OsteoArthritis and Cartilage (2005) **13,** 790–797

doi:10.1016/j.joca.2005.04.015

Osteoarthritis and Cartilage

International Cartilage Repair Society

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Bisphosphonates reduce bone mineral loss at ligament entheses after joint injury[1]

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Summary

Objective: To examine the effects of anterior cruciate ligament (ACL) insufficiency, and subsequent bisphosphonate (BP) antiresorptive therapy, on the bone mineral interface at the enthesis of remaining ligamentous restraints.

Methods: We measured bone mineral geometry (and subsequent adaptation) at the medial collateral ligament (MCL) origin, using micro-computed tomography (μCT). Groups of normal control, 6 and 14 wk anterior cruciate ligament transected (ACLX), and 6 wk ACLX–BP (risedronate) dosed rabbits were evaluated. Samples were then processed histologically, and the results of mineral adaptation and progression of osteoarthritis (OA) compared to joint laxity values obtained from previous biomechanical testing of the MCL-complex.

Results: μCT defined the MCL origin as a symmetrical, metaphyseal depression that contained soft-tissue elements, including fibrocartilage and ligament—as seen in subsequent histological sections. In contrast, the insertions from ACLX animals lost significant bone mineral, with an MCL-insertion volume 1.2 times that of normal controls at 6 wk ACLX, which further increased to 2.3 times that of normal controls at 14 wk ACLX. Significant differences were also measured between 6 and 14 wk ACLX and age-matched normal controls in volume of cortical bone containing the MCL insertion. However, there were no significant differences in the percentage of cortical bone to underlying trabecular bone at the MCL insertion. When comparing μCT mineral adaptation at the MCL-enthesis with historical MCL-complex laxity data, the values for laxity after ACLX increased proportionately as bone mineral at the insertion was lost, and subsequent use of the BP risedronate reduced both mineral loss and MCL-complex laxity.

Conclusion: Compared to the untreated ACLX condition, administering bisphosphonate immediately after loss of the ACL conserved bone mineral at the MCL enthesis, suggesting the potential to therapeutically influence joint-complex laxity and OA progression.

Key words: Osteoarthritis, Risedronate, Micro-computed tomography, Enthesis, Medial collateral ligament, Anterior cruciate ligament transection, Joint laxity.

Introduction

Following anterior cruciate ligament (ACL) rupture in the knee, a rapid phase of periarticular bone loss often occurs, prior to the subchondral sclerosis of end-stage osteoarthritis (OA)[1–3]. That loss occurs, in part, as both trabecular and cortical bone remodel to adapt to altered loading, but also due to the reduced limb usage and related disuse osteopenia. In animal ACL-transection (ACLX) models, significant decreases in femoral and tibial bone mineral density (BMD) were measured for both dogs[1] and rabbits[4], particularly in periarticular cancellous bone. In human studies, the loss of bone mineral in the initial stages after ACL disruption has been well established[5–7], and was measured in the distal femur as a 21% decrease in BMD[5].

As chronic knee instability (and progression in joint passive laxity) often results in the bone–ligament joint complex after loss of the ACL[8,9], we questioned whether the loss of bone mineral at the ligament insertions (or entheses) was a contributing factor to increased joint laxity. We have recently shown that bisphosphonate (BP) antiresorptive therapy can significantly reduce passive laxity in the bone–medial collateral ligament (MCL)–bone complex after ACLX, suggesting that bone mineral adaptation at the MCL insertion was influenced with antiresorptive therapy[10]. We define MCL-complex laxity as the distance between tension and compression of the femur–MCL–tibia complex during axial loading.

Thus, the objective of this study was to assess in an OA model whether antiresorptive therapy altered mineral loss at the bone–ligament insertion. We examined and quantified the changes in bone mineral at the femoral origin of the MCL, using micro-computed tomography (μCT). We also

[1]This research was funded by the Canadian Institutes of Health Research. Presented in part at the Ostearthritis Research Society International (OARSI) Congress in Berlin, Germany, October 2003, and printed in Osteoarthritis and Cartilage 2003;11:S85, P236 (abstract).

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Received 4 November 2004; revision accepted 18 April 2005.

investigated whether short-term antiresorptive therapy (i.e., with risedronate BP) altered bone mineral changes at the MCL enthesis.

Materials and methods

SAMPLES

A total of 28 samples, harvested from the medial femoral condyle of age-matched 1 yr old skeletally mature New Zealand White rabbits, were analyzed. All animals were sourced from the same supplier (Riemens Fur Ranches, St. Agathe, ON) and randomly divided into four groups.

We surgically transected the ACL in three experimental groups of rabbits. The first and second groups remained untreated for 6 wk ($n = 8$) and 14 wk ($n = 6$), respectively, and served as age-matched ACLX controls. The third group was dosed (0.01 mg/kg s.c.) daily with risedronate BP for 6 wk ($n = 6$). The final group of rabbits was evaluated as age-matched, unoperated normal controls ($n = 8$).

