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TITLE: Influence of Bone Remodeling Inhibition on the
Development of Experimental Stress Fractures

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Progress Report**PROGRESS REPORT: DAMD 17-98-1-8515****ADMINISTRATIVE INTRODUCTION**

The grant was transferred in its entirety to Mount Sinai from the Henry Ford Hospital. We have resolved the major progress delays that resulted from the previous arrangement, and have completed all of the histomorphometric analyses that were planned in the original grant. Our results suggest that inhibiting bone resorption using a bisphosphonate has a positive effect on attenuating the stress fracture response in the rabbit model. After discussions with our program officer, Major Rachel Evans, PhD, we shifted a large number of the experimental bones that we were planning to test mechanically to histomorphometric analyses, in order to expand our histomorphometric component and increase our statistical power. In order to complete these extensive additional analyses, we were granted an additional no-cost extension of our project period through 31 November 2004.

INTRODUCTION

Stress fractures result from repetitive loading and have been regarded as a mechanical fatigue-driven process. However, histopathological data and experimental data from our laboratory suggests that increased remodeling precedes the occurrence of bone microdamage and stress fractures, suggesting a central role for increased intracortical remodeling in the pathogenesis of stress fractures. Thus, we propose that stress fracture occurs through a positive feedback mechanism, in which increased mechanical usage stimulates focal bone turnover, resulting in a local increase in porosity. Microdamage accumulation and stress fractures result from continued cyclic loading of this transiently osteoporotic bone. These experiments test the hypothesis that pharmacologically inhibiting the bone remodeling response will attenuate severity of the stress fracture response in an animal model (the rabbit tibial stress fracture model). To test the hypothesis that reactive remodeling within the cortex drives the development of stress fractures, the effect of remodeling suppression using a bisphosphonate on the accumulation of bone microdamage and diminishing the severity of stress fracture was examined. Outcomes were assessed using bone scintigraphy; histomorphometry and biomechanical studies are ongoing.

At the time of our last progress report, we noted that our data showed strong trends in terms of bone remodeling and microdamage changes in loaded tibiae after bisphosphonate treatment, but these were not statistically significant. We were concerned that there was higher variability among bisphosphonate animals than was projected in our original sample size calculations. After discussions with our program officer, MAJ Rachel Evans, we expanded our histomorphometric studies to increase our statistical power in order to definitively answer the question of whether anti-resorptive treatment will attenuate the experimentally induced stress fracture response. We shifted a large number of the experimental bones that we were planning to test mechanically (n=60) to histomorphometric analyses. Since biomechanics was a descriptive aim, but not a critical to testing our hypothesis, this change did not compromise our understanding the stress fracture physiology.

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SUMMARY OF RESEARCH

Our objectives in these experiments were to use the rabbit tibial stress fracture model:

- To determine at the whole bone level whether bisphosphonate inhibition of intracortical remodeling attenuates the increase in focal bone reaction at the periosteal surface.
- To determine at the tissue level whether bisphosphonate inhibition of intracortical remodeling decreases the accumulation of cortical bone microdamage which occurs at the site of stress fracture, and
- To determine how stress fracture compromises mechanical properties of long bones and whether pharmacological inhibition of remodeling can offset that functional deficit.

RESULTS:

Periosteal reactive bone volume – Our previous studies revealed considerable heterogeneity in the size and shape of reactive periosteal woven bone (i.e., “fracture” callus) along the length of the diaphysis, as well as between bones, making measurement from tissue sections problematic.

In order to better account for this heterogeneity, we developed a new CT-based approach to measure reactive periosteal bone volume for the entire diaphysis, rather than 2-D areal measurements from histological sections. This approach we used to determine reactive bone volume in the addition samples that were added to this study this past year.

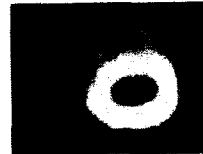
Method: Prior to histological studies, the additional bones incorporated into our studies were scanned through the diaphyseal length using a GE clinical CT scanner at 0.5 mm voxel resolution.

