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In vivo reference point indentation reveals positive effects of raloxifene on mechanical properties following 6 months of treatment in skeletally mature beagle dogs

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ABSTRACT

Raloxifene treatment has been shown previously to positively affect bone mechanical properties following 1 year of treatment in skeletally mature dogs. Reference point indentation (RPI) can be used for *in vivo* assessment of mechanical properties and has been shown to produce values that are highly correlated with properties derived from traditional mechanical testing. The goal of this study was to use RPI to determine if raloxifene-induced alterations in mechanical properties occurred after 6 months of treatment. Twelve skeletally mature female beagle dogs were treated for 6 months with oral doses of saline vehicle (VEH, 1 ml/kg/day) or a clinically relevant dose of raloxifene (RAL, 0.5 mg/kg/day). At 6 months, all animals underwent *in vivo* RPI (10 N force, 10 cycles) of the anterior tibial midshaft. RPI data were analyzed using a custom MATLAB program, designed to provide cycle-by-cycle data from the RPI test and validated against the manufacturer-provided software. Indentation distance increase (IDI), a parameter that is inversely related to bone toughness, was significantly lower in RAL-treated animals compared to VEH (−16.5%), suggesting increased bone toughness. Energy absorption within the first cycle was significantly lower with RAL compared to VEH (−21%). These data build on previous work that has documented positive effects of raloxifene on material properties by showing that these changes exist after 6 months.

Introduction

The ultimate goal in treating patients with osteoporosis is to prevent fracture. How best one achieves this goal can be debated, but it is clear that increasing bone's mechanical properties is an essential component of any treatment regimen. Although we know mechanical properties are important, the challenge lies in their clinical assessment. Most often, bone mineral density (BMD) is used as a surrogate for fracture risk (and by extension bone mechanical properties) but the limitations of BMD on an individual patient basis are clear [1]. One example of this discordance between BMD and fracture risk is observed with raloxifene, which minimally affects BMD yet significantly reduces fracture risk [2,3].

Reference point indentation (RPI) has been recently introduced to the field as a tool for assessing mechanical properties of bone [4]. Pre-clinical studies have documented that a strong correlation exists between RPI outcomes, such as indentation distance increase (IDI) and mechanical property variables (modulus of toughness) estimated by three-point bending mechanical tests [5]. Although the device can be used on specimens *ex vivo*, the novel and exciting aspect is its potential application *in vivo*. Clinically, RPI-assessed IDI has been shown to distinguish between fracture and non-fracture patients [6,7].

Previous work in our laboratory has documented that raloxifene, a selective estrogen receptor modulator, produces a positive effect on the intrinsic biomechanical properties of bone tissue, both cortical and cancellous, independently of bone mass after 1 year of treatment [8,9]. The goal of this study was to use RPI to test the hypothesis that raloxifene-induced improvements in material mechanical properties exist after 6 months of treatment.

Methods

Experimental design

Twelve skeletally mature female beagles (1–2 years old) were separated into two groups (n = 6 per group) by matching body weights. Dogs were treated daily with either oral vehicle (saline, 1 mL/kg) or raloxifene (0.5 mg/kg). Raloxifene was dissolved in 10% hydroxypropyl-β-cyclodextrin and administered at a dose consistent with the clinical

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management of post-menopausal osteoporosis. This dose has been shown previously to alter mechanical properties in this animal model following 1 year of treatment [8,9]. After 6 months of treatment, all animals underwent *in vivo* mechanical property testing using RPI. As this study is part of a larger experiment, these same animals are continuing treatment and will be sacrificed after 12 months of treatment. All procedures were approved by the Indiana University School of Medicine Animal Care and Use Committee prior to the start of the study.

Reference point indentation (RPI)

