indices for both non-PTH treated groups and PTH treated groups after 1 and 2 weeks treatment (Fig. 2). However, after 4 weeks, the local mechanical environment no longer directly correlated to the bone response when mechanical loading was the only stimulus. On the other hand, when PTH and mechanical loading were combined, the direct correlation was sustained even after 4 weeks.

DISCUSSION

Bone formation was significantly increased using either mechanical loading or PTH for 1 week. However, the drop in BFR to the level of control animals after 4 weeks suggests that trabecular bone does not respond to mechanical loading or PTH after 4 weeks and there is a limit in treatment using either mechanical loading or PTH alone. Correlations between tissue level SED and BFR confirm the loss of mechanical responsiveness as the initially present direct correlation after 1 and 2 weeks is lost after 4 weeks when mechanical loading is the only stimulus.

Consistent with previous studies, combined *in vivo* mechanical loading and PTH stimulation shows synergistic increase in bone formation compared to stimulation by mechanical loading or PTH alone [3]. This increase is sustained throughout 4 weeks and the significant direct correlation between tissue level SED and BFR is present throughout the entire treatment duration of this study.

These results indicate that PTH, when combined with mechanical loading, not only elevates the level of bone formation but also helps sustain the increased level and thus the mechanical responsiveness of trabecular bone. This is an important finding in that future treatment for osteoporosis should be designed to incorporate both PTH treatment and an appropriate exercise program to maximize the outcome.

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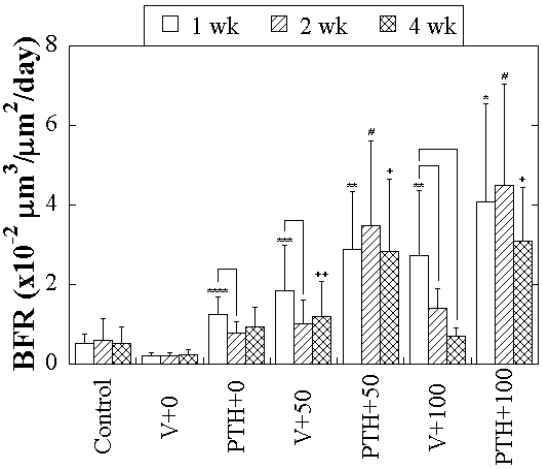

Figure 1. The overall trabecular bone response (BFR) to mechanical and PTH stimulation. The error bars represent the standard deviation and brackets among treatment groups represent significant differences ($p<0.05$). Significantly greater *v.s.* *: 1 wk Control, V+0, PTH+0, V+50, PTH+50, V+100; **: 1 wk Control, V+0, PTH+0; ***: 1 wk Control, V+0; ****: 1 wk V+0; #: 2 wk Control, V+0, PTH+0, V+50, V+100; +: 4 wk Control, V+0, PTH+0, V+50, V+100; ++: 4 wk V+0.

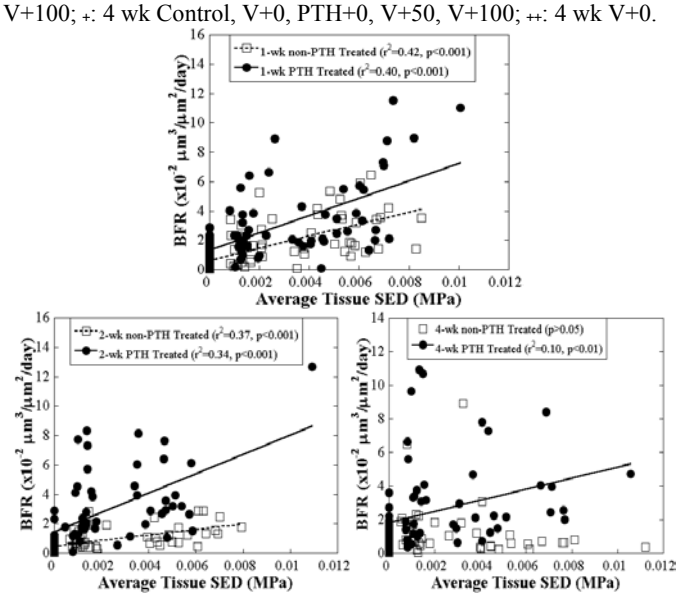

Figure 2. The correlations between regional BFR (top: 1 week, bottom left: 2 week, bottom right: 4 week) and trabecular bone tissue SED. Non-PTH treated groups consist of V+0, V+50, and V+100 groups, whereas PTH treated groups consist of PTH+0, PTH+50, and PTH+100 groups.

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