Figure 1: CT reconstructions showing reactive bone on tibial periosteal surfaces after 6 weeks of chronic loading

Vehicle treated, 6 wk loaded tibia



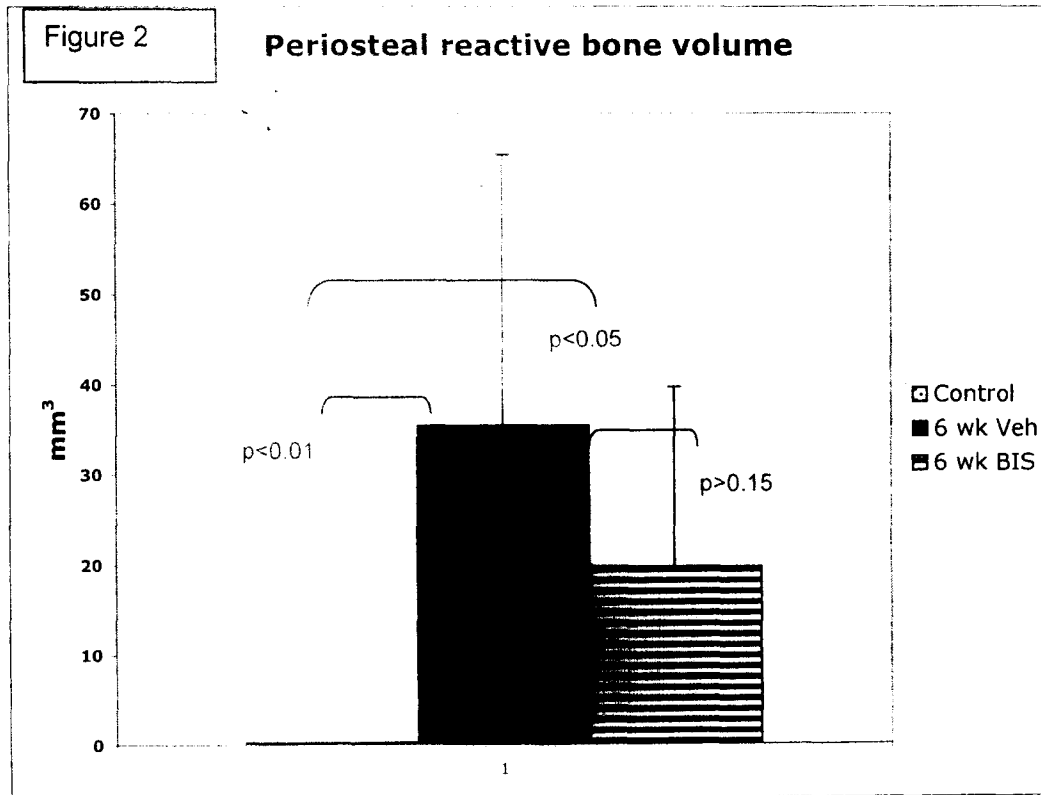
Bisphosphonate-treated, 6 wk loaded tibia



Thresholded diaphyseal cross-sectional images were examined. Reactive (woven) bone on the periosteal surfaces of loaded bones was readily distinguished from the pre-existing cortex. Custom software was developed in MatLab to allow measurement of reactive bone area and normal bone area from each section. This process was repeated at every millimeter at each level for which woven bone could be observed and summation of the reactive bone area over its longitudinal extent were used to determined volumes.

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Bisphosphonate treatment resulted in approximately 40 percent less periosteal reactive bone formation after 6 weeks of chronic loading compared to vehicle treated animals. However, these results were not statistically significant due to the very high variability among animals (coefficients of variation > 100 percent). Thus, while these trends support the hypothesis that suppressing resorption will reduce the severity of the periosteal stress reaction in chronic loading, the results are not definitive.



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Histomorphometry studies: We have now completed the histomorphometry studies on tibias from 92 rabbits subjected to repetitive impact loading for 6 weeks, in order to assess changes in internal remodeling and microdamage with and without antiresorptive treatment. Tibias were fixed in 70% ethanol. Diaphyses were bulk-stained in basic fuchsin, which both stains matrix damage and newly formed bone. After staining, diaphysis were embedded in MMA and serial cross-sections cut at 100µm thickness. Five cross-sections were chosen randomly from within the region of greatest periosteal reaction, and were subjected to histomorphometric analyses. Bone area, resorption space number and size and microcracks were measured.

