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Local bone formation due to combined mechanical loading and intermittent hPTH-(1-34) treatment and its correlation to mechanical signal distributions ☆

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ABSTRACT

We evaluated the local response of cortical bone in the rat tibia due to combined treatment with synthetic parathyroid hormone, hPTH-(1-34), and mechanical stimulation by four-point bending. Forty-eight female retired breeder Sprague-Dawley rats were divided into six groups. Mechanically stimulated animals included the following groups: (1) Bend+PTH, (2) Sham+PTH, (3) Bend+Vehicle, (4) Sham+Vehicle. Non-mechanically stimulated animals included a (5) Control group that received neither loading nor injections, and a (6) PTH group that received only hPTH-(1-34) injections. The right limbs of mechanically loaded animals were exposed to a peak force of 50 N for 36 cycles at 2 Hz, three days per week for four weeks, and PTH-treated animals received injections equivalent to 50 μg/kg BW. Fluorochrome labeling was used to measure local formation at 12 sectors about the endocortical periphery. The distributions of endocortical bone formation were compared to the local formation differences between treatment groups and to a variety of potential mechanical stimuli signals. Results indicated that hPTH-(1-34) exerted a potent anabolic effect with near-uniform formation about the endocortical surface, and that localized formation peaks due to bending were further augmented in the presence of hPTH-(1-34) treatment. Correlation of formation patterns to mechanical signal distributions highlighted several candidate signals including the mid-principal stress, the dilatational strain, and the radial gradient of the local radial strain.

1. Introduction

Bone responds to multiple stimuli, including changes in mechanical environment (Wolff, 1892), variations in hormonal status, and treatment with pharmacologic agents. These stimuli may act independently or in concert to modify bone morphology, microstructure, and/or material properties. Whereas mechanical loading engenders a localized (site-specific) strain environment in bone, most non-mechanical treatments, such as pharmaceutical interventions, act systemically. As new drug treatments are developed to promote bone maintenance and formation, clarifying the interaction between mechanical and non-mechanical signals may suggest new combined treatment strategies.

Following extensive research in animals, synthetic human parathyroid hormone, hPTH-(1-34), has been approved for the treatment of human osteoporosis (Neer et al., 2001; Deal and Gideon, 2003). Experimental studies in rats have shown that intermittent hPTH-(1-34) treatment can exert a potent dose-dependent anabolic response in cortical and trabecular bone (Dempster et al., 1993; Gunness and Hock, 1993; Kimmel et al., 1993; Riond, 1993; Dobnig and Turner, 1997; Cosman and Lindsay, 1998) with concomitant increases in stiffness and bending strength (Ejersted et al., 1993; Mosekilde et al., 1995; Sato et al., 1997). The beneficial effects of hPTH-(1-34) treatment are also reported to be synergistically enhanced by concurrent mechanical stimulation (Chow et al., 1998; Ma et al., 1999; Hagino et al., 2001; Kim et al., 2003; Li et al., 2003).

Animal models of bone mechanotransduction have identified several important aspects of the load history that influence bone

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formation including static versus dynamic loading (Lanyon and Rubin, 1984; Rubin and Lanyon, 1984), peak strain magnitude (Rubin and Lanyon, 1985; Turner et al., 1994a, b), strain rate (O'Connor et al., 1982; Turner et al., 1995; Mosley and Lanyon, 1998), loading frequency (Turner et al., 1994a, b; Rubin et al., 2002; Tanaka et al., 2003; Warden and Turner, 2004), number of loading cycles (Rubin and Lanyon, 1984), and rest insertion (Robling et al., 2002; Srinivasan et al., 2002, 2003). However, the identification of suitable localized, strain-based mechanical signals for use in predictive mathematical models of adaptation is still an open question (Hart, 2001), as experimental bone formation data is seldom reported in a site-specific manner analogous to stress/strain measures. The continued development and validation of predictive mathematical models will depend, in part, on efforts to report site-specific mappings of new bone formation from mechanical loading experiments so that spatial and temporal input–output relationships may be explored (Brown et al., 1990; Qin et al., 1996, 1998, 2003; Gross et al., 1997; Mosley et al., 1997).

The purpose of the present study was two-fold. First, in order to assess the localized interaction between mechanical stimulation and hPTH-(1-34) treatment, we measured the site-specific distribution of new bone formation about the endocortical surface of the rat tibia following exposure to four-point bending and hPTH-(1-34) injections, each applied independently or in combination. The second aim was to compare these endocortical bone formation mappings to a library of candidate mechanical signals generated from a three-dimensional finite element model of the rat tibia and quantify the correlation between observed bone formation and various mechanical signal profiles.

2. Materials and methods

2.1. In vivo animal experiment

Forty-nine Sprague-Dawley female retired breeder rats (aged 10–11 months, approximately 300 g body weight) were acclimated for two weeks and divided into six groups. Four groups (n=8 each) were mechanically treated using a four-point bending device (described below) and were designated as follows: (1) Bending+Vehicle injection, (2) Bending+PTH injection, (3) Sham Bending+Vehicle injection, and (4) Sham Bending+PTH injection. Two additional groups were studied to establish base-line apposition values over the experimental period, in the absence of external mechanical stimulation: a non-treated control group (n=8) and a PTH treatment only group (n=9). All procedures were approved by the Institutional Animal Care and Use Committee of Tulane University.