Animals were sacrificed by barbiturate overdose, and the femur–MCL–tibia complex was dissected free. The MCL was completely transected in its midsubstance, leaving a portion of the MCL at the surface of the insertion as a landmark. The medial and lateral femoral condyles were separated using a bandsaw, and the femoral epiphysis cut in the transverse plane proximal to the patello-femoral groove (Fig. 1). Samples were placed into a polystyrene vial, capped to reduce dehydration, and held in an upright position using a foam jig to standardize the transverse scanning plane for subsequent samples.

MICRO-COMPUTED TOMOGRAPHY (μCT) OF CALCIFIED MINERAL

The medial femoral condyle was scanned in its entirety, using an X-ray microtomograph (μCT, SkyScan 1072, Aartselaar, Belgium). The condyles were scanned at 100 kV through 180° with a rotation step of 0.9°. All samples were scanned at 12× magnification that produced serial cross-sectional images composed of isotropic 19.4 mm^3 voxels. A low-pass Gaussian filter was used to filter the raw image data to reduce noise, and a fixed threshold was used on all samples to extract the mineralized tissue phase. The filtered binary image data were then exported to SCION IMAGE (Scion Image Pty Ltd, Frederick, Maryland, USA) for 2-dimensional (2-D) morphometric measures or to ANALYZE TM 4.0 (Mayo Clinic, Rochester, MN, USA) for 3-dimensional volumetric rendering. Measured indices included cortical bone volume of the insertion, MCL-insertion volume (i.e., space occupied by soft tissue), and percentage of cortical bone to trabecular bone at the MCL insertion.

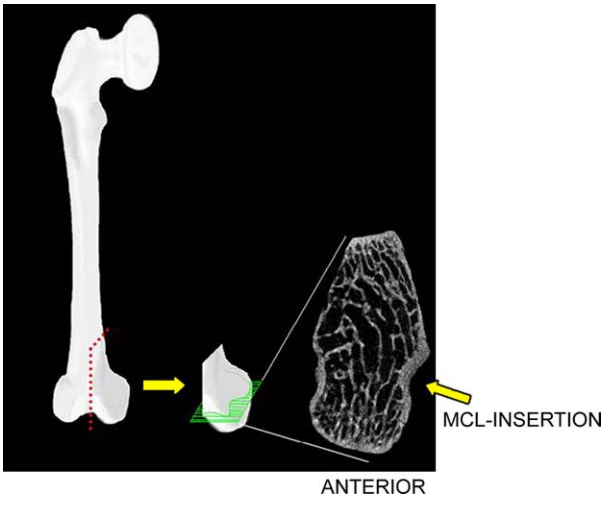

Fig. 1. Diagram illustrating isolation of the medial femoral condyle (red dotted line), and the transverse plane of subsequent μCT slices (green plane). The MCL insertion appeared as a cortical depression.

MORPHOMETRIC MEASUREMENTS (FROM 2-D μCT IMAGES)

MCL-insertion volume

Lines were drawn parallel to the postero-medial and antero-medial surfaces of the medial femoral condyle. The points of intersection between these lines and the outermost region of cortical bone containing the MCL insert were connected. The area defined by that line and the cortical bone containing the MCL insert was defined as the MCL insert area. That process was repeated for every section containing the MCL insertion, summed, and multiplied by μCT section thickness to achieve MCL insert volume. Peri-insertional accreted bone and osteophytes were not included in measurements (Fig. 2).

Sampling of cortical bone volume and percentage cortical/trabecular bone

Perpendicular to the line connecting the anterior and posterior limits of the MCL insert in the transverse plane, a line was extended from the deepest point of the MCL insert. From this newly defined intersection, an arc was drawn 100° from the posterior-medial line, with a radius of 5.25 mm. The region of interest was defined as the area enclosed by this arc. The cortical bone within this arc was separately defined as a region of interest. The area defined by the cortical bone region of interest multiplied by section thickness was defined as the volume fraction of cortical bone containing the MCL insert. The ratio of the volume of cortical bone to the volume of the arc was defined as the percentage of cortical bone defining the MCL insert. This procedure was undertaken for the deepest point of the insert, as well as the twentieth and fortieth sections both proximally and distally to the deepest point of the insert. The measurements from the five sections were summed.

HISTOLOGICAL ASSESSMENT OF FOCAL CARTILAGE DEGRADATION

Following the 30 min μCT scans, bone samples were fixed in 10% neutral buffered formalin for 72 h. Following decalcification in formic acid (Cal-Ex II, Fisher Scientific, ON) for 1 wk, and dehydration in ETOH/xylene, samples were paraffin embedded. Frontal sections (7 μm) were cut using a rotary microtome, and stained using haematoxylin and eosin and safranin-O/fast green, to examine the MCL insertion tidemark characteristics and to grade articular cartilage degradation. For all investigations, a modified Mankin grading system[11,12] (Table I) was used to characterize the superficial surface structure, cellularity, Safranin-O staining characteristics, and tidemark integrity. When examining the histology, the single observer was blinded as to the treatment.