After 6 weeks of daily loading, vehicle treated animals showed a nearly 3-fold increase in intracortical resorption (Figure 3,4). Bisphosphonate (BIS) treatment suppressed this activation of new intracortical remodeling to control levels, but those resorption spaces present in BIS-treated, loaded animals were significantly larger than those in normal bone.

Figure 3

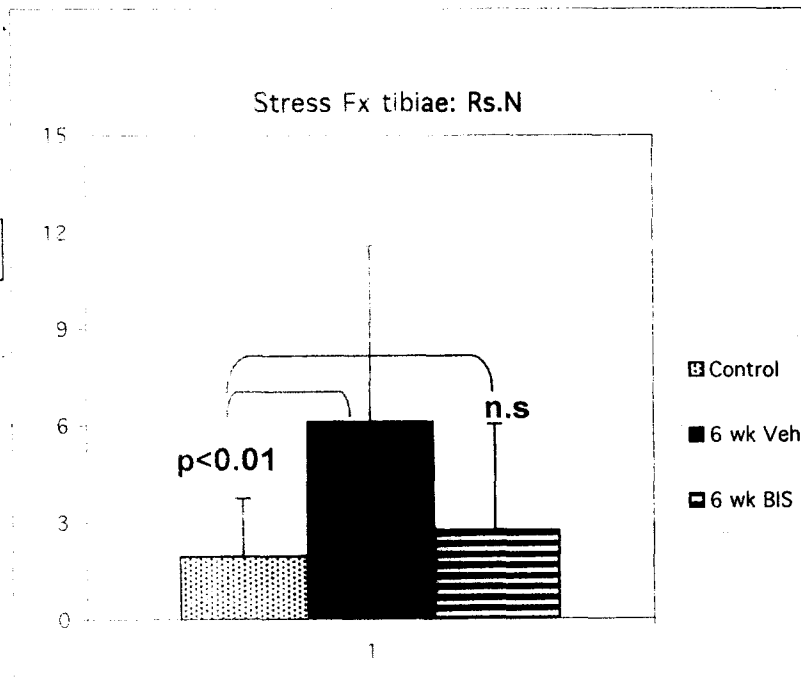
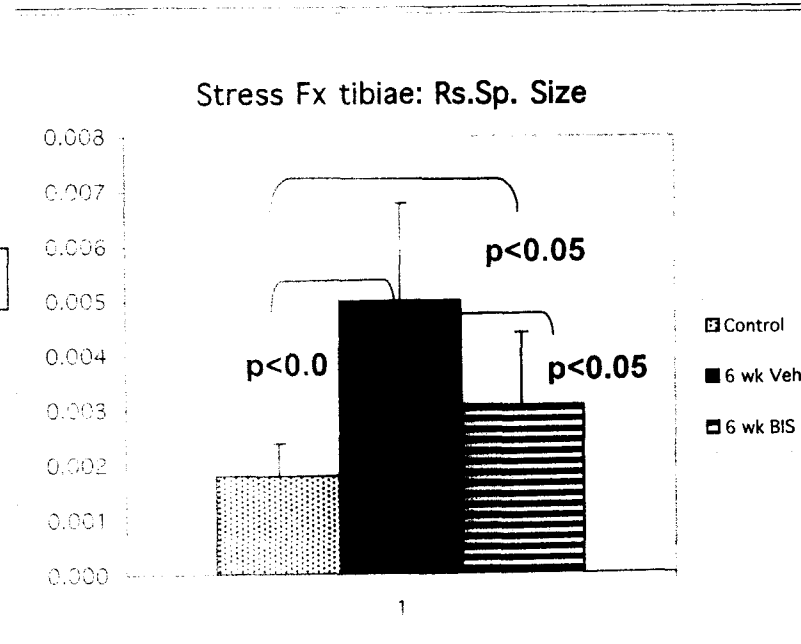