The material-level mechanical properties of the anterior surface in the mid-diaphysis of the tibia cortex were assessed *in vivo* using RPI (Biodent Hfc, Active Life Scientific, Santa Barbara, CA). This site was chosen as it has been utilized previously in human *in vivo* testing [6,7], and its limited soft tissue coverage facilitates easy access to the bone surface. The remodeling rate at this bone site is not known, yet the distal tibia at this age has an intracortical remodeling rate of ~1–2%/year [10] and <5% of the periosteal surface actively forming bone. Dogs were placed under general anesthesia using intravenous propofol and the skin over the right anterior tibia was shaved and aseptically prepared. The tibia mid-diaphysis was identified as the linear midpoint between the superomedial margin of the medial tibia condyle and the distomedial margin of the medial malleolus. A local anesthetic was injected just beneath the skin in the region of testing, just proximal to the midpoint of the tibia. Skin overlying the region was pierced with a sterile BP1 probe contained within the measurement head unit (MHU) attached to a modified holder apparatus (Supplementary Fig. 1). The MHU was lowered vertically, normal to the surface of the bone, until the probe assembly rested on the bone surface (Supplementary Fig. 2). The periosteum was scraped from the underlying cortex by moving the reference probe across the bone surface. After removal of the periosteum, the reference probe was positioned, a reference force of ~13 N was applied to stabilize the MHU, and the measurement protocol was initiated. Measurements began with a series of four preconditioning cycles at a force of 1 N and a frequency of 5 Hz, and concluded with a series of 10 testing cycles at 10 N and 2 Hz. Up to five measurements, within a few mm of each other, were collected on each animal. If a test was found to be unusable during the live animal testing, a replacement was run. In cases where the data were found after the fact to be implausible (for instance a negative IDI that was not caught during the in vivo test), it was not used in the analysis leaving some animals with less than five tests. The coefficient of variation within each animal is presented in Supplementary Table 1. The animals were conscious and mobile within 30 minutes post-testing. There was no sign of pain or discomfort based on pain scoring taken within the first 8–12 h post test, and then again 24 h post test.

MATLAB code

Raw data output from the RPI analysis software (version 2.0) were imported into a customized MATLAB code (Mathworks). The code was internally developed to supplement the RPI software by providing cycle-by-cycle data, which is not available in the manufacturer-supplied software. For example, the manufacturer software provides averages for the unloading slope and energy parameters between cycles 3 and 10. We were interested in the actual values for these parameters (not the averages) and also what the values looked like in the first cycles. To develop the code, both force versus time and distance versus time data were used to produce the points associated with each cycle's curve, from which primary parameters were determined (see Supplementary Fig. 3). The code was validated by comparing its output to the standard RPI analysis software for first cycle indentation distance (ID), total indentation distance (TID), indentation distance increase (IDI), first cycle unloading slope (US), average unloading slope (cycles 1–10), and average energy dissipated (cycles 3–10). Upon validation, the MATLAB program was used to analyze RPI outcomes between the two treatment groups. Primary variables of interest from the MATLAB program are outlined in Fig. 1 and Table 1.

Statistics

RPI data were evaluated using one-tailed independent samples *t-tests* because prior experiments consistently showed improvement

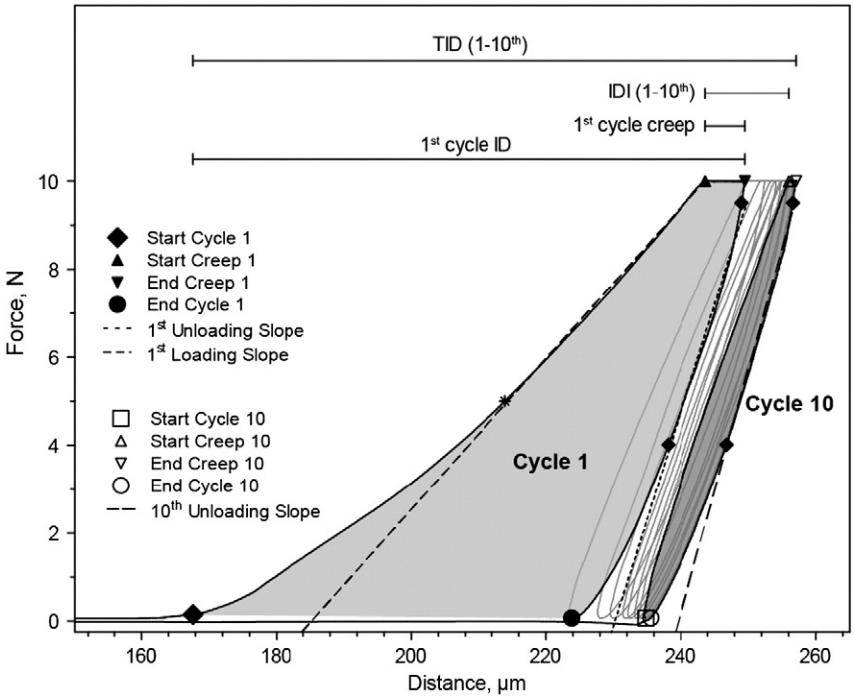

Fig. 1. RPI output from custom MATLAB code used to analyze cycle-by-cycle data. Cycles one and ten are highlighted for reference yet all cycles were included in the analysis.