In vivo mechanical loading of the right tibiae was applied using a custom built four-point bending apparatus (Fig. 1) modeled after the four-point bending apparatus described by Turner et al. (1991) (Akhter et al. 1992; Bober, 1996). The animals were treated three days a week (Monday, Wednesday, Friday) over four weeks and allowed normal cage activity between treatment sessions. This roughly alternate day loading schedule has been shown to be as effective as consecutive daily loading (Raab-Cullen et al., 1994). In a given treatment session, the animals were anaesthetized by ether inhalation and subjected to a 10 to 50 N peak-to-peak load applied at 2 Hz for 36 cycles, with the loading pads positioned to induce a uniform bending moment of 150-Nmm at peak cycle. In the sham configuration, the upper and lower loading pads were aligned opposite to each other, to produce a negligible bending moment during the 10 to 50 N load application (Turner et al., 1994a, b). Thirty to forty-five minutes prior to loading, each rat was weighed and given a subcutaneous injection of vehicle (sham) or hPTH-(1-34) equivalent to 50 μg/kg BW. This timing ensured that the serum level of the drug was near maximal concentration at the time of external load application (Chow et al., 1998). To fluorescently label new bone formation, calcein (7 mg/kg BW i.p.) was given on the Friday of weeks 1 and 2 and alizarin complexone (20 mg/kg i.p.) on weeks 3 and 4. Animals were sacrificed by CO_2 inhalation four days after the final fluorochrome injection.

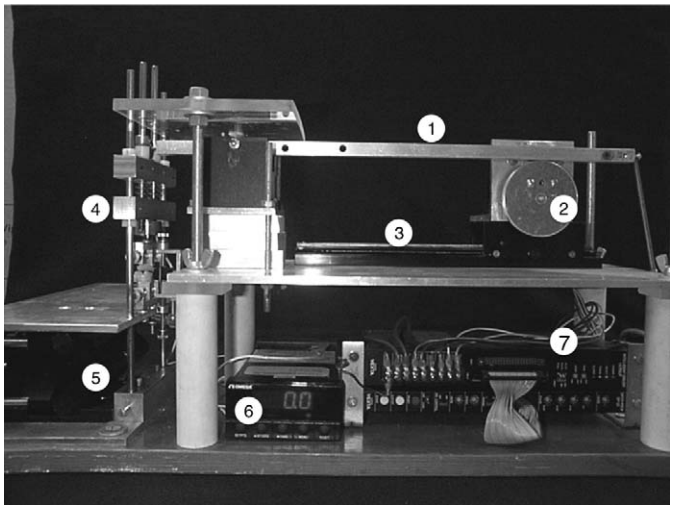

Fig. 1. The four-point bending apparatus is an open loop system consisting of the following components: (1) lever arm, (2) offset circular cam attached to a stepper motor, (3) horizontal track upon which the cam/motor system may be positioned and locked, (4) loading assembly with compression springs and a load cell (driven by the lever arm), (5) vertically adjustable stage where the rat is placed, (6) digital display for load cell output and (7) stepper motor control unit.

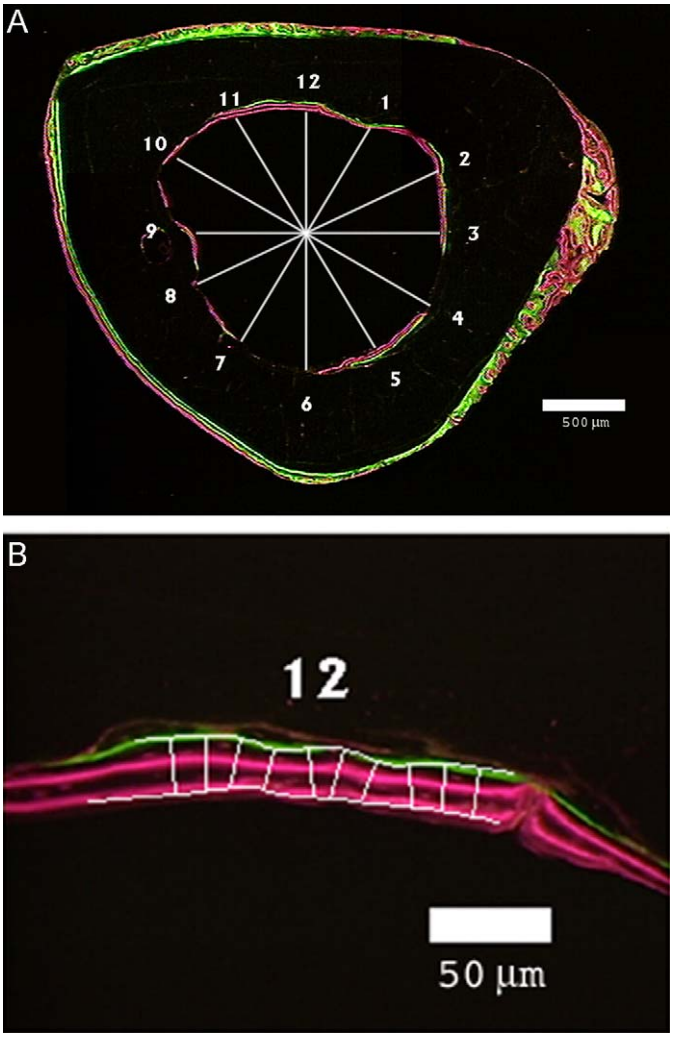

Fig. 2. (A) The endocortical surface was partitioned into 12 measurement sectors by imposing a radial grid onto the low magnification image. The average width across all adjacent fluorochrome labels in a sector was measured by marking points along the inner and outer bounding labels. (B) The average perpendicular distance between these labels was calculated using custom software.