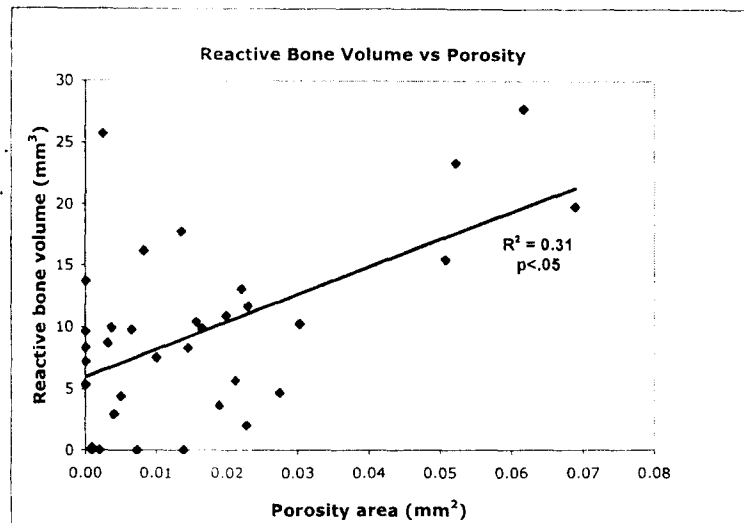
Figure 4



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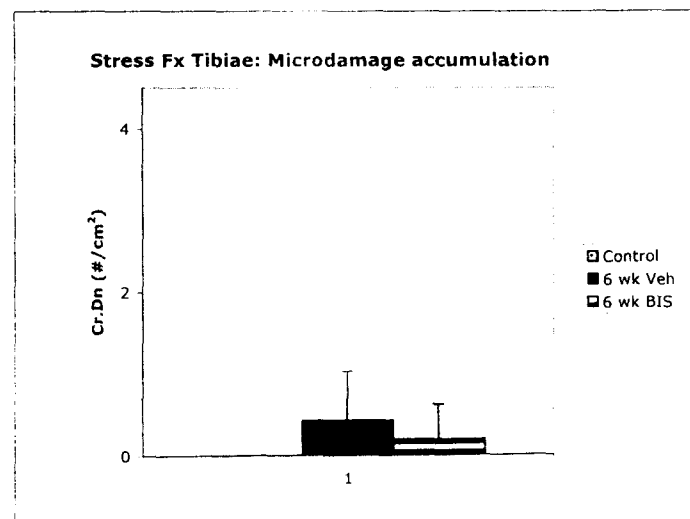
When all loaded (BIS- and Vehicle-treated) specimens were pooled (6 week specimens only, Figure 4), there was a significant correlation between the amount of periosteal reactive bone (CT-determined) and the resorption space (porosity) volume, i.e. diaphyses with more intracortical resorption formed more woven bone at the periosteal surface with chronic loading that did diaphysis with less resorption.

Figure 5



Microdamage: Overall, the amount of microdamage in loaded animals was small (typically less than 0.5 microcracks/cm²). BIS-treated tibiae showed approximately 50 percent fewer microcracks after 6 weeks of loading than did vehicle treated animals (Figure 6). However, these results were not statistically significant due to the very high variability among animals (coefficients of variation > 150 percent). Thus, while these are consistent with the hypothesis that suppressing resorption will prevent microdamage accumulation, the high degree of variability in this animal model prevents a definitive answer.

Figure 6



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Discussion:

Recently, Milgrom and Burr performed a 3-month duration bisphosphonate trial in Israeli army recruits and saw no effect on stress fractures based on ^{99m}Techetium scans. However, in our model, after 6 weeks of loading, bisphosphonate suppression of bone remodeling markedly reduced the amount of woven bone reaction (callus) at the periosteal surface. Further studies are needed to determine if this is a direct result of the treatment on bone formation processes versus an indirect effect, wherein the reduced intracortical porosity within the long bones of treated animals prevents locally elevated bone strain, which in turn prevents the formation of woven bone.

We have discovered a curious, but interesting problem in our loading system, which 1) potentially explains the confounding effects of the high variability in this model, and also 2) potentially provide important insights into the pathogenesis of the stress reaction in these animals.

- 1) First, we have found that some animals developed a extremely florid woven bone reactions during the chronic loading, and these animals occur in both bisphosphonate and vehicle treated groups. Interestingly, these animals appeared to have initially smaller tibial diameters than those that showed less extensive periosteal reactions. This is noteworthy as having a small diaphyseal cross-sectional diameter for body size is well established risk factor for stress fracture in military recruits.
- 2) Second, we found that the amount of periosteal reactive tissue in these smaller bones appears to have been large enough in size so as to interfere with the daily loading. Specifically, after several weeks of loading, these initially smaller tibiae enlarged enough in diameter so as to be compressed by the boot-like splint used to hold the limb during loading, thus superimposing a soft tissue injury onto the bone reaction.

Implications for future studies: Current studies support the hypothesis that increased intracortical porosity is associated with increased periosteal stress reaction in chronic loading. However, because of the higher than expected variability in the rabbit model, and a hitherto unappreciated soft tissue injury in some of the experimental animals, it was not possible to establish statistical significance in this model. These data argue for the development of a new animal model to resolve these concerns (see next section), and allow a more definitive test of the question of whether increased intracortical remodeling is a risk factors for adverse reaction of bone to stress.

New Model

To address concerns about high variability, soft tissue involvement in the rabbit model, we have developed a new model using the adult rat skeleton. This model reproduces the features of stress fracture in humans and in horses. In addition, the bone biology of the rat can be readily manipulated to assess the effect of biologically driven remodeling changes (and osteopenias) as well as gender and age issues in the development and reaction to stress fracture. This model is based on end-loading of the ulna in vivo, which produces a bending moment in the ulnar diaphysis. This model represents a new approach to simulating a naturally occurring fatigue fracture in vivo. This model achieves fracture through the development and

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coalescence of microdamage during controlled, fatigue loading. The loading magnitude per se is hyperphysiological, but it is comparable to that measured in human and horses during very vigorous activities. The loading conditions differ from those occurring during physiological loading, i.e. the load is applied via the flexed carpus and olecranon process. Nonetheless, the distribution of stress and strain along the longitudinal axis of the ulna approximates that occurring physiologically due to the bending moments incurred through axial compression of a curved bone. Furthermore, the fracture configuration is similar to that occurring spontaneously due to fatigue, e.g., as seen in the third metacarpus of racehorses or in the central metatarsal bone of race dogs. Finally, despite being an exogenous loading model, soft tissue surrounding the area of interest is protected from artifactual trauma and damage to the blood supply is minimized

For additional information, please see *Noninvasive fatigue fracture model of the rat ulna* A.E. Tami, P. Nasser, M.B. Schaffler and M.L. Knothe Tate *Journal of Orthopaedic Research* 21 (2003) 1018-1024

KEY RESEARCH ACCOMPLISHMENTS: No Cost Extension

1) Rabbit stress fracture experiments: The results suggest that antiresorptive therapy using a bisphosphonate will reduce the severity of the periosteal stress reaction in chronic loading.

2) New model for stress fractures and healing: Under the aegis of this grant we developed a new model for studying the biology and healing of stress fractures. The resulting fracture is stable and non-displaced and is similar in morphology to that occurring due to fatigue in vivo, e.g., as seen in the third metacarpus of racehorses or in the central tarsal bone of racing dogs. Studies with this new experimental model suggest that healing of stress fractures may differ from other fractures, a finding that may have clinical significance in their treatment. This model can readily be expanded to look at gender issues, age and to superimpose hormonal and pharmacological manipulations.

REPORTABLE OUTCOMES

1) The results to date suggest that antiresorptive therapy using a bisphosphonate reduces the intensity of the bone response to repetitive stress, as indicated by histological evaluation of experimentally loaded bones.

2) We have also developed a novel animal model simulating a naturally occurring fatigue fracture in vivo. This model achieves fracture through the development and coalescence of microdamage during controlled, fatigue loading. The resulting fracture is stable and non-displaced and heals entirely through intramembranous processes. The fracture morphology is similar to that occurring spontaneously due to fatigue in vivo, e.g., as seen in the third metacarpus of racehorses or in the central tarsal bone of racing dogs. Finally, soft tissue surrounding the area of interest appears to be uninjured.